PRE-HARVEST GLYPHOSATE TIMING IN OATS AND FINAL OAT QUALITY

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Pre-harvest glyphosate is often applied to cereal crops, such as oats, to insure uniform grain ripeness at harvest. However, some buyers have claimed that this practice negatively affects oat end product quality. Oat samples were grown in two different growing locations for each of two crop years, and glyphosate was applied at the soft dough, physiological maturity, or not applied. Groat quality and starch quality parameters were analyzed, and rolled oats were produced to analyze end product quality. Groat hardness, groat percentage, and percent plump groats were significantly ($P<0.05$) affected by glyphosate application at the soft dough stage. However, application of glyphosate at physiological maturity did not appear to detrimentally affect groat starch or end product quality. Therefore, pre-harvest glyphosate application is appropriate for oats providing it occurs after plants reach physiological maturity.

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LIST OF ABBREVIATIONS

AMPA	Aminomethylphosphonic Acid
DV	Daily Value
EPA	Environmental Protection Agency
EPSPS	5-Enolpyuvyl-Shikimate-3-Phosphate Synthase
LSD	Least Significant Difference
NIR	Near-Infrared
RVA	Rapid Visco Analyzer
SAS	Statistical Analysis Software
SKCS	Single Kernel Characterization System

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INTRODUCTION

Glyphosate

Glyphosate is the most frequently used herbicide in the world (Benbrook, 2016). In the year 2014, 113.4 million kilograms of glyphosate were applied to crops. Its usage has been increased 15-fold since 1996, when glyphosate-tolerant crops were introduced (Benbrook, 2016).

Glyphosate is considered a broad spectrum herbicide, and thus is effective against a wide variety of weeds (Duke and Powles, 2008). Glyphosate inactivates the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase, which is utilized in the shikimate pathway of plants. Inactivation of this enzyme inhibits the production of aromatic amino acids, which leads to the death of the plant. Since the shikimate pathway is not present in animals, glyphosate is considered non-toxic: the LD_{50} for rats is greater than 5 g kg^{-1}. However, some studies suggest that glyphosate usage may contribute to environmental toxicity and may act as endocrine disruptors in humans (Annett, Habibi, et al., 2014, García-Pérez, Alarcón-Gutiérrez, et al., 2014, Gasnier, Dumont, et al., 2009).

In addition to its use as an herbicide, glyphosate is used as a pre-harvest desiccant on cereal crops such as oats and wheat. This practice kills the plant and allows for uniform drying before harvest. However, there is some debate on whether this practice causes a decrease in quality in crops. Some companies, such as Grain Millers Inc, no longer will purchase oats treated with pre-harvest glyphosate (Cross, 2016).

Oats

64 million bushels of oats were produced in the United States in 2016 (United States Department of Agriculture, 2017). Although the majority of oats produced are used for animal

feed, rolled oat flakes continue to be a popular breakfast choice due to reported health benefits, such as a cholesterol lowering effect (Whitehead, Beck, et al., 2014).

To many consumers, the most recognizable form of oats is the rolled oat flake, which is a popular hot cereal breakfast choice. To produce rolled oat flakes, oat kernels harvested from the field are first cleaned to remove foreign material (Decker, Rose, et al., 2014). Next, the tough, indigestible outer hull is removed, leaving the edible groat behind. Since the groat contains 6-8% fat, compared to the 2-3% found in most other grains, and high levels of lipases, the oats must next be steamed and kiln dried in order to inactivate enzymes and prevent spoilage (Stewart and McDougall, 2014). Additionally, this step results in Maillard browning of the groat, which produces desirable flavors. Depending on the size of rolled oats desired, the oats can then be cut two to four times to produce quick cooking or instant oats or left intact for production of rolled oats. Finally, whole groats or groat pieces are flaked by first steaming the groats to prevent crushing, and then passing them through rollers to produce a flat flake.

LITERATURE REVIEW

Glyphosate

Glyphosate [*N*-(phosphonomethyl)glycine] is a phosphonate derivative of glycine. It is the most commonly used pesticide in the United States and likely, globally (Benbrook, 2016). The volume of glyphosate being used is increasing, mostly due to the development of glyphosate-resistant crops.

Glyphosate was originally synthesized in 1950 by the Swiss chemist Dr. Henri Martin, who was performing pharmaceutical research (Dill, Sammons, et al., 2010). However, its herbicidal activity remained undiscovered and the compound was never developed for commercial use. In the 1960s, the biotechnology company Monsanto synthesized AMPA analogues, which are related to glyphosate, to test as potential water softening agents. The compounds were also tested for herbicidal activity, which was found in low unit activity in two of the compounds. Dr. John Franz began working on synthesizing analogues and derivatives, and in 1970, first synthesized glyphosate. After field testing, glyphosate was introduced to the market under the name Roundup in 1974.

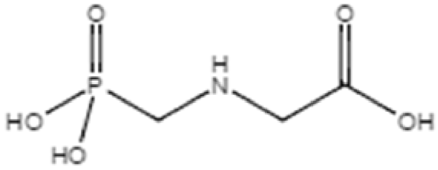

Figure 1: Chemical Structure of Glyphosate

Mechanism

Glyphosate acts on the plant enzyme 5-enolpyuvyl-shikimate-3-phosphate synthase (EPSPS) of the shikimate pathway found in plants (Duke and Powles, 2008). Glyphosate is a transition state analog of the EPSPS substrate phosphoenolpyruvate and thus can bind to EPSPS, acting as a competitive inhibitor. Inhibition of EPSPS prevents the catalysis of shikimate-3-phosphate and phosphoenolpyruvate to 5-enolpyruvyl shikimate-3-phosphate, which is detrimental to the plant in two ways. First, disruption of the shikimate pathway leads to the inability to synthesize the compound chorismate, which is a precursor necessary to produce the three aromatic amino acids Without chorismate, these amino acids cannot be produced. In addition, glyphosate application is shown to reduce carbon fixation in plants (Servaites, Tucci, et al., 1987). This is likely due to an absence of feedback inhibition caused by the decreased synthesis of chorismate, though the exact mechanism of feedback inhibition in the shikimate pathway in plants has not yet been determined. Because the shikimate pathway is conserved in all plants, glyphosate is a non-selective herbicide. However, the shikimate pathway is not found in humans or other animals, so glyphosate is considered non-toxic in normal doses.

Since glyphosate is taken up through the leaves, it can only be used on already growing plants, making it useful for pre-harvest weed management applications (Duke and Powles, 2008). To assist in its uptake by weeds, glyphosate is usually mixed with a surfactant to assist with translocation across the cuticle via diffusion. The rate of translocation varies across plant species. From the leaves, glyphosate is transported to via the phloem to other plant tissues, causing death in any actively growing tissue.

Usage

In the 30 years following its market introduction in 1974, 1.6 billion kilograms of glyphosate active ingredient have been applied to fields in the United states, (Benbrook, 2016). In its first year on the market, 0.36 kilograms of glyphosate were used, which increased steadily to 12.5 million kilograms in 1995, making it the seventh most applied pesticide that year. However, beginning around 1996, genetically engineered crops began gaining market share with

Monsanto's introduction of the Roundup Ready® soybean. Roundup Ready® crops are genetically engineered to be resistant to glyphosate, which allows farmers to apply glyphosate liberally to fields without harming crops. Because of this, usage of glyphosate increased to 36 million kilograms in 2000 and continued to increase to a present yearly usage of 113.4 million kilograms (as of 2014). This represents a 300-fold increase in usage since its introduction.

Glyphosate as a Harvest Aid

In addition to its use as an herbicide, glyphosate is commonly applied pre-harvest to act as a desiccant. Typically, glyphosate is applied when a crop reaches physiological maturity (Griffin, Boudreaux, et al., 2010). This practice will not only kill any weeds present in the field, but also kills the crop, which dries out the foliage. Pre-harvest desiccation provides several advantages to the farmer. For instance, it has been shown to reduce the moisture in the harvested crop. Additionally, pre-harvest desiccation can allow for an earlier harvest (Boudreaux and Griffin, 2008). This is advantageous to farmers, as delaying harvest can be associated with lower yields (Philbrook, 1989).

However, pre-harvest glyphosate treatment is not without drawbacks. Seedlings planted after treatment with glyphosate have been shown to have decreased rates of germination and root growth (Piotrowicz-Cieslak, Adomas, et al., 2010). Wheat treated with pre-harvest glyphosate has been found to contain elevated levels of shikimic acid (Bresnahan, Manthey, et al., 2003). This results in production of flour containing an elevated amount of phenolic acids, which is associated with development of a weaker dough. Application of glyphosate to wheat at the soft dough stage can reduce test weight, kernel size, and kernel weight (Manthey, Chakraborty, et al., 2004). When made into dough, flour produced from wheat treated with pre-harvest glyphosate at the soft dough stage requires longer mix times versus untreated wheat, which is undesirable for bread making.

Environmental Toxicity

After application, the route of glyphosate degradation differs in glyphosate-susceptible and glyphosate-resistant farming systems. In soil, there are two main routes of degradation (Duke, 2011). The first route is via a C-P lyase, which leads to the formation of inorganic phosphate and sarcosine. Glyphosate can also be broken down via breakage of the C-N bond by the enzyme glyphosate oxoreductase, leading to the formation of glyoxylate and aminomethylphosphonic acid (AMPA). AMPA is considered the major metabolite of glyphosate breakdown.

Glyphosate is considered a low dermal and oral toxicity by the Environmental Protection Agency (EPA), and is only considered slightly toxic to wildlife (Environmental Protection Agency, 1993). Despite this, its use remains controversial among some of the general public. Glyphosate and AMPA are non-volatile and will linger in the soil. Half-lives of these compounds have been found to be up to 151 days for glyphosate and 98 days for AMPA (Bai and Ogbourne, 2016). However, these numbers may vary wildly depending on soil type. Studies have drawn mixed conclusions on the effect of glyphosate on the environment. Application of glyphosate in coffee plantations can have detrimental effects on earthworms (García-Pérez, Alarcón-Gutiérrez, et al., 2014). However, contradictory findings show that earthworms appear to be unaffected by glyphosate (Pereira, Antunes, et al., 2009).

Because glyphosate binds tightly to soil, contamination of aquatic environments via runoff, concerns about contamination of aquatic environments are limited (Annett, Habibi, et al., 2014). Additionally, this characteristic minimized the risk of groundwater contamination via runoff. However, AMPA shows lower binding activity in soil which may result in leaching.

Human Toxicity

The target of glyphosate, the shikimate pathway, is not found in humans. Because of this, glyphosate is not considered harmful to humans. With regard to animal consumption, the EPA places glyphosate in the "least toxic" category, category IV (Williams, Kroes, et al., 2000). However, shifts in public opinion and recent research have led many to be skeptical of this classification. Glyphosate has been found to induce breast cancer in human cells *in vitro* via estrogenic activity (Thongprakaisang, Thiantanawat, et al., 2013). Additionally, glyphosate and its metabolites can cause hemolysis and hemoglobin oxidation in human erythrocytes *in vitro* (Kwiatkowska, Huras, et al., 2014). However, the concentration required for these changes to occur would only be present in a poisoning situation and not through incidental exposure via consumption of food treated with glyphosate. Glyphosate has also been found to be an endocrine disrupter in human cell lines (Gasnier, Dumont, et al., 2009). Interestingly, cytotoxic effects generally varied by the type of formulation (for example, surfactant type), and not by concentration of glyphosate.

Oats

Oat (*Avena sativa*) is a cereal crop that is grown around the world for both human and livestock consumption. An oat grain consists of an outer husk, which accounts for about 25-36% of the weight of the grain and covers the caryopsis, which is known as a groat. The groat is the only part of the oat that can be digested by humans and contains the majority of the nutrients. The groat consists of about 59% carbohydrates. 15-20% protein, 7% lipids, and 10% fiber (Gulvady, Brown, et al., 2013).

The groat consists of three major components: bran, germ, and endosperm. The bran makes up 40% of the groat by weight and is the outer layer of the groat. Oat bran is the major source of vitamins and minerals. The endosperm is the largest fraction, making up 57% of the groat's weight (Gulvady, Brown, et al., 2013). The endosperm serves as storage for protein and starch, and also contains 90% of the lipid present in the groat. The germ layer, or embryo, is the minor component, making up 3% of the groat's weight. It contains high levels of protein and lipid.

Figure 2: A Longitudinal Section of an Oat Kernel
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Oats are grown around the world, but the majority of the crop is grown in Europe and the Americas (Marshall, Cowan, et al., 2013). Russia is the global leader in oat production, with 200 million bushels produced in 2010. The United States produced 89.5 million bushels of oats in 2015, with Wisconsin as its largest producer (United States Department of Agriculture, 2016). North Dakota is the 4th largest producer of oats in the US, with 10.3 million bushels produced in 2015.

Human Consumption of Oats

In the United States, annual human consumption of oats per capita sits at about 4.5 pounds (Strychar, 2011). This is down from a peak of 6.5 pounds in 1990. Since they lack gluten, oats are not suitable for use as a bread flour. Instead, they are most often consumed as oatmeal in hot cereal, as oat flour in ready to eat cereal, or as an addition to wheat bread.

Carbohydrates are the primary nutrient found in oats, at 59 g per 100 g of oats (Gulvady, Brown, et al., 2013). This is followed by protein at 17 g per 100 g of oats, fiber at 11 g per 100 g, and lipid at 7 g per 100 g. In comparison, wheat provides protein at 17 g per 100 g and lipid at 3 g per 100g. Additionally, oats are rich in B vitamins. A 100g serving of oats also provides 51% of the USDA recommended daily value (DV) of thiamin, 14% of the DV of folate, and 9% of the DV of riboflavin. Oats are also rich in the minerals phosphorus (52% DV in 100g serving) and potassium (12% DV in 100g serving).

Oats have been found to have many health benefits and are a good addition to the human diet. For instance, oats are high in the polysaccharide β-glucan, which is found in the endosperm and aleurone cell walls at a rate of 2.3-8.5% (Gulvady, Brown, et al., 2013). The β-glucan found in oats has been shown to control blood glucose and cardiovascular disease. Additionally, consumption of oat β-glucan has also been shown to improve cholesterol levels (Butt, Tahir-Nadeem, et al., 2008). The exact mechanism by which cholesterol is lowered is still unresolved, but it is proposed that β-glucan mediates the formation of a viscous layer in the small intestine, which inhibits the uptake of dietary cholesterol and reabsorption of bile (Othman, Moghadasian, et al., 2011). Since bile is not reabsorbed, the synthesis of bile acids from cholesterol is increased, which lowers the circulating LDL cholesterol levels.

Oat Quality Traits

Relevant quality parameters of oats depend on the step in processing being considered. For example, grain yield may be a very important parameter to growers, but food producers are likely more interested in nutrition content and grain uniformity. Therefore, numerous parameters must be analyzed to determine overall oat quality.

Physical Quality

Physical oat quality parameters are of great interest to oat breeders, growers, and millers. This includes groat percentage, which is a measure of the percentage of the weight of a sample of whole oat grain that is attributed to the groat (Ames, Fregeau-Reid, et al., 2014). Groat percentage is calculated by weighing a sample of whole oat grain, dehulling the sample, and then weighing the groats that have been separated from the hulls. A high groat percentage is desired, as hulls are inedible by humans and considered a low-value product. A typical groat percentage is 70-75%, but can vary by cultivar, year, and growing location.

Groat size is another important physical quality parameter. Uniform size is desired for dehulling, as different kernel sizes require different rotor speeds (Doehlert and Wiessenborn, 2007). Larger groats are desired, as large groats are associated with better milling yields and also allow for the production of larger size oat flakes (Ames, Fregeau-Reid, et al., 2014). Oat size can be divided into “plumps” and “thins.” Plump oats are defined as kernels that remain on the top of a 5.5/64” x 0.75” sieve, while thin oats are defined as those that pass through a 5/64” x 0.75” sieve.

Test weight is a measure of the specific volume of a quantity of oats, and is expressed in lb/bu (Ames, Fregeau-Reid, et al., 2014). It is determined by measuring the weight of a fixed volume of oat kernels, and then converting to lb/bu. A high test weight is desired for oats, and is correlated with kernel size and shape, as well as groat density. Starch content is inversely correlated with test weight, as a high starch content reduces density (Doehlert and McMullen, 2000). The minimum test weight for grade No. 1 oats is 36 lb/bu (United States Department of Agriculture, 1988).

Nutritional Quality

Protein content in groats ranges from 12-16%, which is among the highest in cereals (Ames, Fregeau-Reid, et al., 2014). Cultivar, growing location, and weather can contribute to variations in protein content. Protein is an important quality factor in order to meet nutrition claims. Protein content is measured by combusting a sample and measuring the nitrogen released, which can then be used to deduce protein content by using a conversion factor.

The starch content of groats ranges from 40-65% (Ames, Fregeau-Reid, et al., 2014). Starch content is a relevant quality parameter, as starch can affect end product quality. The starch content desired will vary depending on the end product. Oat starch content can be quantified by enzymatic assays, while starch quality can by analyzed by measuring pasting properties. Oat starch is discussed in detail below.

Oat Flake Quality

Oat flake quality is influenced by both the quality of groats used for processing and the processing conditions. For instance, kilning is primarily performed to inactivate peroxidase in flakes to lengthen shelf life. However, kilning groats has been found to increase the specific weight of oat flakes (Gates, Sontag-Strohm, et al., 2008). Tempering groats is necessary before rolling to prevent crushing of the groats, but interestingly, extended tempering times can actually increase the number of fines in oat flakes. Oat flake quality can be assessed using multiple parameters, including flake thickness, flake granulation, and water absorption (Ames and

Rhymer, 2003). Flake thickness and granulation are relevant to meet specifications for whatever product is being produced-for example, instant oats required a thinner flake and smaller granulation than old fashioned rolled oats. Water absorption is dependent on both flake thickness and absorption by the macromolecular components (such starch and β-glucan), and is an indicator of the texture of oat products (Gates, Sontag-Strohm, et al., 2008).

Oat Starch

Starch is the primary component of the groat, accounting for 40-65% of its weight (Kasturi and Bordenave, 2013). Like other plant starches, oat starch is made up of two polymers of glucose: amylose and amylopectin. The proportion of amylopectin to amylose varies among oat varieties, ranging from 20-34% amylose. The amylose found in oat starch is similar in size to that in other cereals, while the amylopectin tends to be of lower molecular weight in comparison. For instance, the average weight of high molecular weight amylopectin for oats is 8.9×10^4 Da, compared to wheat which is 5.6×10^5 Da. The starch forms discrete, irregularly shaped granules, which range from 2-12µm in diameter (Zhou, Robards, et al., 1998). Unlike the starches of other plants, such as wheat, oat starch granules do not have distinct size distributions (such as the A&B distribution in wheat). The granules tend to cluster, forming compound granules that are around 60 µm in diameter.

Since starch is responsible for much of the texture of cooked oats, it is a key factor in oat quality. It has a gelatinization temperature of 57-62ºC, but can very between cultivars (Kasturi and Bordenave, 2013). Starch solubility shows a heightened increase at 90-95ºC, and at 95ºC most of the granules are disintegrated. Oat starch is more sensitive to shearing than other cereals, and its cooled gels are clearer, more elastic, and less susceptible to retrogradation. When cooled, the amylopectin forms a dense network, causing a rapid increase in viscosity at about 70-80ºC.

Oat starch also has a higher proportion of lipids than wheat or maize starch, which contributes to the mentioned pasting properties.

Impact of Processing on Oat Starch

As discussed earlier, oats are rich in lipids, especially compared to other cereals. Oats are also rich in hydrolytic enzymes, such as lipase and lipoxygenase (Hu, Xing, et al., 2010). Thus, over time, degradation of lipids by these enzymes will result in a rancid taste. To combat this, groats are usually heat treated in order to inactivate the enzymes. However, this heat treatment also affects the behavior of oat starch. Steaming and toasting of oat flour results in a reduction in starch damage compared to flour that is not heat treated, likely due to the inactivation of the enzyme amylase, which hydrolyzes starch (Ovando-Martínez, Whitney, et al., 2013). Steaming and toasting also causes an increase in viscosity when cooked at 64˚C for 10 minutes or longer. This treatment also decreases the number of large starch granules, in addition to decreasing the weight averaged molecular weight of amylose, and high and low molecular weight amylopectin.

Oat Processing

To be converted to an edible food for humans, oats must go through several processing steps. Processing specifications may vary due to proprietary methods, but the general steps of oat processing are summarized in Figure 3.

Grading and Dehulling

The first step of oat processing is grading. To operate equipment efficiently, oats must be graded based on size. They are sorted by width, which leads to fractions with similar densities (Decker, Rose, et al., 2014). Sorting is accomplished by passing the oats through a series of perforated cylinders which contain holes of decreasing diameter. After sorting, the inedible outer hull must be removed using a dehuller, which consists of a spinning disk that throws the oats into impact rings, separating the hull from the groat. Once about 85% of the oats are dehulled, the oats are separated from the hulls and the oats with intact hulls are put back into the dehuller (Decker, Rose, et al., 2014). This minimizes breakage, as excess dehulling will cause the groats to break.

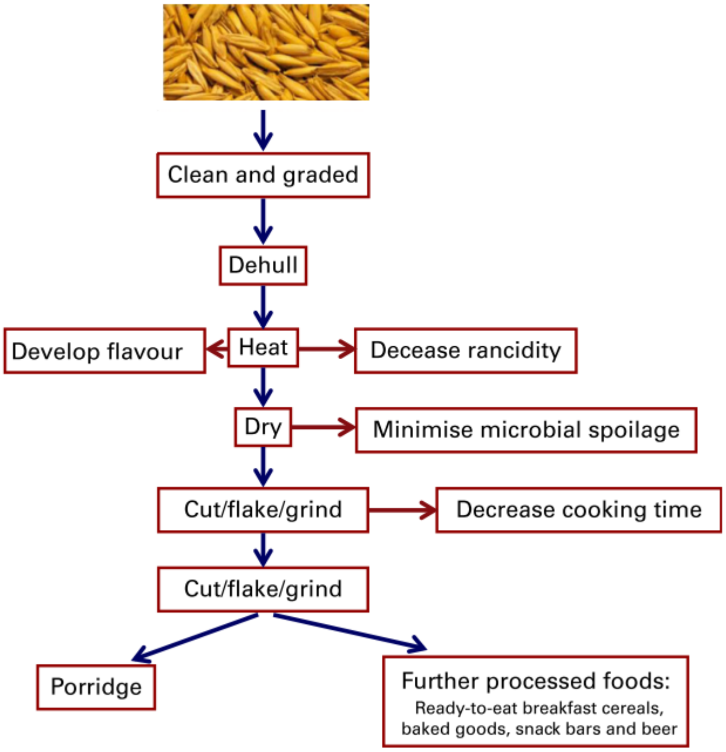

Figure 3: Flowchart of Oat Processing Steps
(Decker, Rose, et al., 2014)

Heat Treatment

In comparison to other cereals, oats are higher in lipids and contain elevated levels of lipid-digesting enzymes. To prevent the breakdown of lipids and the resulting unpleasant taste, the groat lipases must be inactivated. This is done by first passing the oats through long vertical columns and injecting steam to increase the temperature of the groats (Decker, Rose, et al., 2014). After steaming, the groats are exposed to dry heat to decrease the moisture to about 10% to improve conditions for storage. Dry heating also causes the groats to undergo Maillard reactions, which improves the final flavor of the oats. Additionally, the steaming and kiln drying process also acts as a method of decreasing microbial hazards in the groats.

Steel Cut Oats

To produce edible oats, the groats must first be processed into ‘steel cut’ oats via a rotary granulator. This consists of a rotating drum which feeds the groats into a series of knives resulting in the groat being cut two to four times. Steel cut oats can be used for hot cereal but require a longer a cooking time than rolled oats due to their large size and minimal processing. However, steel cut oats are desirable to consumers due to having a low glycemic index, a nuttier flavor, and appealing texture (Ames, Fregeau-Reid, et al., 2014).

Production of Rolled and Quick Cooking Oats

Further processed oat products are produced by rolling either whole groats, for rolled oats, or steel cut oats, for quick cooking or instant oats. The first step in this process is to temper the oats with steam, as the kiln drying process make the oats brittle and prone to crushing (Decker, Rose, et al., 2014). Steaming adds about 3-5% moisture back to the oats. Next, the oats are passed through rollers to produce flakes of the desired thickness: 0.36-0.46 mm for quick cooking oats and 0.51-1.2 mm for rolled oats. After rolling, the flakes are passed through an air stream to bring the moisture back down to 10%. The flaking process (steaming and rolling) pre-gelatinizes the starch in the oats, allowing for rapid water absorption during cooking, which reduces cooking time. Additionally, the properties of flakes can be assessed to determine product quality. Larger and thicker flakes tend to have more positive sensory properties and are also more durable with regards to packaging and transit (Ames, Fregeau-Reid, et al., 2014).

Justification, Objective, and Hypothesis

Justification

Consumer scrutiny in food is growing, especially about the use of pesticides on foods. In 2015, the Canadian firm Grain Millers Inc announced that 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 final oat quality.

Objectives

- To determine the effects of pre-harvest glyphosate application on whole groats
- To examine differences in quality in rolled oats produced from glyphosate treated and untreated groats
- To determine whether the timing of glyphosate application results in quality differences in whole groats and rolled oats

Hypothesis

Application of pre-harvest glyphosate will negatively affect the quality of both whole groats and rolled oats. Earlier application of glyphosate will be associated with a greater decrease in quality.

MATERIALS AND METHODS

Materials

2015 Sample Set

This experiment was performed using two sets of samples. The first, from crop year 2015, consisted of Rockford and Souris cultivar oats. These cultivars were grown in two locations- Prosper, ND, and Minot, ND. At each location, each variety received three treatments: glyphosate application at the soft dough stage, glyphosate application at physiological maturity, and no glyphosate application (Figure 4). Glyphosate was applied in the form of Roundup PowerMAX® (Monsanto, St. Louis, MO) at a concentration of 22 oz/acre (10.7 oz/acre active ingredient). Three replications were preformed of each treatment, for a total of 36 samples.

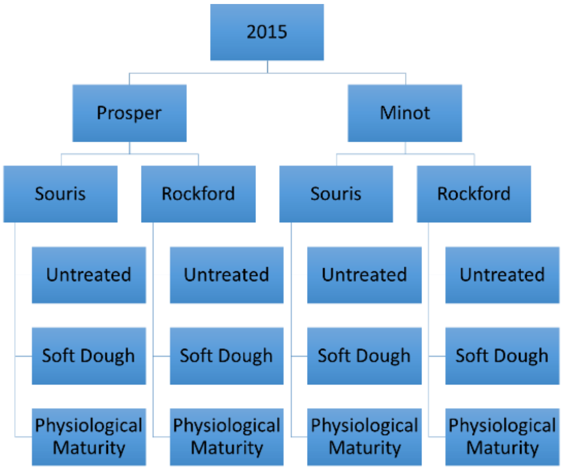

Figure 4: Summary of Crop Year 2015 Sample Set

The oat plots in Prosper were planted on May 22, 2015 and harvested on August 26, 2015. Glyphosate was applied on August 5 for the soft dough treatment and August 12 for the physiological maturity treatment. The average air temperature was within 2 degrees of the 30 year average for each month of the growing season (Table 1). Rainfall was 2.8” above average in May and 1.2” below average in August, while June and July saw near average rainfall levels.

Table 1: Weather Data for Crop Year 2015 in Prosper, ND

Month	Actual Air Temperature (˚F)	30 Year Average Air Temperature (˚F)	Actual Rainfall (inches)	30 Year Average Rainfall (inches)
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 applied on July 31 for the soft dough treatment and August 5 for the physiological maturity treatment. The average air temperature was within 2 degrees of the 30 year average for each month of the growing season (Table 2). Rainfall was within 1” of the 30 year average for all months of the growing season, except for June which saw a rainfall of 2.6” above average.

Table 2: Weather Data for Crop Year 2015 in Minot, ND

Month	Actual Air Temperature (˚F)	30 Year Average Air Temperature (˚F)	Actual Rainfall (inches)	30 Year Average Rainfall (inches)
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)

2016 Sample Set

The second sample set, from crop year 2016, consisted of Shelby and Deon varieties, which were grown in Prosper, ND and Crookston, MN (Figure 5). At each location, each variety either received glyphosate application at physiological maturity or was left untreated. Glyphosate was applied in the form of Roundup PowerMAX® (Monsanto, St. Louis, MO) at a concentration of 22 oz/acre (10.7 oz/acre active ingredient). At each location, there were four replications of each variety and treatment combination, for a total of 32 samples.

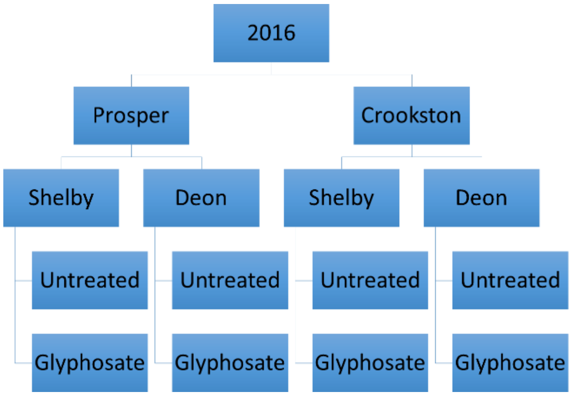

Figure 5: Summary of Crop Year 2016 Sample Set

The Prosper oat plots were planted on April 14, 2016 and harvested on August 1. Glyphosate was applied to the physiological maturity treatment on July 20 and July 25. The average temperature in Prosper for the 2016 growing season was within 3˚F of the 30 year average for April through August (Table 3). Rainfall was 2.5" below average in June and 1.6" below average in August, while April, May and July saw around average rainfall levels.

Table 3: Weather Data for Crop Year 2016 in Prosper, ND

Month	Actual Air Temperature (˚F)	30 Year Average Air Temperature (˚F)	Actual Rainfall (inches)	30 Year Average Rainfall (inches)
April	46	43	0.79	1.45
May	54	56	5.85	3.05
June	67	66	4.32	3.95
July	70	70	3.48	3.46
August	67	69	1.43	2.62

(North Dakota Agricultural Weather Network, 2018)

The plant dates for the Crookston plots were unavailable. The average temperature in Crookston for the 2016 growing season was within 3˚F of the 30 year average for May through August (Table 4). Rainfall was within 0.5" of average for the months of May and August, while June saw rainfall of 2.3" below average and July had rainfall of 1" below average.

Table 4: Weather Data for Crop Year 2016 in Crookston, MN

Month	Actual Air Temperature (˚F)	30 Year Average Air Temperature (˚F)	Actual Rainfall (inches)	30 Year Average Rainfall (inches)
April	45	42	0.49	1.01
May	54	55	3.70	2.36
June	65	64	2.36	3.48
July	70	69	3.21	3.15
August	68	67	2.50	2.88

(National Weather Service, 2018, Weather Underground, 2018)

Sample Preparation

Upon receipt, samples were cleaned on a Carter Day dockage tester (Carter Day International, Minneapolis, MN). The settings for cleaning were as follows:

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

Test weight was determined using a Dickey-John GAC 2100b analyzer (Dickey-John, Auburn, IL), and moisture was determined by Near-Infrared (NIR). The groats were then separated from the hulls using a Codema Laboratory Oat Huller (Codema, Maple Grove, MN) (Figure 6). Whole groats were analyzed for kernel diameter, hardness, and weight using a Single Kernel Characterization System (Perten Instruments, Hägersten, Sweden). For tests necessitating oat flour, whole groats were ground using a udy mill.

Figure 6: Codema Laboratory Oat Dehuller

Groat Analysis

Electron Microscopy

Electron microscopy was performed at the NDSU electron microscopy center. Groats were cut in half transversely with a razor blade and attached to aluminum mounts with high-purity silver paint (SPI Products, West Chester PA, USA). Mounted samples were sputter-coated with a conductive layer of gold (Cressington 108auto, Ted Pella Inc., Redding CA, USA) and then images were obtained immediately using a JEOL JSM-6490LV scanning electron microscope (JEOL USA, Inc., Peabody MA, USA) operating at an accelerating voltage of 15 kV.

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this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Proximate Analysis

Moisture content was measured using AACCI approved method 44-15.02 (AACC International, 1999a). Protein content was analyzed using AACCI approved method 46-30.01 (AACC International, 1999b).

Starch and Viscosity Analysis

Total starch and starch damage were analyzed using AACCI approved methods 76-13.01 and 76-31.01, respectively (AACC International, 1999c and 1999d). Rapid Visco Analysis (RVA) was performed on heat treated groats (process below) using AACCI approved method 76-21.01, standard profile 1 (AACC International, 1999e).

Rolled Oat Preparation

Heat Treatment

To prepare for rolling, whole groats were first heat treated. 100 gram samples of the groats first placed in mesh baskets (Figure 7). Then, samples were steamed at 100% humidity, 100º C for 40 minutes using an Adcraft full size food cooker/warmer 1500W (Admiral Craft, Westbury, NY). Temperature was monitored using a probe thermometer. This was followed by 1 hour of dry heat in a Baxter OV310E mini rotating rack convection oven (Baxter, Orting, WA) at 100º C, stirring the samples every 15 minutes. The groats were removed from the oven and left out to dry overnight to allow the moisture to equilibrate.

Figure 7: Oat Groat Heat Treatment
Groats were steamed (a), toasted (b), and left at room temperature overnight to equilibrate (c)

Rolling

Before rolling, the groats were tempered by steaming for 10 minutes at 100% humidity, 100º C, using an Adcraft full size food cooker/warmer 1500W (Admiral Craft, Westbury, NY). Temperature was monitored using a probe thermometer. After tempering, groats were rolled using a Roskamp Model K roller mill (Roskamp Champion, Waterloo, IA) (Figure 8). The roll gap was set to 0.381 mm using a gap measuring tool. To ensure constant feed rate, a feeder from a Perten LM 3100 mill (Perten Instruments, Hägersten, Sweden) was attached to the roller opening. After rolling, the oats were collected and placed back into a mesh basket. The rolled oats were left out to dry overnight to allow the moisture to equilibrate.

Figure 8: Lab Oat Roller

Rolled Oat Quality Measures

Thickness

Average oat thickness was determined using a Mitutoyo 2416F thickness gauge (Mitutoyo, Aurora, IL). Measurements were taken at the thickest point of 20 individual rolled oats, and averages and standard deviations were calculated for each sample (Figure 9).

Figure 9: Thickness Gauge with Oat Flake

Absorption

Rolled oat absorption was performed using AACC method 56-40.01, modified to a ratio of 25g oats to 100g water, as described in Ames and Rhymer 2003 (AACC International, 1999f, Ames and Rhymer, 2003). 25g of each sample was weighed out into a beaker, to which 100 mL of DI water was added (Figure 10). After 5 minutes, the water and rolled oats were poured out onto a US no. 20 standard sieve and drained for 5 minutes. The weight of the wet oats and sieve was then recorded, and absorption was calculated using the formula:

water absorption (g/25g oats) = weight of sieve and wet oats – (25 + dry sieve weight)

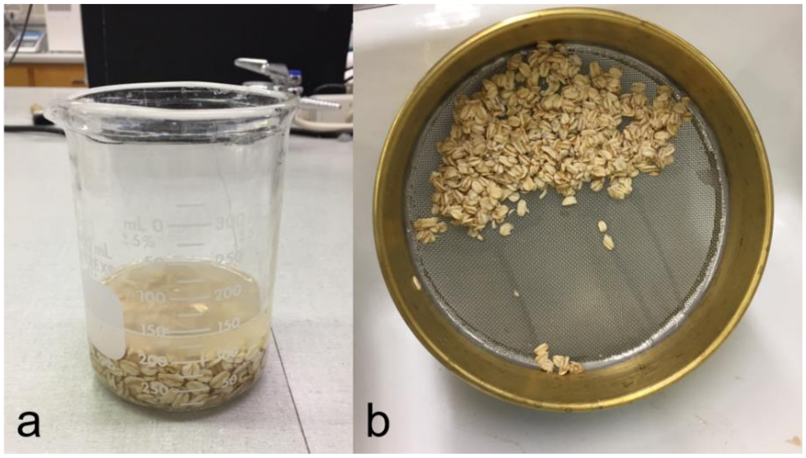

Figure 10: Rolled Oat Absorption Test
25g of rolled oats were placed into a beaker, and 100 mL of water was added (a). After 5 minutes, the contents were poured out onto a sieve and allowed to drain (b).

Granulation

Granulation was measured on 50g of flaked oats using US standard sieves numbers 4, 7, and 10 on a Ro-Tap shaker (W.S. Tyler, Mentor, OH). Samples were shaken for 2 minutes, after which the weight of the sample remaining in each sieve was recorded (Figure 11). The percentage of sample remaining in each sieve from the total sample amount was then calculated.

Figure 11: Sample Remaining on Ro-Tap Sieves
From left: US standard sieves # 4, 7, and 10, bottom pan.

Statistical Analysis

This experiment was designed using a split layout, with location as the main plot and cultivar and glyphosate application as sub-plots. The replications were considered as nested in location. Statistical analysis was performed using Statistical Analysis Software (SAS for Windows 9.4, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

In the 2015 crop year sample set, oats were grown in Minot, ND and Prosper, ND. At each location, Rockford and Souris cultivars were grown. For each location/cultivar combination there were three treatments: application of glyphosate at the soft dough stage, application of glyphosate at physiological maturity, or no glyphosate application.

In the 2016 crop year sample set, oats were grown in Crookston, MN, and Prosper, ND. At each location, Shelby and Deon cultivars were grown. For each location/cultivar combination there were two treatments: application of glyphosate at physiological maturity, or no glyphosate application.

Groat Quality Measures

Groat quality testing can be performed to predict end product characteristics. For instance, plump oats are indicative of a higher test weight and therefore better yield. Groat percentage indicates the percentage of the weight of a sample remaining after dehulling-thus a high groat percentage indicates more useable grain. Protein content is essential to monitor in order to meet nutrition needs, and total starch and starch damage can give indication of product quality after processing. Test weight is a measure of the mass of oats that can be contained within a standard volume.

Overall Results

For the 2015 crop year, plump percentages ranged from 90.56-92.68%. Significant ($P<0.05$) differences were observed between glyphosate treatments (Table 5). Treatment at the soft dough stage resulted in a significantly (P<0.05) lower percentage of plump groats compared to treatment at physiological maturity or untreated samples. This is likely because the samples treated at the soft dough stage did not have as long to develop, resulting in smaller groats.

Application of glyphosate leads to an inability to down regulate the shikimate acid pathway, which results in an increased demand for carbon in that pathway. Thus, this may lead to less carbon being available for grain filling, leading to smaller groats. Groat percentage among the samples ranged from 68.90-70.00%. Groat percentage was significantly ($P<0.05$) lower in the soft dough treatment group versus the other treatment groups. Again, groats treated at the soft dough stage did not have as long to develop as the other two treatment groups, due to the early glyphosate application. This likely led to a lower groat percentage as groat was smaller, which resulted in the hull representing a greater proportion of the kernel weight.

A high degree of starch damage is undesirable in grain, as it increases water absorption, and can affect the swelling and gelatinization properties of starch (Tester and Karkalas, 1996). Starch damage contents of the three treatments ranged from 58.75-60.31% (Table 5) Starch damage was significantly ($P<0.05$) lower in untreated samples versus samples treated at the soft dough stage or physiological maturity. Test weight, protein content, and total starch did not differ significantly ($P>0.05$) between the three treatments. Test weight for the three treatments ranged from 36.24-36.90 lb/bu. The minimum test weight for the various classes of oats ranges between 27.0-36.0 lb/bu (United States Department of Agriculture, 1988).

Table 5: 2015 Groat Quality Parameters

Glyphosate Treatment	Plump (%)	Groat percentage (%)	Test Weight (lb/bu)	Protein* (%)	Total Starch* (%)	Starch Damage* (%)
Untreated	92.32^a	70.28^a	36.24^a	14.80^a	58.75^a	0.43^b
Soft Dough	90.56^b	68.90^b	36.90^a	14.77^a	59.68^a	0.52^a
Physiological Maturity	92.68^a	70.00^a	36.61^a	14.46^a	60.31^a	0.49^a

Values are averages of all locations/cultivars. Values with the same superscript level are not significantly different ($P>0.05$) Least significant difference was used for mean separation. lb/bu: pounds/bushel. *dry weight basis.

The lack of significant difference in test weight between the treatments is curious, especially considering there was a significant difference in groat plumpness, which is correlated with test weight (Ames, Fregeau-Reid, et al., 2014). Additionally, significant differences in test weight in wheat have been observed when glyphosate is applied at the soft dough stage (Manthey, Chakraborty, et al., 2004). The lack of significant difference in protein content, however, is less surprising, as wheat protein also appears to be unaffected by preharvest glyphosate (Darwent, Kirkland, et al., 1994, Manthey, Chakraborty, et al., 2004). This is likely because glyphosate is a slow acting pesticide, so storage protein synthesis (as a percentage of groat weight) likely plateaued before the oat plants were killed by the glyphosate (Peterson and Smith, 1976).

For the 2016 crop year samples, plump groat percentages were 99.45% and 99.38% for untreated oats and oats treated with glyphosate at physiological maturity, respectively (Table 6). These values were not significantly (P>0.05) different. Groat percentage were 72.29% and 71.88% for untreated and glyphosate treated oats, respectively. These values were not significantly (P>0.05) different. Test weight values were 38.83 lb/bu and 38.48 lb/bu for untreated and glyphosate treated oats, respectively. These values were not significantly (P>0.05) different. Protein content was 16.26% for untreated oats and 16.63% for glyphosate treated oats. These values were not significantly (P>0.05) different. Total starch values were 57.66% and 57.53% for untreated and glyphosate treated oats, respectively. These values were not significantly (P>0.05) different. Damaged starch content was 0.68% for glyphosate treated oats and 0.65% for untreated oats. These values were not significantly (P>0.05) different.

Table 6: 2016 Groat Quality Parameters

Glyphosate Treatment	Plump (%)	Groat percentage (%)	Test Weight (lb/bu)	Protein* (%)	Total Starch* (%)	Starch Damage* (%)
Untreated	99.45^a	72.29^a	38.83^a	16.26^a	57.66^a	0.68^a
Physiological Maturity	99.38^a	71.88^a	38.48^a	16.63^a	57.53^a	0.65^a

Values are averages of all locations/cultivars. Values with the same superscript level are not significantly different (*P*>0.05) Least significant difference was used for mean separation. lb/bu: pounds/bushel. *dry weight basis

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Since no significant differences were observed between treatments, it is likely that the oat kernels were fully developed before the glyphosate killed the oat plant. This trend was also seen in the 2015 sample set-no significant differences were observed between the untreated samples and the samples treated at physiological maturity. Conversely, application of glyphosate at the soft dough stage showed significant differences in groat percentage and plump groats, when compared to the untreated samples. Thus, the timing of glyphosate has an impact on groat quality parameters.

Results by Location and Cultivar

When broken down by location and cultivar, more trends can be seen in the 2015 groat quality measurement (Table 7). Plump oat percentage ranged from 96.88%-98.12% for Minot-grown oats, while Prosper-grown oats ranged from 81.18%-88.44%. With regard to plump oats, Rockford cultivar oats grown in Prosper showed the largest effect from glyphosate treatment at the soft dough stage: untreated oats had 87.28% plump, while oats treated at the soft dough stage had only 81.18% plump-a difference that was statistically significant (P<0.05). Groat percentage of Prosper-grown Rockford oats also seemed to be detrimentally affected by treatment at the soft dough stage. While groat percentage for untreated oats was 68.38%, groat percentage for soft dough treated samples was significantly (P<0.05) less, at 64.72%. Protein content appeared to vary greatly by location-protein content for Minot-grown samples ranged from 13.36%-14.69%, while Prosper-grown samples ranged from 14.39%-16.12%. However, within each location x variety interaction, there were no significant ($P>0.05$) differences between glyphosate treatments. For three of the four location x cultivar interactions, the untreated oats had the lowest percentage of total starch of the three treatments. However, none were significantly ($P>0.05$) different from the other two treatments. Likewise, the damaged starch content for all four location x cultivar interactions was lower in the untreated samples versus the soft dough and physiological maturity treatments. Again, however, these results were not significantly ($P>0.05$) lower.

Table 7: 2015 Groat Quality Parameters by Location and Cultivar

Location	Cultivar	Glyphosate Treatment	Plump (%)	Groat percentage (%)	Test Weight (lb/bu)	Protein* (%)	Total Starch* (%)	Starch Damage* (%)
Minot	Rockford	Untreated	97.26	72.54	41.10	14.30	62.49	0.39
		Soft Dough	96.88	71.78	41.70	14.69	59.92	0.43
		Physiological Maturity	97.29	71.54	41.30	13.36	63.01	0.44
	Souris	Untreated	97.85	71.27	40.90	14.31	58.94	0.45
		Soft Dough	97.58	70.27	40.77	13.88	60.11	0.52
		Physiological Maturity	98.12	70.80	41.50	14.26	60.50	0.49
Prosper	Rockford	Untreated	87.27	68.38	31.67	16.12	55.79	0.39
		Soft Dough	81.18	64.72	32.63	15.76	56.57	0.54
		Physiological Maturity	86.85	68.28	32.13	15.82	56.52	0.50
	Souris	Untreated	86.92	68.93	31.30	14.48	57.79	0.48
		Soft Dough	86.62	68.84	32.50	14.75	62.12	0.58
		Physiological Maturity	88.44	69.36	31.50	14.39	61.20	0.54
	LSD within location		1.09	1.64	1.40	2.17	5.59	0.12
	LSD between locations		1.24	1.54	1.51	1.99	5.74	0.12

lb/bu: pounds/bushel. *dry weight basis.

As Table 7 shows, the difference in percentages of plump oats is greatest in for the Rockford-cultivar oats grown in Prosper. Since this trend wasn't seen for the Rockford cultivar in Minot, it is possible that a combination of the glyphosate application, weather, variety, or other location factors all contributed to this observation. For instance, Prosper received below average rainfall in August, which may have compounded with the glyphosate application and other factors unique to the Rockford cultivar to result in a reduced percentage of plump groats. Likewise, these factors may have also contributed to the reduced test weight in those samples.

When broken down by location and cultivar, the 2016 sample set showed very consistent results for plump oats between treatments (Table 8). For every location x cultivar interaction, untreated and glyphosate treated oats showed no significant ($P>0.05$) differences. Groat percentage appeared to be impacted by location, as groat percentage for Crookston-grown samples ranged from 72.13%-74.64% while Prosper-grown samples ranged from 69.32%-71.52%. However, within each location x cultivar interaction, no significant ($P>0.05$) differences were observed. Test weight was similarly impacted by location: Crookston-grown samples ranged from 38.43-41.50 lb/bu, while Prosper samples ranged from 36.05-38.30 lb/bu. Of the location x cultivar interactions, only Crookston x Deon showed a significant ($P<0.05$) difference between treatments-untreated samples averaged 39.45 lb/bu, while glyphosate treated samples averaged 38.43 lb/bu.

Table 8: 2016 Groat Quality Parameters by Location and Cultivar

Location	Cultivar	Glyphosate Treatment	Plump (%)	Groat percentage (%)	Test Weight (lb/bu)	Protein* (%)	Total Starch* (%)	Starch Damage* (%)
Crookston	Deon	Untreated	99.41	72.49	39.45	15.54	58.30	0.76
		Physiological Maturity	99.25	72.13	38.43	15.73	58.43	0.77
	Shelby	Untreated	99.39	74.26	41.50	16.49	57.50	0.52
		Physiological Maturity	99.26	74.64	41.10	17.76	56.75	0.58
Prosper	Deon	Untreated	99.51	70.89	36.05	16.32	57.48	0.72
		Physiological Maturity	99.47	69.32	36.23	15.65	59.73	0.73
	Shelby	Untreated	99.48	71.52	38.30	16.66	57.36	0.73
		Physiological Maturity	99.54	71.44	38.18	17.38	55.20	0.53
	LSD within location		0.27	1.17	0.83	0.98	3.85	0.17
	LSD between locations		0.26	1.58	0.90	0.89	3.62	0.19

lb/bu: pounds/bushel. *dry weight basis

Again, application of glyphosate at physiological maturity of oat plants appears to have no effect on groat quality parameters. As shown in, Table 8, oat cultivar and growing location have a much larger impact. This is likely due to the fact that application at physiological maturity is too late in groat development to have any measurable effects.

Overall, glyphosate application at the soft dough stage may have a detrimental effect on oat plumpness. This could possibly due be to the glyphosate application causing an inability to down regulate the shikimate acid pathway, leading to an increased carbon flow to that pathway and leaving less available for grain filling. This effect seems to disappear when glyphosate is applied later, at physiological maturity. Groat percentage was similarly impacted by glyphosate application at the soft dough stage. This is especially evident in the Prosper x Rockford samples (Table 7). However, test weight did not appear to be affected by glyphosate application, regardless of timing. Likewise, groat protein seemed to be more dependent on growing location than glyphosate application. Total starch did not seem to be affected by glyphosate application regardless of timing. However, application of glyphosate at the soft dough stage did result in a greater percentage of damaged starch (Table 5,Table 7)

Single Kernel Characterization System

Single kernel characterization system (SKCS) testing can be used to measure characteristics that are related to final oat quality. For instance, groat hardness may be related to groat breakage (Doehlert and McMullen, 2000). In this study, groat hardness, weight, and diameter were analyzed via SKCS.

Overall Results

The 2015 sample set showed some differences in SKCS results due to glyphosate treatment (Table 9). Hardness index for the three treatments ranged from -45.34- -42.32. These values are consistent with reported values of groat hardness, though this characteristic is influenced by cultivar and growing location (Doehlert and McMullen, 2000). Oat treated at the soft dough stage had a significantly (*P*<0.05) lower hardness index than either untreated oat or oat treated at physiological maturity, indicating that soft dough-treated groats were the least hard. Since ripening of the kernel occurs between the soft dough stage and physiological maturity, it is possible that the glyphosate application interfered with this process, resulting in softer kernels. Or, it is possible that the glyphosate application killed the plant before the ripening process could finish.

Table 9: 2015 SKCS Results

Glyphosate Treatment	Hardness Index	Weight (mg)	Diameter (mm)
Untreated	-43.11^{a}	26.09^{a}	2.12^{a}
Soft Dough	-45.34^{b}	25.57^{a}	2.08^{b}
Physiological Maturity	-42.32^{a}	25.66^{a}	2.10^{ab}

Values are averages of all locations/cultivars. Values in same column with the same superscript letter are not significantly different (*P*>0.05). Least significant difference was used for mean separation.

Groat weight for the three treatments ranged from 25.66-26.09 mg/groat (Table 9). This measurement is higher than reported by other studies, which report groat weights of around 15-20 mg/groat (Doehlert and McMullen, 2000). No significant (*P*>0.05) differences were observed between the average weights of the three treatments. Since the maximum kernel weight is reached between the soft dough stage and physiological maturity, this suggests that the groats were able to continue maturing for some time after glyphosate application (Bowden, Edwards, et al., 2007). Groat diameter ranged from 2.08-2.12 mm. Groats treated at the soft dough stage had a significantly (*P*<0.05) smaller diameter compared to untreated groats. However, the difference between these diameters was only 0.04mm, so while statistically significant, the difference may not be practically significant. Groats treated at physiological maturity did not exhibit significant (*P*>0.05) differences in diameter compared to untreated or soft dough treated groats.

In the 2016 sample set, average groat hardness index was -44.51 for untreated groats and -45.04 for glyphosate treated groats (Table 10). This indicates that untreated groats were somewhat harder than glyphosate treated groats. However, this result was not statistically significant (*P*>0.05). Untreated groats also were slightly heavier than glyphosate treated groats, at 27.26 mg versus 26.97 mg. However, again, this result was not statistically significant. The average diameter for untreated groats and glyphosate treated groats was nearly identical, at 2.13 mm and 2.12 mm, respectively. These results follow the trend observed in the 2015 sample set, in that application at physiological maturity does not affect hardness, weight, or diameter. This was unsurprising, as the groat is fully developed at physiological maturity, so glyphosate application will not affect development.

Table 10: 2016 SKCS Results

Glyphosate Treatment	Hardness Index	Weight (mg)	Diameter (mm)
Untreated	-44.51^a	27.26^a	2.13^a
Physiological Maturity	-45.04^a	26.97^a	2.12^a

Values are averages of all locations/cultivars. Values in same column with the same superscript letter are not significantly different (*P*>0.05). Least significant difference was used for mean separation.

Results by Location and Cultivar

When broken down by location and cultivar, it appears that glyphosate treatment has a greater effect in some conditions (Table 11). For Minot-grown samples, glyphosate application at either soft dough stage or physiological maturity does not appear to have significant (*P*>0.05) effects on groat hardness for either Rockford or Souris cultivars. However, Prosper-grown groats appear to have been more strongly influenced by glyphosate application. For both Rockford and

Souris cultivars, groats treated at the soft dough stage had a significantly (*P*<0.05) lower hardness index compared to untreated groats, indicating that untreated groats were harder. Within each location x cultivar interaction, no significant (*P*>0.05) differences were observed in groat weight between the three treatments. However, untreated groats did have the heaviest groat weight for three of the four location x cultivar interactions. For groat diameter, the only location x cultivar interaction that showed a significant difference was Prosper x Rockford. For this grouping, untreated groats had a significantly (*P*<0.05) greater diameter than groats treated at the soft dough stage.

Table 11: 2015 SKCS Results by Location and Cultivar

Location	Cultivar	Glyphosate Treatment	Hardness Index	Weight (mg)	Diameter (mm)
Minot	Rockford	Untreated	-50.67	28.47	2.19
		Soft Dough	-48.31	27.25	2.17
		Physiological Maturity	-47.87	27.20	2.15
	Souris	Untreated	-46.08	25.39	2.11
		Soft Dough	-44.78	24.54	2.08
		Physiological Maturity	-43.22	25.07	2.12
Prosper	Rockford	Untreated	-40.44	25.65	2.09
		Soft Dough	-45.73	24.98	2.02
		Physiological Maturity	-39.47	24.66	2.06
	Souris	Untreated	-35.26	24.86	2.06
		Soft Dough	-42.53	25.52	2.06
		Physiological Maturity	-38.72	25.72	2.08
	LSD within location		4.06	1.28	0.04
	LSD between locations		3.79	1.23	0.04

In Table 11, we continue to see the trend that glyphosate application does not affect groat hardness, weight, or diameter. However, treatment with glyphosate at the soft dough stage only significantly affected oats grown in Prosper. Prosper received below average rainfall in August, while Minot received average rainfall, so perhaps the differences in weather caused this pronounced effect. In fact, glyphosate activity is diminished when rainfall occurs soon after application (Molin and Hirase, 2005). Likewise, other differences in growing conditions between the two locations, such as soil quality, could have contributed to this difference.

In the 2016 sample set, no significant ($P>0.05$) differences between treatments. were observed even after breaking down the SKCS data by location and cultivar (Table 12). However, the location x cultivar interaction had a significant ($P<0.05$) effect on groat hardness for Prosper x Shelby compared to the other 3 pairs. Prosper x Shelby had a hardness index of -37.84 for untreated oats and -38.95 for glyphosate treated oats, while the next hardest pair, Prosper x Deon, measured at -45.05 and -47.17 for untreated and glyphosate treated oat, respectively. Although no significant differences between treatments for each location x variety interaction for observed for groat weight, it is worth noting that for each interaction, untreated samples had a greater average weight than glyphosate treated samples. Average groat diameters were nearly identical for all locations, cultivars, and treatments, with only 0.05 mm between the largest and smallest averages.

Table 12: 2016 SKCS Results by Location and Cultivar

Location	Cultivar	Glyphosate Treatment	Hardness Index	Weight (mg)	Diameter (mm)
Crookston	Deon	Untreated	-46.02	28.14	2.12
		Physiological Maturity	-46.44	27.98	2.11
	Shelby	Untreated	-49.12	26.55	2.15
		Physiological Maturity	-47.60	26.24	2.15
Prosper	Deon	Untreated	-45.05	28.56	2.12
		Physiological Maturity	-47.17	28.32	2.10
	Shelby	Untreated	-37.84	25.81	2.12
		Physiological Maturity	-38.95	25.33	2.11
	LSD within location		6.22	1.34	0.03
	LSD between locations		6.39	1.33	0.04

Overall, glyphosate application at the soft dough stage appears to result in softer oat groats, though this impact varied by location. This was likely due to the fact that the groat is not fully hardened during the soft dough stage, so the glyphosate killed the plant before the groats were fully hardened. Since this was not consistent between locations, other factors such as rainfall may contribute to this effect. Glyphosate application at physiological maturity did not have an effect on oat hardness, as the groat was likely fully hardened before the glyphosate affected the plant. Neither application timing showed a significant (*P*>0.05) effect on groat weight. Groat diameter was not affected by glyphosate application, regardless of timing.

Analysis of Oat Samples with Rapid Visco Analyzer

The rapid visco analyzer (RVA) is a test that measures the pasting properties of a slurry. Figure 12 shows an example RVA profile indicating the parameters that are measured over the course of a test. Peak viscosity is the greatest viscosity reached during heating and is achieved when an equilibrium is reached between starch granule swelling and polymer leaching and is an indicator of water binding capacity. Peak time is the time at which peak viscosity is reached. Holding strength is the minimum viscosity measured after peak viscosity is reached and is an indicator of amylose leaching. Breakdown is the difference between the peak viscosity and the holding strength. Final viscosity is the viscosity of the slurry at the end of the cooling cycle of the test, and indicates the degree of reassociation of starch molecules, which is associated with gel formation. Setback is the difference between the final viscosity and the holding strength and is associated with end product starch texture.

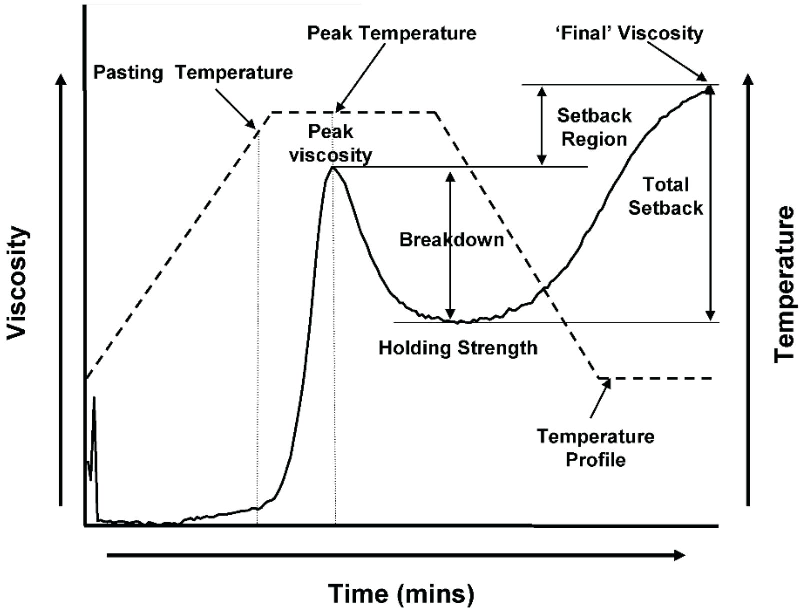

Figure 12: An Rapid visco analyzer (RVA) Profile Showing Recorded Parameters (Saunders, Izydorczyk, et al., 2011)

Overall Results

Slurries of oat flour from each sample were analyzed for pasting properties via RVA. In the 2015 sample set, no significant (*P*>0.05) differences were observed in the RVA parameters of the untreated controls in comparison to the samples treated with glyphosate at physiological maturity (Table 13). However, significant (*P*<0.05) differences were observed in the pasting properties of the untreated oats and the oats treated with glyphosate at the soft dough stage. Peak viscosity was significantly ($P<0.05$) higher in the untreated sample, at 4939 cP versus 4745 cP for the soft dough sample. This implies that the untreated samples would have a thicker texture than the soft dough treated samples when cooked. However, no significant ($P>0.05$) differences were observed between the untreated control and the soft dough treated samples in the holding strength or breakdown measurements. The final viscosity of the untreated control was significantly ($P<0.05$) greater than that of the soft dough treated samples, at 7186 cP versus 6847 cP, respectively. This indicates that if the samples were cooked and cooled, the untreated samples would have a thicker consistency than the samples treated with glyphosate at the soft dough stage. The untreated samples also showed a significantly ($P<0.05$) greater setback than the samples treated with glyphosate at soft dough stage. No significant ($P>0.05$) differences were observed in the peak times of the three treatments.

Table 13: 2015 Rapid Visco Analyzer (RVA) Results

Glyphosate Treatment	Peak Viscosity (cP)	Holding Strength (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Peak Time (min)
Untreated	4939^{a}	3027^{a}	1911^{a}	7186^{a}	4159^{a}	7.8^{a}
Soft Dough	4745^{b}	2902^{a}	1843^{a}	6847^{b}	3945^{b}	7.9^{a}
Physiological Maturity	4992^{a}	3019^{a}	1972^{a}	7095^{a}	4076^{ab}	7.7^{a}

Values are averages of all locations/cultivars. Values in same column with the same superscript letter are not significantly different ($P>0.05$). Least significant difference was used for mean separation. cP: centipoise

Since the amount of starch present was not significantly ($P>0.05$) different between the untreated and soft dough treatment samples (Table 5) the differences in RVA parameters must be caused by differences in starch quality or other parameters. For instance, application of glyphosate may interfere with grain filling that occurs in the soft dough stage, resulting in a lower viscosity starch. Additionally, non-starch components such as β-glucan have an effect on oat viscosity (Anttila, Sontag-Strohm, et al., 2004). It is possible that glyphosate application had an effect on β-glucan concentration, which was not measured in this study.

The pasting properties for the two treatments in the 2016 sample set were very similar. No significant (*P*>0.05) differences were observed in parameters, with the exception of peak time. The untreated samples showed a significantly (*P*<0.05) later peak time of 8.9 minutes, versus 8.7 minutes for the glyphosate treated samples. This indicates that the untreated samples would have to be cooked longer than the glyphosate treated samples to achieve the desired texture. However, such a small difference (12 seconds), while statistically significant, may not be practically significant.

Table 14: 2016 Rapid Visco Analyzer (RVA) Results

Glyphosate Treatment	Peak Viscosity (cP)	Holding Strength (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Peak Time (min)
Untreated	4327^a	2113^a	2214^a	6427^a	4314^a	8.9^a
Physiological Maturity	4288^a	2136^a	2152^a	6413^a	4277^a	8.7^b

Values are averages of all locations/cultivars. Values in same column with the same superscript letter are not significantly different (*P*>0.05). Least significant difference was used for mean separation. cP: centipoise

The 2016 results summarized in Table 14 show a similar trend as the 2015 results summarized in Table 13-application of glyphosate at physiological maturity does not have a significant (*P*>0.05) effect on oat flour viscosity. Presumably, application at this stage is late enough in the kernel development that the glyphosate will not interfere with grain filling or other development that may affect viscosity.

Results by Location and Cultivar

Glyphosate application at the soft dough stage had significant (*P*<0.05) effects on the Prosper-grown Rockford cultivar oats, in comparison to the untreated sample (Table 15). For this interaction, peak viscosity for the untreated oat was significantly (*P*<0.05) greater than that of the oats treated with glyphosate at the soft dough stage. Holding strength was also significantly ($P<0.05$) greater for the untreated oats, while the peak time was significantly ($P<0.05$) earlier. The largest difference was observed in final viscosity, which was significantly ($P<0.05$) greater in the untreated sample at 8298 cP, while the oats treated with glyphosate at the soft dough stage measured 7258 cP. However, for this interaction no significant ($P<0.05$) differences were observed between the untreated oats and the oats treated with glyphosate at physiological maturity.

For the Prosper-grown Souris samples, no significant ($P<0.05$) differences were observed between the untreated oats and the oats treated with glyphosate at the soft dough stage (Table 15). However, some effects were observed when comparing the untreated oats to the oats treated at physiological maturity. Both peak viscosity and breakdown were significantly ($P<0.05$) greater for the oat treated at physiological maturity than the untreated oat. No significant ($P<0.05$) differences between the untreated oats and the glyphosate treated oats were observed for either cultivar when grown in Minot.

Table 15: 2015 Rapid Visco Analyzer (RVA) Parameters by Location and Cultivar

Location	Cultivar	Glyphosate Treatment	Peak Viscosity (cP)	Holding Strength (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Peak Time (min)
Minot	Rockford	Untreated	5057	3254	1803	6854	3601	7.8
		Soft Dough	5161	3447	1714	6892	3445	7.7
		Physiological Maturity	5066	3362	1704	6599	3237	7.6
	Souris	Untreated	4465	2444	2021	6213	3769	8.4
		Soft Dough	4487	2671	1816	6181	3510	8.1
		Physiological Maturity	4598	2744	1855	6388	3644	8.1
Prosper	Rockford	Untreated	5276	3028	2249	8298	5270	7.8
		Soft Dough	4519	2206	2313	7258	5052	8.4
		Physiological Maturity	5067	2718	2349	7973	5255	8.1
	Souris	Untreated	4956	3384	1573	7378	3994	7.4
		Soft Dough	4813	3284	1529	7058	3774	7.4
		Physiological Maturity	5235	3254	1981	7420	4166	7.0
	LSD within location		266.90	443.00	379.54	357.93	380.08	0.53
	LSD between locations		435.47	434.78	374.76	431.68	361.39	0.54

cP: centipoise

The results summarized in Table 15 do not show any clear trends, unfortunately. Prosper x Rockford samples show significantly (*P*<0.05) lower peak and final viscosities in soft dough treated versus untreated samples, while the other three location/cultivar combinations do not show significant differences between these two treatments. Clearly, the results shown cannot be explained by glyphosate alone, and are likely due to a combination of factors, such as cultivar, growing location, and weather.

The 2016 sample set showed very similar pasting profiles between glyphosate treated and untreated oats (Table 16). The only significant (*P*<0.05) difference observed between treatments was that the peak time was significantly later for untreated oats versus glyphosate treated oat for the Deon cultivar grown in Prosper. However, some variance was observed between locations and cultivars. These results reaffirm that application of glyphosate at physiological maturity is too late to have an effect on groat development, and thus produce flours with similar properties.

Table 16: 2016 Rapid Visco Analyzer (RVA) Parameters by Location and Cultivar

Location	Cultivar	Glyphosate Treatment	Peak Viscosity (cP)	Holding Strength (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Peak Time (min)
Crookston	Deon	Untreated	3894	2367	1527	5789	3423	8.3
		Physiological Maturity	3778	2450	1328	5751	3301	8.1
	Shelby	Untreated	4437	2165	2272	6054	3889	8.5
		Physiological Maturity	4433	2155	2278	6049	3894	8.5
Prosper	Deon	Untreated	4363	2149	2214	6757	4608	9.4
		Physiological Maturity	4174	2125	2049	6593	4468	9.1
	Shelby	Untreated	4614	1773	2842	7109	5337	9.4
		Physiological Maturity	4769	1815	2954	7260	5445	9.2
	LSD within location		311	233	258	403	324	0.3
	LSD between locations		280	221	248	369	286	0.3

cP: centipoise

Overall, oats treated with glyphosate at the soft dough stage had lower peak and final viscosities compared to untreated oat, or oat treated at physiological maturity. However, this effect seemed to also be dependent on other factors, such as cultivar and growing location. Because there are no clear trends, it is unclear whether this effect was caused by glyphosate or by chance. Potentially, glyphosate has an effect which can only be seen under certain conditions-for example, the Rockford cultivar may be more susceptible to glyphosate in dry conditions than the Souris cultivar, which would explain the results seen in the 2015 crop year. The 2016 sample set results also show that there are no significant ($P>0.05$) differences in peak or final viscosity between untreated oat samples and oat samples treated with glyphosate at physiological maturity. Low peak viscosity of oat slurries is correlated with unacceptability to consumers (Liu, Bailey, et al., 2010). It is inconclusive whether glyphosate application at the soft dough stage causes lower peak viscosities in oat samples, but producers should ensure to apply only at physiological maturity to minimize the chance of producing a lower quality crop.

Rolled Quality Parameters

Overall Results

The rolled oat thicknesses for the 2015 sample set were nearly identical for all three treatments and ranged from 0.85-0.86 mm (Table 17). This falls within the wide range of industry standard of 0.4-1.2 mm, which varies based on the end product application (Decker, Rose, et al., 2014). Likewise, the standard deviation of the rolled oat thicknesses was nearly identical across all three treatments, indicating that a similar range of thicknesses was achieved for each treatment. Consistent thickness is important in rolled oats to ensure even cooking when prepared. No significant ($P>0.05$) differences were observed in rolled oat water absorption.

Absorption is related to cook time of rolled oats in baked goods or when prepared as a hot cereal (Ames and Rhymer, 2003).

Table 17: Rolled Oat Quality Parameters for 2015 Sample Set

Glyphosate Treatment	Rolled Oat Thickness (mm)	Rolled Oat Thickness Standard Deviation (mm)	Rolled Oat Absorption (mL H_2O/25 g oats)
Untreated	0.86^a	0.14^a	23.48^a
Soft Dough	0.85^a	0.13^a	23.58^a
Physiological Maturity	0.85^a	0.14^a	23.29^a

Values are averages of all locations/cultivars. Values in same column with the same superscript letter are not significantly different (*P*>0.05). Least significant difference was used for mean separation.

Like the 2015 sample set, the rolled oats produced from the 2016 sample set did not show any significant (*P*>0.05) differences in thickness, thickness standard deviation, or absorption (Table 18). Rolled oat thickness was 0.98 mm and 0.97 mm for untreated and glyphosate treated oat, respectively. Thickness standard deviation was 0.15 mm and 0.14 mm for untreated and glyphosate treated oat, respectively. Rolled oat absorption was 21.18 mL H_2O/25 g oats and 21.48 mL H_2O/25 g oats for untreated and glyphosate treated oat, respectively.

Table 18: Rolled Oat Quality Parameters for 2016 Sample Set

Glyphosate Treatment	Rolled Oat Thickness (mm)	Rolled Oat Thickness Standard Deviation (mm)	Rolled Oat Absorption (mL H_2O/25 g oats)
Untreated	0.98^a	0.15^a	21.18^a
Physiological Maturity	0.97^a	0.14^a	21.48^a

Values are averages of all locations/cultivars. Values in same column with the same superscript letter are not significantly different (*P*>0.05). Least significant difference was used for mean separation.

Both the 2015 and 2016 sample sets show that there are no significant (P>0.05) differences in rolled oat quality parameters between treatments (Table 17,Table 18). The lack of significant (P>0.05) differences in thickness is unsurprising, as the groat diameters did not show large variations between treatments (Table 9,Table 10). The lack of significant (*P*>0.05) differences in water absorption suggests that there were no major changes to the hydration of macromolecular components of the oat flakes caused by the glyphosate, which contributes (along with thickness) to absorption.

Results by Location and Cultivar

When examined by location and cultivar, no significant (*P*>0.05) differences were observed between treatments for the rolled oat parameters in the 2015 sampled set (Table 19). However, significant (*P*<0.05) differences were observed between locations. For instance, Prosper-grown rolled oats, which ranged from 0.94 mm-0.98 mm were significantly (*P*<0.05) thicker than Minot-grown rolled oats, which ranged from 0.72 mm-0.79 mm, for all glyphosate treatments. Conversely, Minot-grown rolled oats had significantly (*P*<0.05) higher water absorption than Prosper-grown rolled oats.

Table 19: Rolled Oat Quality Parameters for 2015 Sample Set by Location and Cultivar

Location	Cultivar	Glyphosate Treatment	Rolled Oat Thickness (mm)	Rolled Oat Thickness Standard Deviation (mm)	Rolled Oat Absorption (mL H_2O/25 g oats)
Minot	Rockford	Untreated	0.76	0.16	26.67
		Soft Dough	0.73	0.14	27.97
		Physiological Maturity	0.73	0.14	27.07
	Souris	Untreated	0.78	0.14	29.43
		Soft Dough	0.72	0.12	27.17
		Physiological Maturity	0.79	0.14	27.39
Prosper	Rockford	Untreated	0.94	0.13	17.83
		Soft Dough	0.98	0.13	20.10
		Physiological Maturity	0.95	0.14	18.77
	Souris	Untreated	0.94	0.12	19.97
		Soft Dough	0.97	0.12	19.07
		Physiological Maturity	0.96	0.13	19.93
	LSD within location		0.08	N/A	2.88
	LSD between locations		0.08	N/A	2.78

When the 2016 examined by location and cultivar, no significant (*P*>0.05) differences between treatments were observed in rolled oat thickness standard deviation or rolled oat absorption (Table 20). However, for Deon cultivar oat grown in Prosper, untreated rolled oats were significantly (*P*<0.05) thicker than glyphosate treated rolled oats. For the Shelby cultivar, samples grown in Crookston were significantly (*P*<0.05) thicker than samples grown in Prosper for both treatments. No significant (*P*>0.05) differences were observed between cultivars and growing locations for rolled oat absorption.

Table 20: Rolled Oat Quality Parameters for 2016 Sample Set by Location and Cultivar

Location	Cultivar	Glyphosate Treatment	Rolled Oat Thickness (mm)	Rolled Oat Thickness Standard Deviation (mm)	Rolled Oat Absorption (mL H_2O/25 g oats)
Crookston	Deon	Untreated	1.01	0.16	21.58
		Physiological Maturity	1.03	0.13	21.50
	Shelby	Untreated	0.99	0.15	22.05
		Physiological Maturity	1.00	0.18	21.75
Prosper	Deon	Untreated	0.99	0.14	20.18
		Physiological Maturity	0.95	0.14	20.90
	Shelby	Untreated	0.94	0.15	20.90
		Physiological Maturity	0.92	0.12	21.78
	LSD within location		0.04	N/A	1.89
	LSD between locations		0.04	N/A	1.86

The data summarized in Table 19 and Table 20 indicate that treatment with glyphosate at either soft dough stage or physiological maturity does not have a significant (*P*>0.05) effect on rolled oat quality parameters. On the other hand, quality parameters show significant (*P*>0.05) differences due to factors such as growing location and cultivar. These data indicate that glyphosate application will not influence oat flake thickness or water absorption.

Rolled Oat Granulation

Overall Results

Oat flake granulation is an important parameter to measure product consistency. Additionally, different granulation targets may be necessary depending on the product being produced (e.g. quick cooking versus rolled oats). Significant ($P<0.05$) differences were observed in the rolled oat granulation for the 2015 sample set (Table 21). The untreated rolled oats had the largest percentage of rolled oats remaining in the largest (4750 µm) sieve, at 27.20%. This was significantly ($P<0.05$) greater than the amount remaining for the oat treated at physiological maturity, which had 23.46% remaining. The sample treated at soft dough stage had 24.70% remaining, which was not significantly ($P>0.05$) different from the other two treatments. For the 2800 µm sieve, the oat treated at soft dough stage had the largest amount retained, at 63.18%. This was significantly ($P<0.05$) greater than the untreated oat, which had 61.37% retained. The untreated oat had the least amount of sample retained in the 2000 µm sieve, at 4.03%. This was significantly ($P<0.05$) less than both the oat treated with glyphosate and soft dough stage and physiological maturity, which had 4.68% and 4.86% retained, respectively. The oats treated at physiological maturity had the greatest percentage of breakage, or material that passed through all the sieves, and 9.35%. This was significantly ($P<0.05$) greater than the untreated oat or the oat treated with glyphosate at the soft dough stage, which had breakages of 7.39% and 7.44%, respectively.

Table 21: Granulation of 2015 Sample Set Rolled Oats

Glyphosate Treatment	4750* (%)	2800* (%)	2000* (%)	Breakage† (%)
Untreated	27.20^{a}	61.37^{a}	4.03^{a}	7.39^{a}
Soft Dough	24.70^{ab}	63.18^{b}	4.68^{b}	7.44^{a}
Physiological Maturity	23.46^{b}	62.33^{ab}	4.86^{b}	9.35^{b}

Values are averages of all locations/cultivars. Values in same column with the same superscript letter are not significantly different (*P*>0.05). Least significant difference was used for mean separation. *sieve opening size (µm), † material that passed through 2000 µm sieve

No significant (*P*>0.05) differences in granulation were observed between treatments for the 2016 sampled set rolled oats (Table 22). However, like the 2015 sample set rolled oats, untreated rolled oats did have a larger proportion of the largest size of rolled oats.

Table 22: Granulation of 2016 Sample Set Rolled Oats

Glyphosate Treatment	4750* (%)	2800* (%)	2000* (%)	Breakage† (%)
Untreated	23.86^{a}	72.98^{a}	1.75^{a}	1.46^{a}
Physiological Maturity	21.87^{a}	75.17^{a}	1.69^{a}	1.34^{a}

Values are averages of all locations/cultivars. Values in same column with the same superscript letter are not significantly different (*P*>0.05). Least significant difference was used for mean separation. *sieve opening size (µm), † material that passed through 2000 µm sieve

Results by Location and Cultivar

When divided by growing location and cultivar, similar trends were seen for both cultivars of oats grown in Prosper (Table 23). There were no significant (*P*>0.05) differences in the proportions of flakes remaining in the two largest sieves, or the breakage. However, for both the Rockford and Souris cultivars, the samples treated at the soft dough stage had a significantly (*P*<0.05) greater proportion of the smallest size oat flakes (2000 µm).

For the oats grown in Minot, no significant (*P*>0.05) differences were observed between the untreated control and either glyphosate treatment (Table 23). For the Rockford cultivar, no significant (*P*>0.05) differences were observed between the untreated control and the samples treated with glyphosate at the soft dough stage. However, significant (*P*<0.05) differences were observed between the control and the samples treated at physiological maturity for every granulation size. The untreated sample had a significantly (*P*<0.05) larger proportion of the largest size of rolled oats, which were retained in the 4750 µm sieve. However, the samples treated with glyphosate at physiological maturity had significantly (*P*<0.05) larger proportions of the other granulation sizes: 2800 µm, 2000 µm, and less than 2000 µm.

Table 23: Granulation of 2015 Sample Set Rolled Oats by Cultivar and Location

Location	Cultivar	Glyphosate Treatment	4750* (%)	2800* (%)	2000* (%)	Breakage† (%)
Minot	Rockford	Untreated	59.07	26.96	3.95	10.02
		Soft Dough	54.60	30.22	4.27	10.91
		Physiological Maturity	47.64	31.21	5.41	15.74
	Souris	Untreated	32.37	44.16	6.83	16.63
		Soft Dough	30.69	46.46	6.91	15.94
		Physiological Maturity	28.75	44.76	7.62	18.86
Prosper	Rockford	Untreated	6.35	89.68	2.84	1.13
		Soft Dough	4.41	90.03	4.24	1.32
		Physiological Maturity	5.23	89.80	3.85	1.13
	Souris	Untreated	11.02	84.68	2.50	1.79
		Soft Dough	9.09	86.01	3.31	1.59
		Physiological Maturity	12.24	83.53	2.57	1.66
	LSD within location		5.51	3.45	1.14	2.73
	LSD between locations		5.39	4.46	1.17	2.69

*sieve opening size (µm), † material that passed through 2000 µm sieve

The 2016 sample set showed a significant (*P*<0.05) difference in breakage between treatments for the Shelby cultivar oat grown in Crookston (Table 24). The untreated rolled oats had a higher percentage of breakage, at 1.96%, versus 1.31% for the glyphosate treated rolled oats. However, no other significant differences between treatments were observed. However, significant (*P*<0.05) differences were observed between interactions, especially in the larger granulation sizes. For instance, the percentage of rolled oats retained in the 4750 µm sieve ranged from 16.06%-29.94%, and the percentage retained in the 2800 sieve ranged from 67.45%-80.48%.

Table 24: Granulation of 2016 Sample Set Rolled Oats by Cultivar and Location

Location	Cultivar	Glyphosate Treatment	4750* (%)	2800* (%)	2000* (%)	Breakage† (%)
Crookston	Deon	Untreated	29.94	67.45	1.20	1.56
		Physiological Maturity	29.29	68.20	1.20	1.46
	Shelby	Untreated	25.27	71.57	1.25	1.96
		Physiological Maturity	20.02	77.47	1.35	1.31
Prosper	Deon	Untreated	24.16	72.58	2.05	1.21
		Physiological Maturity	21.81	74.54	2.30	1.35
	Shelby	Untreated	16.06	80.33	2.50	1.11
		Physiological Maturity	16.37	80.48	1.90	1.25
	LSD within location		5.87	5.74	0.78	0.38
	LSD between locations		5.83	5.47	1.04	0.49

*sieve opening size (µm), † material that passed through 2000 µm sieve

Overall, there were no clear trends identified in the relationship between rolled oat granulation and glyphosate application. Again, this is likely due to the fact that all of the groats had similar diameters. There is some evidence that treatment with glyphosate may reduce the proportion of the largest size of oat flakes in a sample, but this is not seen for every cultivar and location. However, this effect was more prominent in the samples treated at physiological maturity, which is not what one would expect: application at the soft dough stage occurs earlier, which should presumably have a greater effect. Additionally, cultivar and growing location have much larger effects on oat granulation. Thus, it is inconclusive whether glyphosate application has an effect on rolled oat granulation.

Scanning Electron Microscopy Studies

Oat starch exists in compound granule clusters that are around 60 µm in diameter (Sayar and White, 2011). The granules can exist in wide variety of shapes, and can be polyhedral, ovoid, or hemispherical. Variations in oat starch granule morphology have been observed in oats that have undergone different heat treatments (Ovando-Martínez, Whitney, et al., 2013). For instance, oats that were autoclaved showed a greater number of clusters of small starch granules, compared to untreated controls.

Scanning electron microscopy was performed on cross sections of untreated groats, groats treated with glyphosate at the soft dough stage, and groats treated with glyphosate at physiological maturity (Figure 13). Both large and small granule clusters can be seen in all three treatments. However, no major differences in the structures of the starch granules were observed in the images. Differences in starch granule morphology could potentially be seen if starch was extracted in from oat flour and visualized. Or possibly, differences in starch caused by glyphosate may only be apparent at the molecular level and not the granular level.

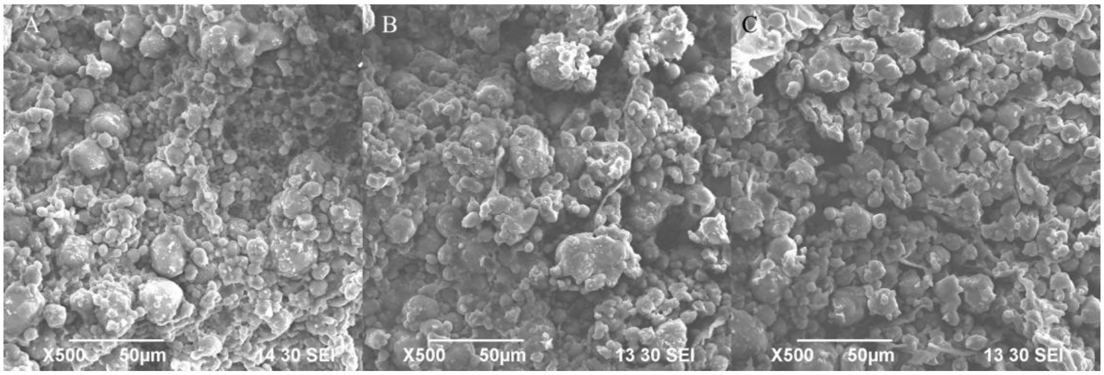

Figure 13: Cross Sections of Heat Treated Groats
A: Untreated B: Treated with glyphosate at soft dough stage. C: Treated with glyphosate at physiological maturity.

CONCLUSIONS

Application of glyphosate to oat during the soft dough stage had a detrimental effect on groat quality. Compared to untreated controls, glyphosate application at the soft dough stage resulted in a lower percentage of plump groats, a lower groat percentage, and higher percentage of damaged starch. Additionally, groats treated at this stage were also less hard than untreated groats, which may not necessarily be detrimental, depending on the final use of the oat. Treatment at the soft dough stage also resulted in a lower viscosity slurry upon heating, which is less preferred by consumers. Although application at the soft dough stage did not impact rolled oat thickness or rolled oat water absorption, glyphosate application had an inconclusive effect on rolled oat size, which could be detrimental depending on the desired end product.

However, application of glyphosate at physiological maturity did not have the profound effect that was seen at application at the soft dough stage. This is supported by both the results of the 2015 and 2016 sample sets. The only noticeable effect produced by treatment at physiological maturity was a change in the size distribution of rolled oats.

In conclusion, preharvest glyphosate application is not detrimental to final oat quality as long as care is taken not to apply glyphosate too early. However, it may be worthwhile for farmers to develop an alternative to preharvest glyphosate, as the practice is falling out of favor with buyers and consumers. Further research can be performed to determine the cause of the differences observed in oat treated with glyphosate at the soft dough stage, and to investigate whether glyphosate application affects other oat macromolecules, such as β-glucan.

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APPENDIX

Table A1: Analysis of Variance for 2015 Groat Quality Parameters

	Source	DF	Mean Square	F Value	Pr>F
Plump	Location	1	1145.54	1022.41	<.0001
	Cultivar	1	19.32	47.34	<.0001
	Treatment	2	15.37	37.66	<.0001
	Location*Cultivar	1	5.19	12.73	0.0019
	Cultivar*Treatment	2	6.66	16.31	<.0001
	Location*Treatment	2	9.51	23.31	<.0001
	Location*Cultivar*Treatment	2	6.36	15.57	<.0001
	Error	20	0.41	-	-
Groat Percentage	Location	1	96.93	276.55	<.0001
	Cultivar	1	1.26	1.36	0.2566
	Treatment	2	6.35	6.87	0.0054
	Location*Cultivar	1	21.51	23.24	0.0001
	Cultivar*Treatment	2	2.16	2.33	0.1230
	Location*Treatment	2	2.68	2.89	0.0788
	Location*Cultivar*Treatment	2	3.61	3.9	0.0372
	Error	20	0.93	-	-
Test Weight	Location	1	770.99	557.45	<.0001
	Cultivar	1	1.07	1.58	0.2226
	Treatment	2	1.31	1.94	0.1701
	Location*Cultivar	1	0.01	0.01	0.9042
	Cultivar*Treatment	2	0.08	0.12	0.884
	Location*Treatment	2	0.78	1.16	0.3328
	Location*Cultivar*Treatment	2	0.51	0.75	0.4848
	Error	20	0.67	-	-
Protein Content	Location	1	10.69	89.36	0.0007
	Cultivar	1	4.00	2.47	0.1315
	Treatment	2	0.44	0.27	0.7651
	Location*Cultivar	1	4.36	2.69	0.1164
	Cultivar*Treatment	2	0.36	0.23	0.8002
	Location*Treatment	2	0.10	0.06	0.9404
	Location*Cultivar*Treatment	2	0.89	0.55	0.5841
	Error	20	1.62	-	-

Table A2: Analysis of Variance for 2016 Groat Quality Parameters

	Source	DF	Mean Square	F Value	Pr>F
Plump	Location	1	0.2321	7.79	0.0315
	Cultivar	1	0.0002	0.01	0.9428
	Treatment	1	0.0396	1.19	0.2893
	Location*Cultivar	1	0.0010	0.03	0.8670
	Cultivar*Treatment	1	0.0086	0.26	0.6165
	Location*Treatment	1	0.0508	1.53	0.2318
	Location*Cultivar*Treatment	1	0.0033	0.10	0.7560
	Error	18	0.0332	-	-
Groat Percentage	Location	1	53.43	21.03	0.0037
	Cultivar	1	24.66	39.88	<.0001
	Treatment	1	1.33	2.15	0.1594
	Location*Cultivar	1	1.15	1.86	0.1892
	Cultivar*Treatment	1	2.48	4.01	0.0605
	Location*Treatment	1	1.38	2.23	0.1523
	Location*Cultivar*Treatment	1	0.27	0.44	0.5156
	Error	18	0.62	-	-
Test Weight	Location	1	68.74	119.22	<.0001
	Cultivar	1	39.83	126.59	<.0001
	Treatment	1	0.95	3.00	0.1001
	Location*Cultivar	1	0.14	0.44	0.5165
	Cultivar*Treatment	1	0.05	0.17	0.6869
	Location*Treatment	1	1.09	3.46	0.0794
	Location*Cultivar*Treatment	1	0.43	1.36	0.2588
	Error	18	0.31	-	-
Protein Content	Location	1	0.117	0.68	0.4406
	Cultivar	1	12.752	29.60	<.0001
	Treatment	1	1.108	2.57	0.1262
	Location*Cultivar	1	0.408	0.95	0.3431
	Cultivar*Treatment	1	3.039	7.06	0.0161
	Location*Treatment	1	1.003	2.33	0.1444
	Location*Cultivar*Treatment	1	0.049	0.11	0.7399
	Error	18	0.431	-	-

Table A3: Analysis of Variance for 2015 Starch Parameters

	Source	DF	Mean Square	F Value	Pr>F
Total Starch	Location	1	56.13	3.63	0.13
	Cultivar	1	10.15	0.94	0.34
	Treatment	2	7.31	0.68	0.52
	Location*Cultivar	1	81.95	7.6	0.01
	Cultivar*Treatment	2	9.99	0.93	0.41
	Location*Treatment	2	8.32	0.77	0.48
	Location*Cultivar*Treatment	2	0.76	0.07	0.93
	Error	20	10.78	-	-
Starch Damage	Location	1	0.026	7.73	0.05
	Cultivar	1	0.035	6.68	0.02
	Treatment	2	0.026	5.04	0.02
	Location*Cultivar	1	0.000	0.03	0.87
	Cultivar*Treatment	2	0.001	0.17	0.85
	Location*Treatment	2	0.003	0.63	0.54
	Location*Cultivar*Treatment	2	0.001	0.23	0.80
	Error	20	0.005	-	-

Table A4: Analysis of Variance for 2016 Starch Parameters

	Source	DF	Mean Square	F Value	Pr>F
Total Starch	Location	1	0.71	0.17	0.70
	Cultivar	1	25.40	3.78	0.07
	Treatment	1	0.14	0.02	0.89
	Location*Cultivar	1	2.33	0.35	0.56
	Cultivar*Treatment	1	13.95	2.07	0.17
	Location*Treatment	1	0.25	0.04	0.85
	Location*Cultivar*Treatment	1	6.20	0.92	0.35
	Error	18	6.73	-	-
Starch Damage	Location	1	0.003	0.13	0.726
	Cultivar	1	0.201	14.99	0.001
	Treatment	1	0.006	0.47	0.502
	Location*Cultivar	1	0.029	2.19	0.156
	Cultivar*Treatment	1	0.012	0.88	0.362
	Location*Treatment	1	0.035	2.60	0.124
	Location*Cultivar*Treatment	1	0.030	2.25	0.151
	Error	18	.013	-	-

Table A5: Analysis of 2015 SKCS

	Source	DF	Mean Square	F Value	Pr>F
Hardness Index	Location	1	375.93	266.10	<.0001
	Cultivar	1	119.81	21.09	0.0002
	Treatment	2	29.41	5.18	0.0154
	Location*Cultivar	1	3.32	0.58	0.4533
	Cultivar*Treatment	2	3.76	0.66	0.5270
	Location*Treatment	2	49.31	8.68	0.0019
	Location*Cultivar*Treatment	2	4.21	0.74	0.4889
	Error	20	5.68	-	-
Weight	Location	1	10.67	31.310	0.0050
	Cultivar	1	12.66	22.310	0.0001
	Treatment	2	0.93	1.640	0.2185
	Location*Cultivar	1	19.07	33.590	<.0001
	Cultivar*Treatment	2	1.48	2.610	0.0985
	Location*Treatment	2	0.84	1.480	0.2508
	Location*Cultivar*Treatment	2	0.22	0.390	0.6831
	Error	20	0.57	-	-
Diameter	Location	1	0.0500	123.46	0.0004
	Cultivar	1	0.0080	12.37	0.0022
	Treatment	2	0.0035	5.43	0.0131
	Location*Cultivar	1	0.0140	21.76	0.0001
	Cultivar*Treatment	2	0.0022	3.40	0.0534
	Location*Treatment	2	0.0001	0.22	0.8007
	Location*Cultivar*Treatment	2	0.0013	2.04	0.1568
	Error	20	0.0006	-	-

Table A6: Analysis of 2016 SKCS

	Source	DF	Mean Square	F Value	Pr>F
Hardness Index	Location	1	203.48	8.58	0.03
	Cultivar	1	62.42	3.56	0.08
	Treatment	1	2.28	0.13	0.72
	Location*Cultivar	1	193.84	11.06	0.00
	Cultivar*Treatment	1	4.36	0.25	0.62
	Location*Treatment	1	9.37	0.53	0.47
	Location*Cultivar*Treatment	1	0.42	0.02	0.88
	Error	18	17.53	-	-
Weight	Location	1	0.389	0.45	0.5265
	Cultivar	1	41.080	50.43	<.0001
	Treatment	1	0.705	0.87	0.3646
	Location*Cultivar	1	2.900	3.56	0.0754
	Cultivar*Treatment	1	0.069	0.08	0.7745
	Location*Treatment	1	0.032	0.04	0.8447
	Location*Cultivar*Treatment	1	0.004	0.01	0.9430
	Error	18	0.815	-	-
Diameter	Location	1	0.0033	1.92	0.216
	Cultivar	1	0.0024	4.69	0.044
	Treatment	1	0.0013	2.52	0.130
	Location*Cultivar	1	0.0013	2.53	0.129
	Cultivar*Treatment	1	0.0002	0.34	0.567
	Location*Treatment	1	0.0002	0.36	0.555
	Location*Cultivar*Treatment	1	9.99×10^{-7}	0.00	0.965
	Error	18	.0005	-	-

Table A7: Analysis of Variance for 2015 RVA Parameters

	Source	DF	Mean Square	F Value	Pr>F
Peak Viscosity	Location	1	267117	1.27	0.3232
	Cultivar	1	633881	25.81	<.0001
	Treatment	2	202567	8.25	0.0024
	Location*Cultivar	1	879531	35.82	<.0001
	Cultivar*Treatment	2	83132	3.39	0.0542
	Location*Treatment	2	246419	10.03	0.0010
	Location*Cultivar*Treatment	2	90799	3.70	0.0430
	Error	20	24557	-	-
Holding Strength	Location	1	576	0.01	0.9274
	Cultivar	1	13767	0.20	0.6568
	Treatment	2	59071	0.87	0.4329
	Location*Cultivar	1	4354178	64.36	<.0001
	Cultivar*Treatment	2	106889	1.58	0.2306
	Location*Treatment	2	345549	5.11	0.0161
	Location*Cultivar*Treatment	2	120552	1.78	0.1940
	Error	20	67650	-	-
Breakdown	Location	1	292501	6.04	0.0699
	Cultivar	1	460815	9.28	0.0064
	Treatment	2	50304	1.01	0.3810
	Location*Cultivar	1	1319818	26.58	<.0001
	Cultivar*Treatment	2	40563	0.82	0.4560
	Location*Treatment	2	113714	2.29	0.1272
	Location*Cultivar*Treatment	2	34482	0.69	0.5110
	Error	20	49657	-	-
Final Viscosity	Location	1	9791684	64.26	0.0013
	Cultivar	1	2620621	59.34	<.0001
	Treatment	2	368696	8.35	0.0023
	Location*Cultivar	1	2970.25	0.07	0.7980
	Cultivar*Treatment	2	135030	3.06	0.0694
	Location*Treatment	2	407838	9.23	0.0014
	Location*Cultivar*Treatment	2	169476	3.84	0.0389
	Error	20	44165	-	-

Table A7: Analysis of Variance for 2015 RVA Parameters (continued)

	Source	DF	Mean Square	F Value	Pr>F
Setback	Location	1	9942460	387.27	<.0001
	Cultivar	1	2254502	45.27	<.0001
	Treatment	2	138821	2.79	0.0855
	Location*Cultivar	1	4584595	92.06	<.0001
	Cultivar*Treatment	2	59441	1.19	0.3238
	Location*Treatment	2	107801	2.16	0.1409
	Location*Cultivar*Treatment	2	4543	0.09	0.9132
	Error	20	49800	-	-
Peak Time	Location	1	0.55	4.05	0.1144
	Cultivar	1	0.30	3.06	0.0954
	Treatment	2	0.10	1.08	0.3585
	Location*Cultivar	1	3.87	39.96	<.0001
	Cultivar*Treatment	2	0.14	1.43	0.2622
	Location*Treatment	2	0.22	2.28	0.1280
	Location*Cultivar*Treatment	2	0.07	0.76	0.4800
	Error	20	.10	-	-

Table A8: Analysis of Variance for 2016 RVA Parameters

	Source	DF	Mean Square	F Value	Pr>F
Peak Viscosity	Location	1	947720	71.91	0.0001
	Cultivar	1	2089479	47.55	<.0001
	Treatment	1	11743	0.27	0.6115
	Location*Cultivar	1	61688	1.40	0.2515
	Cultivar*Treatment	1	103854	2.36	0.1416
	Location*Treatment	1	3549	0.08	0.7795
	Location*Cultivar*Treatment	1	26738	0.61	0.4455
	Error	18	43945	-	-
Holding Strength	Location	1	813450	47.82	0.0005
	Cultivar	1	699745	28.34	<.0001
	Treatment	1	4232	0.17	0.6838
	Location*Cultivar	1	18050	0.73	0.4038
	Cultivar*Treatment	1	378	0.02	0.9029
	Location*Treatment	1	1513	0.06	0.8073
	Location*Cultivar*Treatment	1	12720	0.52	0.4821
	Error	18	24691	-	-
Breakdown	Location	1	3517215	140.16	<.0001
	Cultivar	1	5207571	173.07	<.0001
	Treatment	1	30074	1.00	0.3307
	Location*Cultivar	1	13001	0.43	0.5193
	Cultivar*Treatment	1	116765	3.88	0.0644
	Location*Treatment	1	9695	0.32	0.5773
	Location*Cultivar*Treatment	1	2574	0.09	0.7733
	Error	18	30090	-	-
Final Viscosity	Location	1	8306888	264.92	<.0001
	Cultivar	1	1252945	16.99	0.0006
	Treatment	1	1653.125	0.02	0.8827
	Location*Cultivar	1	103968	1.41	0.2506
	Cultivar*Treatment	1	60726	0.82	0.3762
	Location*Treatment	1	435.125	0.01	0.9396
	Location*Cultivar*Treatment	1	39621	0.54	0.4731
	Error	18	73764	-	-

Table A8: Analysis of Variance for 2016 RVA Parameters (continued)

	Source	DF	Mean Square	F Value	Pr>F
Setback	Location	1	14319276	2037.13	<.0001
	Cultivar	1	3825378	80.37	<.0001
	Treatment	1	11175	0.23	0.6338
	Location*Cultivar	1	208658	4.38	0.0507
	Cultivar*Treatment	1	70688	1.49	0.2387
	Location*Treatment	1	3570	0.08	0.7873
	Location*Cultivar*Treatment	1	7442	0.16	0.6972
	Error	18	47596	-	-
Peak Time	Location	1	7.2835	95.52	<.0001
	Cultivar	1	0.2113	5.75	0.0276
	Treatment	1	0.1701	4.63	0.0452
	Location*Cultivar	1	0.1701	4.63	0.0452
	Cultivar*Treatment	1	0.0401	1.09	0.3098
	Location*Treatment	1	0.0235	0.64	0.4346
	Location*Cultivar*Treatment	1	0.0001	0.00	0.9517
	Error	18	0.0367	-	-

Table A9: Analysis of Variance for 2015 Rolled Oat Quality Parameters

	Source	DF	Mean Square	F Value	Pr>F
Thickness	Location	1	0.3886	143.91	0.0003
	Cultivar	1	0.0011	0.47	0.5029
	Treatment	2	0.0001	0.02	0.9776
	Location*Cultivar	1	0.0012	0.51	0.4814
	Cultivar*Treatment	2	0.0016	0.67	0.5219
	Location*Treatment	2	0.0049	2.12	0.1458
	Location*Cultivar*Treatment	2	0.0006	0.26	0.7758
	Error	20	0.0023	-	-
Thickness Standard Deviation	Location	1	0.0008	1.44	0.2960
	Cultivar	1	0.0011	1.12	0.3029
	Treatment	2	0.0005	0.44	0.6476
	Location*Cultivar	1	0.0001	0.07	0.7997
	Cultivar*Treatment	2	0.0001	0.07	0.9289
	Location*Treatment	2	0.0004	0.40	0.6760
	Location*Cultivar*Treatment	2	0.0002	0.23	0.7932
	Error	20	0.0010	-	-
Absorption	Location	1	625.5835	308.31	<.0001
	Cultivar	1	5.1908	1.82	0.1924
	Treatment	2	0.2525	0.09	0.9156
	Location*Cultivar	1	0.0001	0.00	0.9946
	Cultivar*Treatment	2	8.5013	2.98	0.0736
	Location*Treatment	2	1.4946	0.52	0.6000
	Location*Cultivar*Treatment	2	0.4375	0.15	0.8588
	Error	20	2.8517	-	-

Table A10: Analysis of Variance for 2016 Rolled Oat Quality Parameters

	Source	DF	Mean Square	F Value	Pr>F
Thickness	Location	1	0.0236	51.38	0.0004
	Cultivar	1	0.0086	9.86	0.0057
	Treatment	1	0.0005	0.60	0.4470
	Location*Cultivar	1	0.0010	1.11	0.3068
	Cultivar*Treatment	1	0.0001	0.09	0.7640
	Location*Treatment	1	0.0044	5.09	0.0368
	Location*Cultivar*Treatment	1	0.0002	0.25	0.6211
	Error	18	0.0009	-	-
Thickness Standard Deviation	Location	1	0.0018	2.22	0.1865
	Cultivar	1	0.0004	0.41	0.5303
	Treatment	1	0.0003	0.26	0.6138
	Location*Cultivar	1	0.0011	1.06	0.3176
	Cultivar*Treatment	1	0.0001	0.05	0.825
	Location*Treatment	1	0.0002	0.23	0.6343
	Location*Cultivar*Treatment	1	0.0040	3.77	0.0678
	Error	18	0.0011	-	-
Absorption	Location	1	4.891	2.94	0.1373
	Cultivar	1	2.697	1.67	0.2127
	Treatment	1	0.747	0.46	0.5051
	Location*Cultivar	1	0.385	0.24	0.6314
	Cultivar*Treatment	1	0.003	0.00	0.9683
	Location*Treatment	1	1.955	1.21	0.2858
	Location*Cultivar*Treatment	1	0.069	0.04	0.8382
	Error	18	1.616	-	-

Table A11: Analysis of Variance for 2015 Rolled Oat Granulation

	Source	DF	Mean Square	F Value	Pr>F
4750	Location	1	10485.00	1174.86	<.0001
	Cultivar	1	705.85	67.51	<.0001
	Treatment	2	43.58	4.17	0.0307
	Location*Cultivar	1	1842.75	176.24	<.0001
	Cultivar*Treatment	2	20.57	1.97	0.1660
	Location*Treatment	2	49.94	4.78	0.0201
	Location*Cultivar*Treatment	2	5.59	0.53	0.5942
	Error	20	10.46	-	-
2800	Location	1	22494.00	1218.52	<.0001
	Cultivar	1	251.48	61.28	<.0001
	Treatment	2	9.85	2.40	0.1164
	Location*Cultivar	1	969.73	236.32	<.0001
	Cultivar*Treatment	2	6.04	1.47	0.2532
	Location*Treatment	2	6.70	1.63	0.2205
	Location*Cultivar*Treatment	2	1.19	0.29	0.7507
	Error	20	4.10	-	-
2000	Location	1	61.44	94.72	0.0006
	Cultivar	1	6.73	15.04	0.0009
	Treatment	2	2.29	5.11	0.0161
	Location*Cultivar	1	26.42	59.01	<.0001
	Cultivar*Treatment	2	0.49	1.09	0.3561
	Location*Treatment	2	1.70	3.79	0.0403
	Location*Cultivar*Treatment	2	0.02	0.06	0.9460
	Error	20	0.45	-	-
Breakage	Location	1	1579.71	652.06	<.0001
	Cultivar	1	65.85	25.69	<.0001
	Treatment	2	14.89	5.81	0.0103
	Location*Cultivar	1	44.18	17.24	0.0005
	Cultivar*Treatment	2	2.46	0.96	0.3994
	Location*Treatment	2	15.92	6.21	0.0080
	Location*Cultivar*Treatment	2	2.17	0.85	0.4441
	Error	20	2.56	-	-

Table A12: Analysis of Variance for 2016 Rolled Oat Granulation

	Source	DF	Mean Square	F Value	Pr>F
4750	Location	1	341.19	20.00	0.0042
	Cultivar	1	377.64	24.17	0.0001
	Treatment	1	31.46	2.01	0.1730
	Location*Cultivar	1	0.08	0.00	0.9449
	Cultivar*Treatment	1	1.87	0.12	0.7334
	Location*Treatment	1	7.48	0.48	0.4978
	Location*Cultivar*Treatment	1	26.36	1.69	0.2104
	Error	18	15.63	-	-
2800	Location	1	270.00	24.01	0.00
	Cultivar	1	366.65	24.60	0.00
	Treatment	1	38.27	2.57	0.13
	Location*Cultivar	1	0.05	0.00	0.96
	Cultivar*Treatment	1	5.55	0.37	0.55
	Location*Treatment	1	10.35	0.69	0.42
	Location*Cultivar*Treatment	1	24.23	1.63	0.22
	Error	18	14.90	-	-
2000	Location	1	7.01	6.58	0.0426
	Cultivar	1	0.03	0.12	0.7379
	Treatment	1	0.03	0.12	0.7281
	Location*Cultivar	1	0.01	0.04	0.8440
	Cultivar*Treatment	1	0.28	1.02	0.3259
	Location*Treatment	1	0.11	0.39	0.5395
	Location*Cultivar*Treatment	1	0.45	1.64	0.2160
	Error	18	0.27	-	-
Breakage	Location	1	0.927	4.10	0.0894
	Cultivar	1	0.001	0.02	0.8891
	Treatment	1	0.103	1.59	0.2230
	Location*Cultivar	1	0.100	1.54	0.2300
	Cultivar*Treatment	1	0.151	2.33	0.1442
	Location*Treatment	1	0.547	8.42	0.0095
	Location*Cultivar*Treatment	1	0.151	2.33	0.1446
	Error	18	0.065	-	-