EFFECTS OF HEAT TREATMENTS ON THE SAFETY AND NUTRITIONAL PROPERTIES OF WHOLE GRAIN BARLEY

by

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ABSTRACT

Health claims for barley β-glucan (BG) have prompted the development of more food products using barley. Some new products do not use any form of heat treatment which could become an issue as barley has been found to have high microbial contamination. The aim of this research was to evaluate current commercial barley products for microbial and BG quality and determine the effects of different heat treatments on the safety and physicochemical properties of BG of whole grain barley. Three heat treatments (micronization, roasting and conditioning) were performed on 3 cultivars of barley (CDC Rattan, CDC McGwire and CDC Fibar). The microbial quality was measured with standard plate count (SPC), yeast and mould (MYC), and coliforms/*E. coli*. Only 4 of the 17 commercial barley products tested met acceptable microbial limits used in this study. All 3 heat treatments reduced SPC, MYC and coliforms to acceptable levels. BG was extracted using an *in vitro* digestion method to determine its viscosity, molecular weight (MW) and solubility. Heat-treated barley increased the BG viscosity and MW compared to the untreated barley. The effect of heat treatment on starch pasting, particle size and colour were also evaluated. Overall, heat treatments improved the safety and potential health benefits of whole grain barley.

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LIST OF ABBREVIATIONS

AACCI	American Association of Cereal Chemists International
AAFC	Agriculture and Agri-Food Canada
ANOVA	Analysis of Variance
BG	β-glucan
CFIA	Canadian Food Inspection Agency
CFU	Colony Forming Units
DON	Deoxynivalenol
LSD	Least Significant Difference
MDL	Minimum Detectable Limit
Mp	Peak Molecular Weight
Mw	Weighted Average Molecular Weight
MW	Molecular Weight
MYC	Mold and Yeast Count
PDA	Potato Dextrose Agar
RBC	Rose Bengal Chloramphenicol Agar
RVA	Rapid Visco Analyser
SD	Standard Deviation
SPC	Standard Plate Count

1. INTRODUCTION

North Americans do not typically incorporate barley as a staple in their diet. Most consumers are unaware of its health benefits and its wide range of food uses. Currently, barley is mainly used for animal feed, followed by malt, seed and human food (Newman and Newman, 2008a). Whole grain barley flour and other barley products have been growing in popularity since recent health claims in the United States (U.S. Food and Drug Administration, 2006) and Canada (Health Canada, 2012a). These health claims pertain to β-glucan, a type of soluble fibre found in barley, and its cholesterol-lowering effects. As research and consumer trends develop, processors seek new healthful food applications for barley. Researchers have found ways to create high β-glucan milling fractions, which can be added to food formulations to increase nutritional benefits (Andersson et al. 2003; Izydorczyk et al. 2003; Vasanthan and Temelli, 2008; Román et al. 2010; Sullivan et al. 2010a; Izydorczyk et al. 2011; Srinivasan and Smith, 2012; Gómez-Caravaca et al. 2015).

However, as new whole grain products are developed that may not be subjected to thermal processes, the safety of barley and other cereal grains becomes a concern. Barley has been shown to have high microbial (Ames unpublished data; Kottapalli and Wolf-Hall, 2008) and mycotoxin contamination (Campbell et al. 2000; Lombaert et al. 2003; Castells et al. 2006; Bensassi et al. 2011; Barthel et al. 2012) which poses a threat to consumers. Most people don't consider microbial contamination a problem because usually grain products undergo some kind of heat treatment, for example baking, before consumption. However, over the past 10 years, 3 recorded foodborne illness outbreaks have occurred in the world due to contaminated wheat flour (Zhang et al. 2007; Neil et al. 2012; McCallum et al. 2013).

The use of heat treatments to try to improve the safety, nutritional and physicochemical properties of whole grain barley was the purpose of the present research. The heat treatments explored in this research include micronization, roasting and conditioning. Micronization involves using infrared waves to heat the barley kernel. Conditioning uses steam at atmospheric pressure followed by a drying process. Roasting uses only dry heat, often at a lower temperature and for a longer time. Heat treatments may change the β-glucan viscosity, solubility and molecular weight, which may affect its healthful properties (Izydorczyk et al. 2000; Cenkowski et al. 2006; Hu et al. 2010; Gujral et al. 2011; Ames et al. 2015a). Inactivating β-glucanase enzymes will prevent their breakdown of β-glucan, which is beneficial since high molecular weight, viscosity and solubility are desired for increased nutritional benefits (Theuwissen and Mensink, 2008; Wang et al. 2013). However, this is a difficult task. Researchers have been both successful and ineffective when employing different treatments to try to inactivate β-glucanases (Knuckles and Chiu, 1999; Izydorczyk et al. 2000; Lazaridou et al. 2014; Rieder et al. 2015). Heat treatments could also extend the shelf life of whole grain barley products by inactivating lipase enzymes which can cause rancidity (Gates, 2007). Rancidity can be a problem in whole grain barley products due to the inclusion of the germ, which contains lipids.

Heat treatment of barley can also affect other characteristics of the grain. Starch is a major component of barley, making up 45-60% of the kernel (Newman and Newman, 2008b). Starch pasting is an important functional characteristic as it will affect how barley reacts during food processing. Additional factors that will affect the starch pasting profile include: cultivar and the amylose to amylopectin ratio (Yanagisawa et al. 2006; Gujral et al. 2013; Li et al. 2014).

Roasting has been shown to decrease peak, breakdown, setback and final viscosity of barley flour (Sharma et al. 2011). However, in other research, heat treatments have increased the peak and final viscosity (Zhou et al. 1999; Cenkowski et al. 2006; Emami et al. 2012). Typically, reduction in viscosity in the starch pasting profile is due to partial starch gelatinization in the heat treatment. However, β-glucan largely contributes to the viscosity, even more so than starch, and needs to be accounted for when analyzing whole grain barley flour (Liu and White, 2011).

Particle size is another important aspect that could be changed due to heat treatment. Very little research has been done on the impact of heat treatment on fractionation of milled barley and particle size. Heat treatments can affect kernel hardness (Salmenkallio-Marttila et al. 2004; Murthey et al. 2008), which in turn may change how the barley kernel fractures during milling. Variation in particle size could impact how air classification creates nutrient rich fractions due to different particle densities (Ferrari et al. 2009) and how barley flour reacts in a food system (Ross and Ames, 2005; Izydorczyk et al. 2008; Prasopsunwattana et al. 2009; Lazaridou et al. 2014).

In terms of the safety aspects of whole grain products, research, in general, is not extensive. Very little is known about microbial and mycotoxin accumulation in barley and how to reduce it. Heat treating whole grain barley prior to milling has the potential to be used as a method to decrease microbial and mycotoxin contamination. In addition, it may have nutritional benefits to improve the value to consumers and industry. As knowledge of food safety increases, it has become apparent that cereal grains and grain products are a concern and attention should be brought to the subject.

The goal of this research was to find an effective heat treatment to improve the safety and nutrition of whole grain barley and barley products that could be applied by industry. The specific objectives were as follows:

1) To establish if current commercial whole grain barley products meet microbial safety guidelines and if they have the potential to meet the requirements for the β-glucan health claim
2) To determine if heat treatments (micronization, conditioning and roasting) reduce the microbial contamination of whole grain barley flour
3) To determine what effects the heat treatments have on physicochemical properties of the barley by evaluating β-glucan content, molecular weight, viscosity and solubility, as well as peroxidase activity, particle size, starch pasting and colour.

2. LITERATURE REVIEW

2.1 Barley Production and Utilization

Barley (*Hordeum vulgare* L.) cultivation has been reported as far back in history as 10 000 years ago in the Middle East (Akar et al. 2004) and over time, barley began to be grown all over the world. Canada is one of the top ten producers of barley in the world, along with Russia, Ukraine, France, Germany, the United States of America, Australia, Turkey, the United Kingdom, and Argentina (FAOSTAT, 2013). In 2014, Canada produced over 7.1 million metric tonnes of barley, with Alberta being the highest producing province (Statistics Canada, 2015). Over 90% of Canada's barley production comes from the three Prairie Provinces, Alberta, Saskatchewan and Manitoba (Table 2.1).

Currently the main use for barley in North America is animal feed, followed by malt, seed and human food (Newman and Newman, 2008a). Malted barley has been used to make beer and whiskey for thousands of years and continues to be one of the leading uses for barley. The use of barley as food is low as it is often unavailable and unfamiliar to consumers so they opt for wheat products. Most North American consumers associate barley with pearled barley, which is used in soups or pilaf. However, barley is quickly becoming more popular. The recent establishment of health claims for barley soluble fibre in the United States (U.S. Food and Drug Administration, 2006) and Canada (Health Canada, 2012a), has the potential to increase demand for barley food products.

Table 2.1 Barley production in Canada in 2014

Location	Harvested area (hectares)	Barley Produced (metric tonnes)	Barley Produced (%)
Canada	2 136 100	7 119 000	100.0
Alberta	1 141 200	4 131 300	58.0
Saskatchewan	744 600	2 172 900	30.5
Manitoba	107 200	354 900	5.0
Quebec	52 000	163 500	2.3
Ontario	41 300	151 300	2.1
Prince Edward Island	25 100	78 400	1.1
British Columbia	16 600	42 700	0.6
New Brunswick	6 900	20 100	0.3
Nova Scotia	1 200	3 900	0.1

Data from Statistics Canada (2015).

2.2 Barley Processing

2.2.1 Milling

The barley milling process is similar to other cereal grains. Steps can be added or omitted throughout the milling process to produce a different barley end product. The main barley end products that use milling processes are: whole grain flour, barley flakes, pot barley and pearled barley. Whole grain flour typically uses neither heat treatment nor bran removal as a means to decrease microbial load. An overview of the complete milling process is shown in Fig. 2.1. The milling process starts with cleaning and sizing the grain. Cleaning is done with many different types of equipment such as screens, magnets, de-stoners, separators and aspirators to remove any foreign materials like unwanted grains, dirt, dust, and debris (Newman and Newman, 2008c). Sizing is done by a disk separator which will sort the kernels based on size. Indentations of a certain size in round disks allow kernels of a certain size to sit in them while they are moved to a different area for collection (Newman and Newman, 2008c).

The next step for the cleaned raw barley grain depends on what end product is desired. If pearled or pot barley is the desired end product, the barley is put through a pearling machine where abrasive action removes 30% or 15%, respectively, of the original kernel weight. The portion of the kernel that is removed in this process is called pearlings and contains the outer bran layers of the barley kernel. To achieve whole grain barley flour, no pearling or only very light pearling would be used. Typically, hull-less varieties of barley are used, but if the barley is hulled then the hull would be removed by light pearling. Only removing the hull and retaining almost all of the outer bran layers allows dehulled barley to still be called "whole grain" (Whole Grains Council, 2013). Whole grain barley flour can be made by different types of grinding processes that reduce the particle size of the grain. Traditionally, a stone mill is used to grind whole grains into flour. Stone mills use two to three abrasive stone disks to grind down the grain into flour. However, this method is not as effective at separating the bran from the endosperm and produces large flour particles, so it is no longer commonly used (Newman and Newman, 2008c). A hammer mill is another option that can be used. Hammer mills are equipped with a spinning drum containing swinging hammers to crush the grain against the wall of the drum as it spins through the mill. The milled grain falls through a screen of a specific particle size where it is collected as flour. Hammer mills are effective in reducing the particle size of the grain. However, the flour end product generally has an inconsistent particle size, which is often less desirable (Newman and Newman, 2008c). Pin mills or centrifugal impact mills produce a finer, more consistent particle size than hammer mills. In a pin mill, the grain is spun at high speeds causing them to break upon impact with the pins in the mill (Newman and Newman, 2008c). Currently, roller milling is the most common way to produce flour. It uses a

series of rollers to breakdown the grain. Break rolls, which are corrugated, first crack the grain revealing the endosperm. Then reduction rolls, which are smooth, reduce the particle size of the flour. Screens and sifters are used throughout the system to keep the end product's particle size consistent (Newman and Newman, 2008c). Shorts and bran, two by-products, are produced through the roller milling process. Shorts contain a mixture of endosperm, bran and germ (Newman and Newman, 2008c).

Although there are many ways barley grain can be milled, there are very few specifications that exist to ensure consistent quality like there are for wheat. The composition of barley varies greatly across genotype and is dependent on the environment in which it is grown. This makes it hard for millers to know how the barley grain will react to the milling process and what the end product will be. Any milled product can be air classified in order to create different compositions of flour fractions. Andersson *et al*. (2000) used flour that was pin milled and then air classified to yield different flours with varying physicochemical compositions. This technology could be used to create high fibre or high β-glucan flour fractions. Many researchers have examined the milling process of barley which supplies industry with valuable information, including how to maximize the nutritional content in barley with concentrated fractions (Andersson et al. 2003; Izydorczyk et al. 2003; Vasanthan and Temelli, 2008; Román et al. 2010; Sullivan et al. 2010a; Izydorczyk et al. 2011; Srinivasan and Smith, 2012; Gómez-Caravaca et al. 2015).

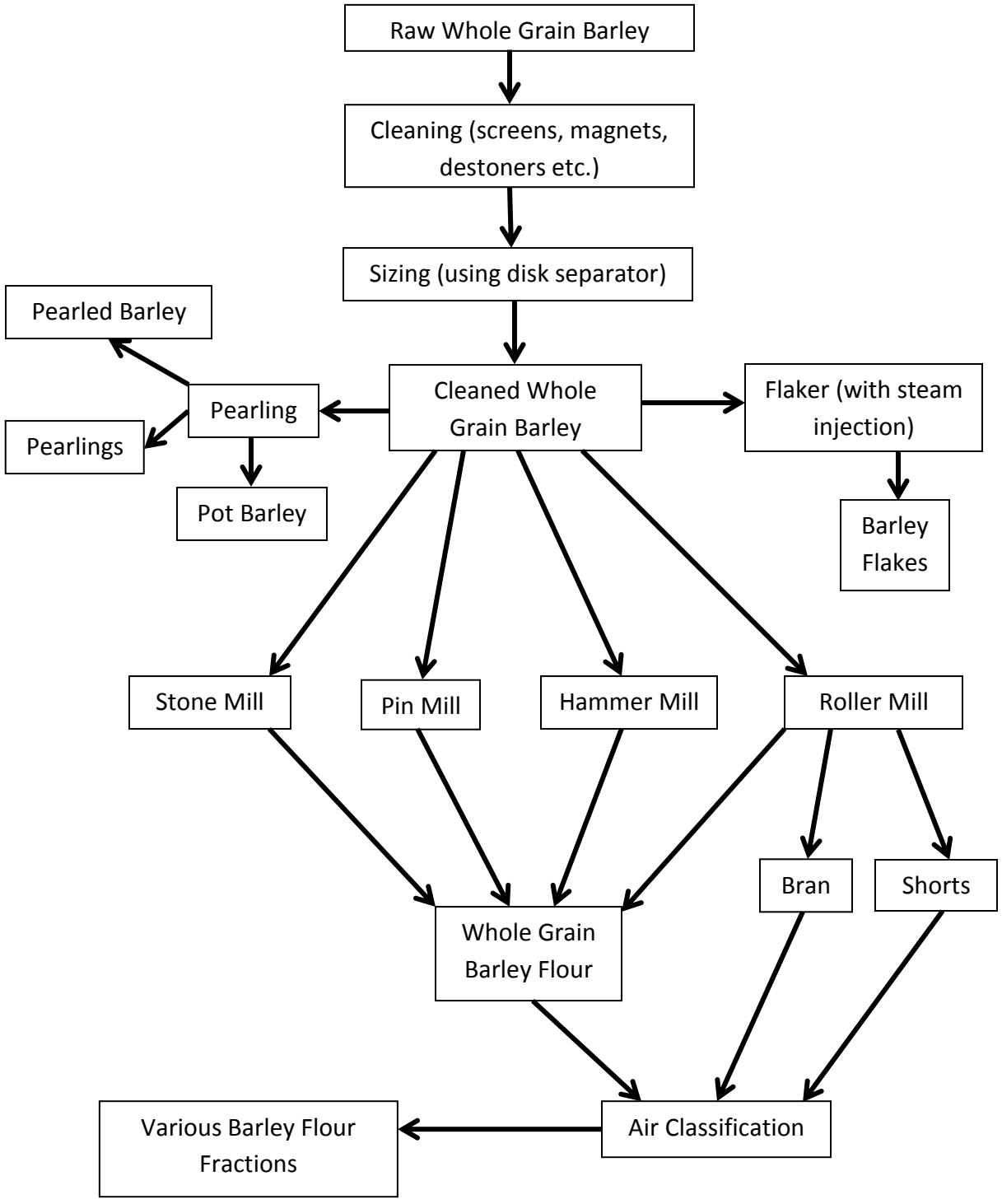


Figure 2.1 Overview of whole grain barley milling processes

2.2.2 Kilning

Kilning usually involves steaming the grain first, which will increase its moisture content, and then the use of dry heat, which will in turn decrease the moisture content. A kiln is a vertical column equipped with steam injection at the top, followed by heating and cooling sections (Girardet and Webster, 2011). In industry, processors may use a variety of moisture addition levels, temperatures, and drying times. A range of kilning times (from 60 to 120 minutes) and temperatures (from 65 to 100°C) are noted by Gates (2007). Girardet and Webster (2011) state the moisture content is often raised to 16-18% using steam before being dried back down to about 9-10% moisture, but not all processors use steam in their kilning process. Steaming mainly inactivates enzymes, while the dry heat treatment is done to develop roasted flavours in the grain (Gates, 2007). Enzyme inactivation is important in oats to prevent rancidity due to lipase activity. Peroxidase activity is a common test used to confirm the kilning procedure was sufficient at inactivating enzymes as it is simpler to test for than other enzymes and peroxidase is the most heat resistant enzyme found in oats (Girardet and Webster, 2011). Barley has a lower fat content (2-5%) than oats so lipase is of less concern but inactivation of other endogenous enzymes like β-glucanases could be beneficial (Newman and Newman, 2008b). Ganßman and Vorwerck (1995) also note that kilning can decrease microbial load on the outside of the kernel. Since kilning is already used in industry to treat oats, it could be a viable option to inactivate enzymes and reduce microbial contamination in barley.

2.2.3 Micronization

Micronization, or infrared heating, uses electromagnetic radiation at wavelengths between 1800 and 3400 nm as a form of heat processing (Fasina et al. 1999; Cenkowski et al. 2006). Near infrared uses wavelengths from 750-3000 nm, mid-infrared from 3000-25000 nm, and far infrared from 25000-100000 nm (Emami et al. 2010). In the food industry, micronization could be used to decrease microbial load, inactivate enzymes, and increase nutrient functionality or digestibility (Cenkowski et al. 2006). The micronization process involves the rapid removal of moisture from the surface of a food, resulting in a final moisture content usually between 7 and 10% for grain (Cenkowski et al. 2006). A micronizer has two key components, a vibrating conveyor and infrared heaters. The vibrating conveyor is what carries the food through the micronizer. The vibrations cause the food to rotate and flip over to ensure even heating (Cenkowski et al. 2003). The infrared heaters are located above the food, a set distance away, and emit infrared waves at a certain wavelength (Cenkowski et al. 2003). Prior to micronization of grains, the moisture content of the raw material is usually increased by tempering, which prevents over drying and burning. A hopper will feed the tempered grain into the vibrating conveyor where it is heated for a set amount of time. The vibration speed and the slope of the conveyor can be adjusted to control the time the grain spends in the micronizer. Different intensities (i.e. voltages) and wavelengths will affect the heating rate and peak temperature of the grain (Cenkowski et al. 2006). Some infrared wavelengths will be absorbed by the food and some will be reflected (Cenkowski et al. 2004). The higher the absorptivity of the food, the less energy it will take to remove moisture because the food will be heated more quickly (Emami et al. 2010). However, if the food is heat-sensitive, low absorptivity is desired to

prevent burning (Emami et al. 2010). Studies have been done on oats (Cenkowski et al. 2006) and barley (Fasina et al. 1999; Emami et al. 2010, 2011) using micronization for various applications.

2.3 Food Safety of Whole Grains

2.3.1 History of Foodborne Illness Outbreaks due to Contaminated Flour

In the past, flour has not been a food product typically associated with foodborne illness. All types of flour are normally considered low-risk foods due to their low moisture content which allows for good shelf stability. However, some pathogens, like *Salmonella,* can remain dormant throughout shelf life and lead to foodborne illness (Eglezos, 2010). In most cases, flour is used as an ingredient when baking or cooking which kills any heat sensitive pathogens present. It is for this reason that no heat treatments are usually applied during milling. However, there are some instances where a raw product is consumed prior to any cooking step, putting the consumer at risk for foodborne illness. Within the last 10 years, there have been 3 recorded foodborne illness outbreaks where contaminated wheat flour was suspected as the cause. The first was in 2005 in the United States where 26 cases of salmonellosis were recorded from cake batter ice cream (Zhang et al. 2007). An epidemiological investigation done by Zhang *et al*. (2007) implicated the raw flour in the cake batter as the source of *Salmonella* Typhimurium. The second outbreak occurred in New Zealand in 2008 from raw wheat flour contaminated with *Salmonella* (McCallum et al. 2013). A specific strain of *Salmonella* was isolated from the raw flour and was directly linked to 67 of the 75 cases of salmonellosis reported (McCallum et al. 2013). The most recent outbreak occurred in 2009 in

the United States from the consumption of raw cookie dough contaminated with *Escherichia coli* O157:H7 (Neil et al. 2012). A total of 77 people fell ill across 30 different states (Neil et al. 2012). Raw wheat flour was never confirmed as the source of *E. coli* O157:H7 but was highly suspected.

2.3.2 Microbial Contamination

In general, people are not concerned with microbial contamination in flours or other raw grain products because they are typically used as ingredients in foods that will be heat treated in some way. However, as the food industry evolves and develops new whole grain products this is not always the case. For example, in the case of barley, concentrated β-glucan flour fractions have been used by researchers to develop functional beverages (Temelli et al. 2004). Products like this one may not be possible without a viable way to heat treat the raw barley ingredient to ensure food safety. Temelli *et al*. (2004) pasteurized the barley β-glucan beverage and conducted shelf stability studies to prove that it was safe to consume. Shelf life is also a concern for raw barley products that could support the survival of bacteria even at low moisture contents.

The microbial contamination of raw flours in cereal grains has not been well researched. There is little literature on the microbial safety of barley grain or how to reduce it. In previous analysis done by Ames, it was discovered to be a problem when developing a smoothie containing barley, which had no heat treatment. Untreated barley flour had a standard plate count (SPC) of 3.1×10^5 colony forming units (CFU)/g, mold and yeast count (MYC) of 2.7×10^2 CFU/g, and 93 most probable number (MPN)/g of coliforms (Ames unpublished data). In this

case, SPC did not conform to published limits. The microbial limits for barley and other grains are not regulated. According to Pedretti and Cerrato (1975), the microbial limits should be < 1 x 10^5 CFU/g for SPC, < 1 x 10^4 CFU/g for MYC and < 500 per gram for coliforms. However in most cases, companies will set their own specifications for the processor to meet. Manthey *et al.* (2004) noted a maximum of 1 x 10^5 CFU/g for SPC is accepted by some millers. In addition, there should be zero tolerance for *Salmonella* (Richter et al. 1993).

Microbiological safety of wheat flour has been more documented than barley. In a study done by Richter *et al.* (1993), over 4000 different wheat flour samples were tested for SPC, MYC, coliforms, *Escherichia coli* and *Salmonella*. Averaging all data together, the SPC was 1.1 x 10^5 CFU/g, MYC was 5.7 x 10^3 CFU/g and coliforms were 150 MPN/g. *E. coli* and *Salmonella* were found in 12.8% and 1.3% of the samples, respectively. In another study, durum wheat samples were found to have 2.5 x 10^6 CFU/g and 2 x 10^3 CFU/g for SPC and MYC, respectively (Manthey et al. 2004). Although some of these results meet published limits, they could be concerning if used in applications not requiring thermal processing.

2.3.3 Processing Effects on Microbial Contamination

The use of different processes to reduce microbial contamination in cereal grains, particularly barley, is not well researched. Grains are typically contaminated by growing conditions, storage or processing. The environmental variables including rainfall, humidity, temperature, insects and soil conditions can all influence the amount of microbial contamination (Bullerman and Bianchini, 2011; Rose et al. 2012). This was shown in a study done by Manthey *et al*. (2004), where durum wheat grown in different regions had various

amounts of microbial contamination. Therefore, it is difficult to predict when there will be a microbial problem. Fungi including *Fusarium*, *Aspergillus* and/or *Penicillium* can grow throughout storage even at low moisture (Bullerman and Bianchini, 2011). Additionally, unclean processing conditions can increase the microbial load.

There are some processes throughout milling that may help reduce the microbial load such as cleaning and sorting the grain. Neagu *et al.* (2012) found that cleaning wheat reduced the mold content by about 77%. Manthey *et al.* (2004) also showed a 1 log reduction in SPC of wheat from cleaning. The SPC content was further decreased in the wheat flour by 1.5 logs. Bran removal also reduces the microbial contamination in wheat as the bacteria and molds are concentrated on the surface layers of the kernel (Laca et al. 2006).

Surface disinfection of wheat kernels using a combination of gaseous ozone, ozonated water and acetic acid was found to be effective in reducing SPC and MYC (Dhillon et al. 2010). However, the chemicals individually were not as effective. Kottapalli and Wolf-Hall (2008) used a hot water treatment at either 45°C or 50°C for variable time periods on malting barley to try to reduce SPC and MYC levels, which were initially 4.4×10^6 CFU/g and 1.75×10^4 CFU/ g, respectively. They were able to reduce these counts, but only by a 1.8 log maximum for each. This treatment is not likely a practical option for food barley as it would have to be dried a great deal afterwards in order to be milled.

Treatments to reduce microbial contamination prior to grains being grown have also been investigated. Pulsed light, which is considered a non-thermal treatment, reduced the mold concentration on wheat seeds by 4 logs without changes in the germination (Maftei et al. 2014). Irradiation at a dose of 12 kGy was able to control microbial contamination in barley

seeds without affecting its germination (Ramakrishna et al. 1991). However, these do not prevent contamination and growth during storage and processing.

2.3.4 Mycotoxins

Mycotoxins are harmful substances that are produced by fungi. Different mycotoxins are produced by different species of the fungi *Fusarium, Aspergillus* and *Penicillium* (Vidal et al. 2013). In cereal grains, the fungal genus most commonly associated with the production of mycotoxins is *Fusarium* (FAO, 1997). Many different species of *Fusarium* can cause fusarium head blight (FHB) in wheat, oats, barley and maize. While FHB is most commonly associated with wheat, barley seems to have become more susceptible now. Most of the barley produced in Canada has FHB, especially in Manitoba (Tekauz et al. 2000). FHB decreases the grain's quality by causing discoloured, smaller kernels and therefore, a decreased yield (Tekauz et al. 2000).The extent of the infestation is dependent on cultivar and environment. Some cultivars of barley may be more resistant than others, and high amounts of precipitation favour the growth of *Fusarium* (Tekauz et al. 2000).

Deoxynivalenol (DON), also known as vomitoxin, is part of the trichothecenes group of mycotoxins, along with T-2 and HT-2 toxins, which are produced by *Fusarium* fungi (Sobrova et al. 2010; Barthel et al. 2012). Barley typically has high levels of DON and ochratoxin A (OTA), but other toxins such as T-2 and HT-2 toxins are also frequently detected (Campbell et al. 2000; Barthel et al. 2012). OTA is different from DON as it is typically produced by *Aspergillus* and *Penicillium* species after harvest and does not have any visible effect on the kernels (Castells et al. 2006; Canadian Grain Commission, 2013a). Various studies state the occurrence of OTA and

DON in barley and barley products is up to 21% and 100% of samples, respectively, at various concentrations (Campbell et al. 2000; Lombaert et al. 2003; Castells et al. 2006; Bensassi et al. 2011; Barthel et al. 2012).

DON is considered less toxic than other trichothecene mycotoxins. However, it can have mild to severe health effects upon consumption (Sobrova et al. 2010; Barthel et al. 2012). It can cause nausea, vomiting, diarrhea, abdominal pain, headaches, dizziness and fever if ingested at high enough levels (Sobrova et al. 2010). In cases where livestock animals have consumed DON contaminated feed, decreased food intake and therefore performance are typically seen as adverse health effects (Canadian Grain Commission, 2013b). There is the potential for DON to be transferred into the foods these animals produce such as eggs and milk but this has not been consistently shown (Sobrova et al. 2010; Canadian Grain Commission, 2013b).

OTA is a potent renal toxin that can cause kidney disease and can have teratogenic, immunosuppressive and carcinogenic effects (Health Canada, 2010). Livestock can also be affected by consuming OTA contaminated feed which causes decreased productivity and weight gain (Duarte et al. 2011). OTA transferred into meat, eggs, and milk produced by the animal has also been found (Skaug, 1999; Guillamont et al. 2005; Dall'Asta et al. 2008; Duarte et al. 2011).

Currently, Health Canada has proposed setting maximum levels for OTA in unprocessed cereal grains, products made from unprocessed cereal grains and infant foods made from cereal grains at 5, 3 and 0.5 µg/kg, respectively (Health Canada, 2010). These levels are the same as those set by the European Commission (European Commission, 2006). The European Commission has also set maximum levels for DON contamination as follows: 1250 µg/kg in raw

cereals (excluding durum wheat, oats and maize), 750 µg/kg in cereals, flours and bran for direct consumption, 500 µg/kg in processed foods using cereals and 200 µg/kg in infant food using cereals (European Commission, 2006). Health Canada (2012b) is currently reviewing maximum limits for DON in uncleaned soft wheat in non-staple foods and baby foods which are 2.0 and 1.0 mg/kg, respectively. Studies have shown that these limits for DON and OTA are frequently exceeded in barley and barley products and therefore reduction in toxins via processing is needed (Fazekas et al. 2002; Park et al. 2002; Abramson et al. 2005; Bensassi et al. 2011; Barthel et al. 2012).

2.3.5 Processing Effects on Mycotoxin Levels

The levels of mycotoxins in raw barley grain and other cereal grains can be reduced through different processing treatments. Milling and heat treatments have been shown to decrease toxin levels in grain. Some studies have found that milling decreases the amount of mycotoxins in the final product (i.e. flour or flakes) by removing the outer layers of the kernel. Cleaning and sorting raw wheat can reduce the amount of DON by up to 49% (Cheli et al. 2013). Edwards *et al.* (2011) showed that comparing cleaned whole grain wheat to milled white flour, DON levels were decreased by 30% in the flour. DON levels were also shown to be 282% higher in the bran fraction than in cleaned whole grain wheat. In oat milling and flaking, dehulling the oat kernel showed the biggest decrease in mycotoxin concentration, followed by kilning and colour sorting (Scudamore et al. 2007, 2009). Dehulling and kilning reduced the DON level in oats to non-detectable (Tekauz et al. 2004). It has been shown that mycotoxin levels are higher in the outer layers of grain and removing them will decrease the amount in the end product

(Manthey et al. 2004; Edwards et al. 2011; Cheli et al. 2013; Khatibi et al. 2014). However, this brings up an issue. The mycotoxins are now concentrated in a by-product fraction typically made up of bran, which is usually used as animal feed. In addition, the bran layer that has been removed contains healthful components, fibre and phytochemicals, that are of value to consumer health. For this reason it is important to find a solution to reduce mycotoxin concentrations in whole grain products and bran fractions.

Different heat treatments on raw grain have also been shown to reduce the amount of mycotoxin contamination. A study by Cenkowski *et al*. (2007) showed that treating wheat grain with superheated steam could decrease the concentration of DON up to 52%. This was attributed to the thermal treatment only and not solubilisation. A different study showed that OTA contamination in barley meal could be decreased up to 86% using a single screw extruder (Castells et al. 2006). In two separate studies, barley was immersed in hot water to decrease the amount of mycotoxins in malting barley (Kottapalli et al. 2003; Kottapalli and Wolf-Hall, 2008). While this application may not be the best for barley intended to be milled, it does show that heat can reduce mycotoxin contamination. A more applicable dry heat treatment was shown to decrease DON levels by 58% by placing a barley sample in a closed conical polypropylene tube in a convection oven at 80°C for 5 days (Abramson et al. 2005).

2.4 Health Benefits of Barley

Barley contains β-glucan which is a type of soluble fibre. β-glucan is a linear polysaccharide composed of repeating D-glucopyranosyl units that are in (1→4) linked blocks with (1→3) linkages. It is the key component of barley that is behind the recent health claims in

the U.S. and Canada. The U.S. health claim, approved in 2006, allows food products that contain at least 0.75 g of barley β-glucan per serving to state the food "may reduce the risk of coronary heart disease" (U.S. Food and Drug Administration, 2006). In 2012, Health Canada approved the health claim regarding the consumption of barley β-glucan and the reduction of blood cholesterol. Food products in Canada can claim they help lower cholesterol if they contain at least 1 g of β-glucan per serving (Health Canada, 2012a). Consumers must eat at least 3 g of barley β-glucan per day to lower their cholesterol and reduce their risk for cardiovascular disease. The European Food Safety Authority (EFSA) has also recognized the cholesterol lowering properties of barley β-glucan and its ability to reduce postprandial glycemic response (European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies, 2011).

In order for Health Canada to approve the health claim on barley β-glucan, substantial scientific evidence from animal and human trials had to be established. Barley has been connected to blood cholesterol reduction in animal studies on broiler chicks (Fadel et al. 1987), pigs (Bird et al. 2004), and hamsters (Ranhotra et al. 1998). Human clinical trials have also successfully shown barley to lower cholesterol, specifically total cholesterol and LDL-cholesterol (Newman et al. 1989; McIntosh et al. 1991; Ikegami et al. 1996; Behall et al. 2004a, 2004b; Wang et al. 2013). However, there are a few studies where barley was not shown to reduce cholesterol (Keogh et al. 2003; Smith et al. 2008). In both these studies, the authors attributed this to the β-glucan being altered in some way during processing to make it less effective. Processes that barley is subjected to in order to produce a food product have the potential to alter the molecular weight, viscosity and/or solubility of the β-glucan (Ames and Rhymer, 2008). Ames *et al*. (2006) showed that heat treatments increased viscosity, and genotype affected the

amount of β-glucan present and its solubility. High molecular weight barley β-glucan is thought to reduce cholesterol better than low molecular weight because it increases viscosity (Theuwissen and Mensink, 2008; Wang et al. 2013). The mechanism by which β-glucan reduces cholesterol is not yet confirmed. An early theory was β-glucan increased intestinal viscosity which slowed the absorption of lipids and cholesterol (Newman and Newman, 2008d). A more recent theory is β-glucan binds with bile salts which cause the body's cholesterol to break down to replace the bound bile salts (Newman and Newman, 2008d). In addition, it is also thought that increased gut viscosity from high molecular weight β-glucan increases the excretion of bile salts which assists in cholesterol reduction (Wang et al. 2013).

Barley has also been shown to reduce postprandial glycemic response in humans, which could benefit those that have or are at risk for type 2 diabetes. In many studies, barley foods were fed to participants to assess how barley affected their blood glucose in comparison to a control food which induced a high glycemic response. Most studies showed that glycemic response was lowered when participants consumed barley foods (Narain et al. 1992; Casiraghi et al. 2006; Poppitt et al. 2007; Thondre et al. 2012; Ames et al. 2015). β-glucan is hypothesized to be the component in barley that reduces glycemic response by increasing gut viscosity (Tosh, 2013). Ames *et al.* (2015b) determined that amylose and insoluble fibre content in barley tortillas did not affect postprandial glucose and insulin levels but β-glucan content did. A study by Aldughpassi *et al*. (2012) showed that genotype and processing both affect the glycemic index of barley. Tosh (2013) suggested a minimum of 3 g of β-glucan per meal is needed to lower glycemic response. However, the author also made note of the importance of molecular weight and how it may affect the functionality of the β-glucan. EFSA has stipulated 4 g of β-

glucan per 30 g available carbohydrate is necessary to reduce postprandial glycemic response (European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies, 2011).

2.4.1 Effect of Heat Treatments on β-Glucan Properties

The physicochemical properties of β-glucan can be altered during processing. Solubility, molecular weight and viscosity are all important characteristics of β-glucan and affect how the body responds. Many studies on oat and barley β-glucan have been conducted to see how different heat treatments, including roasting, steaming, autoclaving and micronization affect the physiological properties of β-glucan (Doehlert et al. 1997; Zhang et al. 1997; Izydorczyk et al. 2000; Cenkowski et al. 2006; Zhang et al. 2009; Hu et al. 2010; Gujral et al. 2011; Sharma et al. 2011; Lazaridou et al. 2014; Ames et al. 2015a).

Roasting involves using dry heat, usually in a convection oven, on the raw grain at low temperatures for a long time. It has been shown that roasting in some cases increased β-glucan solubility (Gujral et al. 2011); viscosity was either unchanged or lower than untreated grain (Doehlert et al. 1997; Izydorczyk et al. 2000; Hu et al. 2010). Steaming uses moist heat, often done at atmospheric pressure in a vegetable steamer in lab scale processes, and has been shown to increase viscosity (Doehlert et al. 1997; Izydorczyk et al. 2000). Autoclaving also uses moist heat but is done in an autoclave at a high pressure (usually 15 pounds force per square inch or 103.4 kilopascals) in order to achieve higher temperatures (121 ^{o}C). Zhang *et al*. (1997) showed that increasing the duration of autoclaving increased the viscosity of oat flour slurries. Autoclaving barley flours for 20 min increased the molecular weight of β-glucan when the flours were tempered to higher moisture content prior to autoclaving due to β-glucanase inactivation

(Lazaridou et al. 2014). Izydorczyk *et al*. (2000) also found that barley β-glucan maintained its high molecular weight after both steaming and autoclaving because β-glucanase was deactivated, thus preventing hydrolysis. However, solubility was not affected by either hydrothermal treatment. An increase in β-glucan solubility was achieved by the addition of enzymes, protease and esterase, and/or physical agitation during extraction.

There is less evidence on how micronization or infrared heating affects β-glucan. It has been established that micronization does not affect the β-glucan content (Cenkowski et al. 2006; Hu et al. 2010). However, the effect on viscosity is variable and appears to be dependent on the specific micronization process used. Cenkowski *et al*. (2006) showed that infrared intensity and/or amount of water added during micronization affected the change in viscosity. The micronization treatments that did produce a significant impact resulted in a slight increase in viscosity when compared to the untreated sample. This may have been because the β-glucan maintained its high molecular weight and was not broken down by endogenous enzymes.

β-glucanase enzymes in barley are made up of 3 different β-D-glucan 4-glucanohydrolases: lichenase ((1,3)(1,4)-β-D-glucan 4-glucanohydrolase EC 3.2.1.73), cellulase ((1,4)-β-D-glucan 4-glucanohydrolase EC 3.2.1.4), and (1,3)-β-D-glucan glucanohydrolase (EC 3.2.1.39) (Woodward and Fincher, 1982). Prior to germination, β-glucanases in food barley are predicted to have a lower activity compared to malting barley and therefore are more difficult to measure (Izydorczyk et al. 2000). Even with a low activity, they decrease β-glucan viscosity and molecular weight which poses a problem when trying to develop more healthful barley products. Therefore, it is important to inactivate them using processing. However, β-glucanases are difficult to inactivate. Knuckles and Chiu (1999) used autoclaving, hot ethanol, hydrochloric

acid, roasting, and trichloroacetic acid to try to eliminate the β-glucanase activity of barley. None of these treatments inactivated β-glucanase but they did reduce its effect on molecular weight. Rieder *et al.* (2015) were also unsuccessful at inactivating β-glucanase completely using a roasting treatment on whole grain barley flour for 1.5 h at 130 °C or 3 h at 150 °C. However, some researchers have been successful using some heat treatments, like autoclaving, to inactivate β-glucanase (Izydorczyk et al. 2000; Lazaridou et al. 2014).

2.5 Effect of Heat Treatment on Starch Pasting Characteristics

Starch is the major constituent in barley, making up approximately 45-60% of the kernel, depending on the cultivar and growing conditions (Newman and Newman, 2008b). Amylopectin and amylose are the two different molecular structures which make up starch. Amylopectin consists of α-(1,4)-D-glucose chains with α-(1,6)-D-glucose linkages to create a branched molecule (Newman and Newman, 2008b). Amylose is typically a linear chain of α-(1,4)-D-glucose but does have a few branches (Newman and Newman, 2008b). In normal starch varieties of barley, about 75% of the starch is amylopectin and 25% is amylose. There are waxy starch and high amylose cultivars as well, which contain 95-100% and 40-70% amylopectin, respectively (Newman and Newman, 2008b). Starch molecules form two different types of starch granules, A- and B-type. B-type granules are smaller in diameter (< 6 µm) than A-type (10-30 µm), and more irregularly shaped (Vasanthan and Hoover, 2009). The ratio of A-type to B-type granules and size depends on the barley cultivar and starch type (waxy, normal, high amylose).

Starch pasting is an important analytical tool that can determine differences between cultivars, processing differences and end-product quality. Many researchers have shown that starch pasting characteristics are affected by barley cultivar and starch type (Yanagisawa et al. 2006; Gujral et al. 2013; Li et al. 2014). In general, waxy barley cultivars have a higher peak viscosity and lower pasting temperature than normal and high amylose cultivars (Vasanthan and Hoover, 2009). A low pasting temperature indicates that the starch granules are more susceptible to swelling and rupturing. High peak viscosities are associated with high breakdown values, which indicate a high degree of swelling and lysing of starch granules during heating (Ragaee and Abdel-Aal, 2006). Waxy cultivars also typically have lower setback and final viscosities. This is due to the low amylose content which is needed to interact with amylopectin to form a stronger gel structure during retrogradation (Yoshimoto et al. 2002; Lee et al. 2011).

Heat treating barley has different effects on the starch pasting properties. Sharma *et al*. (2011) reported treating barley at 280 °C in a sand roaster caused a significant decrease in peak, final, breakdown and setback viscosities. However, the degree at which the viscosities decreased depended on cultivar and β-glucan content. The changes in starch pasting properties were due to the starch becoming gelatinized during roasting causing a decrease in starch swelling. Zhang *et al.* (1997) saw no change in the apparent viscosity of oat flour slurries, measured on a Brookfield viscometer, when oats were roasted at 95 °C for 2 h, but the apparent viscosity decreased significantly when the temperature was increased to 105, 130 and 155 °C. In the same study, steamed and autoclaved oats demonstrated an increase in apparent viscosity. Doehlert *et al.* (1997) found no significant change in the starch pasting of oats when roasted for 120 min at 104 °C. This was due to the heat treatment not being severe enough to

gelatinize the starch. The effect of steam on oat viscosity was also evaluated using a vegetable steamer for 20 min. When held at 30 °C for 1 h in a Rapid Visco Analyser (RVA) during a critical pasting test, the steamed oats had a final viscosity 7-fold greater than raw oats (Doehlert et al. 1997). However, it was determined by the addition of lichenase, the increase in viscosity was not due to starch but β-glucan. Liu and White (2011) determined β-glucan has more influence on the pasting viscosities than starch, and protein has negligible effects. Many researchers have found that β-glucan contributes to the majority of the viscosity observed in pasting properties of oats (Doehlert et al. 1997; Zhou et al. 2000; Colleoni-Sirghie et al. 2004; Yao et al. 2007; Liu et al. 2010; Liu and White, 2011).

Kilning has been shown to have significant effects on the starch pasting profile of oats. Doehlert *et al.* (1997) investigated whether applying a dry heat treatment pre- or post-steaming would affect the starch pasting properties of oats. The apparent viscosity was significantly higher than the raw oats for both combinations of roasting and steaming. However, oats that were steamed after roasting had a higher apparent viscosity than when steamed before roasting. This suggests the addition of moisture and heat has a greater effect on starch than dry heat treatments. Zhou *et al.* (1999) reported an increase in peak and final viscosity of 3 different cultivars of oats after kilning. An increase in peak and final viscosity was also seen in commercially kilned oat flakes by Cenkowski *et al.* (2006). However, kilning did not change the general profile pattern obtained by the RVA (Zhou et al. 1999; Cenkowski et al. 2006).

The effect of micronization on barley starch pasting characteristics has not been studied extensively. Emami *et al.* (2010) showed that the gelatinized starch concentration increased in micronized barley and more gelatinization occurred at a higher initial moisture content and/or

temperature. Micronization of oats was evaluated by Cenkowski *et al.* (2006). There was a slight increase in peak and final viscosity of the standard pasting profile of micronized oat flakes but it was not significantly different from raw oat flakes. However, there was a significant difference in the critical pasting profile of micronized oat flakes compared to raw oat flakes, indicating the starch was altered somehow. This could have been due to higher water absorption capacity. Applying a microwave treatment to 3 different types of barley starch (waxy, normal and high amylose) increased the peak, breakdown, final and setback viscosities of the waxy barley (Emami et al. 2012). The normal and high amylose barleys did not respond in the same way to the microwave treatment and only an increase in final viscosity was seen in the normal starch barley.

There has not been a lot of research on the relationship between barley starch pasting characteristics and end-product quality. Ragaee and Abdel-Aal (2006) substituted wheat flour with barley flour at different levels to evaluate the effect on starch pasting in pita bread, cakes/cookies and snack food product applications. It was determined that barley flour has a similar starch pasting profile to hard wheat flour and could be substituted up to 15% and 30% without detrimental effects to end-product quality in pita bread and cakes/cookies, respectively. Sullivan *et al.* (2010b) found increasing the amount of pearled barley flour in bread flour formulations, increased the peak, breakdown, setback and final viscosities. It was thought the barley-fortified breads may stale quicker due to the higher setback viscosity which indicates increased starch retrogradation.

2.6 Effect of Heat Treatment on Colour and Particle Size

Colour is a key attribute to consider when dealing with food. The appearance can greatly influence consumers' perceptions of foods. The colour of grain products prior to being made into foods can impact the colour of the end food product. Therefore, consideration of how heat treatments affect the colour of barley is important. Research has found that dry heat treatments (roasting) cause a decline in brightness, or the L* value, in barley and oats (Kim et al. 1998; Gujral et al. 2011; Sharma et al. 2011; Yahya et al. 2014). Redness (a*) and yellowness (b*) of the roasted product have been shown to increase (Gujral et al. 2011; Sharma et al. 2011), but only up until a certain duration of heat treatment and then the red and yellow components decreased (Yahya et al. 2014). A study done on the micronization of barley showed a decrease in brightness in all types of barley tested, but only a decrease in redness and yellowness was seen in high amylose and waxy barleys (Emami et al. 2011).

Heat treatments can also affect the texture of the barley kernel and how it fractures during milling. In terms of milling, this is important as the kernel hardness will affect how much energy is needed to mill the barley. In addition, the particle size and its consistency may be affected, which impacts the end product. The deformation force of roasted wheat was studied by Murthey *et al*. (2008) and it was found that increased roasting created a softer wheat kernel. Salmenkallio-Marttila *et al*. (2004) looked at how heat treatments affected the texture of oat kernels and found that autoclaving increased the kernel hardness whereas extrusion decreased it when compared to its native form. Changes in kernel hardness may affect the particle size of barley flour due to different fracturing of the kernel. Particle size can influence many barley flour properties including water absorption (Al-Rabadi et al. 2012), β-glucan viscosity

(Izydorczyk et al. 2014), dough rheology (Ahmed et al. 2015), and starch pasting (Becker et al. 2001). Furthermore, the fractions obtained by air classification are based on particle density and could be affected by the particle size of the flour obtained. This could impact the amount of β-glucan that is able to be concentrated into high fibre fractions. In addition, particle size can impact the quality of barley end products (Ross and Ames, 2005; Izydorczyk et al. 2008; Prasopsunwattana et al. 2009; Lazaridou et al. 2014).

2.7 Food Product Applications of Heat-Treated Barley

Heat treatment of barley may create opportunities for more novel food products. Little research has been done on the application of heat-treated barley into food products. Heat-treated barley flours may alter the functional properties and end product quality attributes of barley products. *Tihni*, traditional Northern Ethiopian roasted barley flour, is used in many applications from breads to beverages (Abraha et al. 2013). In addition, *Kolo* is a whole grain barley snack that is roasted at 140 °C for less than 2 min (Abraha et al. 2013). Ames *et al.* (2005) also developed a whole grain barley product where micronization was yielded a nutritious ready-to-eat snack. There may also be an opportunity to use heat-treated barley in applications which do not require a cooking step. The development of functional beverages is a novel way for barley to be incorporated into new food systems. Temelli *et al.* (2004) were able to create an acceptable orange flavoured β-glucan beverage containing 0.5% β-glucan. A barley tea, high in β-glucan, was created using micronized barley flour by Ross and Ames (2005). Fibre-enriched fruit bars are another application that may not include thermal processing like those created by Shaheen *et al.* (2013) and could benefit from using heat-treated barley.

Heat-treated barley could also be used to enhance the traditional food uses for barley. Pearled barley, flakes and flour that have been heat-treated may create a more desirable flavour and aroma profile (Kim et al 1998; Yahya et al. 2014). In addition, quick cooking barley can be created using heat treatments, which may be more desirable to consumers (Ames et al. 2003). Increasing the product applications and consumer acceptability of barley products will certainly benefit the barley industry. More research is needed on the incorporation of heat-treated barley into different food systems to see how it affects the nutritional functionality and quality of the product.

3. MATERIALS AND METHODS

3.1 Materials and Sample Preparation

3.1.1 Commercial Samples

Barley samples were collected load from a variety of barley processors across Canada to assess microbial and results are summarized in Table 3.1. Processors were asked to provide a representative sample. The sample types included whole grain barley flour, pearled barley, dehulled barley, and barley flakes which may or may not have been subjected to heat treatments. Some processors used micronization and steaming as heat treatments. Unprocessed whole grain barley was also included to determine how microbial contamination changes throughout processing. Samples were milled using a centrifugal mill (Restch ZM-200, Brinkmann Instruments, Westbury, NY, US) to pass through a 0.5 mm screen for most laboratory analyses. Samples were stored at approximately 5 °C throughout the study.

3.1.2 Heat Treatment Samples

Three cultivars (CDC Rattan, CDC McGwire, and CDC Fibar) of whole grain barley (grown in 2013 in Alberta, Canada) were supplied by the Alberta Barley Commission for the heat treatment experiments. All 3 cultivars are 2-row hulless barleys and CDC Fibar and CDC Rattan are waxy starch varieties while CDC McGwire is a normal starch variety (CFIA, 2015a). The barley was commercially cleaned to remove field debris, unwanted grains and insects. Heat treatments were performed on the whole grain barley and then they were milled to pass through a 0.5 mm screen using a centrifugal mill (Restch ZM-200) for most laboratory analyses (microbial analyses were performed prior to milling). Small subsamples of each cultivar that did

not undergo cleaning were also obtained to compare the microbial contamination between cleaned and not cleaned barley. The cleaned barley is referred to as untreated from here on. Samples were stored at approximately 5 °C throughout the study.

Table 3.1 Descriptions of commercial barley samples used in present study

Barley Sample Type	**Comments**
Unprocessed whole grain #1	Organic
Unprocessed whole grain #2	
Unprocessed whole grain #3	
Unprocessed whole grain #4	
Whole grain flour #1[1]	Organic
Whole grain flour #2[1]	
High β-glucan flour[1]	Two lots of high β-glucan barley flour were obtained and analyzed separately
Low β-glucan flour[1]	
Toasted flakes[1]	Toasting refers to the application of micronization as a heat treatment
Rolled[1]	
Pearled & steamed flakes[1]	
Dehulled[1]	
Pearled[1]	
Toasted pearled #1[1]	Toasting refers to the application of micronization as a heat treatment
Toasted pearled #2[1]	Toasting refers to the application of micronization as a heat treatment
Pearlings	Pearlings are a by-product from pearling

[1]Considered end-products in this study

3.2 Heat Treatments

There were three heat treatments (roasting, micronization and conditioning) conducted on each of the barley cultivars. Roasting and micronization were conducted with two levels of tempering, 17% moisture and no tempering. The tempering level of 17% moisture was chosen based on literature stating what moisture level oats are commonly increased to during kilning in industry (Girardet and Webster, 2011). To temper the barley grain the moisture content of the untreated barley grain was determined and then a calculated amount of water was added to the grain to make to final moisture content 17%. The grain and water were weighed into tempering jars and shaken every 15 min for 2 h to ensure even absorption of moisture and then stored at 5°C overnight. Each heat treatment was done in 2 processing replicates.

3.2.1 Micronization

Micronization was done on a MR2 Pilot Scale Micronizer (Micronizing Company UK Ltd., Charnwood Mill, Framlingham, Suffolk, UK) in the Department of Food Science at the University of Manitoba (Fig. 3.1). Barley grain (3 kg) was micronized to an end temperature between 110-130°C. Any barley that was outside of this temperature range was separated and not used for analysis. The slope, and therefore the flow rate, of the micronizer were adjusted throughout processing to keep the end temperature of the barley grain within this range. This temperature range was selected because at higher temperatures the barley grain pops, which was determined to be undesirable for this type of primary processing. A handheld infrared thermometer was used at the end of the micronizer to check the final temperature of the barley kernels.

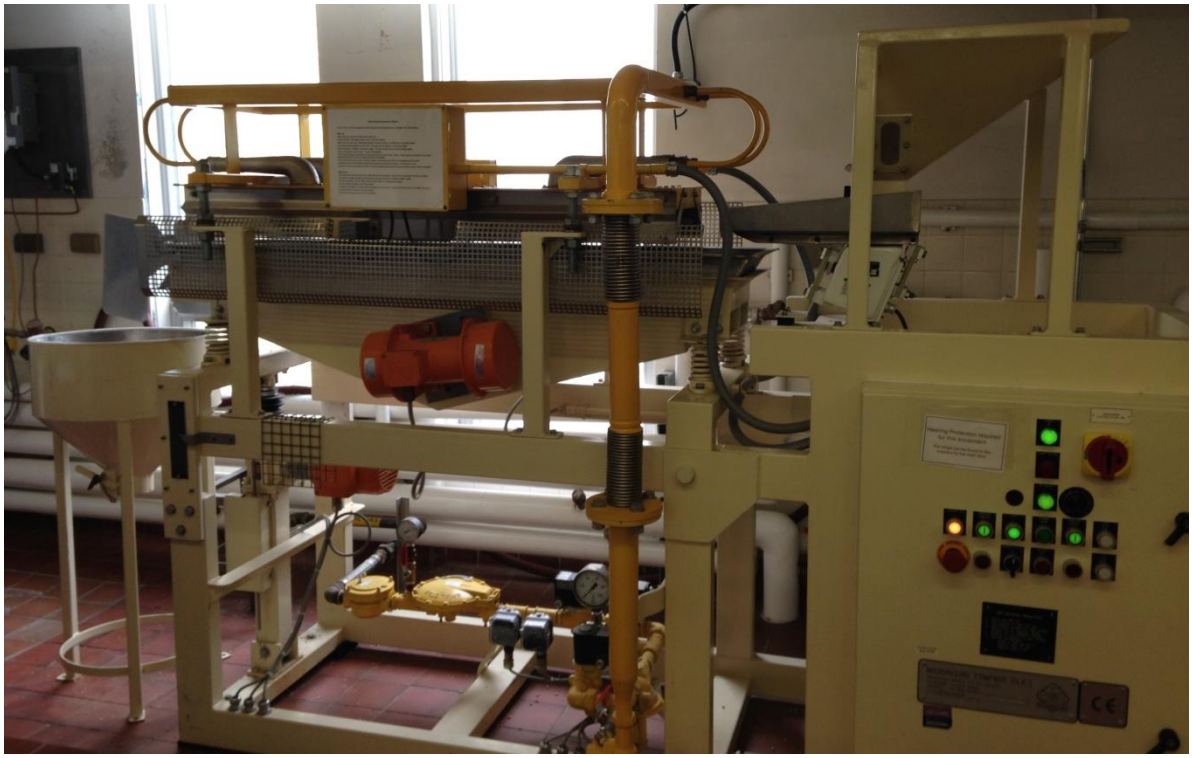


Figure 3.1 Pilot scale micronizer at Department of Food Science, University of Manitoba used for micronization treatment

3.2.2 Conditioning

A laboratory scale conditioning treatment was done to mimic the kilning process used in industry. A small scale conditioning method was adapted from Ames and Rhymer (2003). A handheld steam cleaner (Simoniz, Brampton, ON, Canada) was used to inject steam into 250 mL glass mason jars which contained 70 g of whole grain barley (Fig. 3.2). The jars were shaken throughout the 30 sec steam treatment to allow for even moisture distribution and equal contact with the steam. The steam increased the moisture content of the barley to 17%, which was verified by oven moisture as per AACC International method 44-15.02 (AACC International,

1999a). After steaming, the jars were sealed and placed into a 70°C incubator to keep warm. The maximum amount of time the samples spent in the incubator prior to being put in the oven was 15 min. The jars were placed in a convection oven (Blodgett CTB-1) at 150 °C for 60 min, with shaking every 10 min. The lids were removed at 30 min to allow for the moisture content to be reduced. Once removed from the oven the lids were replaced and the barley was allowed to cool to room temperature. The temperature of the barley was taken using a handheld infrared thermometer throughout the process to ensure consistent heating. The final moisture content achieved was approximately 9-10%, as determined by AACC International method 44-15.02 (AACC International, 1999a).

Figure 3.2 Steaming apparatus used in conditioning heat treatment

3.2.3 Roasting

The roasting treatment was conducted in a convection oven (Blodgett CTB-1, Blodgett Oven Company, Burlington, VT, US) at 110°C for 90 min. The grain (500 g) was spread out on a

sanitized aluminum baking sheet at 1 kernel thickness. The baking sheets were moved throughout the heat treatment to reduce the effect of thermal variation in the oven. The actual temperature of the grain was taken using a handheld infrared thermometer at 4 different spots on the baking sheet every 15 min. The grain was cooled at room temperature before being put into sealed plastic bags.

3.3 Moisture Content

Moisture content of each sample was determined using the AACC International method 44-15.02 (AACC International, 1999a) by weighing 1 g of sample (ground to 0.5 mm) and drying in an oven for 1 h at 130 °C. The sample was then transferred to a desiccator for 1 h. Once cooled to room temperature the dried weight was taken. The moisture content was then calculated as follows:

$$\text{Moisture Content } (\%) = \frac{(\text{MW} + \text{MC} - \text{MD})}{\text{MW}} \times 100$$

Where: MW is weight of wet sample

MC is weight of container

MD is weight of dried sample and container

3.4 Microbial Analyses

Analyses were done on the whole grain of the heat treated samples and the state in which the commercial samples were obtained. Aseptically, 11 g of sample were weighed into a stomacher bag and 99 mL of sterilized diluent were added. The sample was stomached for 1 minute. A stock solution of 0.25 M buffered potassium phosphate (pH 7.2) was prepared. To

make the diluent, an aliquot of the stock solution (1.25 mL) was diluted to 1 L. All serial dilutions were prepared using sterilized phosphate diluent. Diluted sample (0.1 mL) was pipetted onto sterile petri plates containing 15-20 mL of solidified agar and spread evenly across the surface. The diluted samples were used for the determination of SPC, MYC and *E. coli*/coliforms explained in the following sections. The microbial limits that were used in this research were based on industry information and are outlined in Table 3.2.

Table 3.2 Microbial limits used in present study

Analysis	**Limit**
SPC	< $\log_{10}$ 4.7 or 5 x 10^4 CFU/g
MYC	< $\log_{10}$ 3 or 1 x 10^3 CFU/g
Coliforms	< 100 MPN/g
E. coli	< 10 MPN/g

3.4.1 Standard Plate Count

Standard plate count (SPC) was determined using the spread plate method according to AACC International method 42-11.01 (AACC International, 1999b) on plate count agar (Oxoid Ltd., Basingstoke, Hampshire, England). Plates were incubated for 24-48 h at 35 °C. Appropriate dilutions were plated in duplicate. Plates with counts between 30 and 300 colonies were counted and results were reported as $\log_{10}$ CFU/g. The mean and SD were calculated using a Minimum Detectable Limit (MDL) of $\log_{10}$ 2 CFU/g for any plates with results <30 x 10^2 CFU/g.

3.4.2 Yeast and Mould Count

Yeast and mould (MYC) was determined using the spread plate method according to AACC International method 42-50.01 (AACC International, 1999c) on potato dextrose agar (Oxoid Ltd.) acidified with 10% tartaric acid to pH 3.5 and rose bengal chloramphenicol agar base (Oxoid Ltd.) with chloramphenicol supplement (Oxoid Ltd.). Plates were incubated for 5 days at 22-25 °C. Appropriate dilutions were plated in duplicate. Plates with counts of 50 colonies or less were counted and results reported as $\log_{10}$ CFU/g. The mean and SD were calculated using a MDL of $\log_{10}$ 2 CFU/g for any plates with results <1 x 10^2 CFU/g.

The untreated barley was also analyzed for heat resistant mould according to the method outlined by Splittstoesser *et al.* (1970). The grain was heated in the oven at 70°C for 30, 60 and 120 minutes and then plated as described above for MYC enumeration.

3.4.3 *Escherichia coli* and Coliforms

Coliforms and *E. coli* were determined using 3-tube most probable number (MPN) method according to AACC International method 42-15.01 (AACC International, 1999d). An overview of the procedure is outlined in Fig. 3.3. Tubes containing lauryl sulfate tryptose (LST) broth (Oxoid Ltd.) were inoculated at 3 consecutive dilutions in triplicate and incubated at 35 °C for 24-48 h. If gas formation was seen, a 3 mm loop was used to transfer culture from gassing tubes to brilliant green lactose bile (BGLB) broth (Oxoid Ltd.) and E.C. broth (Oxoid Ltd.). BGLB broth was incubated for 24-48 h at 35 °C and E.C. broth was incubated for 24-48 h at 45 °C. Tubes were considered positive for coliforms if gas was produced in BGLB broth and the MPN was determined using the table outlined in the AACC International method 42-15.01. The mean

and SD were calculated using a MDL of 3.0 MPN/g for any results with no positive tubes. If gas was produced in E.C. broth, the sample was streaked on Eosine Methylene Blue (EMB) agar (Oxoid Ltd.). EMB plates were incubated for 24 h at 35 °C. At least two colonies from the EMB plate were then transferred to plate count agar (Oxoid Ltd.) slants. Slants were incubated for 24 h at 35 °C. Growth from the slant was then transferred to tryptone broth (Oxoid Ltd.), Methyl Red-Voges Proskauer (MR-VP) media (Fluka, Sigma-Aldrich Co., St. Louis, MO, US) and Koser's citrate broth (Difco, Becton, Dickinson and Company, Sparks, MD, US) and incubated at 35 °C for 24, 48, and 96 h, respectively. A Gram stain of the growth from the slant was also performed to determine if microorganism was Gram negative or positive. After incubation, Kovac's reagent was added to tryptone broth to test for indole. Two 1 mL aliquots were taken from 48 h MR-VP media. Methyl red was added to one aliquot, and α-naphthol and 40% KOH was added to the other. If a red and pink colour formed, respectively, the sample was positive. If growth was seen in Koser's citrate broth the sample was positive for citrate. Coliforms and *E. coli* were reported as MPN/g. The IMViC classification of *E. coli* was determined from the test for indole, MR-VP, citrate and Gram stain.

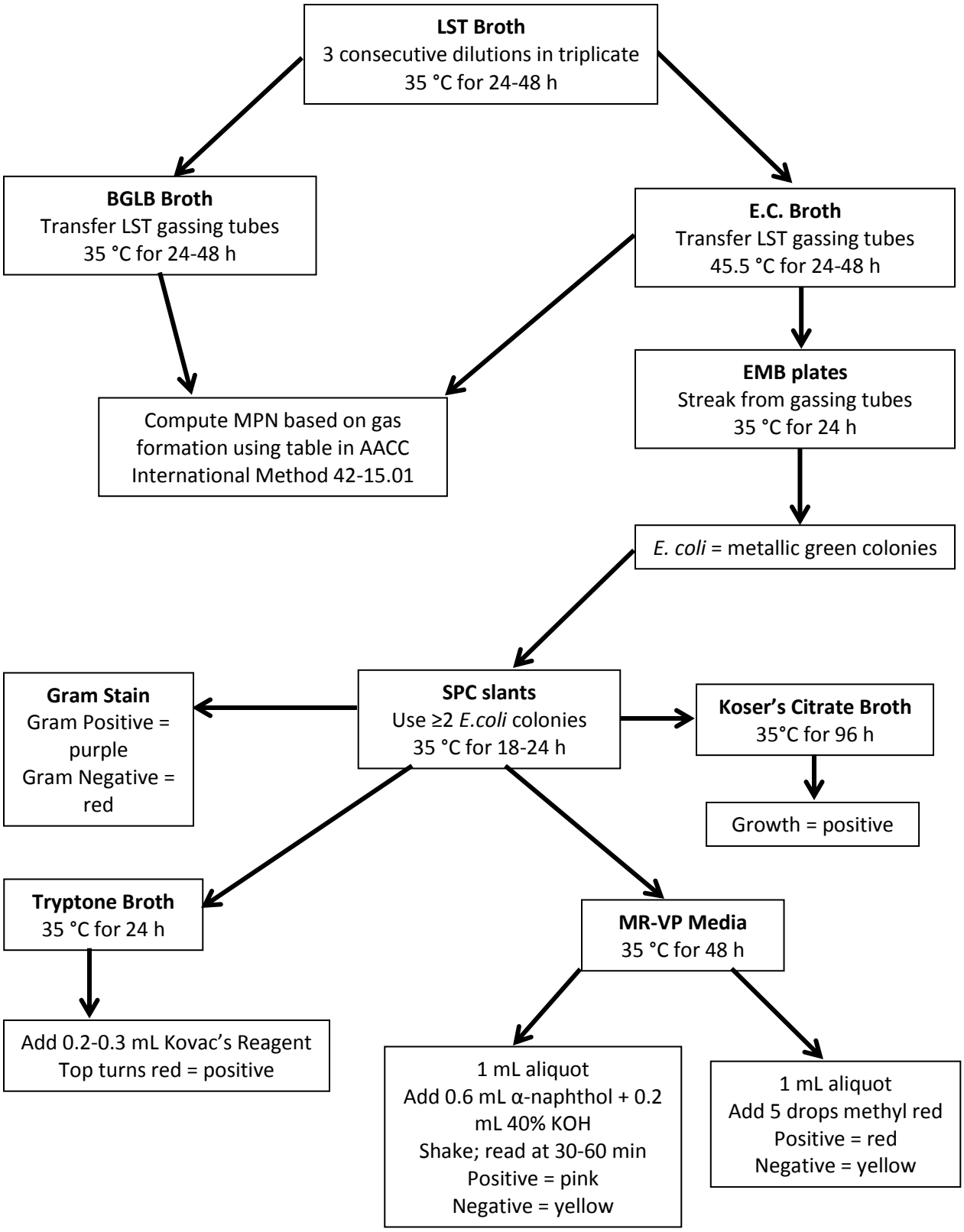


Figure 3.3 Procedure for determination of coliforms/*E. coli* using Most Probable Number method

3.5 Deoxynivalenol Content

Deoxynivalenol (DON) content was analyzed on the untreated barley samples (ground to 0.5 mm) using direct competitive enzyme-linked immunosorbent assays (ELISA). Monoclonal antibodies (MAB) were produced *in vitro* using a 5 L fermenter and the hybridomas prepared according to Sinha *et al.* (1995). The MAB were specific for DON and 15-acetyl-DON, but not for 3-acetyl-DON. A microtiter plate was coated in MAB (100 µL per well) and allowed to air dry overnight at 35 °C. DON was extracted from 1 g of sample by continuous mixing with 10% (v/v) methanol for 1 h. After extraction, the sample was centrifuged for 30 min at 3000 rpm. The supernatant and prepared DON-HRP (horseradish peroxidase) was pipetted (180 µL each) into a microtube and vortexed. DON-HRP was used as the competitor and was prepared to 1 µg/mL in 0.1 M phosphate buffered saline (PBS) with 1% non-fat dairy milk. The sample solution (125 µL) was added to an antibody coated microtiter plate well. The plate was incubated for 1 h at 37 °C, while on a plate shaker at low speed. It was then washed 8 times with 0.1 M PBS/Tween 20 (PBST). Two hundred fifty µL of substrate (0.3 µg/mL o-phenylenediamine dihydrochloride in phosphate buffer containing 2 µL/mL hydrogen peroxide) was added to each well, and then incubated for 30 min at 28 °C. Fifty µL of HCl (5.0 N) was added to each well to stop the reaction. The absorbance of each well was then read on a microtiter plate reader at 490 nm. DON standards with varying concentrations (0.01 – 10 µg/mL) were also analyzed to produce a standard curve. Analysis was carried out in duplicate at the AAFC research lab in Ottawa, Ontario. Results were reported as parts per million (ppm).

3.6 β-Glucan Analyses

3.6.1 β-Glucan Content

β-glucan content was determined using a Megazyme Mixed Linkage β-Glucan assay kit (Megazyme International Ireland, Bray, County Wicklow, Ireland) according to the AACC International method 32-23.01 (AACC International, 1999e). Samples (ground to 0.5 mm) were first boiled with 50% ethanol (v/v) and 20 mM sodium phosphate buffer (pH 6.9). Lichenase and β-glucosidase were used to break down the β-glucan into D-glucose. Glucose oxidase/peroxidase was used to measure the glucose content spectrophotometrically. The absorbance was measured using a UV/Visible spectrophotometer at 510 nm. The total β-glucan content was calculated using the following equation:

$$\text{BG Content (\%w/w)} = \Delta E \times F \times 94 \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

Where: ΔE is the absorbance of the sample minus the blank absorbance

F is a factor (calculated from D-glucose standards) to convert absorbance to μg of D-glucose

$$F = \frac{\text{100 μg of D-glucose}}{\text{absorbance of 100 μg of D-glucose}}$$

94 is the volume correction factor (total volume of extract solution is 9.4 mL)

$\frac{1}{1000}$ is the conversion of μg to mg

$\frac{100}{W}$ is the conversion to mg of sample (based on sample weight as W)

$\frac{162}{180}$ is a factor to convert the amount of D-glucose into amount of BG

3.6.2 β-Glucan Viscosity

The Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) was used to extract the β-glucan according to the *in vitro* method outline by Gamel *et al.* (2012). The temperature of the sample was held constant at 37 °C with a mixing speed at 480 rpm for the first 10 sec, followed by 160 rpm for 30 min or 2 h depending on desired extraction time. Two different extraction times were done to establish if endogenous β-glucanases were present in the samples. An extraction time of 20 min was used instead of 30 min for the commercial samples because the viscosity decreased faster than the heat-treated samples. Slurries were prepared in a RVA canister containing the sample (ground to 0.5 mm and weighed to contain 1% β-glucan) and 25 mL moisture. The moisture present in the sample and the total volume of the digestive enzymes were subtracted from the 25 mL to calculate the amount of 20 mM sodium phosphate with 10 mM NaCl (pH 6.9) to be added. The digestive enzymes were added to the canister as follows: 63 µL salivary amylase (220 U/mL in 2.5 mM $CaCl_2$), 150 µL pepsin (1150 U/mL in 0.9% NaCl), and 300 µL pancreatin (0.5 mg/mL in 20 mM sodium phosphate buffer with 10 mM NaCl, pH 6.9). The RVA final viscosity was noted at the end of 20 or 30 min or 2 h.

Once the extraction was complete, the slurry was centrifuged at 9000 X G for 10 min. An aliquot of the supernatant was boiled for 15 min to inactivate any endogenous β-glucanase enzymes prior to β-glucan molecular weight and solubility analysis. The extract was cooled to room temperature after boiling before further analysis. A separate aliquot of the supernatant was used to measure the extract viscosity on the rheometer (DHR-2, TA Instruments, New Castle, DE, US) using cone (4°, 40 mm diameter) and plate geometry. The Pelletier plate and a

10 sec soak time were used to condition the sample to 37 °C. A flow ramp method was used to increase shear rate to 100.0 s^{-1} logarithmically over 2 min from an initial shear rate of 0.1 s^{-1}. The viscosity at 30 s^{-1} shear rate was noted.

3.6.3 β-Glucan Molecular Weight

β-glucan molecular weight was determined using size exclusion chromatography with calcofluor post-column detection. An aliquot of the boiled and cooled extract was syringe-filtered through a 0.45 µm polyvinylidene fluoride filter prior to injecting into the HPLC (Agilent Technologies, Santa Clara, CA, US). An autosampler injected 5 µL of the sample into the HPLC equipped with Shodex OHpak SB-806M column with an OHpak SB-G guard column, followed by a Waters Ultrahydrogel Linear column. The columns were held at 40 °C. The sample eluted at 1 mL/min in 0.1 M Tris buffer (pH 8.0) using Agilent 1100 pump. A second pump (Agilent 1260) at a flow rate of 1 mL/min was used for post-column addition of calcofluor (20 mg/mL calcofluor in 0.1 M Tris buffer, pH 8.0) to allow for fluorescence detection (excitation at 415 nm, emission at 445 nm) using an Agilent 1100 fluorescence detector. Each extract was injected in triplicate. Peak molecular weight (Mp) and weighted average of molecular weight (Mw) were noted. A standard curve (retention time versus log Mp) was prepared using 5 β-glucan molecular weight standards ranging from 90 to 1000 kDa obtained as a generous gift from Dr. Susan Tosh (Agriculture and Agri-Food Canada, Guelph Food Research Centre). Molecular weight standards (1 mg/mL solution in water containing 0.02% sodium azide) were prepared by boiling for 3 h.

3.6.4 β-Glucan Solubility

β-glucan solubility was determined by analyzing the boiled and cooled extract for β-glucan content using the Megazyme Mixed Linkage β-Glucan assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) and AACC International method 32-23.01 (AACC International, 1999e). An aliquot of 500 µL of each extract was pipetted into tubes instead of weighing each sample. Each extract was analyzed in duplicate. β-glucan solubility was reported as a percentage and calculated as follows:

$$\text{BG content in extract (mg)} = \Delta E \times F \times 69 \times \frac{162}{180} \times \frac{\text{Total liquid volume in RVA slurry(mL)}}{\text{Volume of extract used in BG assay (µL)}}$$

$$\text{Total BG content in RVA slurry (mg)} = \left(W - \frac{W \times M}{100}\right) \times \frac{\text{BG content in sample (\% d.b.)}}{100}$$

$$\text{BG solubility (\%)} = \frac{\text{BG content in extract (mg)}}{\text{Total BG content in RVA slurry}} \times 100$$

Where: ΔE is the absorbance of the sample minus the blank absorbance

F is a factor (calculated from D-glucose standards) to convert absorbance to µg of D-glucose

$$F = \frac{\text{100 µg of D-glucose}}{\text{absorbance of 100 µg of D-glucose}}$$

69 is the volume correction factor (total volume of final extract solution is 6.9 mL)

$\frac{162}{180}$ is a factor to convert the amount of D-glucose into amount of BG

W is the weight of sample in RVA canister (g)

M is the moisture content of the sample (%)

Total liquid volume in RVA slurry = 25 mL

Volume of extract used in BG assay = 500 µL

3.7 Particle Size

3.7.1 Particle Size Distribution using Laser Diffraction

The particle size distribution of the samples (ground to 0.5 mm) was determined using the Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK) with a Scirocco 2000A (Malvern Instruments Ltd., Malvern, UK) dry cell. The Mastersizer uses laser diffraction to measure different particle sizes. A laser beam hits each particle and causes the light to scatter at a different angle dependent on the particle's size. Generally, large particles produce smaller angles and small particles produce larger angles. Analysis was done in duplicate. The following variables were determined:

d(0.1) – size of particle below which 10% of the sample lies

d(0.5) – size at which 50% of the sample is smaller, and 50% is larger

d(0.9) – size of particle below which 90% of the sample lies

D [3, 2] – Surface area mean diameter

D [4, 3] – Volume mean diameter

Span – Width of distribution

Specific surface area – Total area of the particles divided by the total weight

Uniformity – Deviation from the median

3.7.2 Separation of Particle Sizes using Air Jet Sieving

Flour fractions were made from the untreated and heat-treated samples (ground to 0.5 mm) using a 3 min cycle on the Retsch AS 200 Jet Sieve (Brinkmann Instruments, Westbury, NY, US). Two sieves, 125 and 250 µm, were used to separate 75 g of flour into 3 fractions of particles of the following sizes: < 125 µm, 125-250 µm and > 250 µm. The micronized and roasted without tempering samples were not jet sieved as it was determined from previous results they would not be as applicable to industry. The percent yield was calculated for each fraction. Each fraction was analyzed for moisture and β-glucan content in duplicate as outlined in sections 3.3 and 3.6.1, respectively, in this thesis.

3.8 Starch Pasting

The RVA (RVA-4, Newport Scientific, Warriewood, Australia) was used to determine the starch pasting characteristics of the samples. The starch pasting profile standard 1 was used as outlined by AACC International method 76-21.01 (AACC International, 1999f). Samples (ground to 0.5 mm) were weighed to 4.0 g at 14% moisture basis and added to distilled water calculated to give a total water content of 25.0 mL. The profile had a starting temperature of 50 °C, which was held for 1 min, then increased to 95 °C over 3.7 min, held for 2.5 min, then decreased to 50 °C over 3.8 min and held at 50 °C for 2 min. Initially, the sample was stirred at 960 rpm for 10 sec and then at 160 rpm for the remainder of the test. Each analysis took 13 min and was done in duplicate.

To determine the effect β-glucan had on the results obtained by standard starch pasting, a modified standard starch pasting profile was done with the addition of 25 µL

lichenase (*endo*-(1-3),(1-4)-ß-D-glucan 4-glucanohydrolase EC 3.2.1.73, 1000 U/mL, Megazyme International Ireland, Bray, County Wicklow, Ireland). A similar analysis was done by Liu et al. (2010) to evaluate the contribution β-glucan had on starch pasting. Samples (ground to 0.5 mm) were weighed to 4.0 g at 14% moisture basis and added to distilled water calculated to give a total moisture content of 25.0 mL (including 25 µL of lichenase). The standard starch pasting profile was altered to allow the lichenase to break down the β-glucan for 20 min at 50 °C prior to the standard profile used previously. Lichenase is not heat stable so the β-glucan must be broken down prior to increasing the temperature for starch pasting. Following the 20 min period, the temperature was increased to 95 °C over 3.7 min, held for 2.5 min, then decreased to 50 °C over 3.8 min and held at 50 °C for 2 min. Consistent with the standard 1 starch pasting profile, the sample was stirred at 960 rpm for the first 10 sec and then at 160 rpm for the remainder of the test. This analysis took 32 min to complete and was done in duplicate. Peak viscosity, breakdown viscosity, setback viscosity, final viscosity, peak time and pasting temperature were all noted in both starch pasting analyses.

3.9 Peroxidase Analysis

The presence of peroxidase was measured qualitatively using the AACC International method 22-80.01 (AACC International, 1999g). This analysis is routinely used in the oat industry to measure the inactivation of lipase by measuring the more heat-resistant enzyme, peroxidase. Samples (ground to 0.5 mm) were weighed twice, for a blank and reagent. D-isoascorbic acid (1 g/L) and blue dye, 2,6-dichloro-indophenol (0.2 g/L) were added to both the blank and reagent. Hydrogen peroxide (30% v/v) was added to only the reagent in order to measure the colour

change against the blank. Any endogenous peroxidase in the sample used the hydrogen peroxide to oxidize the blue dye and produce a blue colour. Both the blank and reagent flasks were placed in a water bath at 38 °C for 10 min. After 5 min the blank and reagent were compared for any colour change, then stirred and returned to the water bath. The flasks were compared again after 10 min. Results were represented as negative for no colour change, 0 for a grey colour formation and positive for a blue colour formation. Examples of a positive and negative peroxidase result are shown in Appendix A. Only a negative result indicates peroxidase inactivation. Analysis was done in duplicate.

3.10 Colour Analysis

The colour of the samples was determined using a Minolta Chroma Meter CR-410 (Konica Minolta Sensing Americas Inc., Ramsey, NJ, US). Colour was characterized by the L* a* b* colour scale where L* represents lightness from 0 (black) to 100 (white), a* represents green (negative) to red (positive), and b* represents blue (negative) to yellow (positive). The colour of both the barley kernel and flour (ground to 0.5 mm) were measured through the glass of the granular attachment which was filled to the top with the sample. Analysis was done in duplicate and 3 measurements were averaged by the instrument per duplicate.

3.11 Statistical Analysis

The commercial samples analyzed in this thesis were only analyzed for means and standard deviations as no formal statistical analysis could be done due to the low number of

samples obtained in the survey and the large number of sample types, so they could not be grouped.

The heat treatment portion of the study was conducted as a factorial with completely randomized design (CRD) with 2 factors, treatment and cultivar. The cultivars (CDC Fibar, CDC McGwire and CDC Rattan) were grown in 1 location. There were 6 treatments including the control (untreated). The 5 heat treatments (micronization with tempering, micronization without tempering, roasting with tempering, roasting without tempering, and conditioning) were processed in 2 replicates as subsamples from the same lot of grain. Most analyses were done in duplicate.

The data were analyzed using the PROC MIXED and GLIMMIX procedures of SAS (Little et al. 2006; SAS Institute, 2013). A mixed model with cultivar and treatment as fixed effects was used for statistical analysis. Processing replicates were considered as random effects. Contrasts were used to determine the effect of heat treatment for each cultivar and to make comparisons between treatments. A Least Significant Difference (LSD) of 0.05 was determined with the means as a measure of precision and to facilitate means comparisons.

A separate statistical analysis was done on the jet sieved fractions. Data were averaged across sieve and analysis duplicates (subsamples), and mixed model analysis was conducted on these means. The data were analyzed as a 3 factor (cultivar, treatment and fraction) factorial and CRD using the GLIMMIX procedure of SAS (Littell et al. 2006; SAS Institute 2013). Cultivar, heat treatment, and fraction were fixed effects and processing replicates were random effects. An LSD of 0.05 was determined with means as a measure of precision and to facilitate means

comparisons. Only the control (untreated), micronization with tempering, roasting with tempering and conditioning treatments were used in this portion of the study.

There were 2 groups of response variables analyzed to determine their correlation. First, all the β-glucan variables, excluding β-glucan content, were assessed to determine the relationship between the different devices and extraction times used. These variables included all β-glucan viscosity, molecular weight and solubility data. Second, the relationships between the starch pasting (only with lichenase addition) and particle size variables were determined. Pearson correlation analysis was also determined for selected response variables using the CORR procedure of SAS (SAS Institute, 2013).

4. RESULTS AND DISCUSSION

4.1 Survey of the Safety and Nutritional Properties of Current Canadian Commercial Barley Products

4.1.1 Microbial Contamination

At the beginning of this study, a number of Canadian barley processors were contacted in order to gain a better understanding of current practices used in the barley industry. The objective of this was to understand what types of processes were being used, if heat treatments were being applied and if microbial and mycotoxin testing was done. Ten processors were willing to supply information and 5 supplied samples. These samples included unprocessed grain, by-products and end products (Table 3.1). For the purposes of this study, "end product" will be used to describe the product obtained at the end of primary processing; these include barley flour, barley flakes and pearled barley. In total, 17 samples were collected and 12 of these were considered end products for this study. Of the 10 processors contacted, 4 performed heat treatments on their barley products. These heat treatments included micronization, boiling, kilning and steaming. From the samples obtained for this study, 4 were heat-treated, 3 of which were micronized and the other steam-treated. The majority of the processors contacted did not test for microbiology or mycotoxins. Three stated they will perform microbial tests if their customers request it but only 2 actually had limits in place, which were determined by the processing company. Four perform mycotoxin analyses at the customer's request. However, one of these companies indicated that they test for DON upon receiving barley to ensure it meets their requirements.

The overall microbial results are summarized in Table 4.1. It is important to note that there are 2 results for MYC reported due to the use of 2 different agars, acidified potato

dextrose agar (PDA) and rose bengal chloramphenicol (RBC). The 2 agars did not produce similar results consistently, and therefore could not be averaged together. Generally, RBC had higher counts than PDA. This was not due to bacteria growth as chloramphenicol and tartaric acid were added to RBC and PDA, respectively, to inhibit bacteria. The difference was likely due to RBC supporting injured yeast and mold growth better than PDA (Beuchat, 1992). In addition, different yeast and mould species may have grown better on one media than the other due to different media supplying different nutrients, which may be a more favourable environment (Beuchat, 1992). Evaluating the SPC results, 5 of 17 barley samples had less than $\log_{10}$ 4.7 CFU/g and 9 samples exceeded the limit by greater than 1 log. Considering both MYC results, 12 of 17 samples had values greater than $\log_{10}$ 3 CFU/g and 6 samples exceeded the limit by greater than 1 log. Coliforms had a quite variable response among samples. Five samples had < 3.0 MPN/g which means there was no positive growth in any BGLB tubes. Of the samples with positive growth, 5 were greater than 100 MPN/g. Overall, only 4 out 17 samples met the microbial limits (Table 3.2) outlined for this study. These samples were toasted pearled barley #2, pearled and steamed barley flakes, rolled barley and whole grain barley flour #2. Interestingly, the two other heat-treated samples, toasted barley flakes and toasted pearled barley #1, did not meet the limits for SPC or coliforms, despite being micronized. This could be due to many factors including inconsistent heating or cross-contamination from unclean processing equipment or storage containers.

In addition, one of the two different lots of high β-glucan flour tested positive for *E. coli*. The microorganism was determined to be Gram negative and rod-shaped from the Gram stain. The IMViC test indicated the microorganism was positive for indole and methyl red, and

negative for Voges-Proskauer and citrate. These characteristics confirmed the microorganism was typical *E. coli*. Using the MPN method and averaging all duplicates, the mean *E. coli* count was 9.67 MPN/g, which is just under the acceptable limit used in this study. However, the tests used in the study cannot determine the pathogenicity of the *E. coli* found in this sample. These results show that barley and other grains are just as susceptible to contamination from potentially harmful bacteria as other types of foods and procedures should be implemented to ensure the safety of grain products.

For most samples at least a 1 log reduction in SPC and MYC was seen through processing, from unprocessed barley to an end product. The greatest reduction seen was in toasted pearled barley #2 and pearled & steamed barley flakes. From the microbial results in Table 4.1, it appears that some microbial reduction may be possible without applying heat treatments. The pearlings have the highest counts for SPC, MYC and coliforms and exceed the limits used in this study. This may have been due to increased contamination in the outer bran layers. Mycotoxins have been shown to be concentrated on the outer layers of barley kernels (Manthey et al. 2004; Edwards et al. 2011; Cheli et al. 2013; Khatibi et al. 2014), so the same could be true for microbes. While pearlings are not typically used as an end product, they are often used in animal feed which makes the high microbial counts a concern. Air classification is new way companies are creating nutrient and fibre rich barley fractions. However, these microbial results indicate they may be concentrating the microbes as well. They also suggest that any type of processing, not just heat treatments, can cause microbial reductions. While this may be true, direct conclusions cannot be made from this data as the samples obtained were from a variety of processors. They do, however, indicate the importance of processing and heat

treatments of barley in order to improve the safety of barley products and allow for novel product development. In addition, they show an opportunity for more research in the area to explore how the microbial population may differ throughout the barley kernel and the effect of other processes, like pearling and air classification, on the safety of barley and its products.

Table 4.1 Summary of mean standard plate count (SPC), mold and yeast count (MYC), coliforms and *E. coli* in commercial barley samples

Sample type	**SPC** (log_{10} CFU/g) ± SD	**MYC** (log_{10} CFU/g) ± SD		**Coliforms** (MPN/g) ± SD	***E. coli*** (MPN/g) ± SD
		PDA	RBC		
Unprocessed whole grain barley #1	6.67 ± 0.13	5.64 ± 0.32	5.48	< 3.0	< 3.0
Unprocessed whole grain barley #2	6.97 ± 0.19	4.71 ± 0.28	4.79 ± 0.28	20.3 ± 16.2[2]	< 3.0
Unprocessed whole grain barley #3	6.95 ± 0.23	4.96 ± 0.27	5.14 ± 0.19	185.3 ± 242.1[2]	< 3.0
Unprocessed whole grain barley #4	6.05 ± 0.04	4.54 ± 0.35	4.75 ± 0.19	16.3 ± 23.1[2]	< 3.0
Whole grain barley flour #1[3]	5.89 ± 0.13	4.34 ± 0.09	4.38 ± 0.25	600.0 ± 446.8	< 3.0
Whole grain barley flour #2[3]	≤ 2.00[1]	2.08 ± 0.15	≤ 2.00[1]	< 3.0	< 3.0
High β-glucan barley flour – lot A[3]	5.25 ± 0.04	3.13 ± 0.33	3.38 ± 0.36	36.3 ± 11.6	9.7 ± 11.6[2]
High β-glucan barley flour – lot B[3]	4.93 ± 0.07	2.66 ± 0.29	3.19 ± 0.45	65.1 ± 48.4	< 3.0
Low β-glucan barley flour[3]	4.56 ± 0.19	3.39 ± 0.29	3.37 ± 0.19	11.9 ± 10.0	< 3.0
Toasted barley flakes[3]	5.86 ± 0.22	≤ 2.00[1]	3.40 ± 0.37	11.7 ± 10.2[2]	< 3.0
Rolled barley[3]	≤ 2.00[1]	≤ 2.00[1]	2.32 ± 0.41	< 3.0	< 3.0
Pearled & steamed barley flakes[3]	2.64 ± 1.00[1]	2.14 ± 0.38[1]	2.05 ± 0.12[1]	< 3.0	< 3.0
Dehulled barley[3]	6.64 ± 0.13	3.71 ± 0.20	4.07 ± 0.17	118.7 ± 148.7	< 3.0
Pearled barley[3]	6.18 ± 0.15	3.35 ± 0.30	3.59 ± 0.26	17500.0 ± 9192.4	< 3.0
Toasted pearled barley #1[3]	5.64 ± 0.33	2.15 ± 0.17[1]	2.60 ± 0.21	23.2 ± 19.7	< 3.0
Toasted pearled barley #2[3]	2.38 ± 0.77[1]	≤ 2.00[1]	≤ 2.00[1]	< 3.0	< 3.0
Barley pearlings	7.59 ± 0.61	5.47 ± 0.23	5.80 ± 0.41	88000.0 ± 105655.7	< 3.0

[1]Mean and SD calculated using MDL for duplicates showing <30 x 10^2 CFU/g for SPC or <1 x 10^2 CFU/g for MYC (MDL = log_{10} 2 CFU/g)

[2]Mean and SD calculated using MDL for duplicates showing negative results (MDL = 3.0 MPN/g)

[3]Considered end-products in this study

No SD listed indicates a value of 0

PDA = potato dextrose agar; RBC = rose bengal chloramphenicol agar

4.1.2 β-glucan Content

The average β-glucan contents of the commercial barley products obtained in this study are shown in Table 4.2. Generally, a product that contains the whole grain will have more β-glucan than a product which removes some of the kernel, like pearled barley. However, this does not mean whole grain products will always have more β-glucan as other factors affect the β-glucan content as well. The β-glucan content will vary in a product depending on cultivar, environmental conditions, agronomic practices and product type (Pérez-Vendrell et al. 1996; Ames et al. 2006; Hang et al. 2007; Dickin et al. 2011). In addition, β-glucan in barley is distributed throughout the kernel, unlike oats where it is in the bran layers only (Miller and Fulcher, 1994; Zheng et al. 2000). The samples obtained in this study were a variety of cultivars and product types collected from across Canada. The β-glucan content in the commercial end products ranged from 3.63-6.68% (d.b.), excluding the high β-glucan flour samples which had approximately 25% (d.b.) of β-glucan.

The β-glucan content of the commercial samples was determined in order to assess the ability of current barley products to meet the health claim for cholesterol-lowering (Health Canada, 2012a). Table 4.3 shows the estimated amount of β-glucan per serving of a variety of barley foods that could be made using the commercial products obtained. In general, the commercial products appear to be able to meet the health claim requirements put forth by Health Canada. With the exception of pearled barley, all the pearled or dehulled barley products will supply over the 3 g daily requirement for cholesterol lowering in 1 serving of pilaf or soup. All of the barley flour products meet the health claim of 1 g β-glucan per serving when used in a waffle formulation. But, a muffin or slice of pizza would not meet the health claim

with the flour products, except for the high β-glucan flour. However, a high β-glucan product like this one would not typically be used in the same amount as regular barley flour due to its different functional properties. Therefore, this may be a misrepresentation of this product's ability to meet the health claim. A food application like a smoothie is more suitable to a highly concentrated product due to the low amount of barley flour used. If the high β-glucan flour was used in a smoothie it would meet the required amount of β-glucan per serving for Health Canada's cholesterol lowering health claim. The barley flake products also would be able to meet the health claim when used in porridge or granola bar applications, with the exception of rolled barley in the granola bar. The rolled barley and whole grain flour #2 had the lowest β-glucan contents and therefore had the least potential to meet the health claim with the products shown in Table 4.3.

Table 4.2 Mean β-glucan (BG) content of commercial barley end products

Sample Type	**BG content** (% d.b.)	**SD**
Whole grain barley flour #1	4.53	0.15
Whole grain barley flour #2	3.63	0.10
High β-glucan barley flour – lot A	25.62	0.42
High β-glucan barley flour – lot B	24.41	1.29
Low β-glucan barley flour	4.14	0.15
Toasted barley flakes	4.74	0.10
Rolled barley	3.74	0.12
Pearled & steamed barley flakes	5.29	0.07
Dehulled barley	6.68	0.28
Pearled barley	5.74	0.18
Toasted pearled barley #1	6.02	0.25
Toasted pearled barley #2	6.65	0.01

Table 4.3 Estimated amounts of barley β-glucan (g per serving) in barley foods potentially made from commercial barley products

Sample Type	BG content (% as is)	Pilaf[1] 140 g	Soup[2] 250 mL	Waffle[2] 1 waffle	Muffin[3] 1 muffin	Pizza[2] 1 slice	Smoothie[4] 250 mL	Porridge[1] 250 mL	Granola Bar[5] 1 bar
Whole grain barley flour #1	4.07	n/a	n/a	2.04*	0.81	0.81	0.20	n/a	n/a
Whole grain barley flour #2	3.19	n/a	n/a	1.59*	0.64	0.64	0.16	n/a	n/a
High BG barley flour – lot A	24.06	n/a	n/a	12.03**	4.81**	4.81**	1.20*	n/a	n/a
High BG barley flour – lot B	23.33	n/a	n/a	11.66**	4.67**	4.67**	1.17*	n/a	n/a
Low BG barley flour	3.83	n/a	n/a	1.91*	0.77	0.77	0.19	n/a	n/a
Toasted barley flakes	4.34	n/a	n/a	n/a	n/a	n/a	n/a	1.73*	1.04*
Rolled barley	3.28	n/a	n/a	n/a	n/a	n/a	n/a	1.31*	0.79
Pearled & steamed barley flakes	4.64	n/a	n/a	n/a	n/a	n/a	n/a	1.86*	1.11*
Dehulled barley	5.90	3.54**	3.42**	n/a	n/a	n/a	n/a	n/a	n/a
Pearled barley	4.95	2.97*	2.87*	n/a	n/a	n/a	n/a	n/a	n/a
Toasted pearled barley #1	5.40	3.24**	3.13**	n/a	n/a	n/a	n/a	n/a	n/a
Toasted pearled barley #2	6.03	3.62**	3.50**	n/a	n/a	n/a	n/a	n/a	n/a

[1]Serving size based on CFIA reference amount (CFIA, 2015b)

[2]Serving size based on recipes developed by Agriculture and Agri-Food Canada (AAFC, 2015)

[3]Serving size based on recipes in *Go Barley: Modern Recipes for an Ancient Grain* cook book (Inglis and Whitworth, 2014)

[4]Serving size based on CFIA reference amount (CFIA, 2015b) and the addition of 5 g flour per reference amount

[5]Serving size based on recipe developed by Dr. Nancy Ames (Ames unpublished)

*Satisfies 1 g of barley BG per serving requirement for cholesterol-lowering health claim (Health Canada, 2012a)

**Satisfies entire daily requirement of 3 g of barley BG in one serving for cholesterol-lowing health claim (Health Canada, 2012a)

BG = β-glucan

4.1.3 β-glucan Viscosity, Molecular Weight and Solubility

While the commercial products have been shown to have acceptable β-glucan contents in terms of Health Canada's health claim for cholesterol-lowering, other physicochemical properties of β-glucan also influence its cholesterol-lowering ability. Barley products containing β-glucan with a high viscosity, molecular weight and solubility are more successful at cholesterol-lowering (Ames and Rhymer, 2008; Theuwissen and Mensink, 2008; Ames et al. 2015a; Wang et al. 2013). The mean β-glucan *in vitro* digest extract viscosity, molecular weight and solubility of the commercial barley end-products are summarized in Table 4.4. Despite their β-glucan contents, almost all of the commercial products did not have the β-glucan characteristics to be effective if they were used in an uncooked application. Only one commercial product had a high β-glucan extract viscosity and molecular weight. The pearled and steamed barley flakes had an average β-glucan extract viscosity of 1372 cP and an average Mp of 2 552 kDa, which exceeded the desired properties. The drawback was its low solubility of 35.67%. The β-glucan solubility of the commercial barley samples ranged from 35.67-74.67%. The low β-glucan flour had the highest solubility, followed by pearled barley. Typically, higher molecular weight chains of β-glucan are harder to solubilize and therefore are negatively correlated to solubility (Klamczynski et al. 2004; Ames et al. 2015a). The β-glucan viscosity and molecular weight of other heat-treated commercial samples did not appear any different from the non-heat treated samples, and did not have a higher viscosity and molecular weight as expected. This could be because the heat treatments were inadequate to inactivate β-glucanases, or different barley cultivars could have different β-glucan polymer sizes.

Table 4.4 Mean β-glucan viscosity, molecular weight and solubility of 2 h *in vitro* digest extracts of commercial barley end products

Sample Type	**RVA viscosity** (cP) ± SD	**Rheometer viscosity** at 30 s^{-1} (mPa.s) ± SD	**Molecular weight** (kDa) ± SD		**Solubility** (%) ± SD
			Mp	**Mw**	
Whole grain barley flour #1	77 ± 7.78	10.31 ± 0.06	132 ± 46	166 ± 57	51.44 ± 1.95
Whole grain barley flour #2	81 ± 10.61	8.75 ± 0.07	100 ± 27	124 ± 32	46.76 ± 1.13
High β-glucan barley flour – lot A	132 ± 34.35	80.00 ± 14.34	435 ± 175	501 ± 196	60.42 ± 2.06
High β-glucan barley flour – lot B	119 ± 13.03	52.06 ± 9.65	329 ± 144	403 ± 173	62.08 ± 2.36
Low β-glucan barley flour	105 ± 13.44	22.40 ± 0.45	205 ± 59	262 ± 74	74.67 ± 4.52
Toasted barley flakes	70 ± 3.54	8.62 ± 0.03	57 ± 22	65 ± 24	64.10 ± 1.10
Rolled barley	95 ± 2.83	9.36 ± 0	105 ± 27	124 ± 31	55.45 ± 0.65
Pearled & steamed barley flakes	1372 ± 50.20	869.77 ± 25.51	2,552 ± 630	2,226 ± 538	35.67 ± 1.24
Dehulled barley	36 ± 19.09	8.27 ± 0.07	55 ± 20	64 ± 22	55.67 ± 1.48
Pearled barley	55 ± 9.90	8.71 ± 0.23	70 ± 27	75 ± 27	68.83 ± 2.08
Toasted pearled barley #1	39 ± 6.36	9.09 ± 0.01	100 ± 32	122 ± 38	51.40 ± 1.80
Toasted pearled barley #2	83 ± 9.90	12.05 ± 0.10	204 ± 56	270 ± 72	49.79 ± 0.30

RVA = Rapid Visco Analyser; Mp = peak molecular weight; Mw = weighted average molecular weight

Based on the RVA profiles over the 2 h extraction period, β-glucanase activity was suspected (Appendix B). The viscosities of all the samples, except the pearled and steamed flakes, decreased during the extraction. To confirm the viscosity drop was due to β-glucanases, another β-glucan *in vitro* extraction was carried out but for only 20 min to assess if larger molecular weights were present earlier in the extraction. The viscosity, molecular weight and solubility of the 20 min β-glucan *in vitro* digest extracts were also measured. All of the samples, except the pearled and steamed flakes, had higher viscosities and molecular weights after the shorter extraction time. This indicated there was β-glucanase activity present causing the β-glucan to break down. This caused a lower molecular weight and therefore, viscosity. For some samples the viscosity and molecular weight were still low after the 20 min extraction indicating β-glucanases may have been less of a factor causing the low viscosities and molecular weights in the results that were obtained after 2 h. In these cases, the β-glucan may innately have a low extract viscosity and molecular weight due to cultivar. However, there was a larger decrease in molecular weight and viscosity for some samples (Fig. 4.1). The samples most affected by β-glucanases were the high β-glucan flours and low β-glucan flour, as their molecular weight and viscosity decreased by approximately 500 kDa and 400 cP, respectively. This substantial reduction in β-glucan viscosity and molecular weight may affect the nutritional benefits of these products. As well, if the β-glucanase activity is variable it may become challenging for processors to create a consistent product due to the impact the β-glucan has on product functionality.

Considering all the β-glucan results, majority of the samples met the β-glucan content requirements set by Health Canada (2012a) for most food applications. However, the

commercial barley products collected may not contain the functional characteristics to impart health benefits for cholesterol-lowering when used in a food application without heat treatment. A sufficient heat treatment is need to inactivate β-glucanases to maintain a high β-glucan *in vitro* digest extract viscosity and molecular weight. Only one product, pearled and steamed flakes, had a high β-glucan viscosity and molecular weight and was the only one that appeared not to be affected by β-glucanases based on the RVA profiles.

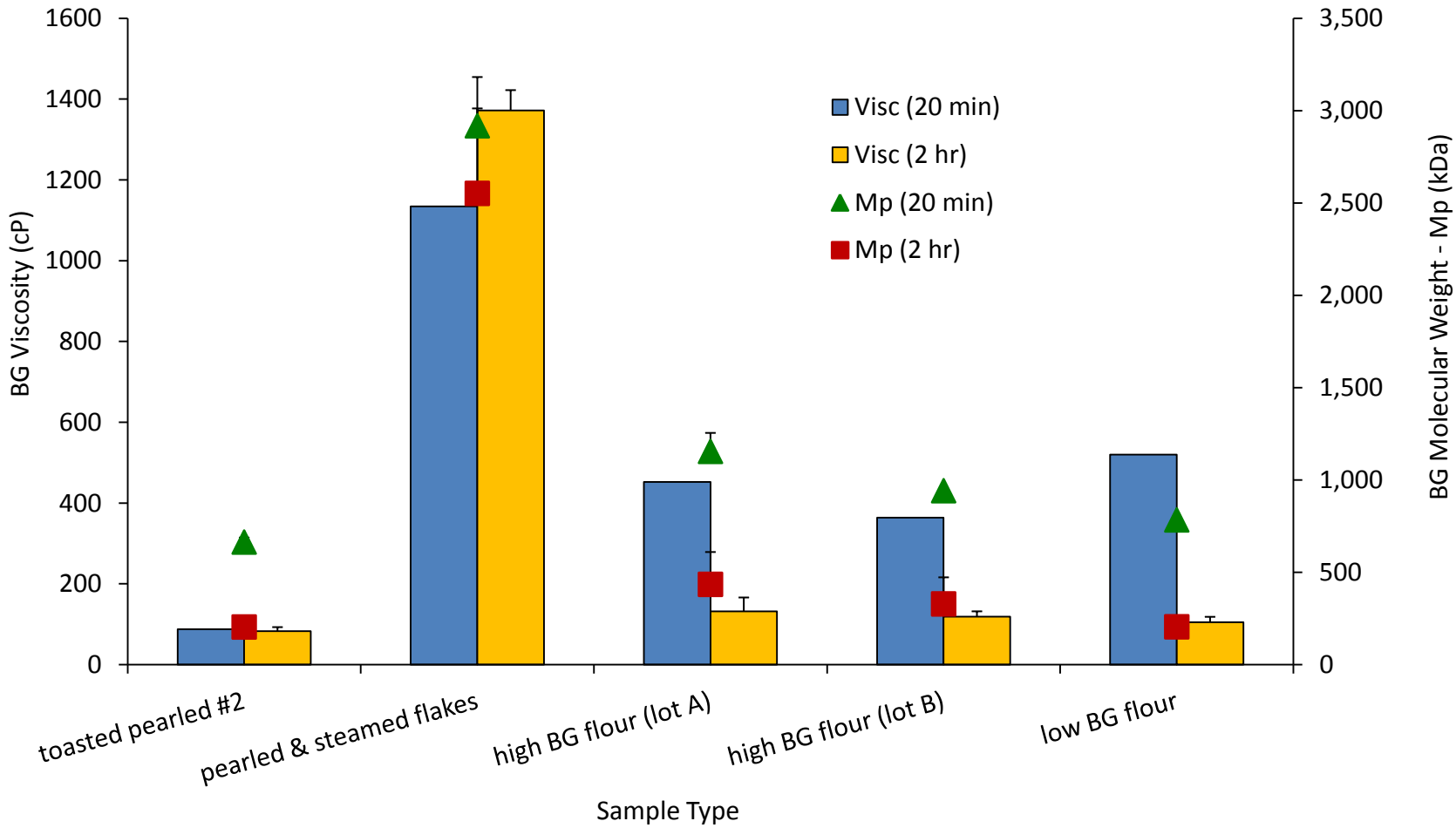


Figure 4.1 Comparison between 20 min and 2 h *in vitro* digest extraction times of mean β-glucan (BG) extract viscosity (RVA) and molecular weight (Mp) of selected commercial barley end products

4.1.4 Peroxidase Activity

The peroxidase activity of the commercial samples was measured using a qualitative test. While peroxidase was not the target enzyme to inactivate, it is more heat stabile than lipase and easier to measure (Girardet and Webster, 2011). All samples tested positive for peroxidase activity except for pearled and steamed barley flakes (Table 4.5). Therefore, lipase was not inactivated in all of the positive commercial samples causing them to be susceptible to rancidity. Even though barley has low oil content (2-5%), this may decrease shelf life (Newman and Newman, 2008b). Interestingly, the peroxidase results were similar to what was found in the β-glucan results, with regards to β-glucanase activity. The pearled and steamed barley flakes was the only sample to not experience a decrease in viscosity over the 2 h extraction time and only had a very small reduction in molecular weight. The steam process used in this sample was most likely what caused the enzyme inactivation as enzymes are more susceptible to moist heat than dry heat (Gates, 2007). It is possible that the β-glucanases could have been inactivated along with peroxidase, lipase and other enzymes in this sample. More research is needed to assess if peroxidase activity could be used to evaluate the inactivation of β-glucanases.

Table 4.5 Peroxidase activity of commercial barley samples

Sample Type	Peroxidase Activity (- or +)
Whole grain barley flour #1	+
Whole grain barley flour #2	+
High β-glucan barley flour – lot A	+
High β-glucan barley flour – lot B	+
Low β-glucan barley flour	+
Toasted barley flakes	+
Rolled barley	+
Pearled & steamed barley flakes	-
Dehulled barley	+
Pearled barley	+
Toasted pearled barley #1	+
Toasted pearled barley #2	+

- Result with a negative (no colour change) reaction
+ Results with a positive (blue colour formation) reaction

4.2 Effect of Heat Treatments on the Safety of Whole Grain Barley

4.2.1 Microbial Contamination

The microbial results examined in this section have two mean values reported for MYC because the two media used, PDA and RBC, failed to produce similar results consistently. Therefore, they could not be averaged together. Generally, RBC had higher counts than PDA. This was not due to bacteria growth as chloramphenicol and tartaric acid were added to RBC and PDA, respectively, to inhibit bacteria. The difference was likely due to RBC supporting injured yeast and mould growth better than PDA (Beuchat, 1992). In addition, some yeast and mould species may have grown better on one media than the other due to different media supplying different nutrients, which may be a more favourable environment (Beuchat, 1992).

Prior to heat treatment, barley that had not been cleaned was tested for microbial contamination and compared to untreated (cleaned) barley from the same lot of grain. These results are summarized in Table 4.6. Uncleaned barley was tested in order to evaluate if cleaning the grain reduced the initial microbial count. It was expected that untreated barley would have a lower microbial load than uncleaned barley because dirt, insects and other field debris were removed. Neagu *et al.* (2012) found that cleaning wheat reduced mould and aerobic bacteria by 70 and 62.5%, respectively. However, in this study, microbial contamination increased in the untreated barley with the exception of coliforms and *E. coli* which did not change from cleaning. SPC increased by approximately 1 log CFU/g in Fibar and Rattan, while McGwire only increased approximately 0.2 log CFU/g. MYC increased by approximately 0.6-0.7-log CFU/g in Fibar and Rattan as well, but there was minimal change in McGwire. The increase in SPC and MYC that occurred in the untreated barley could be due to contamination from the

cleaning equipment. Dirt and debris are constantly moving through the cleaning equipment which means the barley could have been contaminated from other lots of barley or cereal grains that were previously cleaned. In addition, the barley cultivars used in this study are hulless. Therefore, dehulling would not contribute greatly to microbial reduction, even though hulless cultivars are not completely hull-free. The reduction of microbial contamination from hull removal has not been studied. However, hull removal has been previously shown to reduce DON contamination in oats (Tekauz et al. 2004; Scudamore et al. 2007, 2009). The increase in microbial load in the untreated barley may be due to storage as well. Improper storage conditions can allow for microbial growth, especially *Fusarium*, *Aspergillus* and/or *Penicillium,* which can grow at low moisture contents (Bullerman and Bianchini, 2011). Both the uncleaned and untreated barley were stored under the same conditions throughout the study. However, before barley was received for the current project, it was stored offsite. Overall, it is unclear exactly why an increase in SPC and MYC were seen after cleaning in the 3 barley cultivars tested. In addition, more research would need to be done to assess if no change or an increase in microbial load is seen after cleaning repeatedly, or if in some cases a reduction is seen.

The baseline microbial load was determined from untreated barley (Table 4.6). All 3 cultivars exceeded the SPC limit ($\log_{10}$ 4.7 CFU/g) for this study. Rattan had the lowest mean SPC at $\log_{10}$ 5.16 CFU/g and Fibar had the highest at $\log_{10}$ 5.98 CFU/g. The untreated barley cultivars also exceeded the limit for MYC ($\log_{10}$ 3 CFU/g). The Fibar MYC-PDA result was slightly less than the limit with $\log_{10}$ 2.94 CFU/g, but the MYC-RBC result for Fibar surpassed the limit with $\log_{10}$ 3.50 CFU/g. Therefore, Fibar was considered to be outside the acceptable range for MYC. Coliforms for all 3 cultivars were below the limit of <100 MPN/g with an average of 3.2

MPN/g. *E. coli* was also within the acceptable limits of <10 MPN/g with an average of < 3.0 MPN/g for each cultivar.

Table 4.6 Mean standard plate count (SPC), mold and yeast count (MYC) and coliforms of uncleaned and untreated (cleaned) barley

Treatment	Cultivar	SPC (log_{10} CFU/g) ± SD	MYC – PDA (log_{10} CFU/g) ± SD	MYC – RBC (log_{10} CFU/g) ± SD	Coliforms (MPN/g) ± SD	*E. coli* (MPN/g) ± SD
Uncleaned	Fibar	5.01 ± 0.11	2.26 ± 0.58[1]	2.93 ± 0.78[1]	3.2 ± 0.35[2]	< 3.0 ± 0
	McGwire	5.21 ± 0.27	3.41 ±0.29	3.87 ± 0.34	3.2 ± 0.35[2]	< 3.0 ± 0
	Rattan	4.96 ± 0.13	2.30 ± 0.45[1]	2.88 ± 0.59	3.2 ± 0.35[2]	< 3.0 ± 0
Untreated	Fibar	5.98 ± 0.11	2.94 ± 0.09	3.50 ± 0.45	3.2 ± 0.35[2]	< 3.0 ± 0
	McGwire	5.78 ± 0.12	3.45 ± 0.23	3.68 ± 0.16	3.2 ± 0.35[2]	< 3.0 ± 0
	Rattan	5.16 ± 0.30	3.17 ± 0.23	3.50 ± 0.21	3.2 ± 0.35[2]	< 3.0 ± 0

[1]Mean and SD calculated using MDL for duplicates showing < 1 x 10^2 CFU/g (MDL = log_{10} 2 CFU/g)

[2]Mean and SD calculated using MDL for duplicates showing negative results (MDL = 3.0 MPN/g)

Values reported as means within treatment and cultivar

PDA = potato dextrose agar; RBC = rose bengal chloramphenicol agar

Heat treatments were conducted on each of the 3 barley cultivars to assess their ability to reduce microbial contamination. *E. coli* results are not presented as all samples had no positive reaction and therefore a mean value of < 3.0 MPN/g. The ANOVA results are summarized in Table 4.7. The main effect for cultivar was not significant for MYC-PDA, MYC-RBC and coliforms indicating that none of the cultivars were more susceptible to yeast, mould and coliform contamination. A significant treatment effect was seen for SPC, MYC-PDA, MYC-RBC and coliforms. A summary of the treatment means is shown in Table 4.8. All heat treatments reduced the microbial contamination to below the limits used in this study and were significantly different from the untreated sample. Conditioning was the most successful because the mean result was the minimum detectable limit (MDL) for all variables. This indicates there was no growth seen during any of the analyses. Micronization without tempering was not as effective at reducing SPC as the other heat treatments. While the mean SPC result was less than the acceptable limit, it was significantly different than the other treatments. Considering both the PDA and RBC results for MYC, all heat treatments were significantly different from untreated. Conditioning was significantly different from all the other heat treatments for the RBC data. However, for the PDA data conditioning was only significantly different from roasting with tempering. It was unexpected to see yeast and mould organisms survive the roasting and micronization treatments as most yeast and mould organisms are heat sensitive. Therefore, the untreated samples were also analyzed for heat resistant mould. The untreated barley had no growth after heating samples at 70 °C for 30, 60 and 120 min (results not shown). This indicates there was no heat resistant mould present prior to heat treatment and therefore could not be present in the heat-treated samples. For this reason, the positive

MYC results found in the micronized and roasted samples were probably due to inconsistent heating during the processes. The pilot and lab scale nature of the micronization and roasting treatments may not have applied a consistent level of heat to each barley kernel allowing some organisms to survive. All of the heat treatments had significantly different mean coliforms from the untreated samples. However, the untreated samples were already well within the acceptable limits for coliforms.

Table 4.7 Summary of ANOVA for microbial results

	P-values			
Effect	SPC	MYC - PDA	MYC - RBC	Coliforms
Cultivar	0.006*	0.844	0.991	0.505
Treatment	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Cultivar x Treatment	0.026*	0.384	0.540	0.618

*Significant effect at P (<0.05)

SPC = standard plate count; MYC = mold and yeast count; PDA = potato dextrose agar; RBC = rose bengal chloramphenicol agar

Table 4.8 Effect of heat treatments on microbial contamination of whole grain barley

Treatment	SPC[3] (log_{10} CFU/g)	MYC – PDA[3] (log_{10} CFU/g)	MYC – RBC[3] (log_{10} CFU/g)	Coliforms[4] (MPN/g)
Micronized (T)[1]	≤ 2.00 a	2.13 (0.39) ab	2.20 (0.43) a	< 3.0 a
Micronized (N)[2]	2.68 (0.94) b	2.18 (0.39) ab	2.19 (0.39) a	3.0 (0.16) a
Roasted (T)[1]	≤ 2.00 a	2.20 (0.41) b	2.27 (0.49) a	< 3.0 a
Roasted (N)[2]	≤ 2.00 a	2.09 (0.31) ab	2.24 (0.42) a	< 3.0 a
Conditioned	≤ 2.00 a	≤ 2.00 a	≤ 2.00 b	< 3.0 a
Untreated	5.64 (0.43) c	3.19 (0.28) c	3.56 (0.32) c	3.2 (0.30) b

[1]Barley tempered to 17% moisture prior to treatment

[2]Barley was not tempered prior to treatment

[3]Means except untreated calculated using MDL for duplicates showing < 30 x 10^2 CFU/g for SPC or < 1 x 10^2 CFU/g for MYC (MDL = log_{10} 2 CFU/g)

[4]Means calculated using MDL for duplicates showing negative results (MDL = 3.0 MPN/g)

SPC = standard plate count; MYC = mold and yeast count; PDA = potato dextrose agar; RBC = rose bengal chloramphenicol agar

Values reported as means among all cultivars within a treatment

Values presented as mean (SD); No SD listed indicates a value of 0

Values within same column followed by same letter are not statistically significant (LSD, P ≥ 0.05)

SPC also had significant cultivar and interactional (cultivar X treatment) effects (Table 4.7). The mean SPC for each cultivar and treatment are summarized in Table 4.9. The significant cultivar effect was likely due to the difference in SPC between cultivars in the untreated barley. This difference between cultivars could be due to many variables including cultivar, environmental conditions, agronomic practices and storage conditions (Manthey et al. 2004; Bullerman and Bianchini, 2011). The amount of reduction seen for each treatment was consistent considering the baseline SPC of each cultivar. For each cultivar, all heat treatments achieved a mean SPC of ≤ $\log_{10}$ 2.00 CFU/g, except micronization without tempering. The micronized without tempering Fibar and McGwire samples were significantly different from all other treatments. However, tempering had no effect on SPC when roasting was applied as a heat treatment. This indicated that adding moisture prior to micronizing barley improved the reduction in SPC.

Overall, micronization, roasting and conditioning reduced the SPC and MYC to within acceptable limits. Conditioning had the greatest reduction and was the most consistent. Tempering improved the micronization treatment in terms of SPC reduction but had no effect on roasting. Therefore, heat treatments are an acceptable method to reduce microbial load and improve the safety of barley.

Table 4.9 Effect of heat treatments on mean standard plate count (log_{10} CFU/g) of different cultivars of barley

Treatment	Fibar	McGwire	Rattan
Micronized (T)[1]	≤ 2.00 a	≤ 2.00 a	≤ 2.00 a
Micronized (N)[2]	2.94 (0.99) b	2.97 (1.03) b	2.13 (0.51) a
Roasted (T)[1]	≤ 2.00 a	≤ 2.00 a	≤ 2.00 a
Roasted (N)[2]	≤ 2.00 a	≤ 2.00 a	≤ 2.00 a
Conditioned	≤ 2.00 a	≤ 2.00 a	≤ 2.00 a
Untreated	5.98 (0.11) c	5.78 (0.12) c	5.16 (0.30) b

[1]Barley tempered to 17% moisture prior to treatment
[2]Barley was not tempered prior to treatment
Values reported as means with cultivar and treatment
Values presented as mean (SD); No SD listed indicates a value of 0
Values within same column followed by same letter are not statistically significant (LSD, $P \geq 0.05$)
All means and SD except untreated were calculated using MDL for duplicates showing $< 30 \times 10^2$ CFU/g (MDL = log_{10} 2 CFU/g)

4.2.2 Deoxynivalenol

The effect of heat treatments on DON content in whole grain barley was not assessed because the DON concentration in the 3 untreated cultivars would meet the limits for any food type outlined by the European Commission (2006) and those proposed by Health Canada (2012b) for soft wheat. As well, the concentration of the untreated barley was close to the lowest detectable limit of 0.1 ppm so minimal differences due to heat treatment would be detected. The DON concentration results for the untreated barley samples are summarized in Table 4.10.

Table 4.10 Mean Deoxynivalenol (DON) concentration of untreated barley cultivars

Cultivar	DON Concentration (ppm)	SD
Fibar	0.2	0
McGwire	0.2	0
Rattan	0.2	0

4.3 Effect of Heat Treatments on Nutritional and Physicochemical Properties of Whole Grain Barley

4.3.1 β-glucan

β-glucan is an important constituent of barley as it influences functionality and nutritional benefits. The effects of heat treatment on barley β-glucan were assessed by analyzing the samples for β-glucan content, *in vitro* digest extract viscosity, molecular weight and solubility. Each of these variables affects the nutritional value of barley and how it will respond in different food matrices. The ANOVA is summarized in Table 4.11 and with contrasts in Appendix C. Significant cultivar and treatment effects were found for β-glucan content. It was

expected that there would be a difference in β-glucan content between cultivars as many researchers have found that cultivar, growing conditions and agronomic practices have a significant impact on barley β-glucan content (Pérez-Vendrell et al. 1996; Ames et al. 2006; Hang et al. 2007; Dickin et al. 2011). The cultivar and treatment means for β-glucan content are summarized in Table 4.12. The average β-glucan content for Fibar, McGwire and Rattan were 9.59, 4.79 and 7.04 % (d.b.), respectively. While the treatment effect was less significant (P-value 0.010) than the cultivar effect, β-glucan content was not anticipated to be significantly affected by treatment. Conditioning was the only treatment significantly different in β-glucan content from the other treatments. This result indicated that heat treatments may have affected the ability to measure β-glucan as it is unlikely the content actually changed. Wang (2014) also noted a significant treatment effect ($P < 0.05$) on β-glucan content when pilot scale kilning and steam/flake treatments were applied to oats. The mean β-glucan contents increased when both heat treatments were applied. However, this same effect was not seen on commercially kilned and steamed/flaked oats. More research would be required to assess if the β-glucan content in barley is actually affected by steam treatment.

Table 4.11 Summary of ANOVA for β-glucan (BG) results

	P-values					
Effect	BG content	RVA BG Viscosity[1]	Rheometer BG Viscosity[1]	BG MW - Mp [1]	BG MW - Mw [1]	BG Solubility[1]
Cultivar	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Treatment	0.010*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Cultivar x Treatment	0.434	< 0.001*	< 0.001*	0.001*	0.002*	< 0.001*

*Significant effect at P (<0.05)

[1]Based on 2 h BG *in vitro* digest extraction data

RVA = Rapid Visco Analyser; MW = molecular weight; Mp = peak molecular weight; Mw = weighted average molecular weight

Table 4.12 Effect of cultivar and treatment on β-glucan (BG) content

Effect		**BG content** (% d.b.)	**SD**
Cultivar	Fibar	9.59 a	0.25
	McGwire	4.79 c	0.15
	Rattan	7.04 b	0.15
Treatment	Micronized (T)[1]	7.13 a	2.04
	Micronized (N)[2]	7.12 a	2.07
	Roasted (T)[1]	7.00 a	2.01
	Roasted (N)[2]	7.11 a	2.17
	Conditioned	7.35 b	2.10
	Untreated	7.14 a	2.04

[1]Barley tempered to 17% moisture prior to treatment

[2]Barley was not tempered prior to treatment

Cultivar effect reported as means of all treatments within a cultivar

Treatment effect reported as means of all cultivars within a treatment

Values within same column and effect followed by same letter are not statistically significant (LSD, $P \geq 0.05$)

The β-glucan *in vitro* digest extract viscosity, molecular weight and solubility all had statistically significant main effects and interactional effects between cultivar and treatment (Table 4.11). This indicates the β-glucan polymers in each cultivar reacted differently to the treatments applied in this study. The β-glucan content was controlled to 1% during the *in vitro* extraction so it would not interfere with the viscosity and solubility results (Gamel et al. 2012). Molecular weight is presented using two variables, peak molecular weight (Mp) and weighted average molecular weight (Mw), which were both obtained from the same standard curve. It should be noted that molecular weight results exceeding 1 000 kDa have been determined by extrapolation as no larger standards were available.

The means of the viscosity, molecular weight and solubility of the β-glucan *in vitro* digest extracts are summarized in Table 4.13. For all 3 cultivars, conditioning produced the highest viscosity and molecular weights followed by micronization with tempering. Tempering had a significant effect ($P < 0.05$) on the viscosity and molecular weight of micronized Fibar and Rattan, but not roasted. Untreated and roasting without tempering had the lowest viscosity and molecular weight for all cultivars. The roasted treatments were not significantly different in viscosity and molecular weight compared to untreated. Doehlert *et al.* (1997) noted roasted oats had a lower viscosity than untreated and suggested that roasting may affect the β-glucan somehow that impedes its ability to absorb water. Many studies have focused on β-glucan dose when assessing nutritional benefits of barley but more recent research has found that nutritional benefits obtained are influenced more when β-glucan has a high viscosity and molecular weight regardless of content (Wang et al. 2013). Conditioning was the only treatment to exceed 1 000 kDa and had the highest viscosities for each cultivar. Rattan,

micronized with tempering, also exceeded a molecular weight of 1 000 kDa. Fibar, micronized with tempering, and Rattan, roasted with tempering, were just under 1 000 kDa and just over 1000 kDa for Mp and Mw, respectively. These samples were the least affected by β-glucanases and were able to maintain a high molecular weight.

The solubility of β-glucan also has a big impact on the nutritional benefits barley is able to provide. A high solubility is better nutritionally because the body is able to access and utilize more β-glucan (Ames et al. 2015a). In this study, the high molecular weight samples, conditioned and micronized with tempering, had the lowest solubility. Overall, solubility ranged from 33.2-72.5%. McGwire exhibited the highest solubility, followed by Rattan and then Fibar. This suggests different cultivars have different β-glucan solubility. Other researchers have also showed genotypic differences in solubility (Izydorczyk et al. 2000; Ames et al. 2006; Sharma et al. 2011). The untreated samples had the highest solubility compared to the heat treatments for Fibar and Rattan.

β-glucan *in vitro* digest extract viscosity was measured by two instruments, the RVA and rheometer. The RVA viscosity describes the β-glucan extract in the flour slurry, while the rheometer viscosity describes the supernatant of the extract obtained in the RVA. Both instruments gave reliable results and were significantly ($P < 0.05$) positively correlated to one another with a Pearson correlation coefficient of 0.977 (Table 4.14). However, more statistical differences between treatments were obtained with the rheometer data. The solubility was found to have a significant ($P < 0.05$) negative correlation to molecular weight, both Mp and Mw, with a Pearson correlation coefficient of -0.76 (Table 4.14). Viscosity was also significantly ($P < 0.05$) negatively correlated to solubility but less than molecular weight. Intuitively, more

soluble β-glucan in the extract should mean a higher extract viscosity. However, due to high molecular weight polymers causing a high viscosity despite having a low solubility, a negative correlation was found. Therefore, even though all 3 variables had significant ($P < 0.05$) correlations to one another, molecular weight and solubility appear to have a greater influence on viscosity than viscosity has on them.

Table 4.13 Interaction effect between cultivar and treatment on mean β-glucan viscosity, molecular weight and solubility from 2 h *in vitro* digest extraction data

		RVA	**Rheometer Viscosity**	**Molecular Weight** (kDa)		
Cultivar	**Treatment**	**Viscosity** (cP)	(mPa.s at $30s^{-1}$)	**Mp**	**Mw**	**Solubility** (%)
Fibar	Micronized (T)[1]	311 (16.8) a	133.0 (6.76) b	921 (230.7) b	1038 (220.1) b	42.2 (2.21) bc
	Micronized (N)[2]	155 (16.5) b	38.2 (5.62) c	540 (126.8) c	617 (131.4) c	45.4 (2.33) b
	Roasted (T)[1]	128 (3.5) b	41.3 (0.53) c	552 (42.4) c	618 (40.0) c	39.4 (2.74) c
	Roasted (N)[2]	76 (12.0) b	23.6 (1.36) c	344 (10.9) c	412 (10.3) c	40.6 (3.30) bc
	Conditioned	383 (7.8) a	249.7 (1.10) a	1956 (243.9) a	2142 (221.1) a	34.0 (0.56) d
	Untreated	87 (3.5) b	25.7 (1.45) c	459 (14.1) c	529 (1.96) c	51.6 (1.39) a
McGwire	Micronized (T)[1]	115 (16.6) a	17.4 (0.83) b	221 (48.0) a	265 (48.6) a	72.5 (3.69) a
	Micronized (N)[2]	74 (6.4) a	12.1 (0.02) b	143 (5.9) a	180 (3.5) a	71.3 (1.71) a
	Roasted (T)[1]	108 (0) a	13.2 (0.09) b	172 (13.9) a	229 (12.9) a	62.6 (1.82) b
	Roasted (N)[2]	71 (2.1) a	10.5 (0.32) b	114 (27.2) a	142 (35.8) a	63.5 (1.48) b
	Conditioned	708 (60.8) b	336.9 (52.13) a	1775 (198.0) b	1827 (28.2) b	33.2 (0.81) c
	Untreated	91 (32.5) a	9.7 (0.06) b	129 (19.0) a	142 (13.9) a	65.9 (1.27) b
Rattan	Micronized (T)[1]	711 (117.4) a	382.5 (109.37) b	1221 (34.6) b	1360 (25.5) b	48.6 (2.75) ab
	Micronized (N)[2]	324 (59.9) b	139.7 (29.81) c	813 (179.6) cd	930 (172.5) c	44.8 (2.75) bc
	Roasted (T)[1]	237 (2.0) bc	97.2 (6.80) cd	946 (103.0) c	1055 (124.3) c	40.2 (1.11) cd
	Roasted (N)[2]	144 (14.8) c	45.5 (0.41) d	595 (26.3) de	675 (22.9) d	43.5 (1.49) bc
	Conditioned	720 (55.2) a	480.3 (19.05) a	1826 (106.0) a	1950 (111.7) a	35.6 (0.71) d
	Untreated	157 (17.0)c	47.8 (4.61) d	529 (35.2) e	607 (11.5) d	52.3 (1.66) a

[1]Barley tempered to 17% moisture prior to treatment

[2]Barley was not tempered prior to treatment

RVA = Rapid Visco Analyser; Mp = peak molecular weight; Mw = weighted average molecular weight

Values reported as means (SD) within a cultivar and treatment

Values within same column and cultivar followed by same letter are not statistically significant (LSD, $P \geq 0.05$)

Table 4.14 Relationship between β-glucan viscosity, solubility and molecular weight from 2 h *in vitro* digest extractions using Pearson correlation coefficients (r)

	RVA viscosity	Rheometer viscosity	Mp	Mw	Solubility
RVA viscosity	1				
Rheometer viscosity	0.977*	1			
Mp	0.859*	0.881*	1		
Mw	0.850*	0.874*	0.998*	1	
Solubility	-0.546*	-0.561*	-0.756*	-0.760*	1

*Significant effect at P (<0.05)

RVA = Rapid Visco Analyser; Mp = peak molecular weight; Mw = weighted average molecular weight

From the RVA profiles, it was clear that β-glucanases had a large impact on the β-glucan *in vitro* digest extract viscosity results (Figs. 4.2, 4.3 and 4.4). The conditioned barley was the only treatment to not experience a drop in viscosity over the 2 h extraction period in the RVA. Micronization with tempering showed a slight decrease in RVA viscosity over 2 h, but only for Fibar and Rattan (Figs. 4.2 and 4.4). McGwire exhibited a large viscosity drop, only 10-15 min into the 2 h extraction, in all treatments except conditioning (Fig. 4.3). A similar RVA profile was obtained by Doehlert *et al.* (1997) that showed steamed oats did not decrease in viscosity over 60 min at 30 °C, while untreated and roasted oats did. To confirm the breakdown of β-glucan due to β-glucanase activity was the cause of the viscosity decrease, a second *in vitro* digest extract was performed but only for 30 min. In general, the 30 min extracts had a higher viscosity and molecular weight than the 2 h extracts indicating β-glucanase activity (Figs. 4.5 and 4.6). Conditioning was the only treatment for all 3 cultivars that showed a higher viscosity after the 2 h extraction period compared to the 30 min extraction. However, conditioned McGwire and Rattan had higher molecular weights after 30 min compared to 2 h. This may mean there was still some β-glucanase activity to reduce the size of the β-glucan during the extraction, but not enough to reduce the viscosity. The micronized, roasted and untreated samples all experienced decreases in viscosity and molecular weight after 2 h extractions compared to 30 min, except Rattan micronized and roasted with tempering, which had a slightly higher molecular weight after 2 h. The higher molecular weights in these Rattan samples may have occurred due to a greater inactivation of β-glucanases, or the longer extraction time allowed for larger β-glucan polymers to become soluble in the extract. Izydorczyk *et al.* (2000) noted steam treatment was more effective than roasting at inactivating

β-glucanases due to moist heat entering the kernel more efficiently. Overall, McGwire had the greatest decrease in molecular weight and viscosity over the 2 h extraction period for every treatment except conditioning, followed by Fibar and then Rattan. This was likely due to a higher β-glucanase activity in McGwire than the other cultivars, which indicates there could be variability between cultivars. More research is needed on the direct analysis of β-glucanase activity in barley, how it changes with cultivar and processing, and its impact on the physicochemical properties of β-glucan in food products.

Overall, the β-glucan content differed significantly between cultivars and a significant interactional effect was seen for β-glucan *in vitro* digest extract viscosity, molecular weight and solubility. Conditioning provided the largest β-glucan extract viscosity and molecular weight but the lowest solubility across all cultivars. It was also the only treatment that exhibited no viscosity drop over the 2 h RVA extraction, which suggested no β-glucanase activity. Viscosity, molecular weight and solubility were all found to be significantly correlated to one another.

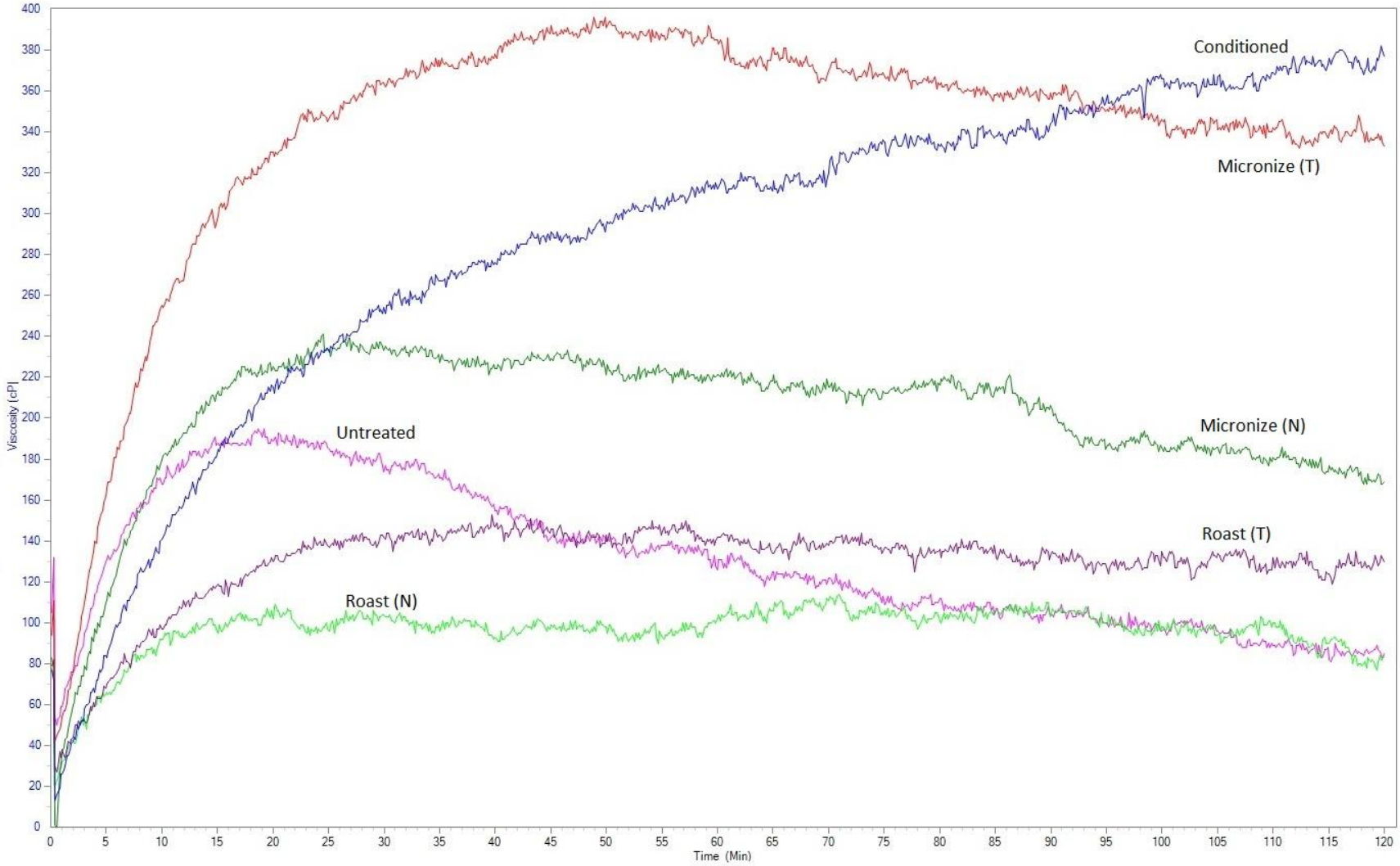


Figure 4.2 RVA profiles of 2 h *in vitro* digest extractions of Fibar treatments (Note: only one replicate and duplicate shown)
T = tempered to 17% moisture prior to treatment; N = not tempered prior to treatment

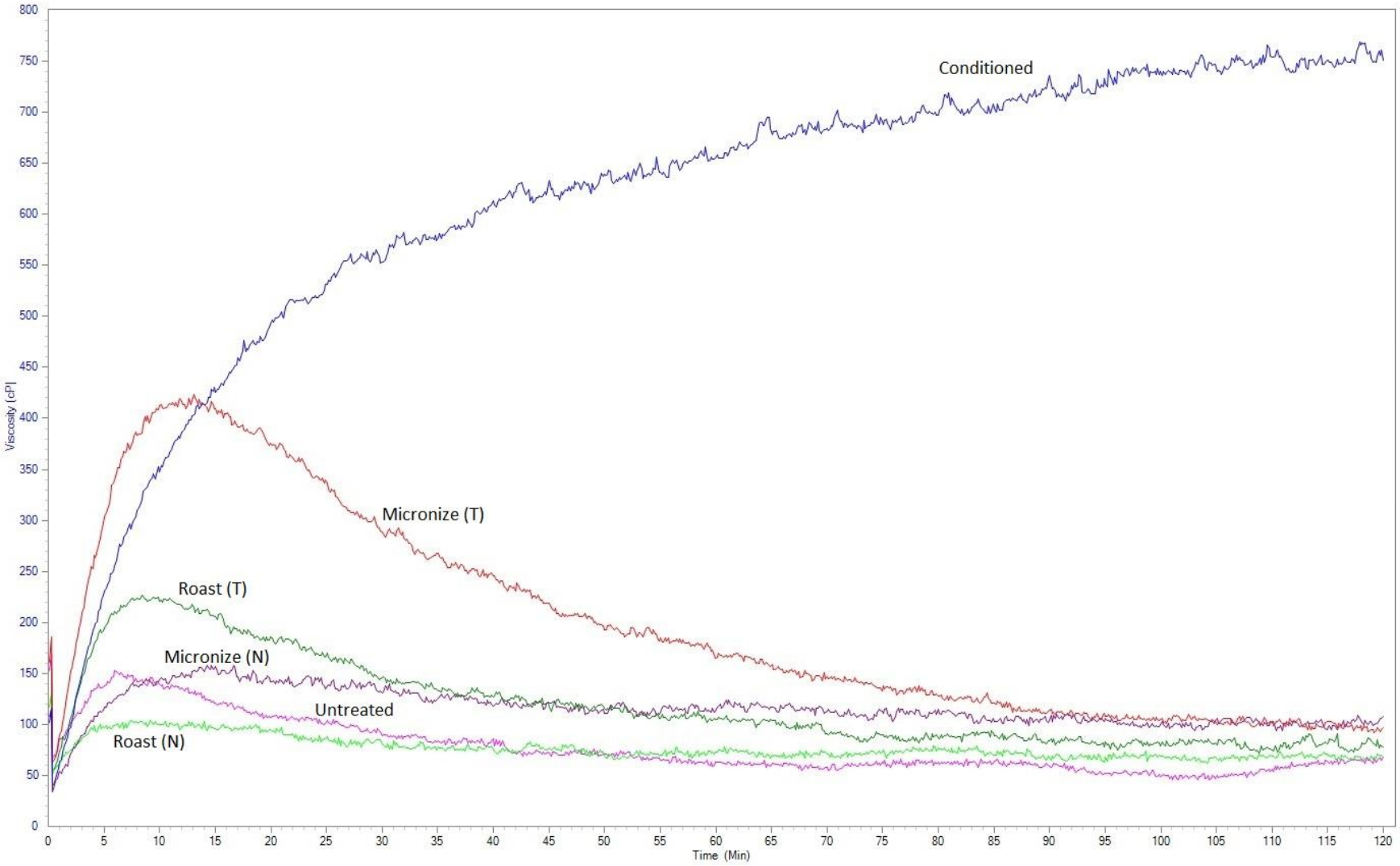


Figure 4.3 RVA profiles of 2 h *in vitro* digest extractions of McGwire treatments (Note: only one replicate and duplicate shown)
T = tempered to 17% moisture prior to treatment; N = not tempered prior to treatment

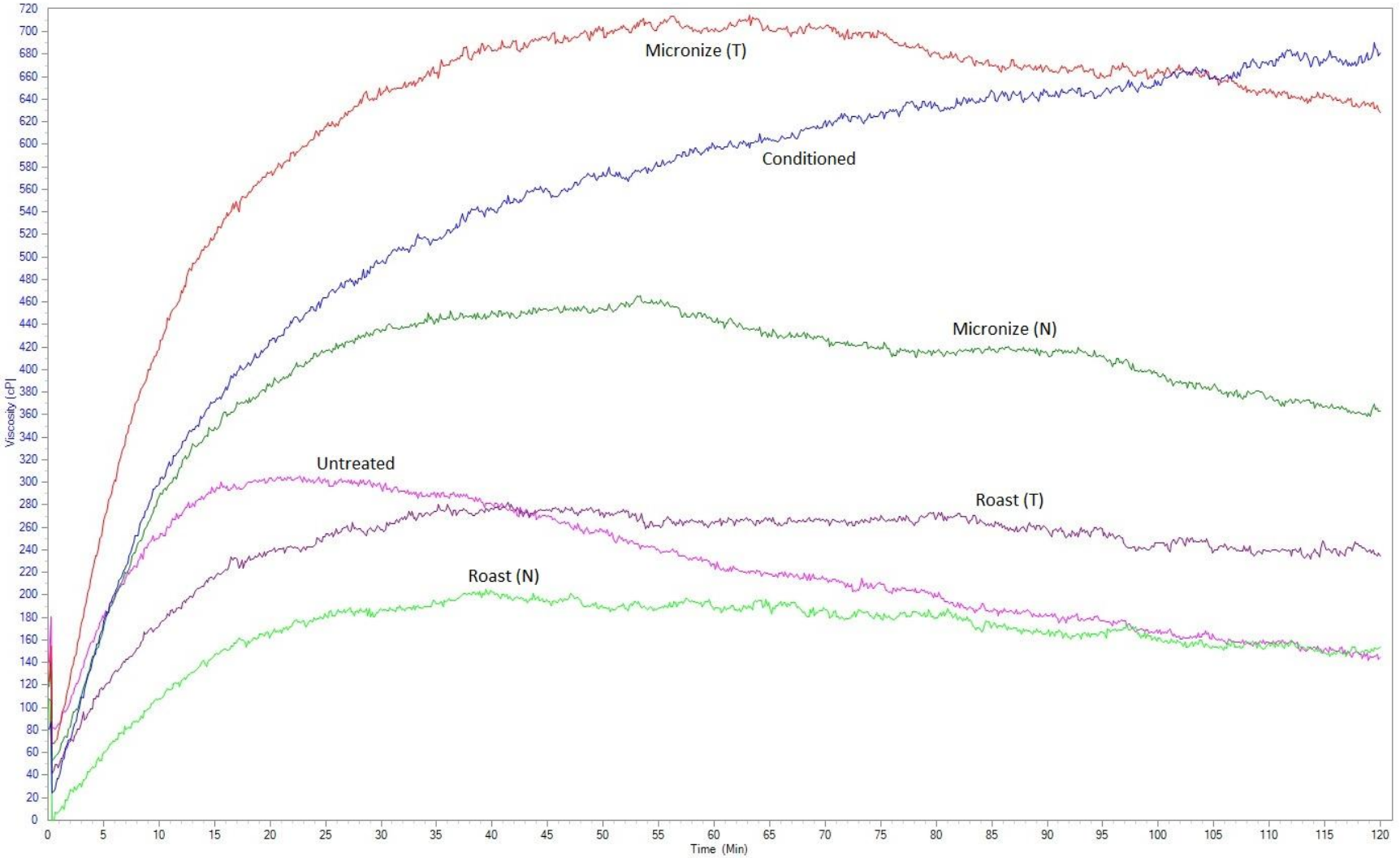


Figure 4.4 RVA profiles of 2 h *in vitro* digest extractions of Rattan treatments (Note: only one replicate and duplicate shown)
T = tempered to 17% moisture prior to treatment; N = not tempered prior to treatment

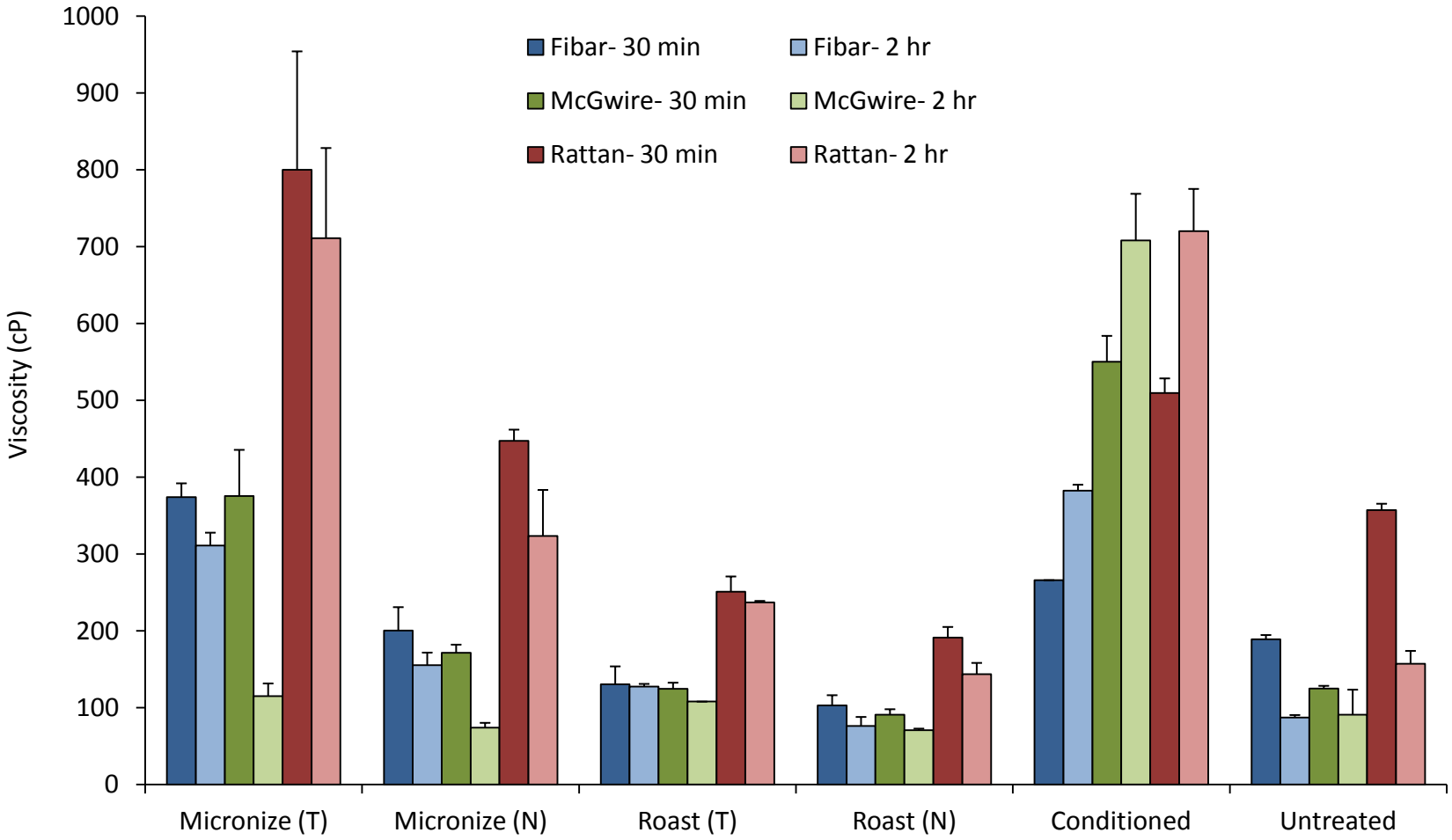


Figure 4.5 Comparison of 30 min and 2 h β-glucan *in vitro* digest extract RVA viscosities
T = tempered to 17% moisture prior to treatment
N = not tempered prior to treatment
Values represent means within cultivar and treatment

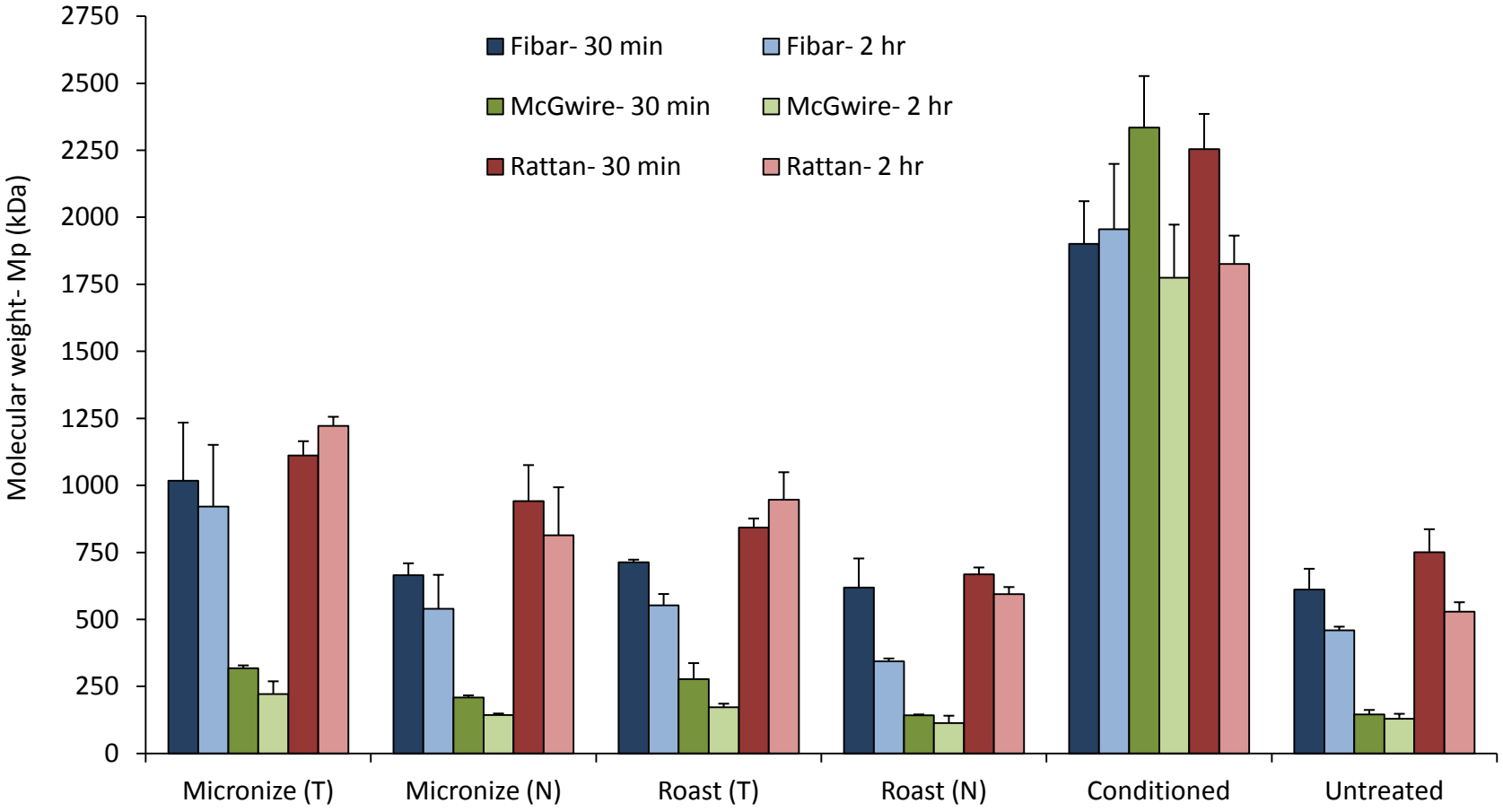


Figure 4.6 Comparison of 30 min and 2 h β-glucan *in vitro* digest extract molecular weights (peak molecular weight)
T = tempered to 17% moisture prior to treatment
N = not tempered prior to treatment
Values represent means within cultivar and treatment

4.3.2 Particle Size Distribution

Different particle sizes can be obtained by different types of milling, pre-treatments or sieving. It is important to consider heat treatments may change how the barley kernel fractures during milling and create different particle sizes. The particle size of barley flour influences many different physicochemical properties including water absorption, starch pasting, nutrient digestion and solubility, colour and end product quality (Becker et al. 2001; Izydorczyk et al. 2008; Prasopsunwattana et al. 2009; Al-Rabadi et al. 2012; Izydorczyk et al. 2014; Lazaridou et al. 2014).

The particle size was assessed using laser diffraction on samples that had been Retsch milled at 0.5 mm. This type of analysis supplies the results based on volume. All of the samples showed a bimodal distribution, meaning there were two large populations of particle sizes that made up the majority of the sample. However, the conditioned samples had a less defined trough between the two peaks compared to the other treatments. There are two important details to consider when evaluating the following particle size data. First, the laser diffraction method used assumes all particles are spherical. This means when the laser hits a particle and diffracts at a specific angle then used to describe its size, it assumes all sides of that particle are equivalent. Secondly, some of the milled samples, specifically the roasted samples, had a tendency to clump, which could have affected the results if the particles were still stuck together at time of measurement. The conditioned and untreated samples had minimal clumping and flowed more easily out of the sample tray. Both of these reasons can explain why particle sizes greater than the sieve size used to mill the samples (0.5 mm) were observed.

The ANOVA results are summarized in Table 4.15. There were significant cultivar effects for all variables except span and uniformity, and significant treatment effects for all variables. Significant interactional effects were observed for d(0.9), D[3,2], span and specific surface area. The cultivar and treatment means are listed in Table 4.16. Across all treatments, Fibar had the largest median diameter (d(0.5)) and volume weighted mean (D[4,3]), followed by Rattan and then McGwire. This could have been because different barley cultivars have different kernel hardness values, which may affect how they fracture during milling (Nair et al. 2010; Nair et al. 2011). Waxy barleys (Fibar and Rattan) may fracture differently than normal starch barleys (McGwire) even though Nair *et al.* (2011) did not find a significant relationship between amylose content and hardness.

Considering all the treatments, roasting and conditioning caused the greatest effect on particle size. They produced the lowest volume weighted means (D[4,3]) and were significantly different from micronized and untreated (Table 4.16). The volume weighted mean represents the mean size of particle that makes up the majority of the sample. Roasted without tempering was significantly different from roasting with tempering and had the lowest volume weighted mean at 198 µm. Both tempering levels of micronized and untreated did not have significantly different volume weighted means. The distribution of particle size was described using the particle size in which 10% (d(0.1)), 50% (d(0.5)) and 90% (d(0.9)) of the sample lies below. Micronized with tempering was most comparable to untreated and only differed significantly in d(0.1). Tempering had significant effects on the mean d(0.1) and d(0.5) values for the micronization treatments but not for roasting. Conditioning produced similar particle size distribution results to roasting, but did have a significantly different median (d(0.5)) and d(0.1).

These differences in particle sizes could be due to variation in kernel hardness. Roasting has been found to decrease kernel hardness in wheat (Murthey et al. 2008). Softer barley kernels produce smaller particle size flours because the endosperm has a lower density and the starch kernels are more easily released (Nair et al. 2011).

Table 4.15 Summary of ANOVA for particle size parameters

	P-values							
Effect	d(0.1)	d(0.5)	d(0.9)	D[3,2]	D[4,3]	Span	Specific Surface Area	Uniformity
Cultivar	0.014*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.108	< 0.001*	0.792
Treatment	< 0.001*	< 0.001*	0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Cultivar x Treatment	0.534	0.284	0.039*	< 0.001*	0.083	0.016*	< 0.001*	0.149

*Significant effect at P (<0.05)

d(0.1) = size of particle below which 10% of the sample lies; d(0.5) = size of particle below which 10% of the sample lies; d(0.9) = size of particle below which 90% of the sample lies; D[3,2] = surface are mean diameter; D[4,3] = volume mean diameter

Table 4.16 Effect of cultivar and treatment on mean particle size parameters

	d(0.1) (μm)	d(0.5) (μm)	d(0.9) (μm)	D[3,2] (μm)	D[4,3] (μm)	Span	Specific Surface Area (m^2/g)	Uniformity
Cultivar								
Fibar	13.3 (2.9) b	214 (31.5) a	589 (97.5) a	42.5 (9.0) a	265 (33.9) a	2.72 (0.55) a	0.147 (0.03) c	0.88 (0.17) a
McGwire	14.2 (2.7) a	165 (25.2) c	444 (82.2) c	33.9 (6.2) c	205 (36.9) c	2.61 (0.28) ab	0.182 (0.03) a	0.88 (0.14) a
Rattan	13.0 (2.7) b	197 (31.9) b	512 (74.1) b	36.4 (10.3) b	239 (35.5) b	2.54 (0.17) b	0.175 (0.04) b	0.86 (0.10) a
Treatment								
Micronized (T)[1]	17.9 (1.4) a	228 (32.7) a	562 (81.6) a	52.2 (7.5) a	266 (39.8) a	2.39 (0.09) d	0.117 (0.02) f	0.76 (0.04) c
Micronized (N)[2]	13.6 (0.9) c	206 (17.9) b	567 (65.2) a	38.4 (4.3) c	261 (24.6) a	2.70 (0.31) bc	0.158 (0.02) d	0.91 (0.11) b
Roasted (T)[1]	11.2 (0.3) de	161 (24.2) d	514 (167.0) ab	30.9 (3.4) e	225 (55.0) b	3.07 (0.58) a	0.196 (0.02) b	1.04 (0.15) a
Roasted (N)[2]	10.6 (0.5) e	155 (15.4) d	432 (68.6) c	28.6 (2.3) f	198 (32.7) c	2.71 (0.22) b	0.211 (0.02) a	0.94 (0.11) b
Conditioned	15.4 (1.2) b	186 (23.0) c	472 (52.6) bc	41.2 (2.6) b	220 (23.9) bc	2.46 (0.07) cd	0.146 (0.01) e	0.80 (0.05) c
Untreated	12.3 (0.7) d	220 (21.6) a	544 (56.4) a	34.2 (5.9) d	248 (26.2) a	2.42 (0.04) d	0.179 (0.03) c	0.78 (0.01) c

[1]Barley tempered to 17% moisture prior to treatment

[2]Barley was not tempered prior to treatment

d(0.1) = size of particle below which 10% of the sample lies; d(0.5) = size of particle below which 10% of the sample lies; d(0.9) = size of particle below which 90% of the sample lies; D[3,2] = surface are mean diameter; D[4,3] = volume mean diameter

Cultivar effect reported as means (SD) of all treatments within a cultivar

Treatment effect reported as means (SD) of all cultivars within a treatment

Values within same column and main effect followed by same letter are not statistically significant (LSD, $P \geq 0.05$)

To further understand the effect of heat on particle size, the span, which describes the width of the distribution, must be evaluated. Looking only at the treatment effect, roasting with tempering had the largest span and micronization with tempering had the smallest. However, span had a significant interactional effect between cultivar and treatment. The treatment means for each cultivar are shown in Fig. 4.7. Generally, the spans were fairly consistent between treatments within cultivar. However, Fibar, roasted with tempering, had a significantly higher span than all the other treatments, but the same was not true for McGwire and Rattan. In fact, none of the treatments had a significant effect on the span of Rattan. The biggest difference in the span of the McGwire samples occurred between the two tempering levels of micronization. There was an overall increase in the span of the heat-treated samples for each cultivar, but micronization with tempering and conditioning gave similar results to untreated. The uniformity results also confirmed this. Uniformity can also be used to explain particle size as it describes the deviation from the median. Therefore, the smaller the value, the more uniform the particle size throughout the sample. Micronized with tempering, conditioned and untreated had the lowest mean uniformity values and were not significantly different from each other (Table 4.16). Roasted with tempering was the least uniform and was significantly different from all the other treatments. Considering both span and uniformity results, micronization with tempering and conditioning fractured more consistently during milling. However, the roasted samples had a tendency to clump, which may have overestimated the actual particle size distribution. This could explain why there was such a large increase in the span of the Fibar roasted with tempering sample.

Overall, roasting and conditioning produced the smallest particle-sized flours and micronization had a similar particle size to untreated. Conditioning and micronization were not significantly different from untreated in terms of span and uniformity, and therefore produced more consistent particle sized flours. The interpretation on what these results mean to food product applications and industry is difficult because there has been minimal previous research on the effects of heat on the milling and particle size of barley. A few researchers have examined the effects of different particle-sized barley flours on the quality of barley tea (Ross and Ames, 2005), two-layer flat bread (Izydorczyk et al. 2008), tortillas (Prasopsunwattana et al. 2009), extruded puffs (Al-Rabadi et al. 2011) and rusks (Lazaridou et al. 2014) with varying results. There may also be implications regarding the solubility and digestibility of nutrients, like β-glucan, due to particle size. It has been found that lower particle size contributes to increased β-glucan solubility in barley flours (Izydorczyk et al. 2008; Lazaridou et al. 2014). This was not found in this study but the effect of the heat treatments on β-glucan viscosity and molecular weight likely overshadow any relationship between particle size and β-glucan solubility. Further investigation on the effect of heat treatments on particle size of barley flours with respect to kernel structure, milling practices, and nutrient functionality is needed.

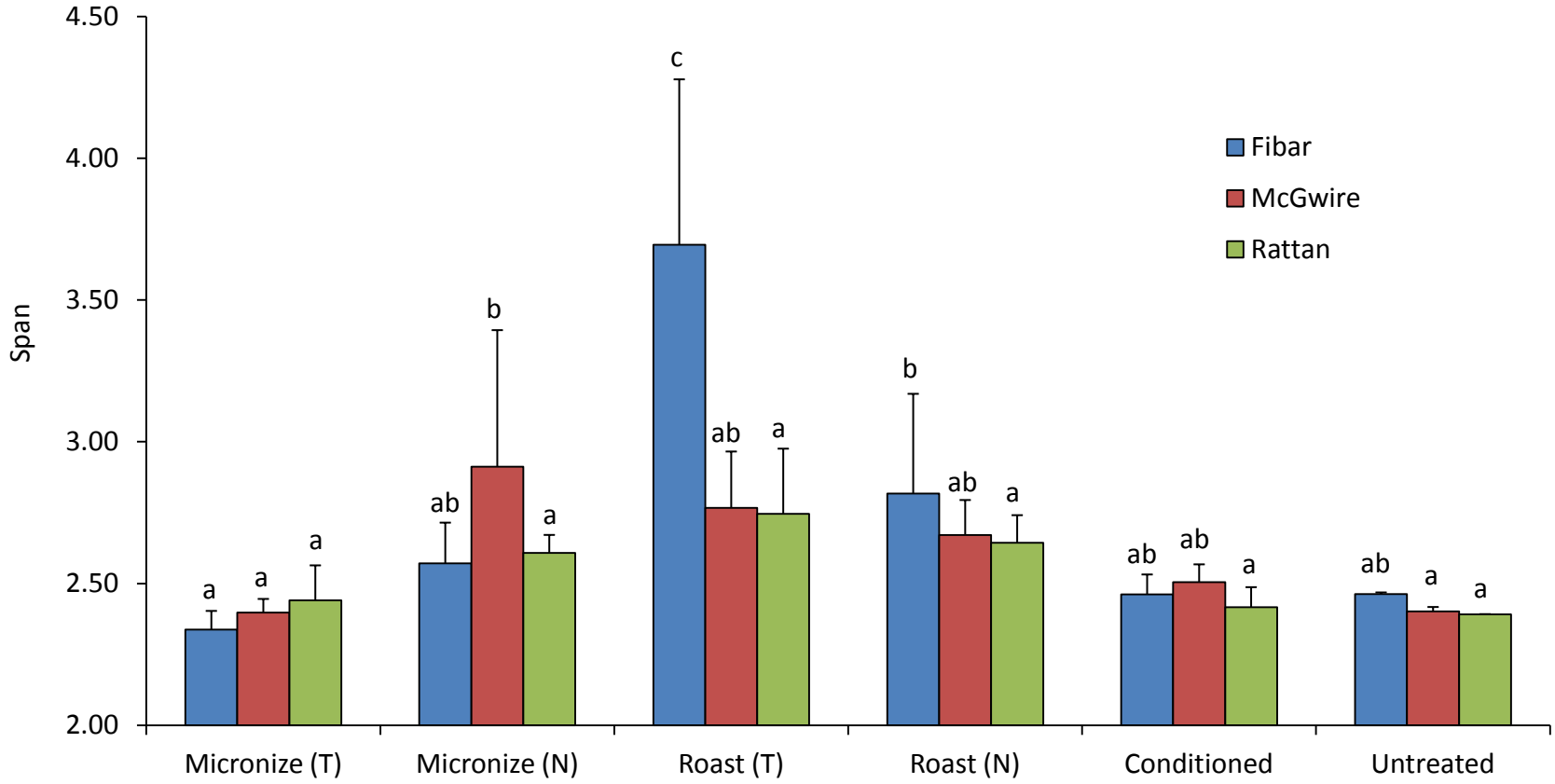


Figure 4.7 Effect of heat treatment and cultivar on mean span of particle size
T = tempered to 17% prior to treatment
N = not tempered prior to treatment
Values reported as means within treatment and cultivar
Different letters within same variable are significantly different from one another (LSD, $P < 0.05$)

4.3.3 β-glucan Concentration in Particle Size Fractions

It has been determined that heat treatments affected the particle size distribution of the flour after centrifugal milling. Heat treatments may also impact how the kernels fracture during milling and produce different β-glucan concentrations in fractions separated based on particle size. This is important if the barley flour will be air classified or sieved to get a β-glucan rich fraction.

The percent yield of each fraction is shown in Fig. 4.8. It is important to note that the yield results do not add up to 100% due to losses during sieving. The amount of loss was between 4.21 and 18.44% depending on the sample. McGwire had the lowest amount of loss compared to Fibar and Rattan, and the untreated samples for all cultivars had the largest loss percentages. The smallest fraction (< 125 µm) had the largest yield of the 3 fractions for each sample ranging from 37.19 to 56.82%. McGwire had a larger yield of < 125 µm fraction than Fibar and Rattan. Sundberg and Åman (1994) also found that waxy cultivars had lower yield of flour fines when compared to normal starch cultivars. Fibar had the largest yield percentage of the large fraction (> 250 µm). As expected based on the particle size distribution, the roasted with tempering and conditioned samples for all cultivars had the largest yield of < 125 µm fraction. Across all the cultivars, micronized with tempering and untreated samples had similar yields for all fractions. The medium (125-250 µm) and large (> 250 µm) fractions produced similar amounts with the large fraction having a slightly larger yield.

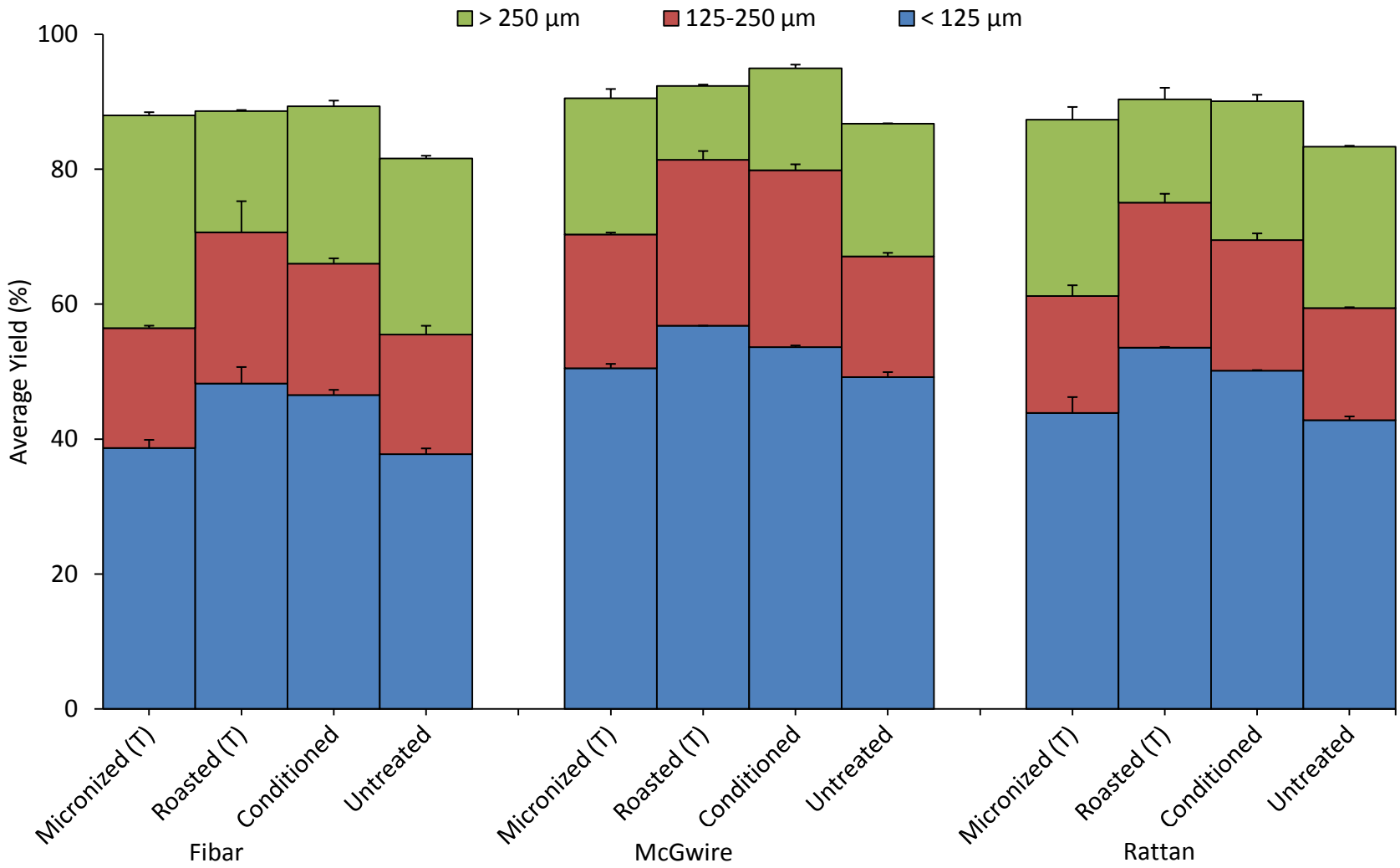


Figure 4.8 Effect of cultivar and heat treatment on average yield of flour fractions obtained from air jet sieving
T = tempered to 17% prior to treatment

Each fraction obtained was analyzed for β-glucan content to assess if the concentrations differed in the fractions. The ANOVA results are summarized in Table 4.17. The 3-way interaction between cultivar, treatment and fraction was found to be significant with a p-value of 0.033. This indicates each cultivar was influenced by the heat treatments causing the kernels to fractionate differently and produce different concentrations of β-glucan in the particle size fractions. It was expected for cultivar to have a significant effect due to the different starting β-glucan contents. The smallest particle size fractions had the lowest β-glucan contents which was also seen by a few other researchers who looked at barley milled in a different way to produce different particle sizes (Izydorcyzk et al. 2003; Román et al. 2010; Gómez-Caravaca et al. 2015). The mean β-glucan contents of each fraction within cultivar and treatment are shown in Table 4.18. The 3 heat treatments evaluated had a significant effect ($P < 0.05$) on the β-glucan content of all cultivar by fraction combinations except for McGwire and Rattan 125-250 µm. The untreated > 250 µm fraction for all 3 cultivars was significantly higher in β-glucan content than the treatments where heat was applied. Rattan roasted with tempering was also significantly lower in β-glucan than micronized with tempering. The β-glucan content of the < 125 µm Fibar fraction was only significantly affected by the roasted with tempering treatment, which had the highest value. Roasting with tempering and conditioning significantly increased the β-glucan content of the < 125 µm McGwire and Rattan fraction compared to untreated.

These results indicated heat treatments will affect how the β-glucan is distributed throughout different particles sizes of flour. Therefore, it could affect the concentration of barley concentrate products produced in industry. As discussed in the previous section, roasting and conditioning had the smallest particle size distribution. The higher β-glucan concentrations

found in the < 125 µm fractions of roasted with tempering and conditioned treatments compared to untreated shows that not just the starch particles are being reduced to a smaller size. The results also showed that with heat treatment the > 250 and 125-250 µm fractions were more similar in β-glucan content. This could have been due to more starch in the larger fractions or the fiber rich particles reducing in size more due to the heat treatment. Although air classification is based on particle density not size, the differences in β-glucan content shown in this work indicated the yield and β-glucan concentration achieved during air classification may differ if heat treatments are applied to barley. Flour particle density has an inverse relationship with β-glucan content because the endosperm cells have released some of the starch content (Ferrari et al. 2009). Roasting with tempering and conditioning may have damaged or made the cell walls more susceptible to breaking during milling which increased the amount of starch released. This could explain the high yield and low β-glucan content in the < 125 µm fraction. If heat treatments can cause more starch to release from the cells, it may be easier to separate the β-glucan rich particles out to create a high value product.

It has been shown that heat treatments impact how the barley kernel fractures during milling and the concentration of β-glucan in the different particle size fractions. However, more research is needed to evaluate the effect of heat treatment on the particle fractionation with different types of milling and determine if they can improve the creation of high β-glucan flour products.

Table 4.17 Summary of ANOVA for β-glucan (BG) content of air jet sieved fractions

Effect	**BG Content** (P-Value)
Cultivar	< 0.001*
Fraction	< 0.001*
Cultivar x Fraction	< 0.001*
Treatment	< 0.001*
Cultivar x Treatment	0.057
Treatment x Fraction	< 0.001*
Cultivar x Treatment x Fraction	0.033*

*Significant effect at P (<0.05)

Table 4.18 Interaction effect between cultivar, fraction and treatment on mean β-glucan content (% d.b.) of particle size fractions

	Treatment			
	Micronized (T)[1]	Roasted (T)[1]	Conditioned	Untreated
Fibar				
>250 µm	15.97 b	15.64 bc	16.16 b	19.00 a
125-250 µm	13.43 e	14.83 cd	15.46 bc	14.01 de
<125 µm	4.49 g	6.04 f	4.84 g	4.66 g
McGwire				
>250 µm	7.22 bc	6.36 d	6.42 cd	8.30 a
125-250 µm	7.24 b	6.99 bcd	7.06 bcd	7.78 ab
<125 µm	2.68 f	3.62 e	3.51 e	2.39 f
Rattan				
>250 µm	11.72 b	10.60 c	11.17 bc	14.50 a
125-250 µm	11.31 bc	11.20 bc	11.34 bc	11.61 b
<125 µm	3.22 e	4.59 d	3.88 ed	2.96 e

[1]T = tempered to 17% moisture prior to heat treatment

Values reported are treatment means within cultivar and fraction

Values within same row or column within cultivar followed by same letter are not statistically significant (LSD, $P \geq 0.05 = 0.91$)

4.3.4 Starch Pasting

Starch pasting is an important property of barley that affects processing of barley food products and their quality. Ragaee and Abdel-Aal (2006) and Sullivan *et al.* (2010b) used starch pasting to determine the effect barley flour had on the quality of different food products like pitas, cakes and bread. While barley flour has a similar starch pasting profile to hard wheat flour (Ragaee and Abdel-Aal, 2006), it is important to determine its specific starch pasting characteristics, especially if heat treatments were to be applied prior to secondary processing. In addition, starch pasting is a simple method that could be used in industry for quality control purposes.

In this study, starch pasting was evaluated twice in order to determine the influence β-glucan has on the starch pasting profile. A standard starch pasting profile (standard 1) was used on each of the treated barley flours. However, these results (Appendix D) were hard to decipher because it was unclear how much of the viscosity was from starch and how much was from β-glucan. Liu *et al.* (2010) determined that β-glucan contributed the most to starch pasting followed by starch and then protein. Therefore, lichenase was added to the sample in order to break down the β-glucan and remove its influence. An example of the difference between the starch pasting results with and without lichenase addition on the untreated flours is shown in Fig. 4.9. Without lichenase addition the viscosities obtained were consistently 1000-3000 cP higher, depending on the viscosity variable, than when lichenase was added. This trend was the same for all samples. Pasting temperature was also affected by β-glucan (Table 4.19). The β-glucan caused the viscosity to increase too quickly, which generated a much lower pasting temperature than without the influence of β-glucan. The starch pasting characteristics of the

samples without breaking down β-glucan, are important in terms of how the different flours would react in an actual food system. They indicated that there would be an overall increase in viscosity, which may affect many secondary processing parameters including equipment, processing time and amount of energy required for mixing. All of these variables are important to processors and industry. However, for the purposes of this research the effect of heat on starch was the focus of the experiment. Therefore, further discussions will only be referring to starch pasting results in which lichenase was added.

The summary of the ANOVA results is shown in Table 4.20. All of the starch pasting variables displayed significant cultivar, treatment and interactional effects ($P < 0.05$). It was not surprising cultivar had a significant effect ($P < 0.05$) as both waxy and normal starch cultivars were used. Many researchers have shown that barley cultivar, starch type, and ratio of A-type to B-type starch granules affect starch pasting characteristics (Yanagisawa et al. 2006; Gujral et al. 2013; Li et al. 2014). Typically, waxy cultivars show higher peak and breakdown viscosity and lower setback and final viscosity due to the lower amount of amylose compared to normal starch cultivars (Yoshimoto et al. 2002; Lee et al. 2011). This was true of the 3 cultivars used in this study. Fibar and Rattan (waxy starch cultivars) had higher peak and breakdown viscosities, while McGwire (normal starch cultivar) had higher final and setback viscosities (Fig. 4.10). In addition, McGwire had higher pasting temperature than Fibar and Rattan. This was consistent with the finding by Lee *et al.* (2011) that amylose content has a strong positive correlation with pasting temperature. The lower amylose content in waxy cultivars causes a weaker gel structure upon cooling which causes lower final and setback viscosities during pasting (Ragaee and Abdel-Aal, 2006).

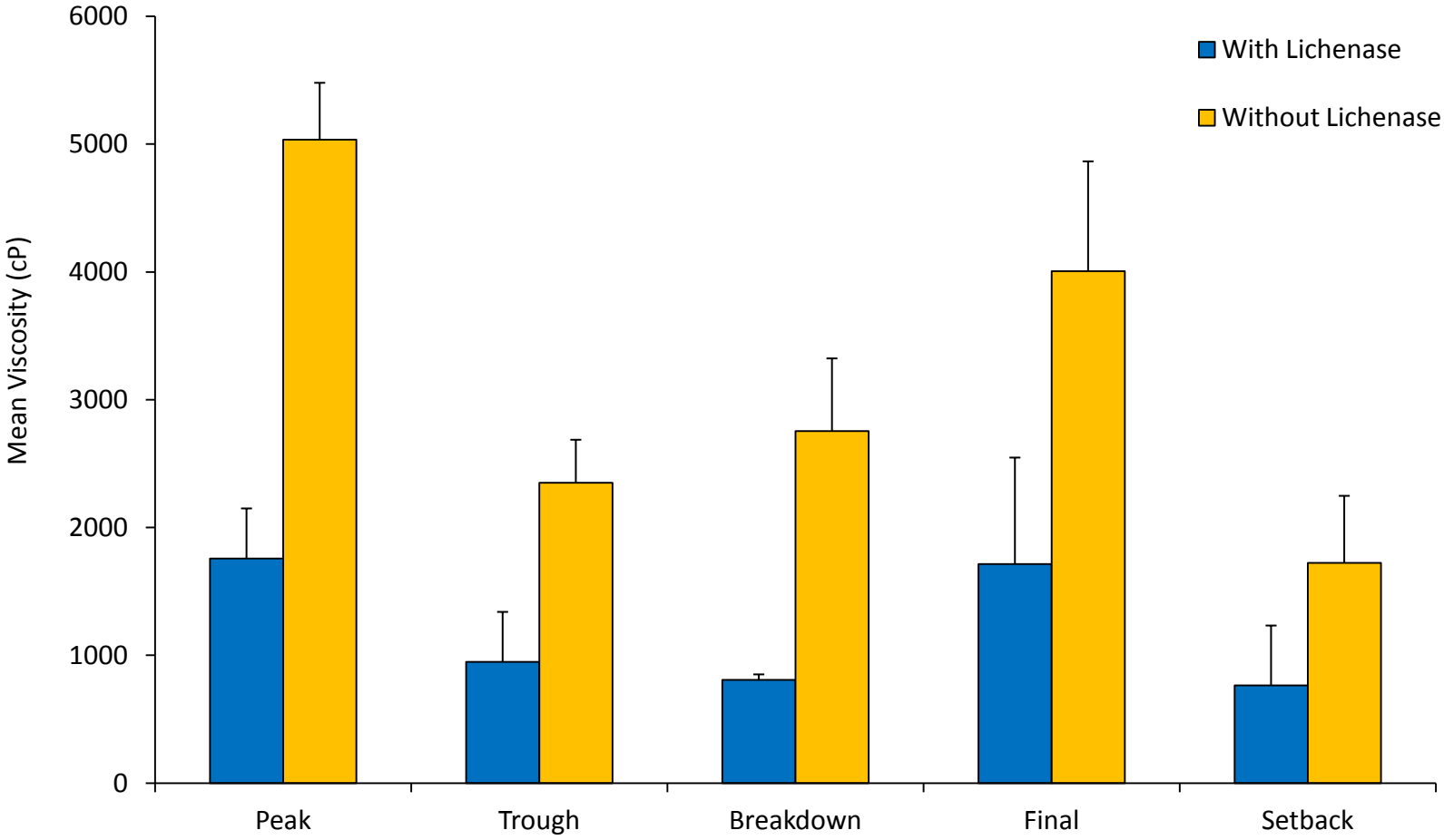


Figure 4.9 Comparison of starch pasting profiles of untreated barley with and without lichenase addition
Values reported as means among all cultivars

Table 4.19 Comparison of mean pasting temperatures (PT) with and without lichenase addition between treatments

Treatment	**PT with lichenase** (°C)	**PT without lichenase** (°C)
Micronized (T)[1]	73.8 (8.2) a	58.9 (7.4) a
Micronized (N)[2]	73.6 (8.0) a	62.3 (5.4) b
Roasted (T)[1]	73.7 (6.8) a	67.1 (1.3) c
Roasted (N)[2]	73.4 (7.2) a	67.2 (1.7) c
Conditioned	75.0 (6.1) b	65.7 (4.9) c
Untreated	73.2 (7.6) a	62.2 (5.9) b

[1]Barley tempered to 17% moisture prior to treatment

[2]Barley was not tempered prior to treatment

Values reported are treatment means (SD) across all cultivars

Values within same column followed by same letter are not statistically significant (LSD, $P \geq 0.05$)

Table 4.20 Summary of ANOVA for starch pasting results (with lichenase addition)

	P-values					
Effect	Peak	Breakdown	Final	Setback	Pasting Temp.	Peak Time
Cultivar	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Treatment	< 0.001*	< 0.001*	< 0.001*	0.005*	0.001*	< 0.001*
Cultivar x Treatment	< 0.001*	< 0.001*	< 0.001*	0.003*	0.001*	< 0.001*

*Significant effect at P (<0.05)

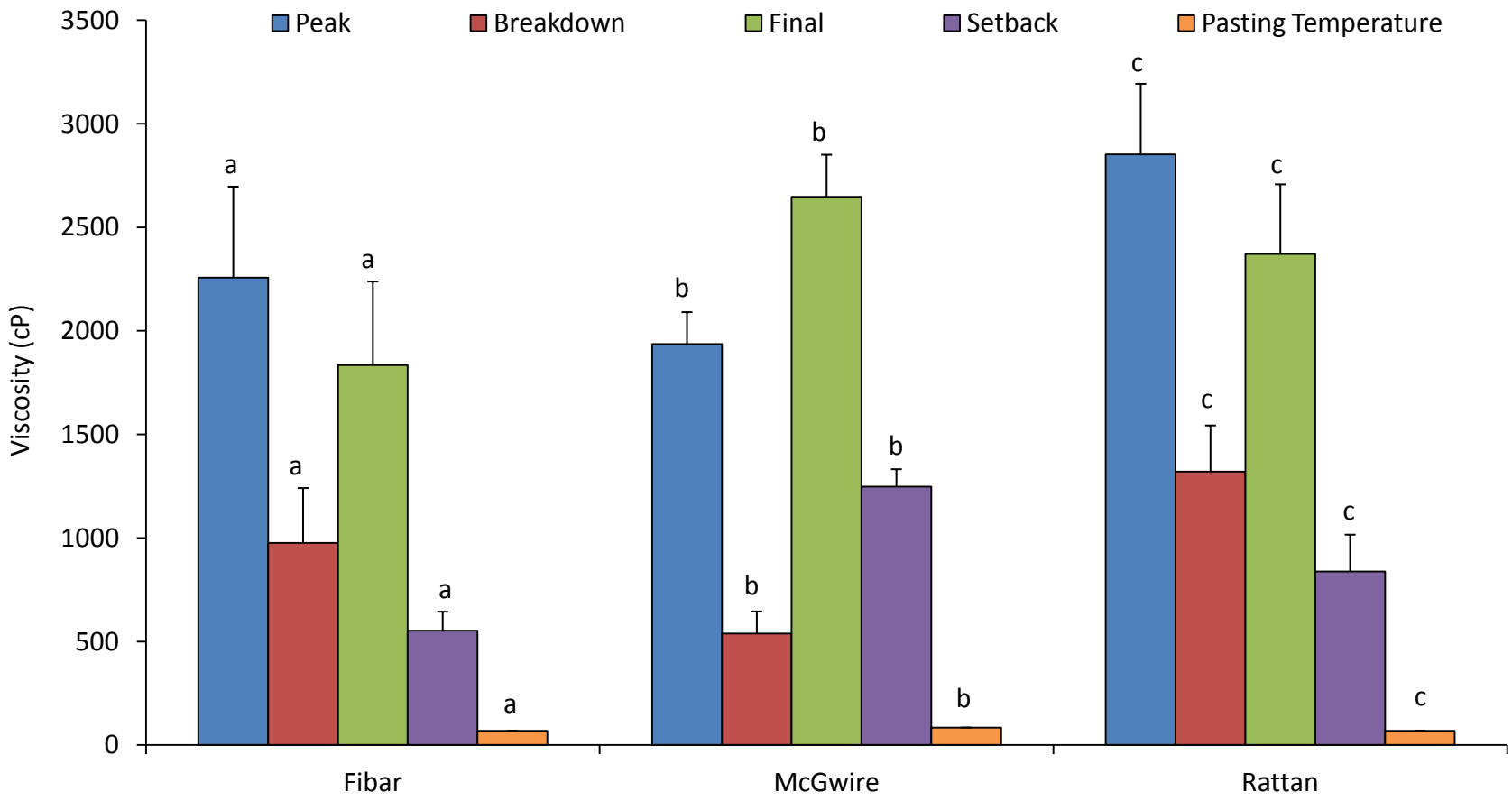


Figure 4.10 Effect of cultivar on mean starch pasting viscosities and pasting temperature (with lichenase addition)
Values represent means within cultivar and across all treatments
Different letters within same variable are significantly different from one another (LSD, $P < 0.05$)

The treatment means within cultivars are summarized in Table 4.21. In general, the heat treatments had significant effects on all viscosity parameters compared to untreated for Fibar and Rattan. However, McGwire was less affected by the heat treatments. Visual representations of how the starch pasting profiles differ among treatments and cultivar are shown in Figs. 4.11, 4.12 and 4.13. Conditioning and micronization increased the peak and breakdown viscosity the most for Fibar and Rattan, while roasting had the highest final and setback viscosities. This indicates that all of the heat treatments allowed the Fibar and Rattan starch granules to swell more but also caused them to be more susceptible to high temperatures and lysing. In addition, the heat treatments aided in starch retrogradation and formed stronger gels compared to the untreated Fibar and Rattan. Increased starch pasting viscosities of steamed or kilned oats were also seen by Zhang *et al.* (1997), Doehlert *et al.* (1997) and Cenkowski *et al.* (2006). Micronized oats also increased in peak and final viscosity according to Cenkowski *et al.* (2006).

Previous research done on the effect of heat treatments on the starch pasting of barley or oats was consistent with the results seen for Fibar and Rattan but McGwire behaved differently. Unlike the distinct increase in peak and final viscosity from heat treatments seen in the waxy cultivars, a significant decrease occurred in the roasted McGwire samples. The McGwire starch granules may have been damaged during the roasting treatment, which hindered their swelling and gelling ability. Sharma *et al.* (2011) also noted a significant decrease in peak, breakdown and final viscosity of various roasted barley cultivars due to partially gelatinized starch. The micronized and conditioned McGwire samples were not significantly different from the untreated in peak, final and setback viscosity. The biggest impact the heat

treatments had on the McGwire samples was on breakdown viscosity. All the heat treatments significantly decreased the breakdown viscosity compared to the untreated McGwire. This indicated the starch in the heat-treated McGwire samples was more stable at a high temperature and resistant to lysing.

Conditioning was the only treatment to significantly affect the pasting temperature of Rattan. The pasting temperature of Fibar was significantly affected by conditioning, while the pasting temperature of McGwire was only significantly affected by micronization. The higher pasting temperatures obtained by the conditioning treatment means the starch absorbs water and swells at a slower rate. Sharma *et al.* (2011) noted a significant increase in pasting temperature of roasted barley. In the present study, the pasting temperature of the roasted samples was not significantly different from untreated. However, a much lower roasting temperature was used in the present study which could explain the difference. There was minimal change in starch pasting due to tempering level.

There was no significant difference ($P \geq 0.05$) between tempering levels for both micronization and roasting for all starch pasting parameters for Rattan. A similar trend was noted for McGwire and Fibar but there were significant differences seen for some variables between tempering levels. There were significant differences ($P < 0.05$) between treatments for peak time for all 3 cultivars but the actual differences were minimal.

Typically, starch gelatinization occurs when heat is applied. Therefore, it was expected that some starch gelatinization occurred during the heat treatments of the barley grain. However, the results did not indicate that gelatinization transpired in most samples. Gelatinized starch should have increased the viscosity during the first 20 min of the test, prior to the

temperature increasing. However, as shown in Figs. 4.11-4.13, there was no increase in viscosity in the 20 min time period. Reduced peak viscosity is also an indication that partial gelatinization has occurred because the starch granules rapidly swell and lyse (Sharma et al. 2011). A reduction in peak viscosity only occurred in the roasted McGwire samples (Table 4.21). Since all heat treatments were above gelatinization temperature, partial gelatinization likely did not occur in most samples due to the lack of excess moisture available in the grain (Vasanthan and Hoover, 2009). Therefore, the differences in starch pasting were probably from the starch granules being altered by the heat. Further investigation is required to examine what happens at a starch granule level that causes the pasting characteristics to be altered by heat.

Table 4.21 Interaction effect between treatment and cultivar on starch pasting parameters (with lichenase addition)

	Peak (cP)	**Breakdown** (cP)	**Final** (cP)	**Setback** (cP)	**Pasting Temp.** (°C)	**Peak Time** (s)
Fibar						
Micronized (T)[1]	2720 (154.2) a	1315 (66.4) a	1987 (104.6) b	582 (8.2) a	67.7 (0.09) c	22.6 (0.03) c
Micronized (N)[2]	2531 (36.9) b	1269 (30.2) a	1758 (26.5) c	495 (3.3) ab	67.9 (0.50) bc	22.4 (0.04) d
Roasted (T)[1]	2276 (17.9) c	765 (30.5) cd	2157 (18.9) ab	645 (23.5) a	69.0 (0.51) b	22.9 (0.12) b
Roasted (N)[2]	2048 (57.3) d	668 (52.5) d	2003 (29.7) b	623 (20.9) a	68.4 (0.42) bc	22.6 (0.09) c
Conditioned	2710 (61.1) a	1046 (70.8) b	2292 (53.3) a	628 (11.9) a	70.6 (0.46) a	23.1 (0.04) a
Untreated	1261 (1.4) e	794 (1.4) c	809 (6.4) d	342 (3.5) b	68.0 (0.53) bc	22.4 (0) d
McGwire						
Micronized (T)[1]	1978 (74.1) a	400 (42.4) d	2802 (204.4) a	1224 (176.3) a	84.9 (1.55) a	25.0 (0.09) a
Micronized (N)[2]	2084 (34.6) a	530 (22.8) bc	2831 (43.3) a	1277 (35.9) a	84.5 (0.41) ab	24.9 (0.05) a
Roasted (T)[1]	1765 (29.4) b	496 (23.0) bcd	2473 (45.3) b	1205 (30.6) a	82.8 (1.56)c	24.5 (0.06) c
Roasted (N)[2]	1725 (41.9) b	576 (34.1) b	2364 (41.5) b	1215 (34.2) a	83.1 (0.68) c	24.5 (0.04) c
Conditioned	1976 (93.5) a	451 (57.3) cd	2742 (95.3) a	1217 (47.0) a	83.3 (0.71) bc	24.7 (0.09) b
Untreated	2095 (7.07) a	775 (19.1) a	2671 (10.6) a	1350 (22.6) a	83.1 (1.17) c	24.9 (0) a
Rattan						
Micronized (T)[1]	3116 (66.0) a	1613 (29.9) a	2315 (75.3) b	813 (145.9) b	68.8 (0.40)a	23.8 (0.04) c
Micronized (N)[2]	3035 (26.5) ab	1532 (40.3) ab	2268 (13.4) b	765 (30.3) bc	68.5 (0.04) a	23.8 (0.04) c
Roasted (T)[1]	3012 (25.2) ab	1284 (23.9) c	2794 (12.3) a	1066 (8.8) a	69.4 (0.11) a	24.0 (0.04) b
Roasted (N)[2]	2931 (30.0) b	1215 (31.2) c	2782 (14.5) a	1066 (19.5) a	68.7 (0.49) a	24.0 (0.04) b
Conditioned	3109 (84.9) a	1421 (105.7) b	2405 (44.7) b	717 (19.7) bc	71.2 (0.33) b	24.0 (0.05) b
Untreated	1915 (37.5) c	856 (41.0) d	1663 (9.9) c	605 (6.4) c	68.6 (0.11) a	24.2 (0.04) a

[1]Barley tempered to 17% moisture prior to treatment

[2]Barley was not tempered prior to treatment

Values reported as means (SD) within a treatment and cultivar

Values within same column and cultivar followed by same letter are not statistically significant (LSD, $P \geq 0.05$)

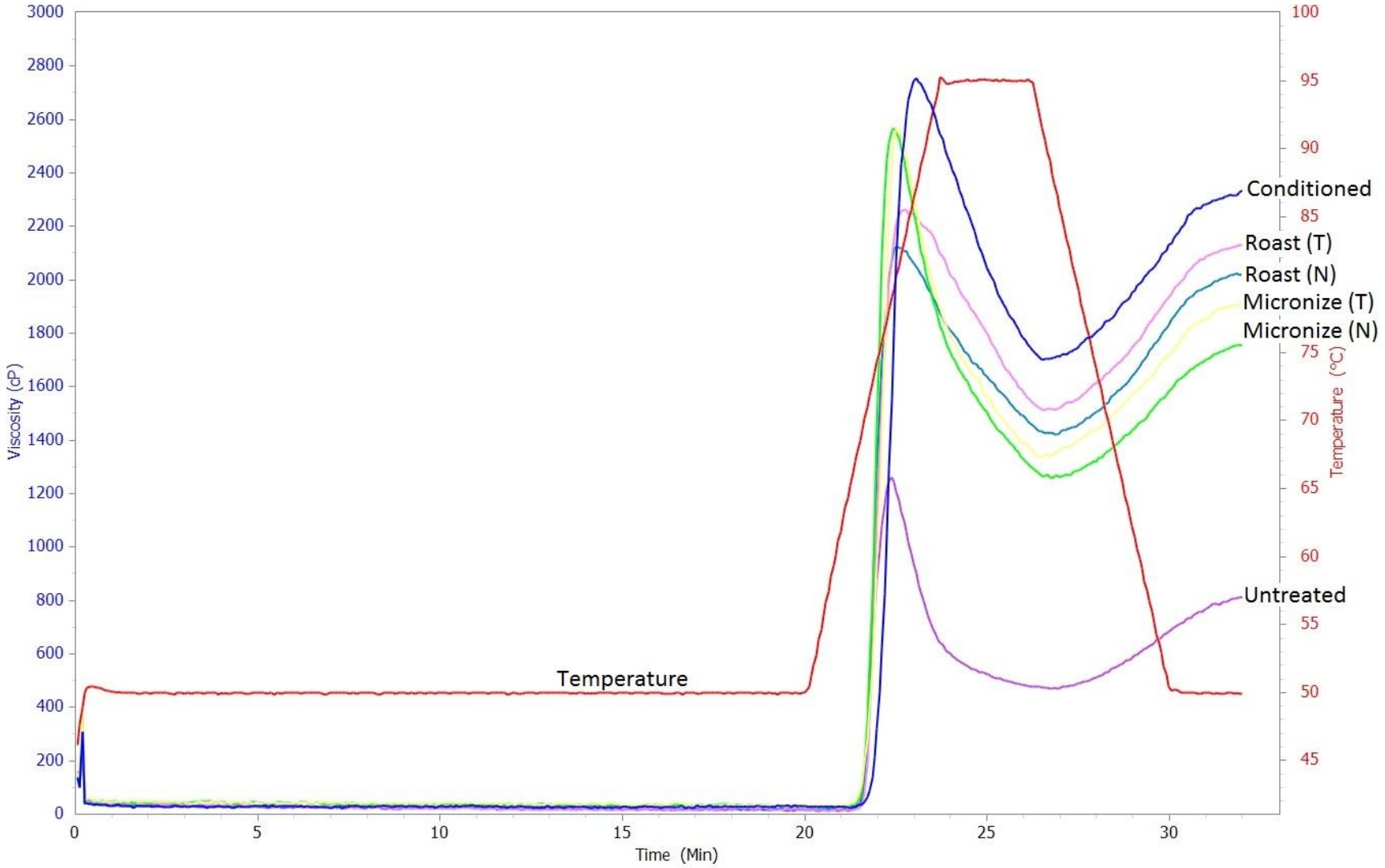


Figure 4.11 Starch pasting profiles (with lichenase addition) of heat treated and untreated Fibar
T = barley tempered to 17% moisture prior to treatment
N = barley not tempered prior to treatment

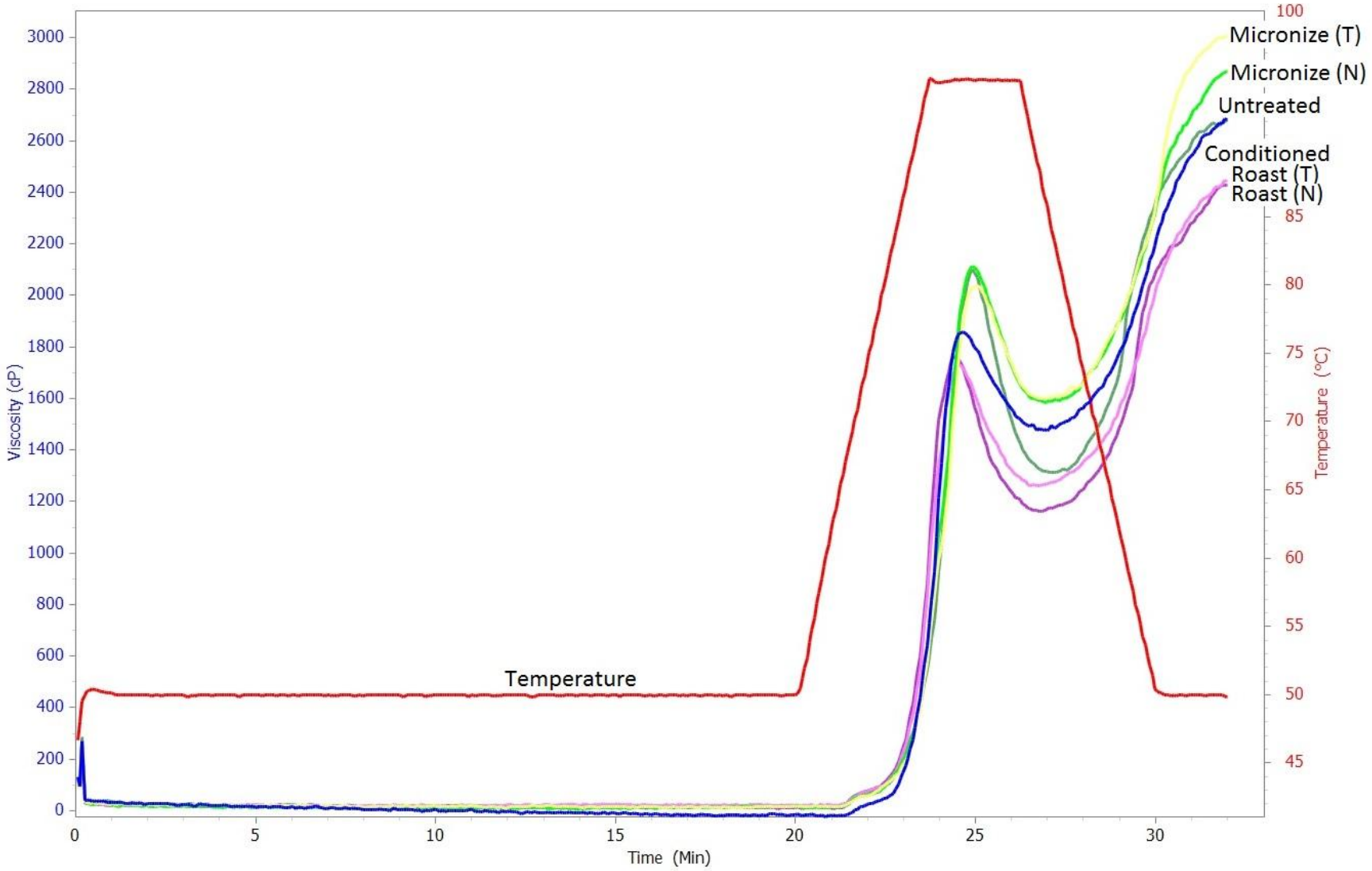


Figure 4.12 Starch pasting profiles (with lichenase addition) of heat treated and untreated McGwire
T = barley tempered to 17% moisture prior to treatment
N = barley not tempered prior to treatment

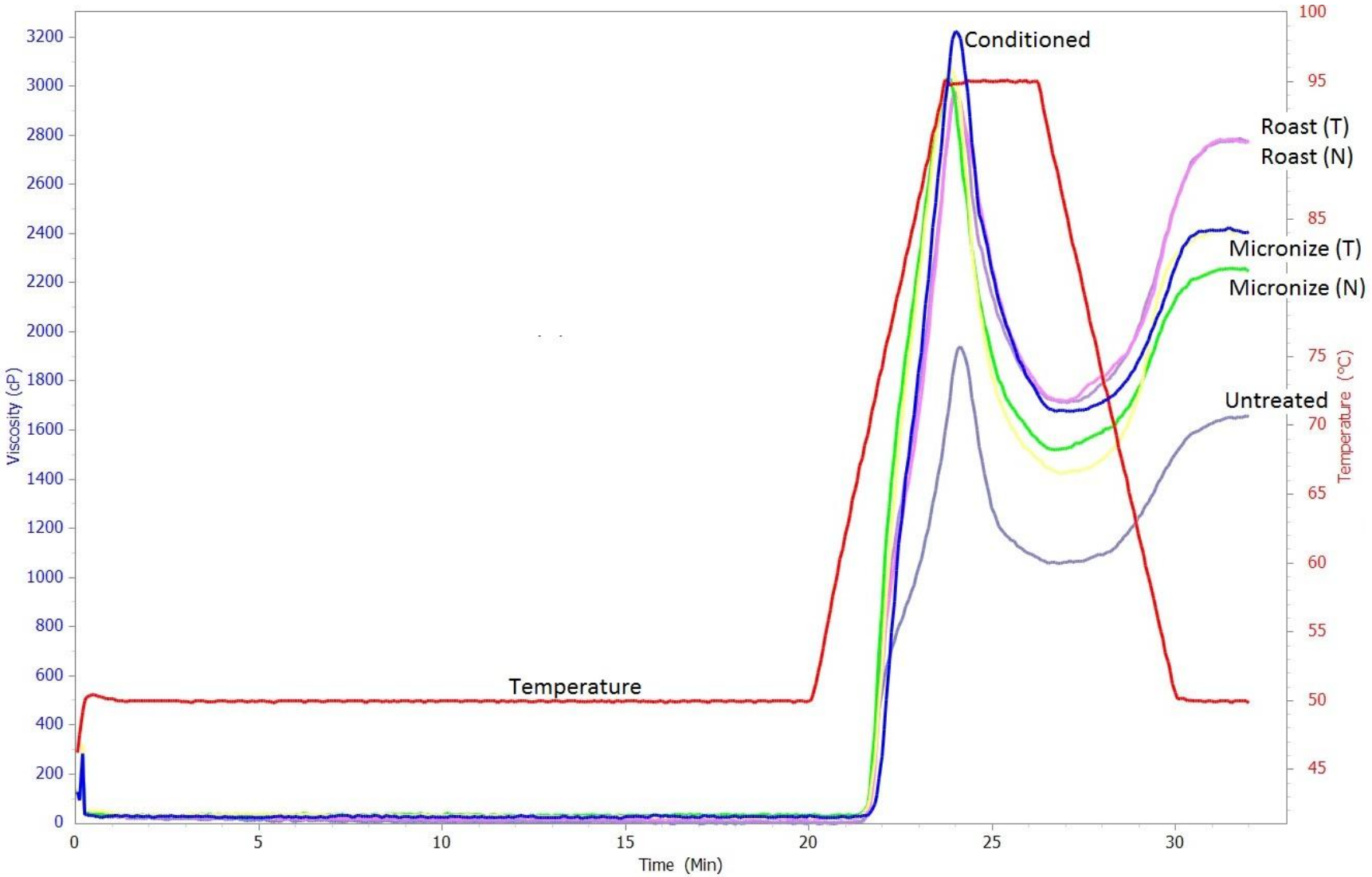


Figure 4.13 Starch pasting profiles (with lichenase addition) of heat treated and untreated Rattan
T = barley tempered to 17% moisture prior to treatment
N = barley not tempered prior to treatment

The relationship between particle size and starch pasting was also determined. It is known that particle size can affect water absorption, which can influence the starch pasting profile (Al-Rabadi et al. 2012). The Pearson correlation coefficients (r) that are summarized in Table 4.22 help explain the correlations between particle size and starch pasting. Generally, the correlation coefficients were low. This was likely due to the large differences found between cultivar and heat treatment so the effect of particle size was probably less influential on the starch pasting results. However, there were significant effects found. The peak viscosity had no significant correlations ($P \geq 0.05$) with any of the particle size parameters. However, the large differences between the cultivars in peak viscosity due to starch type may have influenced the correlation values. Breakdown viscosity had a significant ($P < 0.05$) positive correlation with the following particle size parameters: d(0.5), d(0.9) and D[4,3]. But, final and setback viscosity, pasting temperature and peak time had significant ($P < 0.05$) negative correlations with those same particle size parameters. No significant correlations were made between starch pasting and span, uniformity or d(0.1). This indicated that the samples with a larger particle size were less stable at high pasting temperatures and caused the starch granules to lyse easier. Starch granules often rupture easier when they have absorbed more water (Ragaee and Abdel-Aal, 2006). Therefore, the micronized and untreated samples which had larger particles likely had an increased swelling capacity which caused them to be less stable at high temperatures. This is consistent with findings of Al-Rabadi *et al.* (2012) where it was shown that barley with a particle size of 0.25 and 0.50 mm had a larger water absorbency index than barley of a smaller particle size. In addition, the micronized and untreated samples formed weaker gels upon cooling, which research by Al-Rabadi *et al.* (2012) supported.

Table 4.22 Relationship between starch pasting (with lichenase addition) and particle size variables represented by Pearson correlation coefficients (r)

	d(0.1)	d(0.5)	d(0.9)	D[3,2]	D[4,3]	Span	Specific Surface Area	Uniformity
Peak	0.171	0.338	0.228	0.331	0.332	-0.151	-0.293	-0.127
Breakdown	0.100	0.555*	0.359*	0.409*	0.485*	-0.248	-0.349*	-0.245
Final	0.123	-0.546*	-0.469*	-0.281	-0.473*	0.050	0.289	0.191
Setback	0.012	-0.651*	-0.608*	-0.448*	-0.627*	0.006	0.471*	0.175
PT	0.206	-0.551*	-0.539*	-0.279	-0.580*	-0.042	0.251	0.022
Peak Time	0.146	-0.538*	-0.586*	-0.376*	-0.584*	-0.132	0.370*	0.008

*Significant effect at P (<0.05)

PT = Pasting Temperature; d(0.1) = size of particle below which 10% of the sample lies; d(0.5) = size of particle below which 10% of the sample lies; d(0.9) = size of particle below which 90% of the sample lies; D[3,2] = surface are mean diameter; D[4,3] = volume mean diameter

4.3.5 Peroxidase Activity

The peroxidase activity of the untreated and heat-treated barley was determined qualitatively. The results are summarized in Table 4.23. The presence of lipase is assumed when peroxidase is present because peroxidase is more heat resistant (Girardet and Webster, 2011). However, it must be noted that the literature is still unclear on whether peroxidase is an accurate test for lipase even though it is used routinely in the oat industry (Ekstrand et al. 1992; Gates, 2007). The untreated sample for each of the 3 cultivars had a positive reaction for peroxidase indicating lipase activity was likely present, as well as other enzymes. Only conditioning, for all cultivars, produced a negative peroxidase result. The micronized and roasted samples produced a blue colour indicating the presence of peroxidase and therefore lipase. Hu et al. (2010) also found that roasting did not inactivate peroxidase in oats, but peroxidase was inactivated with micronization. Although barley has low oil content (2-5%), the presence of lipase may decrease the shelf life due to rancidity (Newman and Newman, 2008b). Interestingly, the conditioned barley samples were the only ones to not experience a decline in β-glucan *in vitro* digest extract viscosity and only a slight decrease in molecular weight over the 2 h extraction period. This indicates that the β-glucanases were possibly inactivated by conditioning as well. These results suggest the opportunity of using peroxidase activity in the future to determine if a heat treatment was sufficient to inactivate β-glucanases as well as lipase. This is beneficial because the prevention of the breakdown of β-glucan is important to ensure high β-glucan viscosity and molecular weight in barley end products in order to maximize nutrition.

Table 4.23 Effect of heat treatment on peroxidase activity of different barley cultivars

Heat Treatment	**Cultivar**	**Peroxidase Activity** (- or +)
Micronize (T)[1]	Fibar	+
	McGwire	+
	Rattan	+
Micronize (N)[2]	Fibar	+
	McGwire	+
	Rattan	+
Roast (T)[1]	Fibar	+
	McGwire	+
	Rattan	+
Roast (N)[2]	Fibar	+
	McGwire	+
	Rattan	+
Conditioned	Fibar	-
	McGwire	-
	Rattan	-
Untreated	Fibar	+
	McGwire	+
	Rattan	+

[1]Barley tempered to 17% moisture prior to treatment

[2]Barley was not tempered prior to treatment

- Result with a negative (no colour change) reaction

+ Results with a positive (blue colour formation) reaction

4.3.6 Colour

Colour is an important variable when considering barley quality. The colour of the kernel and flour will affect the colour and acceptability of the food products produced from them. The effect of heat treatments on barley flour and kernel colour was assessed using the L* a* b* scale. L* represents the brightness value on a scale of 0 (black) to 100 (white). The a* value describes the degree of green and red colours in the sample with green samples providing a negative value and red samples providing a positive value. The b* values represent the amount blue (negative value) and yellow (positive value) in the sample.

The ANOVA results are summarized in Table 4.24. Flour and kernel colour was assessed on milled barley (0.5 mm) and the whole barley kernel, respectively. There was significant treatment effect ($P < 0.05$) on barley flour colour for L*, a* and b* and a significant cultivar effect for L*. A significant interactional effect was also noted for b* of barley flour but with lower P-value of 0.022. Although Rattan was found to have significantly different flour L* mean (59.7) than McGwire (59.2) which caused a significant cultivar effect, the visual difference would be imperceptible. The treatment means of flour colour are shown in Table 4.25. Overall, the colour values of the flours were comparable to the range described by Sharma and Gujral (2010), except L* which was much lower in the present study. The brightness (L*) of the heat treated flours decreased and were all significantly different ($P < 0.05$) than untreated, except for micronization without tempering. Conditioning was significantly different ($P < 0.05$) from all other treatments and showed the greatest decrease in L*. The decrease in brightness seen in all the heat treatments is consistent with literature which noted dry heat treatments and micronization reduced the L* value of barley (Kim et al. 1998; Emami et al. 2011; Sharma et al.

2011; Yahya et al. 2014). Tempering had no effect ($P \geq 0.05$) on the brightness of barley flour in the roasting and micronizing treatments.

The redness (a*) of the heat treated flours increased and were all significantly different ($P < 0.05$) from untreated, except for micronization without tempering. Again, conditioning was significantly different ($P < 0.05$) from all other treatments and showed the greatest increase in a*. Tempering only showed a significant difference ($P < 0.05$) in a* in the roasting treatment, but the actual numerical difference was minimal. The yellowness (b*) of the heat-treated barley flours increased and were significantly different ($P < 0.05$) from untreated. Roasting with tempering had the highest b* value at 9.85 and was significantly ($P < 0.05$) different from all other treatments. Tempering had a significant effect ($P < 0.05$) on the b* value of the roasted samples, but there was no effect ($P \geq 0.05$) on the micronized samples. The added moisture from tempering likely allowed for greater heat absorption, which caused more browning reactions. The increase in redness (a*) and yellowness (b*) of the heat-treated barley flours was consistent with colour changes seen by Sharma *et al.* (2011) in roasted barley.

Table 4.24 Summary of ANOVA for colour of barley kernels and flour

	P-values					
	Flour			Kernel		
Effect	L*	a*	b*	L*	a*	b*
Cultivar	0.005*	0.509	0.567	0.002*	< 0.001*	< 0.001*
Treatment	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Cultivar x Treatment	0.362	0.281	0.022*	0.349	0.002*	0.292

*Significant effect at P (<0.05)

Table 4.25 Effect of heat treatment on barley flour colour

Treatment	L*	a*	b*
Micronized (T)[1]	59.6 (0.40) bc	0.71 (0.04) d	8.40 (0.25) c
Micronized (N)[2]	59.9 (0.41) ab	0.65 (0.03) de	8.24 (0.23) c
Roasted (T)[1]	59.4 (0.12) c	1.05 (0.07) b	9.85 (0.29) a
Roasted (N)[2]	59.6 (0.27) bc	0.94 (0.06) c	9.52 (0.19) b
Conditioned	58.0 (0.67) d	1.15 (0.08) a	9.55 (0.28) b
Untreated	60.2 (0.46) a	0.62 (0.01) e	7.74 (0.20) d

[1]Barley tempered to 17% moisture prior to treatment

[2]Barley was not tempered prior to treatment

Values reported as means (SD) among all cultivars within a treatment

Values within same column followed by same letter are not statistically significant (LSD, $P \geq 0.05$)

There were significant cultivar and treatment effects on the barley kernel colour for L*, a* and b*, and an interactional effect for a* (Table 4.24). The main effect means are summarized in Table 4.26. For L*, a* and b*, McGwire was significantly different ($P < 0.05$) from Fibar and Rattan. Emami *et al.* (2011) noted waxy barleys had a greater decrease in L*, a* and b* values when exposed to micronization than normal starch barleys. In this study, McGwire (normal starch barley) had an overall lower L*, a* and b* value than Fibar and Rattan (waxy barleys). However, this difference was likely from untreated McGwire having lower L*, a* and b* values than Fibar and Rattan to begin with.

The brightness (L*) of the kernel colour was significantly different ($P < 0.05$) from untreated for the micronized without tempering, roasting with tempering and conditioning treatments. Micronization was the only treatment to have no significant effect (with tempering) and significantly increase (without tempering) the brightness value compared to untreated, while the other treatments decreased it. This differs from what was found by Emami *et al.* (2011). Conditioning had the largest decrease in L*. A higher temperature (150 °C) was used for the oven treatment part of conditioning compared to roasting which likely caused more toasting in the kernel from Maillard and browning reactions. Yahya *et al.* (2014) noted at temperatures over 150 °C kernel brightness dropped significantly. Tempering did not have an effect ($P \geq 0.05$) on L* within the micronization and roasting treatments. The a* and b* (redness and yellowness) values of the kernels reacted similarly for each of the treatments. All of the heat treatments were significantly different ($P < 0.05$) from untreated, and roasted with tempering showing the largest increase in a*and b*. Tempering had a significant effect ($P < 0.05$) on micronization and roasting treatments for both a* and b*.

There was a significant interactional effect ($P < 0.05$) for a* because Fibar and Rattan reacted differently to the treatments than McGwire (Fig. 4.14). Larger increases in a* values were seen in the roasting and conditioning treatments for Fibar and Rattan, likely due to browning reactions. Higher amounts of phenolic compounds in the grain can cause larger colour changes, which may be why there was a significant cultivar effect (Quinde-Axtell and Baik, 2006; Baik and Ullrich, 2008).

Overall, the kernel colour was more affected by heat treatments than the flour colour. Micronization, roasting and conditioning all were significantly different in L*, a* and b* values. Conditioning produced the greatest difference in kernel and flour colour compared to untreated, and micronization the least. However, it is important to note that while there were measurable differences between all the treatments, some difference may be visually imperceptible to the human eye.

Table 4.26 Effect of cultivar and treatment on barley kernel colour

	L*	a*	b*
Cultivar			
Fibar	56.1 (2.36) a	5.74 (1.14) a	22.6 (0.92) a
McGwire	54.8 (1.88) b	5.35 (0.74) b	21.6 (1.14) b
Rattan	55.8 (2.52) a	5.81 (0.96) a	22.5 (1.20) a
Treatment			
Micronized (T)[1]	57.1 (1.12) ab	5.05 (0.21) c	23.2 (0.53) a
Micronized (N)[2]	57.6 (1.45) a	4.77 (0.15) d	22.6 (0.74) b
Roasted (T)[1]	54.9 (0.74) d	7.07 (0.40) a	23.6 (0.65) a
Roasted (N)[2]	55.7 (0.61) cd	6.26 (0.34) b	22.0 (0.48) c
Conditioned	51.7 (1.33) e	6.21 (0.48) b	21.3 (0.77) d
Untreated	56.5 (1.31) bc	4.44 (0.26) e	20.6 (0.95) e

[1]Barley tempered to 17% moisture prior to treatment

[2]Barley was not tempered prior to treatment

Cultivar effect reported as means (SD) of all treatments within a cultivar

Treatment effect reported as means (SD) of all cultivars within a treatment

Values within same column and effect followed by same letter are not statistically significant (LSD, $P \geq 0.05$)

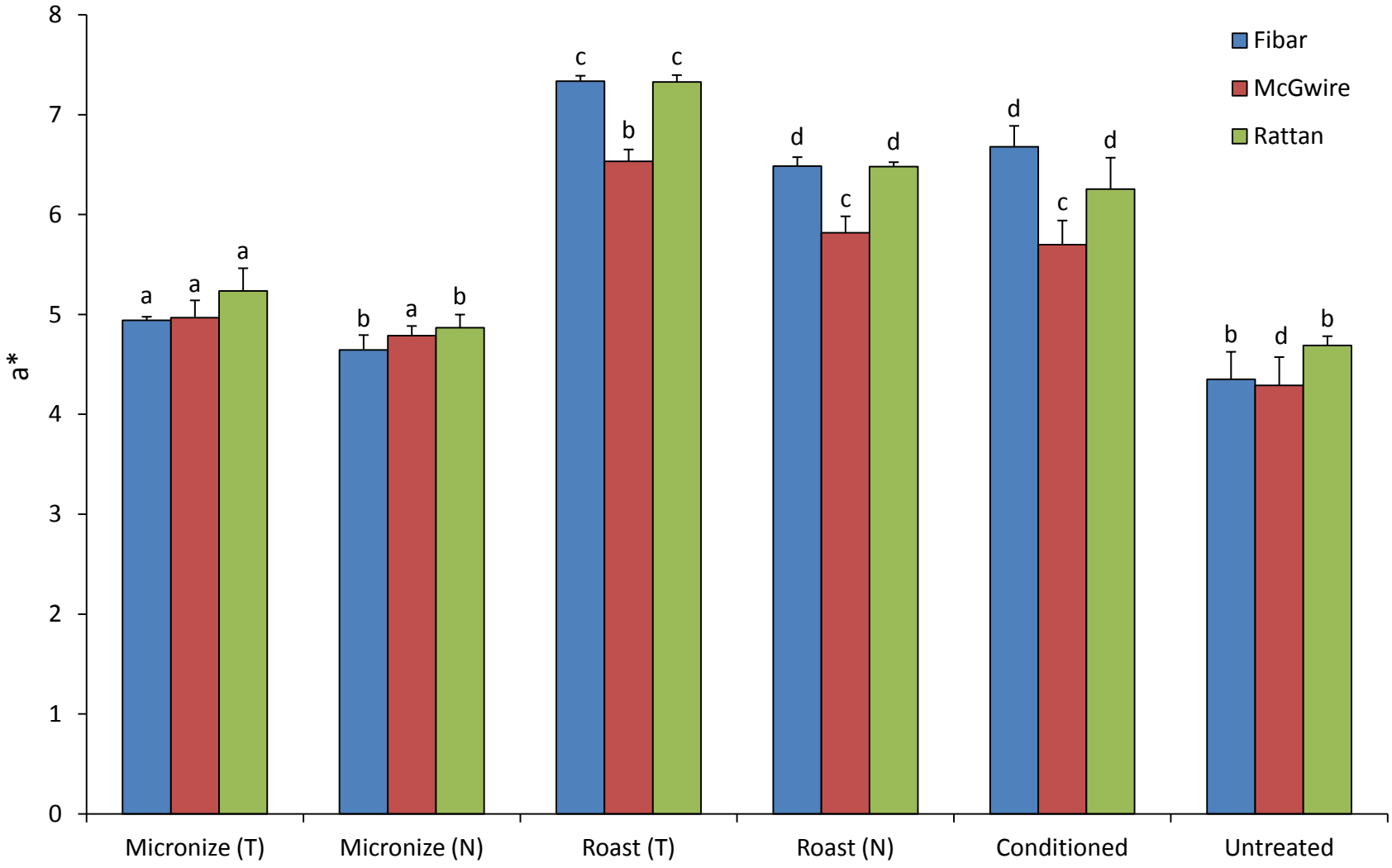


Figure 4.14 Effect of heat treatments on redness (a*) of barley kernel colour for different barley cultivars
Values reported as means within a treatment and cultivar
Different letters within same cultivar are significantly different from one another (LSD, $P < 0.05$)

5. CONCLUSIONS

5.1 Summary

The first part of this research was to assess current commercial barley products for microbial safety and potential to meet the β-glucan health claim. Of the 17 barley samples collected, 12 of these were considered end-products and only 4 met the microbial limits for SPC, MYC and coliforms/*E. coli* established for this study. There also appeared to be a reduction in microbial load from the raw barley to the end product through processing. The β-glucan contents of the commercial products ranged from about 3.5-7% (d.b.) excluding the high β-glucan flours which contained approximately 25% (d.b.). All of these products had the potential to meet the health claim set by Health Canada for cholesterol-lowering depending on the food application. However, the viscosity and molecular weight of the β-glucan in the commercial products were not high enough to provide the best possible health effects if used in an uncooked food application. The pearled and steamed barley flakes was the only sample to have a high mean β-glucan viscosity and molecular weight that were more than 1300 cP and 2 000 kDa, respectively. All the other samples appeared to be affected by β-glucanase activity and were positive for peroxidase activity.

The second part of this research was to evaluate the effects of micronization, roasting and conditioning on the safety, nutritional and other physicochemical properties of whole grain barley. There was a significant treatment effect ($P < 0.05$) on SPC, MYC and coliforms. SPC also had significant cultivar and interactional effects ($P < 0.05$) but this was likely due to the cultivars having different baselines of contamination. All treatments reduced the microbial load to within acceptable limits used in this study and were significantly different ($P < 0.05$) from

untreated. Conditioning provided the greatest reduction in MYC and appeared to be the most consistent.

In order to evaluate the physicochemical properties of the β-glucan, the content, viscosity, molecular weight and solubility were measured. There were significant cultivar and treatment effects ($P < 0.05$) on the β-glucan content. The conditioned samples consistently had a higher level of β-glucan. However, this was likely from variations within the barley lot and not necessarily from the conditioning treatment. β-glucan *in vitro* digest extract viscosity, molecular weight and solubility all had significant interactional effects ($P < 0.05$). The conditioned samples had the highest viscosity and molecular weight but the lowest solubility. All other treatments appeared to be affected by β-glucanase activity, which reduced their viscosity and molecular weight but increased their solubility. In addition, conditioning was the only treatment that inactivated peroxidase. A significant ($P < 0.05$) negative correlation was found between β-glucan solubility and molecular weight, and a significant ($P < 0.05$) positive correlation between β-glucan molecular weight and viscosity.

The heat treatments also impacted the particle size of the flour once milled. There were significant cultivar and treatments effects ($P < 0.05$) for most of the particle size parameters including the median and volume weighted mean. Roasting and conditioning had the lowest median and volume weighted mean values and were significantly different ($P < 0.05$) from untreated. Micronization had no effect ($P \geq 0.05$) on the particle size median or volume weighted mean when compared to untreated. There was a significant interactional effect ($P < 0.05$) on the particle size span. Conditioning and micronization with tempering within each

cultivar had no effect ($P \geq 0.05$) on span. Neither did any of the treatments on Rattan, indicating it may mill more consistently than Fibar or McGwire.

A significant interactional effect was found between cultivar, treatment and fraction on the β-glucan content of various flour fractions. Generally, the β-glucan content was higher in the large fraction and lower in the small fraction regardless of cultivar or treatment. However, the > 250 and 125-250 μm fractions became similar in β-glucan content when heat treatments were applied. The results showed that heat treatments will affect how barley β-glucan is concentrated in differently sized flour fractions.

Starch pasting was also altered by heat treatments. Significant interactional effects ($P < 0.05$) on all starch pasting parameters were found. Fibar and Rattan (waxy starch) reacted differently to the heat treatments than McGwire (normal starch). Peak, breakdown, final and setback viscosity were all significantly ($P < 0.05$) increased by heat treatments in Fibar and Rattan. However, McGwire was only significantly ($P < 0.05$) affected by roasting which decreased its peak, breakdown and final viscosity. The breakdown viscosity also had a significant ($P < 0.05$) positive correlation to median and volume weighted mean, while final and setback viscosity, and pasting temperature had significant ($P < 0.05$) negative correlations to median and volume weighted mean.

There were significant cultivar and treatments effects ($P < 0.05$) on barley kernel colour but only a significant treatment effect ($P < 0.05$) on flour colour. McGwire was significantly different ($P < 0.05$) in kernel colour from both Fibar and Rattan. For both kernel and flour colour, conditioning and roasting decreased L* and increased a* and b* the greatest.

Micronization changed the colour the least compared to untreated. Visually, these changes may be unnoticeable and the change in flour colour was less than kernel colour.

5.2 Overall Conclusions

The main purpose of the research presented here was to assess the safety of barley products and use heat processes to reduce the microbial contamination. Many commercial barley products exceeded predetermined microbial limits used in this study. This is a concern as new food trends develop and thermal processes are not always applied during the production of barley products. When heat treatments were applied in this study, conditioning reduced the microbial contamination the most effectively. Roasting and micronization also reduced the microbial contamination to within the predetermined limits but with less consistent results.

The secondary goal of this research to determine the heat treatment effects on the β-glucan characteristics. Conditioning also produced the highest viscosity and molecular weight of β-glucan by inactivating β-glucanase enzymes. The degree of inactivation of β-glucanase enzymes appeared to be an important role in the β-glucan viscosity and molecular weight results. A high β-glucan viscosity and molecular weight is desired for the best health benefits. Therefore, in uncooked food applications, heat treatments can improve the health benefits provided by barley β-glucan.

The last part of this research was to determine the effect the heat treatments had on other characteristics of barley. The heat treatments caused the kernels to fracture differently, resulting in different particle sized flours. Roasting and conditioning had the greatest impact on particle size compared to untreated. All heat treatments had an effect on the β-glucan content

of different particle-sized fractions obtained by air jet sieving, which indicated the kernel fractured differently during milling after heat treatment. Heat treatments also altered the starch pasting profiles but the results were dependant on starch type. Waxy cultivars (Fibar and Rattan) showed an increase in starch pasting viscosities while the normal starch cultivar (McGwire) had no change or reduction in viscosities. There were overall colour changes to the kernel and flour but visually, they were minimal. However, they may become more noticeable when used in other applications. In comparison with untreated, conditioning displayed the best overall results but micronization behaved in a similar manner for many properties. For all these reasons, heat treatments could be a useful process for the barley industry to use to improve the safety of their products as well as their physicochemical properties.

5.3 Impact of Research

Cereal grains, barley included, are generally not considered as a food safety concern. However, this research shows there is a potential problem, especially if barley is used in uncooked food applications. From this, industry can gain knowledge of safety risks associated with barley and implement procedures to reduce the risk. The heat treatments used in this study show that processing can be beneficial. Not only do heat treatments reduce the microbial contamination, they can improve the viscosity and molecular weight characteristics of β-glucan. Enzymes, which degrade important components in barley, can also be inactivated using heat. This may improve the quality and stability of the product. This research also showed that different barley cultivars can react differently to heat treatments and some may be better than others in certain applications. Understanding the processing variation of different barley

cultivars, will help industry optimize processes in order to produce a consistent product. They can also use heat treatments to their benefit and create more nutritious products that will be more desired by consumers. In addition, it is hoped that heat treatments of barley will allow for unique food development opportunities that will increase the number of barley products on the market.

5.4 Strengths and Limitations

This was the first study to evaluate commercial barley products for microbial contamination and examine the effects of heat treatments on microbial safety of whole grain barley. In addition, there are minimal studies evaluating the microbial safety of other cereal grains and the effect of processing on microbial load. The results of this study confirm that the barley industry should not assume barley products are safe and supply the industry with applicable methods to address the issue of food safety. This study was also one of few to determine the effects of heat treatments, applied during primary processing, on a number of physicochemical properties of barley. The results supply alternative benefits, like nutritional and physical changes, to implementing heat treatments during primary barley processing.

A limitation of this study was that the 3 cultivars of barley were only collected from one location. The growing location can affect the microbial contamination, as well as, the barley composition which could have impacted the results. This work should be repeated on multiple cultivars from multiple locations to better understand the microbial safety of barley and assess if the processing effects are consistent across locations.

Another limitation of this study was that it was difficult to draw conclusions from the commercial barley product data because of the low number of samples and little information supplied by industry on the processes used. There are few barley processors in Canada and not all were willing to supply samples and/or processing information. General trends were able to be evaluated but statistical analysis was not performed due to the variability of the samples. A survey on the microbial safety of all different types of grains from different processors across Canada may be able to supply more comprehensive analysis on the current safety of all grains, not just barley.

5.5 Future Research

The heat processes done in this study were on either lab or pilot scale. To confirm the results found in this study, commercial scale experiments with larger lots of barley are necessary. This will assess the potential use of heat treatments in the barley industry and determine if they produce consistent results at a larger scale. In addition, investigation of how the microbial contamination changes throughout non-heat processes used in industry currently, like milling, pearling and air classification is needed.

Further investigation on the effects of heat treatments on whole grain barley is needed to more thoroughly determine how and why the physicochemical attributes change. The effects on starch were not completely explained by the starch pasting results obtained in this research. More research on how the starch granules themselves are altered is needed. Physical characteristics, like kernel hardness and kernel density, and their effect on particle size are also important to determine as they may affect how it is milled and the milling energy required.

Lastly, β-glucan is an important bioactive in barley and researching methods to increase its solubility while maintaining a high molecular weight and viscosity are important to increase barley's nutrition.

Product development using heat-treated barley is also an important area to explore. The reduction in microbial contamination obtained by the heat treatments allows for more novel food applications that do not require a cook step to be developed. In addition, the functional characteristics may change with heat treatment and could alter how barley can be used in different food systems. As well, the effect of heat treatments on sensory characteristics, especially flavour and aroma, should be determined. Heat treatments during primary processing may impact the sensory attributes of the end product and any foods made with that product. In order to increase the use of barley, consumer acceptability is important and should be investigated.

6. REFERENCES

AACC International (1999a). Approved Methods of Analysis, 11th Ed. Method 44-15.02. Moisture – Air-oven Methods. Approved November 3, 1999. AACC International, St. Paul, MN, U.S.A. http://dx.doi.org/10.1094/AACCIntMethod-44-15.02.

AACC International (1999b). Approved Methods of Analysis, 11th Ed. Method 42-11.01. Aerobic Plate Count. Approved November 3, 1999. AACC International, St. Paul, MN, U.S.A. http://dx.doi.org/10.1094/AACCIntMethod-42-11.01.

AACC International (1999c). Approved Methods of Analysis, 11th Ed. Method 42-50.01. Mold and Yeast Counts. Approved November 3, 1999. AACC International, St. Paul, MN, U.S.A. http://dx.doi.org/10.1094/AACCIntMethod-42-50.01.

AACC International (1999d). Approved Methods of Analysis, 11th Ed. Method 42-15.01. Coliform – *E. coli*. Approved November 3, 1999. AACC International, St. Paul, MN, U.S.A. http://dx.doi.org/10.1094/AACCIntMethod-42-15.01.

AACC International (1999e). Approved Methods of Analysis, 11th Ed. Method 32-23.01. β-Glucan Content of Barley and Oats – Rapid Enzymatic Procedure. Approved November 3, 1999. AACC International, St. Paul, MN, U.S.A. http://dx.doi.org/10.1094/AACCIntMethod-32-23.01.

AACC International (1999f). Approved Methods of Analysis, 11th Ed. Method 76-21.01. General pasting method for wheat or rye flour or starch using Rapid Visco Analyser. Approved November 3, 1999. AACC International, St. Paul, MN, U.S.A. http://dx.doi.org/10.1094/AACCIntMethod-76-21.01.

AACC International (1999g). Approved Methods of Analysis, 11th Ed. Method 22-80.01. Qualitative Test for Peroxidase in Oat Products. Approved November 3, 1999. AACC International, St. Paul, MN, U.S.A. http://dx.doi.org/10.1094/AACCIntMethod-22-80.01.

AAFC (2015). Recipes. Available at: http://www.agr.gc.ca/eng/about-us/publications/recipes/?id=1353011564794 Accessed June 26, 2015.

Abraha, A., Uhlen, A.K., Abay, F., Sahlstrøm, S. and Bjørnstad, Å. (2013). Roasted barley foods: processing and varietal differences affecting *Kolo* and *Tihni*, traditional grain products in Northern Ethiopia. *Cereal Foods World*, 58:71-79.

Abramson, D., House, J.D. and Nyachoti, C.M. (2005). Reduction of deoxynivalenol in barley by treatment with aqueous sodium carbonate and heat. *Mycopathologia,* 160:297-301.

Ahmed, J., Thomas, L. and Al-Attar, H. (2015). Oscillatory rheology and creep behavior of barley β-D-glucan concentrate dough: Effect of particle size, temperature, and water content. *Journal of Food Science*, 80:E73-E83.

Akar, T., Avci, M. and Dusunceli, F. (2004). Barley: post-harvest operations. Food and Agriculture Organization of the United Nations. Available at: http://www.fao.org/inpho/content/compend/text/ch31/ch31.htm Accessed September 10, 2013.

Aldughpassi, A., Abdel-Aal, E.S.M. and Wolever, T.M.S. (2012). Barley cultivar, kernel composition, and processing affect the glycemic index. *Journal of Nutrition,* 142:1666-1671.

Al-Rabadi, G.J., Torley, P.J., Williams, B.A., Bryden, W.L. and Gidley, M.J. (2012). Particle size heterogeneity in milled barley and sorghum grains: Effects on physico-chemical properties and starch digestibility. *Journal of Cereal Science*, 56:396-403.

Ames, N., Blewitt, H., Storsley, J., Thandapilly, S.J., Zahradka, P. and Taylor, C. (2015b). A double-blind randomised controlled trial testing the effect of a barley product containing varying amounts and types of fibre on the postprandial glucose response of healthy volunteers. *British Journal of Nutrition,* 113:1373-1383.

Ames, N.P. and Rhymer, C.R. (2003). Development of a laboratory-scale oat flaking machine for oat end product testing. *Cereal Chemistry*, 80:699-702.

Ames, N.P. and Rhymer, C.R. (2008). Issues surrounding health claims for barley. *The Journal of Nutrition,* 138:237S-243S.

Ames, N., Rhymer, C. and Rhyland, D. (2005). Optimizing end-product utilization of whole grain barley by infrared processing. Poster session at: Whole Grains Summit, Minneapolis, MN.

Ames, N., Rhymer, C., Rossnagel, B. and Arntfield, S. (2003). Properties of instant whole grain barley products created by infrared heat treatment. Poster session at: AACC International Annual Meeting, Portland, OR.

Ames, N., Rhymer, C., Rossnagel, B., Therrien, M., Ryland, D., Dua, S. and Ross, K. (2006). Utilization of diverse hulless barley properties to maximize food product quality. *Cereal Foods World*, 51:23-28.

Ames, N., Storsley, J. and Tosh, S. (2015a). Effects of processing on physicochemical properties and efficacy of β-glucan from oat and barley. *Cereal Foods World*, 60:4-8.

Andersson, A.A.M., Andersson, R. and Åman, P. (2000). Air Classification of Barley Flours. *Cereal Chemistry*, 77:436-467.

Andersson, A.A.M., Courtin, C.M., Delcour, J.A., Fredriksson, H., Schofield, J.D., Trogh, I., Tsiami, A.A. and Åman, P. (2003). Milling Performance of North European Hull-less Barleys and Characterization of Resultant Millstreams. *Cereal Chemistry,* 80:667-673.

Baik, B.K. and Ullrich, S.E. (2008). Barley for food: Characteristics, improvement and renewed interest. *Journal of Cereal Science*, 48:233-242.

Barthel, J., Gottschalk, C., Rapp, M., Berger, M., Bauer, J. and Meyer, K. (2012). Occurrence of type A, B and D trichothecenes in barley and barley products from the Bavarian market. *Mycotoxin Research*, 28:97-106.

Becker, A., Hill, S.E. and Mitchell, J.R. (2001). Milling – A further parameter affecting the Rapid Visco Analyser (RVA) profile. *Cereal Chemistry,* 78:166-172.

Behall, K.M., Scholfield, D.J. and Hallfrisch, J. (2004a). Lipids significantly reduced by diets containing barley in moderately hypercholesterolemic men. *Journal of the American College of Nutrition*, 23:55-62

Behall, K.M., Scholfield, D.J. and Hallfrisch, J. (2004b). Diets containing barley significantly reduce lipids in mildly hypercholesterolemic men and women. *American Journal of Clinical Nutrition,* 80:1185-1193.

Bensassi, F., Rjibac, I., Zarrouka, A., Rhouma, A., Hajlaoui, M.R. and Bachaa, H. (2011). Deoxynivalenol contamination in Tunisian barley in the 2009 harvest. *Food Additives and Contaminants: Part B Surveillance,* 4:205-211.

Beuchat, L.R. (1992). Media for detecting and enumerating yeasts and molds. *International Journal of Food Microbiology*, 17:145-158.

Bird, A.R., Jackson, M., King, R.A., Davies, D.A., Usher, S. and Topping, D.L. (2004). A novel high-amylose barley cultivar (*Hordeum vulgare* var. Himalaya 292) lowers plasma cholesterol and alters indices of large-bowel fermentation in pigs. *British Journal of Nutrition,* 92:607-615.

Bullerman, L.B. and Bianchini, A. (2011). The Microbiology of Cereals and Cereal Products: Variety of media and methods available for detection and enumeration of molds in cereal products. *Food Quality & Safety Magazine*. Available at: http://www.foodquality.com/details/article/1023787/The_Microbiology_of_Cereals_and_Cereal_Products.html?tzcheck=1 Accessed September 12, 2013.

Campbell, H., Choo, T.M., Vigier, B. and Underhill, L. (2000). Mycotoxins in barley and oat samples from eastern Canada. *Canadian Journal of Plant Science,* 80:977-980.

Canadian Grain Commission. (2013a). Prevent Ochratoxin A in stored grain. Available at: http://www.grainscanada.gc.ca/storage-entrepose/ota/ota-eng.htm Accessed September 12, 2013.

Canadian Grain Commission. (2013b). Grading tolerances for *Fusarium*-damaged grain and DON feeding guidelines. Available at: http://www.grainscanada.gc.ca/guides-guides/don/don-1-eng.htm Accessed September 12, 2013.

Casiraghi, M.C., Garsetti, M., Testolin, G. and Brighenti, F. (2006). Post-prandial responses to cereal products enriched with barley β-glucan. *Journal of the American College of Nutrition,* 25:313-320.

Castells, M., Pardo, E., Ramos, A.J., Sanchis, V. and Marín, S. (2006). Reduction of ochratoxin A in extruded barley meal. *Journal of Food Protection,* 69:1139-1143.

Cenkowski, S., Ames, N. and Muir, W.E. (2006). Infrared processing of oat groats in a laboratory-scale electric micronizer. *Canadian Biosystems Engineering,* 48:3.17-3.25.

Cenkowski, S., Hong, J.T., Scanlon, M.G. and Arntfield, S.D. (2003). Development of a mathematical model for high-intensity infrared processing (micronization) of peas. *Transactions of the American Society of Agricultural Engineers*, 46:705-713.

Cenkowski, S., Hong, J.T., Scanlon, M.G. and Arntfield, S.D. (2004). Mathematical modeling of heat and mass transfer during continuous infrared micronization. *Drying Technology*, 22:2255-2272.

Cenkowski, S., Pronyk, C., Zmidzinska, D. and Muir, W.E. (2007). Decontamination of food products with superheated steam. *Journal of Food Engineering,* 83:68-75.

CFIA. (2015a). Barley. Available at: http://www.inspection.gc.ca/english/plaveg/pbrpov/cropreport/bare.shtml Accessed May 28, 2015.

CFIA. (2015b). Information within the nutrition facts table: reference amounts. Available at: http://inspection.gc.ca/food/labelling/food-labelling-for-industry/nutrition-labelling/information-within-the-nutrition-facts-table/eng/1389198568400/1389198597278?chap=5 Accessed June 26, 2015.

Cheli, F., Pinotti, L., Rossi, L. and Dell'Orto, V. (2013). Effect of milling procedures on mycotoxin distribution in wheat fractions: a review. *LWT - Food Science and Technology,* 54:307-314.

Dall'Asta, C., Lindner, J.D., Galaverna, G., Dossena, A., Neviani, E. and Marchelli, R. (2008). The occurrence of ochratoxin A in blue cheese. *Food Chemistry*, 106:729–734.

Dhillon, B., Wiesenborn, D., Dhillon, H. and Wolf-Hall, C. (2010). Development and evaluation of a fluidized bed system for wheat grain disinfection. *Journal of Food Science*, 75:E372-E378.

Dickin, E., Steele, K., Frost, G., Edwards-Jones, G. and Wright, D. (2011). Effect of genotype, environment and agronomic management on β-glucan concentration of naked barley grain intended for health food use. *Journal of Cereal Science*, 54:44-52.

Doehlert, D.C., Zhang, D. and Moore, W.R. (1997). Influence of heat pretreatments of oat grain on the viscosity of flour slurries. *Journal of the Science of Food and Agriculture,* 74:125-131.

Duarte, S.C., Lino, C.M. and Pena, A. (2011). Ochratoxin A in feed of food-producing animals: An undesirable mycotoxin with health and performance effects. *Veterinary Microbiology,* 154:1-13.

Edwards, S.G., Dickin, E.T., MacDonald, S., Buttler, D., Hazel, C.M., Patel, S. and Scudamore, K.A. (2011). Distribution of *Fusarium* mycotoxins in UK wheat mill fractions. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment,* 28:1694-704.

European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies. (2011). Scientific opinion on the substantiation of health claim related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post-prandial glycemic responses (ID 821, 824), and "digestive function" (ID 850) pursuant to article 13(1) of regulation (EC) No 1924/2006. *EFSA Journal*, 9:2207.

Eglezos, S. (2010). Microbiological quality of wheat grain and flour from two mills in Queensland, Australia. *Journal of Food Protection,* 73:1533-1536.

Ekstrand, B., Gangby, I. and Åkesson, G. (1992). Lipase in oats-distribution, pH dependence, and heat inactivation. *Cereal Chemistry*, 69:379-381.

Emami, S., Meda, V., Pickard, M.D. and Tyler, R.T. (2010). Impact of micronization on rapidly digestible, slowly digestible, and resistant starch concentrations in normal, high-amylose, and waxy barley. *Journal of Agricultural and Food Chemistry,* 58:9793-9799.

Emami, S., Meda, V. and Tyler, R.T. (2011). Effect of micronisation and electromagnetic radiation on physical and mechanical properties of Canadian barley. *International Journal of Food Science and Technology,* 46:421-428.

Emami, S., Perera, A., Meda, V. and Tyler, R.T. (2012). Effect of microwave treatment on starch digestibility and physico-chemical properties of three barley types. *Food and Bioprocess Technology*, 5:2266-2274.

European Commission. (2006). Commission Recommendation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union,* L364:5-24.

Fadel, J.G., Newman, R. K., Newman, C.W. and Barnes, A.E. (1987). Hypocholesterolemic effects of beta-glucans in different barley diets fed to broiler chicks. *Nutrition Reports International*, 35:1049-1058.

FAO. (1997). Mycotoxins in Grain. Technical Leaflet No. 3. Available at: http://www.fao.org/wairdocs/x5008e/X5008e00.htm#Contents Accessed September 12, 2013.

FAOSTAT. (2013). Food and Agricultural Commodities Production. Available at: http://faostat3.fao.org/browse/rankings/countries_by_commodity/E Accessed May 28, 2015.

Fasina, O.O., Tyler, R.T., Pickard, M.D. and Zheng, G.H. (1999). Infrared heating of hulless and pearled barley. *Journal of Food Processing and Preservation,* 23:135-51.

Fazekas, B., Tar, A.K. and Zomborszky-Kovacs, M. (2002). Ochratoxin A contamination of cereal grains and coffee in Hungary in the year 2001. *Acta Veterinaria Hungarica,* 50: 177-188.

Ferrari, B., Finocchiaro, F., Stanca, A.M. and Gianinetti, A. (2009). Optimization of air classification for the production of β-glucan-enriched barley flours. *Journal of Cereal Science*, 50:152-158.

Gamel, T.H., Abdel-Aal, E.S.M., Wood, P.J., Ames, N.P. and Tosh, S.M. (2012). Application of the rapid Visco Analyzer (RVA) as an effective rheological tool for measurement of β-glucan viscosity. *Cereal Chemistry*, 89:52-58.

Ganßmann, W., and Vorwerck, K. (1995). Oat milling, processing and storage. In: *The Oat Crop* (pp. 369-408). Springer Netherlands.

Gates, F. (2007). Role of heat treatment in the processing and quality of oat flakes. EKT series 1380. University of Helsinki, Department of Food Technology, 69 pp.

Girardet, N. and Webster, F.H. (2011). Oat milling: specifications, storage, and processing. In: *Oats: Chemistry and Technology* (Second Edition). (pp. 301-319) St. Paul, Minnesota: AACC International, Inc.

Gómez-Caravaca, A.M., Verardo, V., Candigliota, T., Marconi, E., Segura-Carretero, A., Fernandez-Gutierrez, A. and Caboni, M.F. (2015). Use of air classification technology as green process to produce functional barley flours naturally enriched of alkylresorcinols, β-glucans and phenolic compounds. *Food Research International*, 73:88-96.

Guillamont, E.M., Lino, C.M., Baeta, M.L., Pena, A.S., Silveira, M.I.N. and Vinuesa, J.M. (2005). A comparative study of extraction apparatus in HPLC analysis of ochratoxin A in muscle. *Analytical and Bioanalytical Chemistry*, 383:570–575.

Gujral, H.S., Sharma, P., Kaur, H. and Singh, J. (2013). Physiochemical, pasting, and thermal properties of starch isolated from different barley cultivars. *International Journal of Food Properties*, 16:1494-1506.

Gujral, H.S., Sharma, P. and Rachna, S. (2011). Effect of sand roasting on beta glucan extractability, physicochemical and antioxidant properties of oats. *LWT - Food Science and Technology,* 44:2223-2230.

Hang, A., Obert, D., Gironella, A.I.N. and Burton, C.S. (2007). Barley amylose and β-glucan: Their relationships to protein, agronomic traits, and environmental factors. *Crop Science*, 47:1754-1760.

Health Canada. (2010). Summary of Comments Received on Health Canada's Proposed Maximum Limits for Ochratoxin A in Certain Foods - August 2008 to June 2009. Available at: http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/toxin-natur/summary-resume-eng.php Accessed September 12, 2013.

Health Canada. (2012a). Summary of Health Canada's Assessment of a Health Claim about Barley Products and Blood Cholesterol Lowering. Bureau of Nutritional Sciences, Food Directorate, Health Products and Food Branch.

Health Canada. (2012b). Canadian Standards (Maximum Levels) for Various Chemical Contaminants in Foods. Available at: http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/contaminants-guidelines-directives-eng.php Accessed October 10, 2013.

Hu, X., Xing, X. and Ren, C. (2010). The effects of steaming and roasting treatments on β-glucan, lipid and starch in the kernels of naked oat (*Avena nuda*). *Journal of the Science of Food and Agriculture,* 90:690-695.

Ikegami, S., Tomita, M., Honda, S., Yamaguchi, M., Mizukawa, R., Suzuki, Y., Ishii, K., Ohsawa, S., Kiyooka, N., Higuchi, M. and Kobayashi, S. (1996). Effect of boiled barley-rice-feeding in hypercholesterolemic and normolipemic subjects. *Plant Foods for Human Nutrition,* 49:317-328.

Inglis, P. and Whitworth, L. (2015). Go Barley: Modern recipes for an ancient grain. Alberta, Canada: TouchWood Editions.

Izydorczyk, M.S., Chornick, T.L., Paulley, F.G., Edwards, N.M. and Dexter, J.E. (2008). Physicochemical properties of hull-less barley fibre-rich fractions varying in particle size and their potential as functional ingredients in two-layer flat bread. *Food Chemistry*, 108:561-570.

Izydorczyk, M.S., Dexter, J.E., Desjardins, R.G., Rossnagel, B.G., Lagasse, S.L. and Hatcher, D.W. (2003). Roller milling of Canadian hull-less barley: optimization of roller milling conditions and composition of mill streams. *Cereal Chemistry*, 80:637-44.

Izydorczyk, M.S., McMillan, T., Bazin, S., Kletke, J., Dushnicky, L., Dexter, J., Chepurna, A. and Rossnagel, B. (2014). Milling of Canadian oats and barley for functional food ingredients: oat bran and barley fibre-rich fractions. *Canadian Journal of Plant Science,* 94:573-586.

Izydorczyk, M.S., McMillan, T.L., Kletke, J.B. and Dexter, J.E. (2011). Effects of pearling, grinding conditions, and roller mill flow on the yield and composition of milled products from hull-less barley. *Cereal Chemistry,* 88:375-384.

Izydorczyk, M.S., Storsley, J., Labossiere, D., MacGregor, A.W. and Rossnagel, B.G. (2000). Variation in total and soluble β-glucan content in hulless barley: Effects of thermal, physical, and enzymatic treatments. *Journal of Agricultural and Food Chemistry*, 48:982-989.

Keogh, G.F., Cooper, G.J.S., Mulvey, T.B., McArdle, B.H., Coles, G.D., Monro, J.A. and Poppitt, S.D. (2003). Randomized controlled crossover study of the effect of a highly β-glucan-enriched barley on cardiovascular disease risk factors in mildly hypercholesterolemic men. *American Journal of Clinical Nutrition,* 78:711-718.

Khatibi, P.A., Berger, G., Wilson, J., Brooks, W.S., McMaster, N., Griffey, C.A., Hicks, K.B., Nghiem, N.P. and Schmale III, D.G. (2014). A comparison of two milling strategies to reduce the mycotoxin deoxynivalenol in barley. *Journal of Agricultural and Food Chemistry,* 62:4204-4213.

Kim, Y., Lee, Y.C. and Kim, K.O. (1998). Optimum roasting and extraction conditions and flavour characteristics of roasted malt extract. *Cereal Chemistry*, 75:282-288.

Klamczynski, A.P., Knuckles, B.E. and Glenn, G.M. (2004). Puffing and jet cooking affect solubility and molecular weight of barley β-glucans. *Journal of Food Processing and Preservation*, 28:433-441.

Knuckles, B.E. and Chiu, M.M. (1999). β-glucanase activity and molecular weight of β-glucans in barley after various treatments. *Cereal Chemistry*, 76:92-95.

Kottapalli, B. and Wolf-Hall, C.E. (2008). Effect of hot water treatments on the safety and quality of *Fusarium*-infected malting barley. *International Journal of Food Microbiology,* 124:171-178.

Kottapalli, B., Wolf-Hall, C.E., Schwarz, P., Schwarz, J. and Gillespie, J. (2003). Evaluation of hot water and electron beam irradiation for reducing *Fusarium* infection in malting barley. *Journal of Food Protection,* 66:1241-6.

Laca, A., Mousia, Z., Díaz, M., Webb, C. and Pandiella, S.S. (2006). Distribution of microbial contamination within cereal grains. *Journal of Food Engineering*, 72:332-338.

Lazaridou, A., Marinopoulou, A., Matsoukas, N.P. and Biliaderis, C.G. (2014). Impact of flour particle size and autoclaving on β-glucan physicochemical properties and starch digestibility of barley rusks as assessed by *in vitro* assays. *Bioactive Carbohydrates and Dietary Fibre*, 4:58-73.

Lee, M.J., Lee, N.Y., Kim, Y.K., Park, J.C., Choi, I.D., Cho, S.G., Kim, J.G., Park, H.K., Park, K.H., Kim, K.J. and Kim, H.S. (2011). Physicochemical properties of pearled hull-less barley cultivars with normal and low amylose content. *Food Science and Biotechnology*, 20:55-62.

Li, W., Xiao, X., Zhang, W., Zheng, J., Luo, Q., Ouyang, S. and Zhang, G. (2014). Compositional, morphological, structural and physicochemical properties of starches from seven naked barley cultivars grown in China. *Food Research International*, 58:7-14.

Littell, R. C., Milliken, G. A., Stroup, W. W. and Wolfinger, R. D. 2006. SAS® System for Mixed Models. Second ed. SAS Institute Inc., Cary, NC.

Liu, Y., Bailey, T.B. and White, P.J. (2010). Individual and interactional effects of β-glucan, starch, and protein on pasting properties of oat flours. *Journal of Agricultural and Food Chemistry*, 58:9198-9203.

Liu, Y. and White, P.J. (2011). Molecular weight and structure of water soluble (1→3),(1→4)-β-glucans affect pasting properties of oat flours. *Journal of Food Science*, 76:C68-C74.

Lombaert, G.A., Pellaers, P., Roscoe, V., Mankotia, M., Neil, R. and Scott, P.M. (2003). Mycotoxins in infant cereal foods from the Canadian retail market. *Food Additives and Contaminants,* 20:494-504.

Maftei, N.A., Ramos-Villarroel, A.Y., Nicolau, A.I., Martín-Belloso, O. and Soliva-Fortuny, R. (2014). Pulsed light inactivation of naturally occurring moulds on wheat grain. *Journal of the Science of Food and Agriculture*, 94:721-726.

Manthey, F.A., Wolf-Hall, C.E., Yalla, S., Vijayakumar, C. and Carlson, D. (2004). Microbial loads, mycotoxins, and quality of durum wheat from the 2001 harvest of the Northern Plains region of the United States. *Journal of Food Protection,* 67:772-780.

McCallum, L., Paine, S., Sexton, K., Dufour, M., Dyet, K., Wilson, M., Campbell, D., Bandaranayake, D. and Hope, V. (2013). An outbreak of *Salmonella* Typhimurium phage type 42 associated with the consumption of raw flour. *Foodborne Pathogens and Disease,* 10:159-164.

McIntosh, G.H., Whyte, J., McArthur, R. and Nestel, P.J. (1991). Barley and wheat foods: Influence on plasma cholesterol concentrations in hypercholesterolemic men. *American Journal of Clinical Nutrition,* 53:1205-1209.

Miller, S.S. and Fulcher, R.G. (1994). Distribution of (1-3),(1-4)-β-D-glucan in kernels of oats and barley using micro spectrofluorometry. *Cereal Chemistry*, 71:64-68.

Murthey, K.V., Ravi, R., Bhat, K.K. and Raghavarao, K.S.M.S. (2008). Studies on roasting of wheat using fluidized bed roaster. *Journal of Food Engineering*, 89:336-342.

Nair, S., Ullrich, S.E. and Baik, B.-K. (2011). Association of barley kernel hardness with physical grain traits and food processing parameters. *Cereal Chemistry*, 88:147-152.

Nair, S., Ullrich, S.E., Blake, T.K., Cooper, B., Griffey, C.A., Hayes, P.M., Hole, D.J., Horsley, R.D., Obert, D.E., Smith, K.P., Muehlbauer, G.J. and Baik, B.-K. (2010). Variation in kernel hardness and associated traits in U.S. barley breeding lines. *Cereal Chemistry,* 87:461-466.

Narain, J.P., Shukla, K., Bijlani, R.L., Kochhar, K.P., Karmarkar, M.G., Bala, S., Srivastava, L.M. and Reddy, K.S. (1992). Metabolic responses to a four week barley supplement. *International Journal of Food Sciences and Nutrition,* 43:41-46.

Neagu, C., Maftei, N.A., Banu, I., Nicolau, A. and Aprodu, I. (2012). Effects of industrial cleaning on wheat microbial burden and deoxynivalenol levels. *Environmental Engineering and Management Journal*, 11:1857-1863.

Neil, K.P., Biggerstaff, G., MacDonald, J.K., Trees, E., Medus, C., Musser, K.A., Stroika, S.G., Zink, D. and Sotir, M.J. (2012). A novel vehicle for transmission of *Escherichia coli* O157:H7 to humans: Multistate outbreak of *E. coli* O157:H7 infections associated with consumption of ready-to-bake commercial prepackaged cookie dough-United States, 2009. *Clinical Infectious Diseases*, 54:511-518.

Newman, R.K. and Newman, C.W. (2008a). Current status of global barley production and utilization. In: *Barley for Food and Health: Science, Technology, and Products* (pp. 204-209). Hoboken, New Jersey: John Wiley & Sons, Inc.

Newman, R.K. and Newman, C.W. (2008b). Barley: genetics and nutrient composition. In: *Barley for Food and Health: Science, Technology, and Products* (pp.56-94). Hoboken, New Jersey: John Wiley & Sons, Inc.

Newman, R.K. and Newman, C.W. (2008c). Barley Processing: Methods and Product Composition. In: *Barley for Food and Health: Science, Technology, and Products* (pp. 95-132). Hoboken, New Jersey: John Wiley & Sons, Inc.

Newman, R.K. and Newman, C.W. (2008d). Health benefits of barley foods. In: *Barley for Food and Health: Science, Technology, and Products* (pp. 178-203). Hoboken, New Jersey: John Wiley & Sons, Inc.

Newman, R.K., Newman, C.W. and Graham, H. (1989). The hypocholesterolemic function of barley beta-glucans. *Cereal Foods World,* 34:883-886.

Park, J.W., Kim, E.K., Shon, D.H. and Kim, Y.B. (2002). Natural co-occurrence of aflatoxin-B1, fumonisin-B1 and ochratoxin A in barley and corn foods from Korea. *Food Additives and Contaminants,* 19:1073-1080.

Pedretti, G. and Cerrato, A. (1975). Microbiological characteristics of wheat flours. Ind. Aliment. 14:60-64.

Pérez-Vendrell, A.M., Brufau, J., Molina-Cano, J.L., Francesch, M. and Guasch, J. (1996). Effects of cultivar and environment on β-(1,3)-(1,4)-D-glucan content and acid extract viscosity of Spanish barleys. *Journal of Cereal Science*, 23:285-292.

Poppitt, S.D., Van Drunen, J.D.E., McGill, A.T., Mulvey, T.B. and Leahy, F.E. (2007). Supplementation of a high-carbohydrate breakfast with barley β-glucan improves postprandial glycaemic response for meals but not beverages. *Asia Pacific Journal of Clinical Nutrition,* 16:16-24.

Prasopsunwattana, N., Omary, M.B., Arndt, E.A., Cooke, P.H., Flores, R.A., Yokoyama, W., Toma, A., Chongcham, S. and Lee, S.P. (2009). Particle size effects on the quality of flour tortillas enriched with whole grain waxy barley. *Cereal Chemistry*, 86:439-451.

Quinde-Axtell, Z. and Baik, B.K. (2006). Phenolic compounds of barley grain and their implication in food product discolouration. *Journal of Agricultural and Food Chemistry*, 54:9978-9984.

Ragaee, S. and Abdel-Aal, E.M. (2006). Pasting properties of starch and protein in selected cereals and quality of their food products. *Food Chemistry*, 95:9-18.

Ramakrishna, N., Lacey, J. and Smith, J.E. (1991). Effect of surface sterilization, fumigation, and gamma irradiation on the microflora and germination of barley seeds. *International Journal of Food Microbiology*, 13:47-54.

Ranhotra, G.S., Gelroth, J.A., Leinen, S.D. and Bhatty, R.S. (1998). Dose response to soluble fiber in barley in lowering blood lipids in hamster. *Plant Foods for Human Nutrition,* 52:329-336

Richter, K.S., Dorneanu, E., Eskridge, K.M. and Rao, C.S. (1993). Microbiological quality of flours. *Cereal Foods World*, 38:367-369.

Rieder, A., Ballance, S. and Knutsen, S.H. (2015). Viscosity based quantification of endogenous β-glucanase activity in flour. *Carbohydrate Polymers,* 115:104-111.

Román, O.B., Sánchez, E.A., Bautista, F.J.C., Calvo, M.A.S. and Yagüe, S.L. (2010). Beta-glucan enriched products obtained from different barley milling fractions and their mixtures. *Chemical Engineering Transactions,* 21:691-696.

Rose, D.J., Bianchini, A., Martinez, B. and Flores, R.A. (2012). Methods for reducing microbial contamination of wheat flour and effects on functionality. *Cereal Foods World*, 57:104-109.

Ross, K.A. and Ames, N. (2005). The effect of micronization, milling fraction, and variety on the extractability of beta glucan for utilization in functional barley beverages. Poster session at: Whole Grains Summit, Minneapolis, MN.

Salmenkallio-Marttila, M., Heiniö, R.-L., Myllymäki, O., Lille, M., Autio, K. and Poutanen, K. (2004). Relating microstructure, sensory and instrumental texture of processed oat. *Agricultural and Food Science*, 13:124-137.

SAS Institute. 2013. SAS/STAT® 13.1 User's Guide. SAS Institute Inc., Cary, NC.

Scudamore, K.A., Baillie, H., Patel, S. and Edwards, S.G. (2007). Occurrence and fate of *Fusarium* mycotoxins during commercial processing of oats in the UK. *Food Additives and Contaminants,* 24:1374-1385.

Scudamore, K.A., Patel, S. and Edwards, S.G. (2009). HT-2 toxin and T-2 toxin in commercial cereal processing in the United Kingdom, 2004-2007. *World Mycotoxin Journal*, 2:357-365.

Sharma, P. and Gujral, H.S. (2010). Milling behaviours of hulled barley and its thermal and pasting properties. *Journal of Food Engineering*, 97:329-334.

Sharma, P., Gujral, H.S. and Rosell, C.M. (2011). Effects of roasting on barley β-glucan, thermal, textural and pasting properties. *Journal of Cereal Science*, 53:25-30.

Shaheen, B., Nadeem, M., Kauser, T., Mueen-ud-Din, G. and Mahmood, S. (2013). Preparation and nutritional evaluation of date based fiber enriched fruit bars. *Pakistan Journal of Nutrition*, 12:1061-1065.

Sinha, R.C., Savard, M.E. and Lau, R. (1995). Production of monoclonal antibodies for the specific detection of deoxynivalenol and 15-acetyldeoxynivalenol by ELISA. *Journal of Agricultural and Food Chemistry*, 43:1740-1744.

Skaug, M.A. (1999). Analysis of Norwegian milk and infant formulas for ochratoxin A. *Food Additives and Contaminants*, 16:75–78.

Smith, K.N., Queenan, K.M., Thomas, W., Fulcher, R.G. and Slavin, J.L. (2008). Physiological effects of concentrated barley β-glucan in mildly hypercholesterolemic adults. *Journal of the American College of Nutrition,* 27:434-440.

Sobrova, P., Adam, V., Vasatkova, A., Beklova, M., Zeman, L. and Kizek, R. (2010). Deoxynivalenol and its toxicity. *Interdisciplinary Toxicology,* 3:94-99.

Splittstoesser, D.F., Kuss, F.R. and Harrison, W. (1970). Enumeration of *Byssochlamys* and other heat-resistant molds. *Applied Microbiology*, 20:393-397.

Srinivasan, R. and Smith, K. (2012). Additional wet milling step for fractionation of barley flour after hull separation. *Journal of Cereal Science,* 56:248-252.

Statistics Canada. (2015). Table 001-0010: Estimated areas, yield, production and average farm price of principal field crops, in metric units. Available at: http://www5.statcan.gc.ca/cansim/a26?lang=eng&retrLang=eng&id=0010010&paSer=&pattern=&stByVal=1&p1=1&p2=35&tabMode=dataTable&csid= Accessed May 28, 2015.

Sullivan, P., O'Flaherty, J., Brunton, N., Arendt, E. and Gallagher, E. (2010b). Fundamental rheological and textural properties of doughs and breads produced from milled pearled barley flour. *European Food Research and Technology,* 231:441-453.

Sullivan, P., O'Flaherty, J., Brunton, N., Gee, V.L., Arendt, E. and Gallagher, E. (2010a). Chemical composition and microstructure of milled barley fractions. *European Food Research and Technology,* 230:579-595.

Sundberg, B. and Åman, P. (1994). Fractionation of different types of barley by roller milling and sieving. *Journal of Cereal Science*, 19:179-184.

Tekauz, A., McCallum, B., Ames, N. and Mitchell-Fetch, J. (2004). *Fusarium* head blight of oat – current status in Western Canada. *Canadian Journal of Plant Pathology*, 26:473-479.

Tekauz, A., McCallum, B. and Gilbert, J. (2000). *Fusarium* head blight of barley in Western Canada. *Canadian Journal of Plant Pathology,* 22:9-16.

Temelli, F., Bansema, C. and Stobbe, K. (2004). Development of an orange-flavored barley β-glucan beverage. *Cereal Chemistry,* 81:499-503.

Theuwissen, E. and Mensink, R.P. (2008). Water-soluble dietary fibers and cardiovascular disease. *Physiology & Behavior,* 94:285-292.

Thondre, P.S., Wang, K., Rosenthal, A.J. and Henry, C.J.K. (2012). Glycaemic response to barley porridge varying in dietary fibre content. *British Journal of Nutrition,* 107:719-724.

Tosh, S.M. (2013). Review of human studies investigating the post-prandial blood-glucose lowering ability of oat and barley food products. *European Journal of Clinical Nutrition,* 67:310-317.

U.S. Food and Drug Administration. (2006). FDA Finalizes Health Claim Associating Consumption of Barley Products with Reduction of Risk of Coronary Heart Disease. Available at: http://www.fda.gov/newsevents/newsroom/pressannouncements/2006/ucm108657.htm Accessed September 10, 2013.

Vasanthan, T. and Hoover, R. (2009). Barley starch: production, properties, modification and uses. In: Starch: Chemistry and Technology (Third Edition). (pp. 601-628). Academic Press.

Vasanthan, T. and Temelli, F. (2008). Grain fractionation technologies for cereal beta-glucan concentration. *Food Research International,* 41:876-881.

Vidal, A., Marín, S., Ramos, A.J., Cano-Sancho, G. and Sanchis, V. (2013). Determination of aflatoxins, deoxynivalenol, ochratoxin A and zearalenone in wheat and oat based bran supplements sold in the Spanish market. *Food and Chemical Toxicology*, 53:133-138.

Wang, X. (2014). The effects of processing on the nutritional characteristics of oat fibre. (Master's Thesis, University of Manitoba, Winnipeg, Manitoba, Canada). Retrieved from http://hdl.handle.net/1993/23812.

Wang, Y., Thandapilly, S.J., Harding, S.V., Tosh, S.M., Jones, P.J. and Ames, N.P. (2013). Barley β-Glucan Consumption Decreases Serum Cholesterol Levels and Increases 7 alpha-Hydroxy-4-Cholesten-3-One Levels in Moderately Hypercholesterolemic Adults. Poster session at: Experimental Biology, Boston, MA.

Whole Grains Council. (2013). Types of Barley. Available at: http://wholegrainscouncil.org/whole-grains-101/types-of-barley Accessed October 1, 2013.

Woodward, J.R. and Fincher, G.B. (1982). Substrate specificities and kinetic properties of two (1→3),(1→4)-β-D-glucan endo-hydrolases from germinating barley. *Carbohydrate Research*, 106:111-122.

Yahya, H., Linforth, R.S.T. and Cook, D.J. (2014). Flavour generation during commercial barley and malt roasting operations: A time course study. *Food Chemistry*, 145:378-387.

Yanagisawa, T., Domon, E., Fujita, M., Kiribuchi-Otobe, C. and Takayama, T. (2006). Starch pasting properties and amylose content from 17 waxy barley lines. *Cereal Chemistry*, 83:354-357.

Yoshimoto, Y., Takenouchi, T. and Takeda, Y. (2002). Molecular structure and some physicochemical properties of waxy and low-amylose barley starches. *Carbohydrate Polymers*, 47:159-167.

Zhang, D., Doehlert, D.C. and Moore, W.R. (1997). Factors affecting viscosity of slurries of oat groat flours. *Cereal Chemistry,* 74:722-726.

Zhang, M., Liang, Y., Pei, Y., Gao, W. and Zhang, Z. (2009). Effect of process on physicochemical properties of oat bran soluble dietary fiber. *Journal of Food Science,* 74:C628-C636.

Zhang, G., Ma, L., Patel, N., Swaminathan, B., Wedel, S. and Doyle, M.P. (2007). Isolation of *Salmonella* Typhimurium from outbreak-associated cake mix. *Journal of Food Protection*, 70:997-1001.

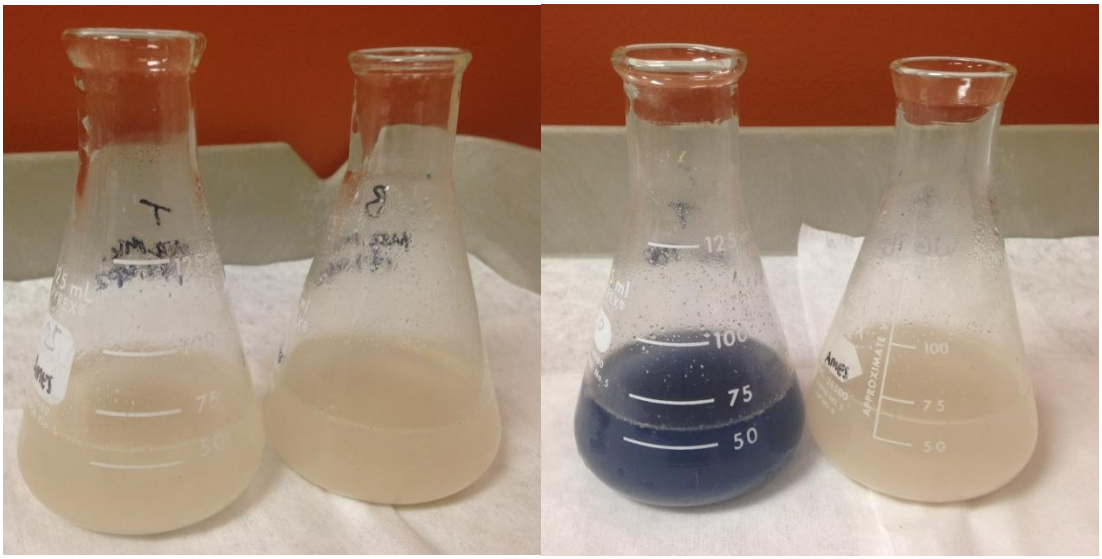
Zheng, G.H., Rossnagel, B.G., Tyler, R.T. and Bhatty, R.S. (2000). Distribution of β-glucan in the grain of hulless barley. *Cereal Chemistry*, 77:140-144.

Zhou, M., Robards, K., Glennie-Holmes, M. and Helliwell, S. (1999). Analysis of volatile compounds and their contribution to flavor in cereals. *Journal of Agricultural and Food Chemistry*, 47:3941-3953.

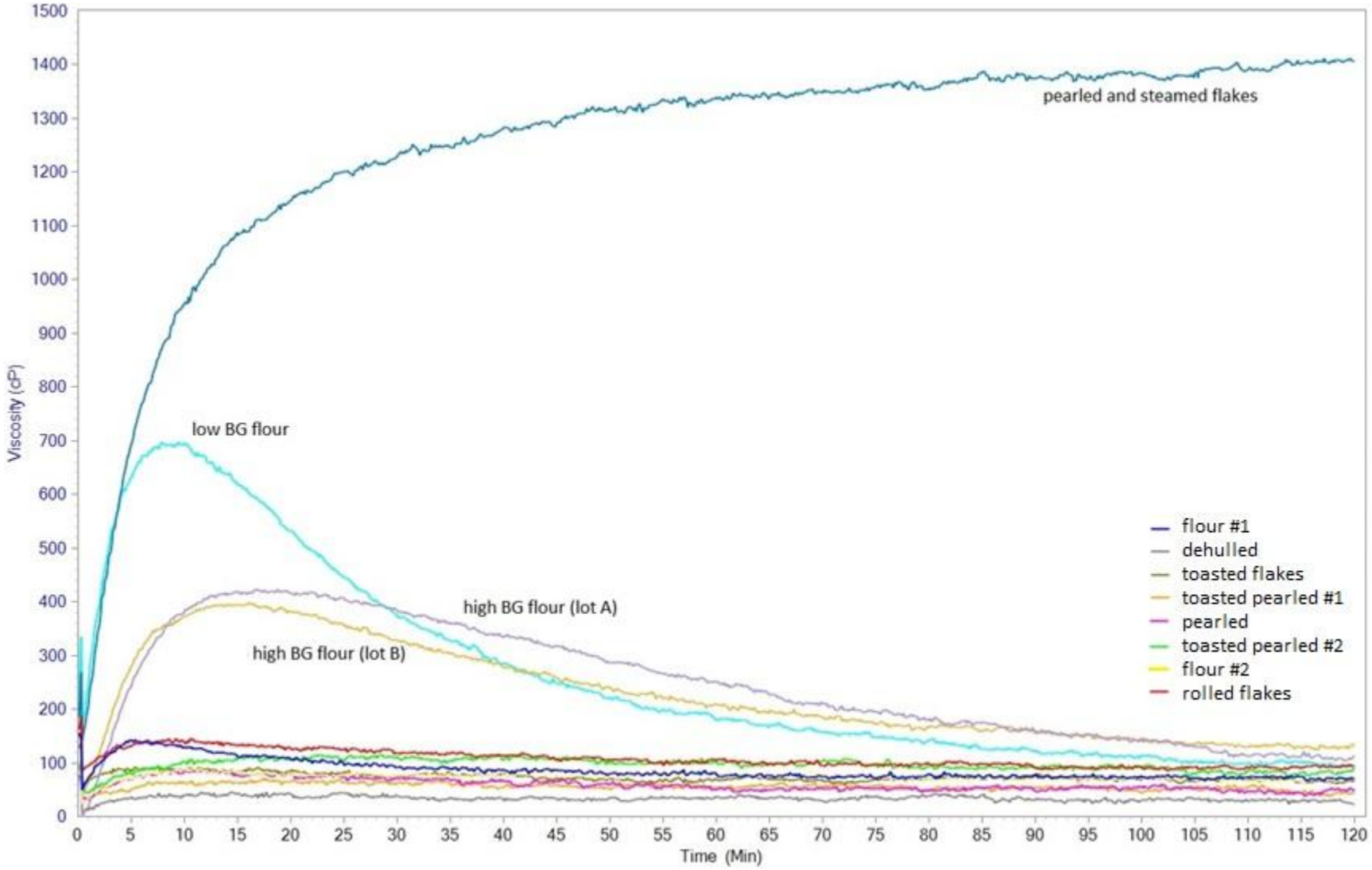
Zhou, M., Robards, K., Glennie-Holmes, M. and Helliwell, S. (2000). Contribution of volatiles to the flavour of oatmeal. *Journal of the Science of Food and Agriculture,* 80:247-254.

7. APPENDICES

Appendix A – Example of negative (left) and positive (right) peroxidase activity reaction



Appendix B – RVA profiles of 2 h *in vitro* digest extractions of commercial barley samples



Appendix C – Summary of ANOVA for β-glucan (BG) results with contrasts

	P-values					
Effect	BG content	RVA BG Viscosity	Rheometer BG Viscosity	BG MW - Mp	BG MW - Mw	BG Solubility
Cultivar	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Treatment	0.010*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Micronize vs. untreated	0.812	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.116
Roast vs. untreated	0.317	0.586	0.608	0.231	0.191	< 0.001*
Conditioned vs. untreated	0.043*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Tempered vs. not tempered	0.418	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Cultivar x Treatment	0.434	< 0.001*	< 0.001*	0.001*	0.002*	< 0.001*
Fibar: micronize vs. untreated	0.302	0.008*	0.115	0.030*	0.026*	0.008*
Fibar: roast vs. untreated	0.598	0.771	0.852	0.927	0.909	< 0.001*
Fibar: conditioned vs untreated	0.020*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Fibar: Tempered vs. not tempered	0.877	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.008*
McGwire: micronize vs. untreated	0.547	0.946	0.889	0.646	0.517	0.032*
McGwire: roast vs. untreated	0.109	0.973	0.952	0.906	0.727	0.286
McGwire: conditioned vs untreated	0.699	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
McGwire: Tempered vs. not tempered	0.094	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Rattan: micronize vs. untreated	0.397	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.045*
Rattan: roast vs. untreated	0.539	0.493	0.521	0.050	0.050	< 0.001*
Rattan: conditioned vs untreated	0.414	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Rattan: Tempered vs. not tempered	0.855	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.084

*Significant effect at P (<0.05)

Viscosity, molecular weight and solubility values from 2 hour BG extraction data

Contrasts represent the comparison of multiple means

RVA = Rapid Visco Analyser; MW = molecular weight; Mp = peak molecular weight; Mw = weighted average molecular weight

Appendix D – Effects of heat treatments on starch pasting properties of different barley cultivars without lichenase addition

Cultivar	**Treatment**	**Peak** (cP)	**Breakdown** (cP)	**Final** (cP)	**Setback** (cP)	**Pasting Temp.** (°C)	**Peak Time** (s)
Fibar	Micronize (T)[1]	6084 b	3214 a	4713 c	1843 ab	50.1 d	3.78 bc
	Micronize (N)[2]	5650 c	2863 b	4430 d	1642 c	55.6 c	3.80 bc
	Roast (T)[1]	6248 ab	2671 c	5464 a	1887 a	65.9 a	3.87 b
	Roast (N)[2]	5709 c	2292 d	5219 b	1802 ab	65.5 a	3.73 c
	Conditioned	6419 a	2906 b	5267 b	1754 bc	60.2 b	4.22 a
	Untreated	4549 d	2475 d	3512 e	1438 d	55.4 c	3.78 bc
McGwire	Micronize (T)[1]	5324 a	1839 c	5840 a	2355 b	67.1 a	6.03 a
	Micronize (N)[2]	5327 a	2105 b	5669 b	2447 ab	66.8 a	5.88 b
	Roast (T)[1]	4616 c	1885 c	5183 d	2452 ab	68.6 a	5.70 c
	Roast (N)[2]	4536 c	2004 bc	5021 e	2489 a	69.1 a	5.63 c
	Conditioned	4726 c	1647 d	5420 c	2341 b	69.5 a	5.95 ab
	Untreated	5020 b	2305 a	5110 de	2395 ab	66.1 a	5.89 b
Rattan	Micronize (T)[1]	7490 a	4924 a	4035 c	1470 b	59.6 a	4.24 b
	Micronize (N)[2]	7224 b	4784 a	3902 c	1462 b	64.5 b	4.14 b
	Roast (T)[1]	7232 b	4139 b	4806 a	1714 a	67.0 b	4.38 a
	Roast (N)[2]	7127 b	4095 b	4862 a	1829 a	66.9 b	4.35 a
	Conditioned	7131 b	4164 b	4309 b	1342 c	67.5 b	4.45 a
	Untreated	5539 c	3483 c	3396 d	1340 c	65.2 b	4.35 a
	LSD (0.05)	215	186	134	116	3.4	0.10

[1]Barley tempered to 17% moisture prior to treatment

[2]Barley was not tempered prior to treatment

Values reported as means within a treatment and cultivar

Values within same column and cultivar followed by same letter are not statistically significant (LSD, $P \geq 0.05$)