Effect of Pre-processing on the Nutritive, Physical, and Sensory Properties of Proso Millet

by

Abir Sarker

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ABSTRACT

EFFECT OF PRE-PROCESSING ON THE NUTRITIVE, PHYSICAL, AND SENSORY PROPERTIES OF PROSO MILLET

Abir Sarker
University of Guelph, 2015

Advisor: Dr. Lisa Duizer

Millet is a small seeded grain that is highly nutritious, gluten free and has a low glycemic index. However, it is underutilized in North America as consumers find the taste unappealing. This study examined the use of pre-processing on proso millet and its effect on the nutritive, physical, and sensory properties. Germination maintained the nutritive value of millet. The expected glycemic index increased as rate of germination increased, from 46.5-60.8. Descriptive analysis showed decortication led to mild flavour. The germinated samples were rated highest for sweetness; however this was not enough to mask the bitterness, which also increased. Phenolic content in decorticated millet significantly reduced (p>0.05), whereas they increased in the germinated samples. This could have contributed to the stronger bitter taste perceived. Germination was not successful in improving the flavour of millet, however decorticated millets flavour was enhanced by reducing negative sensory properties.
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CHAPTER 1: INTRODUCTION

With the increasing prevalence of celiac disease and type II diabetes mellitus in today’s society, there is greater interest in studying different grains that may be nutritionally beneficial for individuals with these health issues. Millet is a type of cereal grain that has gained recognition as a healthy alternative to more prominent grains on the market (Omary, Fong, Rothschild, & Finney, 2012). It is gluten free, low on the glycemic index and a rich source of protein, fat and minerals (Kalinova & Moudry, 2006). Millet is an important crop in semiarid and tropical regions, such as Africa and Asia, as it produces sustainable yields in drought-like environments (Devi, Vijayabharathi, Sathyabama, Malleshi, & Priyadarisini, 2014).

Recently, millet has grown in significance in North America, with production occurring in eastern Colorado and western Nebraska (Ragaee, Abdel-Aal, & Noaman, 2006). Delhi, Ontario has also begun producing millet for the Ontario region. However, most of the millet produced in North America is used as bird and poultry feed. Current studies focus mostly on the major millet varieties of pearl and finger millet. Little research has been conducted on the minor millet variety, proso. As this is a variety grown in North America and more accessible to North American food processors than those grown in other regions of the world, more research is needed to better understand proso millet and its functionality.

Pre-processing techniques, such as germination and decortication, have traditionally been used in households to create specific food products. These pre-processing methods have been shown to improve nutritive value, enhance bioavailability of minerals, as well as improve the sensory properties of millet. Although this has been studied in millet common to African and Asian countries, proso millet, common in North America has not been extensively studied.
Therefore, the objective of this research was to observe the effects of pre-processing on the nutritive, physical and sensory properties of proso millet.

The aim of germination is to activate hydrolytic enzymes that can structurally modify grain components such as starch and protein and synthesize new compounds (Singh, Rehal, Kaur, & Jyot, 2013). Limited research has been conducted to study the effect of germination of proso millet on physical and nutritive properties, especially in vitro starch digestibility. Most digestibility studies have used millet composite flours and the effect of 100% millet products on blood glucose levels is not well understood. Chapter 3 examines the use of various conditions for germination and how this affects the starch digestibility of millet.

Decortication involves the removal of the pericarp and germ of the grain, which leaves only the endosperm. As most phytates and polyphenols are located in the pericarp and aleurone layers of millet, decortication drastically reduces these antinutrient levels (Saleh, Zhang, Chen, & Shen, 2013). Germination has also been used as a method for reducing antinutrients in millet (Chethan, Sreerama, & Malleshi, 2008). Antinutrients are associated with negative sensory properties and their reduction can lead to a more acceptable food product. There has not been extensive research conducted evaluating the sensory properties of millet and many studies use consumer testing to measure liking of the product. Chapter 4 investigates the effect of decortication and germination on the taste and flavours of proso millet. Descriptive analysis was used to identify the specific sensory attributes that are found in decorticated and germinated millet.
CHAPTER 2: LITERATURE REVIEW

2.1 Millet and its Production

The name given to millet is a broad term applied to several small seeded grains from several genera of Poaceae family of grasses. There are many species and varieties of millet grown around the world. These include proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*), kodo millet (*Paspalum scrobiculatum*), little millet (*Panicum sumatrense*) and pearl millet (*Pennisetum glaucum*) (Singh & Raghuvanshi 2012).

Millets are some of the oldest crops and have been cultivated for thousands of years. It has been recorded that crops of proso and foxtail millet were grown from 2000 to 1000 BC in China (Oelke et. al. 1990). Foxtail is the oldest of the millet varieties and originated in southern Asia. Proso millet began growing in the United States in the 18th century when it was brought over from Europe. Growth in North Dakota has ranged from 50,000 to 100,000 acres, with a few thousand acres in Minnesota (Oelke et. al. 1990). Proso millet is also being grown in Ontario, Canada. Of the many types of millet grown, pearl millet is the most widely produced type of millet worldwide.

Approximately 90% of the world’s millet production is being used in the developing nations. The remaining 10% is being used by developed countries mostly as livestock feed (FAO 1990). India is the largest producer of millet with 38.6% of the world’s millet being grown. Pearl millet accounts for two-thirds of this production. Millet is mostly grown as a subsistence crop for the local market, with some produced for the commercial market in India (FAO 1995). It ranks as the sixth most important cereal crop in terms of production, worldwide (Saleh et al., 2013).
Millet is a staple in the diets of those from poorer regions of the world. It is deemed “the poor man’s grain” and is often stigmatized for this reason. However, it has played a major role in food security in Africa and Asia. Millet is a short season crop that is usually ready to sow in 60 to 90 days. Millet is adaptable to various types of soil and climates and requires little water and tending for optimal growth. This ability to grow in dry and unfertilized soil conditions makes millet relatively drought resistant. Interest in drought resistant crops is growing as water scarcity and climate change becomes an issue (Ashraf, Ashfaq, & Ashraf, 2002; Baltensperger, 2002; Saleh et al., 2013). They can survive with less than 300 mm of rainfall compared to corn, which needs a minimum of 600 mm (Léder 2004). Millet is considered to be more nutrient dense than more popular grain varieties available in North America, such as rice, wheat and corn (Asharani, Jayadeep and Malleshi, 2010). It is underutilized in the West because of it unappealing flavour. However, it is a gluten free alternative with a low glycemic index (Mani et al., 1993), both of which are appealing qualities in the North American food industry. More research on varieties of millet is needed to get a better understanding of its nutritional and health benefits.

2.2 Physiology of Common Millet Types

There are many varieties of millet found around the world. The basic structure of the grain is similar among all millet types, including a pericarp, endosperm and germ. The pericarp is the outermost layer of the grain and aids in the control of water loss during growth. It is the protective layer that forms around the endosperm and germ of the seed (Bradbury, MacMasters, & Cull, 1956). Fibres are mainly concentrated into the outer layers of the grain. The endosperm is made up of two layers, the starchy endosperm and the aleurone layer. The starchy endosperm is the largest component of the grain and consists of peripheral, floury and corneous zones. It is mostly comprised of starch granules within a matrix of protein bodies. The aleurone layer
surrounds the starchy endosperm, with concentrations of protein, lipids, vitamins and minerals. The germ of the grain includes the embryo and scutellum. The germ is rich in lipids, proteins and minerals (Evers & Millar, 2002).

The size, structure and shape of the grain can vary depending on the type of millet and where it was grown. Within millet types, cultivars can differ based on production region, breeding and genetics (Colosi & Schaal, 1997). Pearl millet is about 2 mm in length and has a 1000 kernel average weight of 8 grams. They tend to have an ovoid shape. The seed coat of this millet is 0.4 µm in thickness. In pearl, the pericarp is caryopsis type, meaning the pericarp is completely bound to the endosperm. Pearl millet characteristically has a larger germ (Taylor 2004). The endosperm to germ ratio of pearl millet is 4.5:1. Pearl has a larger floury than corneous zone which creates a softer texture. The starch granules in the corneous zone of the endosperm are polyhedral in shape and about 6.4 µm in size. The starch granules found in the floury zone are spherical and larger than those found in the corneous at 7.6 µm. Starch that is larger is hydrolyzed more slowly (FAO, 1995).

Finger millet is a small grain about 2 mm long and globose in shape. The 1000 kernel weight of this millet is 2.6 grams. It has a utricle type of pericarp, meaning the pericarp is loosely bound to the endosperm at one point and breaks away quite easily. Finger millet differentiates from other types by the 5 layers of seed coat, creating a greater thickness of 10.8-24.2 pm (Baltensperger 2004). The endosperm to germ ratio of finger is 11:1. Therefore, the germ is larger in comparison to proso but much smaller than that of pearl. The starch granules in the corneous region are 3-19 µm and those in the floury zone are 11-21 µm (FAO, 1995).
The shape of proso is usually oval and about 3 mm in length and 2 mm wide. It has a 1000 kernel weight of 4.7 to 7.2 grams, depending on the variety. The pericarp in proso is a utricle type, similar to finger millet. The seed coat is 0.2-0.4 µm in thickness. The endosperm to germ ratio of proso is 12:1 (Baltensperger 2004). Proso millet has similar sizes of floury and corneous zones, making the texture in between soft and hard (FAO 1995). The starch granules in both the floury and corneous zones range from 4.1-8.0 µm in size. The starch granules are mostly small spherical or large polygonal in shape. Large spherical granules also exist but are rare (Kumari & Thayumanavan, 1998).

2.3 Nutritive Value of Millet

Millet has been used in developing nations to solve problems of food insecurity and malnutrition. Along with its appealing drought resistance and short growing season, millet is also a nutritionally dense food (Saleh et al., 2013). It is often ground into flour and used to make porridge, roti and beverages. The nutrient composition of millet can vary by type and variety. Nutritionally, it has been found to be superior when compared to other common grains (Parameswaran & Sadasivam, 1994). It is a good source of protein, fat, fibre and many micronutrients (Léder, 2004). The use of millet worldwide is important as it feeds many low income families and provides proper nutrition to those in need.

2.3.1 Nutrient composition

Protein content of millet ranges from 7.7-12.5% in varieties of proso, pearl and finger. Kumar & Parameswaran (1998) found that millet contains five protein fractions. These include Fraction I: albumin+globulin, Fraction II: true prolamin, Fraction III: prolamin-like, Fraction IV: glutenin-like, and Fraction V: true glutenin. Prolamin is considered to be the fraction directly associated with protein quality and is the most prevalent fraction in millet (FAO, 1995). The
albumin+globulin content ranges from 22.6-26.6% and 17.3-27.6% in pearl millet and finger millet, respectively. Prolamin ranges from 22.8-31.7% in pearl millet and is the highest in finger millet with 24.6-36.2%. Glutenin content is the lowest protein fraction with 16.4-19.2% in pearl and 12.4-28.2% in finger millet (FAO, 1995). Millets have been found to contain high levels of essential amino acids, particularly sulphur containing amino acids of methionine and cysteine (Amadou, Gounga, & Le, 2013). In comparison to sorghum and maize, pearl millet contains higher amounts of lysine (Ejeta, Hassen, & Mertz, 1987).

Lipids are concentrated in the germ, pericarp and aleurone layers of the millet grain. Free lipids in many varieties of millet have been found to range from 2.8-8.0% (Rooney, 1978). Pearl millet has a free lipids content range of 5.6-7.1% and bound lipids range of 0.57-0.90% (Lai & Varriano-Marston, 1980). The free lipid fraction is comprised of hydrocarbons, triglycerides, monoglycerides, diglycerides and free fatty acids, while the bound lipid fraction is made up of lecithin and other components but no free fatty acids (Rooney, 1978). The free lipid content of pearl millet is high in unsaturated fatty acids, accounting for 70.3% of the total free lipid content (Lai & Varriano-Marston, 1980). The main fatty acids found in free lipids are linoleic, oleic and palmitic (Lai & Varriano-Marston, 1980; Rooney, 1978).

The dietary fibre in millet is found mostly in the outer pericarp and decreases in quantity to the endosperm of the grain. Finger millet is known to have higher than average dietary fibre content. It contains about 72% carbohydrates and the non-starchy polysaccharides amount to about 15-20% of seed matter as dietary fibre (Devi et al., 2014). Dharmaraj & Malleshi (2011) investigated the dietary fibre content of finger millet and found the soluble dietary fibre content to be 1.4% and insoluble dietary fibre levels to be 15.7%. In comparison to many other cereal grains, the soluble dietary fibre content of pearl millet has been shown to be 1.45% and an
insoluble fibre content of 13.5% (Ragaee et al., 2006). The total dietary fibre of millet is higher than that of wheat; however it is lower than the values of rye and sorghum grains (Ragaee et al., 2006).

Finger millet has a total carbohydrate content ranging from 72-79.5%. Starch comprises between 59.4 and 70.2% of the carbohydrate content of millet with the majority of this being amylopectin. For instance, about 80-85% of starch is amylopectin and 15-20% making up the amylose content (Singh & Raghuvanshi, 2012). The free sugars in millet include glucose, fructose and sucrose. Sucrose is the main sugar found in the highest amounts in millet, ranging from 60 to 68% of the total free sugars (Malleshi and Desikachar 1986; Subramanian, Jambunathan, and Suryaprakash 1981). The total free sugars in pearl millet range from 2.16-2.78%. Glucose and fructose contents range from 3.2 to 6.3% of the total sugars in pearl millet (Subramanian et al., 1981).

2.3.2 Starch Digestibility

There is evidence that healthy plant based diets can be beneficial to those with type 2 diabetes. Millets have been shown to have a low effect on blood glucose levels based on low starch digestibility. In comparison to rice and wheat, consumption of a whole finger millet diet produces the lowest plasma glucose levels, with the rice diet producing the highest effect on blood glucose (Kumari & Sumathi, 2002). Glycemic index studies involving biscuits made with foxtail millet-refined wheat composite flour and burfi made with a foxtail millet-gram flour mix showed that both the biscuits and burfi were low GI products and were able to significantly reduce serum glucose levels compared to baseline (Singh, Srivastava, & Thathola, 2011). Another study using a composite flour of millet and legumes (bengal, green and black gram) lowered blood glucose levels in healthy subjects (Shobana, Kumari, Malleshi, & Ali, 2007).
There are studies examining the effects that composite flours containing millet have on blood glucose levels (Kumari & Sumathi, 2002; Shobana et al., 2007; Singh et al., 2011). However, there is limited research on the *in vivo* and *in vitro* starch digestibility of 100% whole grain millet products. Whole grain products have been shown to have lower glycemic index values compared to refined products. Whole grains are high in dietary fibres and resistant starch, which can delay carbohydrate absorption, leading to lower starch digestibility (Hernot, Boileau, Bauer, Swanson, & Fahey, 2008; Kumari & Sumathi, 2002).

After ingestion, starch is hydrolyzed by amylases and α-glucosidases before absorption and entering blood circulation. Phenolic acids can work as non-competitive inhibitors of amylase and α-glucosidase, regulating glucose uptake in the body. Prevention of carbohydrate absorption after intake is an approach for decreasing postprandial hyperglycemia (Shobana, Sreerama, & Malleshi, 2009).

2.3.3 Antinutrients

Millets contain antinutrients, including polyphenolics, phytic acid and tannins, that can interfere with the bioavailability of some nutrients and minerals. Polyphenolics are located in the pericarp and endosperm of all millets. Ferulic acid is the major phenolic acid found in millets (Dykes & Rooney, 2006). Phenolic content is higher in millet than in wheat. Total phenolic content of wheat ranges from 500-560 µg/g, whereas millets contain approximately 1387 µg/g phenolic content (Ragaee et al., 2006). Phenolics also have an antioxidative capacity. They help prevent free radical formation in the body and can prevent undesirable changes in flavour and nutrients (Pushparaj & Urooj, 2014; Subba Rao & Muralikrishna, 2002). Proso millets contain 6.3-4.2 mM TE/g of total antioxidant capacity, which is superior to other cereal contents. Finger
millet has a larger range with an average of 15.3 mM TE/g (Asharani, Jayadeep, & Malleshi, 2010).

Phytic acid is present in all cereal grains. However, all grains contain different levels of phytates. These phytates tend to bind to certain minerals, including iron, decreasing their absorption by the body. Although phytic acid levels are slightly higher in wheat, millets still contain levels that are high enough to decrease mineral bioavailability (Simwemba, Hoseney, Varriano-Marston, & Zeleznak, 1984). Millets also contain tannins that tend to bind to proteins and carbohydrates. Consequently, this reduces the digestibility of the nutrients within the body. According to Ramachandra, Virupaksha, & Shadaksharaswamy (1977), tannin levels are higher in those millets with a darker coat.

In addition to their antinutrient properties, phenolic acids are associated with the sensory properties of millet and can directly contribute to undesirable tastes and aromas (Ho, Lee, & Huang, 1992). Off flavours, such as bitterness and astringency, occur as a result of high levels of phenolic acids within the millet grain (Sosulski, Krygier, & Hogge, 1982). These flavours tend to be unappealing to North American consumers.

2.4 Pre-processing Methods

There are many pre-processing methods that have been used to change the nutritional value and sensory properties of millet. They aim to increase nutrients, decrease antinutrients and make micronutrients more bioavailable within the body. Soaking, fermentation, germination and decortication are some traditional pre-processing methods that have been used for many years (Saleh et al., 2013).
2.4.1 Decortication

The process of decortication of millet involves the removal of the pericarp and germ of the grain, leaving only the endosperm. There are two types of pericarp, caryopsis and utricle. Utricle pericarps are loosely bound at one end and easily removed with abrasion. Caryopsis pericarps, on the other hand, are completely bound to the endosperm, making them difficult to remove (FAO, 1995). The endosperm of millet is made up of a large floury zone and crumbles into grits when the pericarp is removed (Shobana & Malleshi, 2007). Hydrothermal pre-processing techniques, such as parboiling, have been employed in order to harden the endosperm before removal of the seed coat (Dharmaraj, Ravi, & Malleshi, 2013). The use of dry milling has also been applied to millet, removing the pericarp through abrasion. Anderson (2014) successfully tested dry milling procedures at a commercial level and produced a fully intact endosperm that yielded a high grit size.

Decortication removes the pericarp and germ of a grain which can drastically change the nutrient profile. Dharmaraj & Malleshi (2011) decorticated finger millet and assessed the changes that occurred in the compositional properties. The protein and ash content decreased from 7.0% to 4.4% and from 2.0% to 1.0%, respectively. The protein fraction of prolamin drastically decreased after decortication from 1.26% to 0.35%. Decortication increased the amount of carbohydrate from 61% to 73% and the insoluble fibre present from 1.4% to 2.3%. Approximately 30-40% of the nutrients are found within the seed coat of millet. Therefore, the removal of the seed coat will decrease the nutritional value but increase the starch content. The pericarp is also mostly made up of cellulose and its removal decreases insoluble fibre but increases soluble fibre in millet (Dharmaraj & Malleshi, 2011).
Decortication can lead to a reduction in the antinutrient content found within millet, which can improve the flavour profile of the grain. Lestienne, Buisson, Lullien-Pellerin, Picq, & Trèche (2007) found that decortication led to decreasing amounts of phytates, phenolics and fibres; however this did not lead to greater bioavailability of iron and zinc. This reduction could be due to the location of the antinutrients and nutrients in the outer layers that are removed during decortication (Saleh et al., 2013). The seed coat of millet gives off a musty odour and chewy texture that can lead to low acceptability amongst consumers. Much of the phenolics are found in the pericarp of millet, which can directly contribute to off flavours and aromas (Ho et al., 1992). Sensory testing of decorticated and cooked finger millet showed that the millet imparted a very mild flavour which consumers rate as like moderately on a 9-point hedonic scale (Dharmaraj, Ravi, & Malleshi, 2014). Therefore, decortication can be a means of creating a more acceptable product and therefore increasing the utilization of millet (Shobana & Malleshi, 2007).

2.4.2 Germination

The terms ‘germination,’ ‘malting’ and ‘sprouting’ are all used interchangeably to describe a pre-processing method used for grains. Germination has been shown to improve the nutritional content of grains and decrease the antinutrients that may be present (Mbithi-Mwikya, Van Camp, Yiru, & Huyghebaert, 2000). It is a natural pre-processing method that has gained new interest as a practical way of increasing the health benefits of whole grains, while improving palatability. Germination can change the physicochemical properties of a grain, however these changes can vary between seed varieties and the germination conditions the grains are exposed to (Nelson, Stojanovska, Vasiljevic, & Mathai, 2013).

The first phase of germination is breaking the seed’s dormancy. About 30-35% moisture content in the millet grain is required for this to occur (Malleshi & Desikachar, 1986). Steeping
the mature seeds in water is essential for them to be able to imbibe water and break dormancy. The grain then undergoes many processes and resumes metabolic activity and respiration during phase two. Many enzymes that are present within the dry seed become activated, including α-amylase which hydrolyzes starch (Helland, Wicklund, & Narvhus, 2002). Germination is completed after radicle elongation occurs in phase three (Bewley, 1997). It is a method performed at the household and industrial level in India and African nations. Generally, steeping occurs for 24 hours in order to imbibe enough water to increase moisture content. The seeds are then laid in a single layer in controlled conditions for at least 24 hours. They are then dried at 50°C and the root growth is removed manually by gentle brushing (Swami, Thakor, & Gurav, 2013).

According to Garcia-Huidobro, Monteith, & Squire (1982), the leading factor that determines how well germination occurs is temperature. It has been shown that the rate of germination increases with temperature, however there is a narrow range of optimal temperature. Their results show that sprouting conditions are best between the temperatures of 18-38 °C (Garcia-Huidobro et al., 1982). The second most important variable for germination is time. The yield of viable germinated seeds decreased with increased sprouting time (Badau, Nkama, & Jideani, 2006). Those seeds that require an increased amount of time to germinate will usually not survive as a crop (Garcia-Huidobro et al., 1982). The most ideal germination time is 72 hours. The least amount of sprouting occurs at the 24 hour mark as the millet seeds do not have enough time to fully germinate (Badau et al., 2006).

2.4.3 Effects of Germination on Nutritional Properties of Millet

Germination alters nutritional properties of millet, specifically protein, carbohydrate and antinutrient content. Protein content of millet has been shown to increase after germination. This
could be attributed to dry matter loss that occurs during germination. Parameswaran & Sadasivam (1994) saw an increase of protein content in proso millet from 12.32% to 14.30% after seven days, while Swami et al. (2013) also found protein content to increase from 14% to 17.5% after 24 hours of germination of finger millet. During this process, degradation of the protein matrix that surrounds starch granules occurs. The protease enzyme becomes activated after imbibition and protein changes follow through proteolysis and transamination (Belton & Taylor, 2004). The conversion to soluble peptides and amino acids can help to synthesize proteins within the embryo (Swami et al., 2013).

Through proteolysis, amino acids also become more available. Total free amino acid has been shown to increase from 0.086% to 2.64% after seven days of germinating proso millet. Lysine and methione also increased from 1.64% to 4.40% and 2.0% to 2.12%, respectively (Parameswaran & Sadasivam, 1994). Lysine is the limiting essential amino acid within millet grains and germination has been used as a method to increase its content (Malleshi & Klopfenstein, 1998; Parameswaran & Sadasivam, 1994).

The carbohydrates in millet are mainly starch. Starch is a storage polysaccharide that is made up of two parts, amylose and amylopectin. Amylose is a polymer that is made of α-1,4 glucan linkages, whereas amylopectin is a linear chain of α-1,4 glucan linkages with branched α-1,6 linkages every 24-30 glucose units. Starch degradation can occur in the presence of cereal amylases (Muralikrishna & Nirmala, 2005). During germination, α-amylase is synthesized by a plant growth hormone called gibberellic acid (GA3) that becomes activated during this process (Muralikrishna & Nirmala, 2005). Gibberellic acid is released into the endosperm and new enzymes are synthesized in the aleurone layer (Zarnkow, Mauch, Back, Arendt, & Kreisz, 2007). Once α-amylase is synthesized, it works to breakdown starch polymers. The enzyme attacks the
surface of starch and begins to tunnel inside the granule. Then, α-amylase hydrolyzes the starch from the inside (Zarnkow et al., 2007). It randomly hydrolyzes α-1,4 linkages of amylose and amyllopectin to release monosaccharides and polysaccharides. Glucose is the major sugar released, along with maltose and α-limit dextrins. This action degrades starch granules, thereby reducing water binding capacity and leading to low viscosity (Helland et al., 2002).

During germination, starch granules are hydrolyzed by the action of α-amylase, leading to a release of free sugars. Raw finger millet containing 1.5% total free sugars showed an increase in total free sugars to 16.0% after four days of germination (Nirmala, Subba Rao, & Muralikrishna, 2000). Concurrent with this sugar increase is a reduction in starch content. Starch content of germinated finger millet has been shown to decrease from 65% to 43% after 4 days (Nirmala et al., 2000). As a result of this starch degradation, Mbithi-Mwikya et al. (2000) found that the viscosity of germinated finger millet drastically decreased after two days of germination.

Germination has been shown to have contradictory effects on the antinutrients present in grains. Many studies on wheat, barley and rice have found that germination can both increase and decrease antinutrient values (Nelson et al., 2013). Studies involving germination of finger millet have shown a phenolic content reduction of 44% after one day of germination (Chethan et al., 2008). Specific phenolic acids that have been affected by germination include protocatechuic acid, showing a threefold decrease and caffeic acid, with a fourfold decrease (Subba Rao & Muralikrishna, 2002). Free phenolic acid contents of coumaric, gallic and ferulic acids have been shown to drastically increase after four days of germination, while the bound phenolic acid contents of caffeic, coumaric and ferulic acids decreased twofold after four days (Subba Rao & Muralikrishna, 2002). Germination has provided an effect on the phenolic content of grains, however there is no clear trend as to how it is affected.
The reduction of antinutrients, such as phenolic acids, tannins and phytates due to germination increases the bioavailability of minerals. Phenolics and phytates are known to bind minerals and as their concentration decreases, minerals become more readily available. Micronutrient deficiencies are prevalent in developing countries, especially in iron, zinc and calcium, and germination has been utilized as a method of increasing the bioavailability of these nutrients in plant foods. Platel et al. (2010) compared the mineral content of brown and white finger millet after germination. The mineral content for both varieties was not affected by pre-processing, with only slight but not significant decreases observed. However, the bioaccessibility of calcium, iron, copper and manganese increased. The largest increase was seen in the iron levels of both varieties, showing a fourfold increase in bioavailability. Hemalatha, Platel, & Srinivasan (2007) germinated finger millet for two days and found that as tannin and phytate levels decreased, the bioavailability of iron increased by 20%. More research involving proso millet is required in order to determine how pre-processing can affect the antinutrients present.

2.4.4 Effect of Germination on Sensory Properties of Millet

There are few studies examining the effect of germination of millet on its sensory properties and those tests that have been conducted have only examined consumer liking. Inyang & Zakari (2008) germinated pearl millet for two days and made a traditional Nigerian cereal called instant fura. Various sensory attributes were tested using a 7-point hedonic scale. The germinated fura sample was rated higher than native millet in colour, texture and colour. It was rated highest amongst all samples for overall acceptability of the product and indicated its preference. Balasubramanian, Kaur, & Singh (2014) prepared a weaning formula using variations of pearl millet and barley extrudates in combination with pearl millet and barley malted flour. A 9-point hedonic scale was used to test for overall acceptability of the product.
Acceptability ranged from like moderately to like very much and those formulas using a higher concentration of malted pearl millet flour were rated on the higher end of the scale.

Phenolic acids have been known to contribute to the bitterness and astringency of a product (Drewnowski & Gomez-Carneros, 2000). Sorghum varieties that contained a higher phenolic content were also rated higher for bitterness and astringency using a trained panel (Kobue-Lekalake, Taylor, & de Kock, 2007). Whether germination increases or decreases the amount of phenolic acids present in millet, there will be an apparent effect on the sensory properties. The increase of free sugars attributed to germinated grains can also play a role in the flavour profile, making the sample sweeter. Current studies have yet to show whether this increase of free sugars can help to mask the off flavours associated with germinated proso millet.

2.5 Conclusion

Millets are an underutilized grain that offers many health benefits to consumers. They are gluten free grains that are low on the glycemic index, which are valuable characteristics in the North American food industry. They are known to be nutritionally comparable or superior to more prominent grains on the market; however they have received less attention. More current research is needed in this area to generate interest in millet and the benefits of its use. Pre-processing techniques can be used to increase its nutritional value, as well as improve palatability, which could lead to increased usage in food products. The increase in nutrients and antinutrients can also make it a functional food for those with celiac disease, type II diabetes mellitus and general consumers.
2.6 Objectives

The objectives of this study were:

1. To germinate proso millet using various environmental conditions to determine its effect on the nutritive and physical properties

2. Examine the use of two pre-processing techniques on the sensory properties and phenolic contents of proso millet

The goal of this research is to gain more insight into the effect that pre-processing has on proso millet. Research has been conducted on many varieties of millet, mostly pearl and finger millet. This study uses a variety that is grown in North America and is more accessible than those varieties grown in India and regions of Africa. Current studies have investigated decortication and germination of millet and their effects on the nutritive and physical properties, however there is a lack of information regarding the effects on the sensory properties of millet. Millet has an unappealing, bitter taste and identifying factors contributing to it, as well as ways to decrease this taste, may increase utilization of the grain. There is a consumer demand for healthy, gluten free grain alternatives and this research proposes to evaluate the functional properties of germinated proso millet.
CHAPTER 3: EFFECT OF GERMINATION UNDER VARIOUS CONDITIONS ON $\alpha$-AMYLASE ACTIVITY, NUTRITIVE VALUE AND THE PHYSICAL PROPERTIES OF PROSO MILLET

3.1 Abstract

Germination is a natural pre-processing method that has been shown to improve the nutritive value of grains. Germination initiates seed growth and resumes metabolic processes, including enzyme activation. The enzyme $\alpha$-amylase is synthesized during germination and breaks down starch polymers within the grain to release sugars. Exposing the grains to different conditions can result in various levels of enzyme synthesis. Proso millet was germinated for 48, 72 and 96 h, as well as three temperatures of 20, 25 and 30°C, for a total of nine treatments. An ungerminated whole grain control was also tested. The present study examined the use of germination under various conditions to observe the effect it had on the physical properties of proso millet. Proximate analysis was conducted and the nutritive value was maintained. Alpha-amylase activity increased as germination time and temperature increased. Glucose content increased and was correlated with $\alpha$-amylase activity. eGI values increased as the rate of germination increased with values ranging from 44.8 to 60.8. Higher levels of $\alpha$-amylase activity hydrolyze starch granules at a faster rate, therefore causing a loss of water binding capacity. Peak and final viscosity decreased as germination time increased. Those samples that were germinated at higher times and temperatures underwent the most significant changes.
3.2 Introduction

In the 21st century, increasing world population, rising food prices, climate change and water shortage are soon to affect the food security of the world. It is important for researchers to begin investigating other food sources (Saleh, Zhang, Chen, & Shen, 2013). Millet has been an important crop in developing nations and helps to solve issues of malnutrition and food insecurity (Amadou, Gounga, & Le, 2013). Along with wheat and barley, proso millet is one of the oldest summer cereals. Nutritionally, proso is comparable to other common grains. It is rich in protein, fat, vitamins, minerals and shown to be low on the glycemic index (Kalinova & Moudry, 2006). Not only is millet highly nutritious, it is also a drought resistant crop. Millets are resistant to pests and disease, have a short growing season and can provide sustainable yields in drought-like conditions. Presently, millet is used primarily as animal and birdfeed in developed areas, like North America (Devi, Vijayabharathi, Sathyabama, Malleshi, & Priyadarisini, 2014). However, more research on this grain can increase its utilization in food products.

In grains, pre-processing techniques have been used to improve nutritional value and reduce antinutrients contents, thereby reducing negative sensory properties (Tian et al., 2010). Germination is a natural pre-processing method and has been shown to improve the nutritional properties of grains, such as increasing protein content and decreasing carbohydrate content (Inyang & Zakari, 2008; Malleshi & Desikachar, 1986; Marero et al., 1988). Alpha-amylase becomes activated when a seed undergoes steeping and leaves the dormancy phase. It acts to randomly hydrolyze the α-1, 4-glucan linkages in the starch polymers of amyllose and amylopectin. This leads to a degradation of starch and many physicochemical changes can occur (Helland, Wicklund, & Narvhus, 2002).
Germination also leads to an increase in free sugars, including glucose, which is the major sugar found in germinating grains (Nomura, Kono, & Akazawa, 1969). Current studies have yet to observe the effect of germination on the in vitro digestibility of proso millet. The modifications occurring due to α-amylase activation during germination can alter the starch digestibility of a grain along with increasing the amount of free sugars (Chungcharoen, Prachayawarakorn, Tungtrakul, & Soponronnarit, 2014). Germination has been found to increase starch digestibility as time of sprouting increases (Bravo, Siddhuraju, & Saura-Calixto, 1998; Preet & Punia, 2000). However, the extent of the increase can vary based on the type of grain used. This study aims to uncover how germination affects the in vitro starch digestibility of proso millet in different environments.

Studies examining the effects of germination on millet are limited, especially using a variety found in North America, such as proso millet. The duration and conditions of germination can lead to highly variable results, with changes seen in nutritional and physical properties at different time intervals (Amadou et al., 2013). Proso millet has grown in importance in North America, with production largely occurring in eastern Colorado and western Nebraska (Ragaei, Abdel-Aal, & Noaman, 2006). Using a variety that is highly accessible in this region and germinating under various conditions can lead to consistent and reproducible results that can be useful in the North American food industry.

The objective of this study was to evaluate the changes occurring in the nutritive and physical properties of proso millet germinated under various conditions. It will focus on α-amylase activity and how it can affect pasting properties and final viscosity of starch. Free sugars and in vitro starch digestibility were also measured to see how germination affects these properties in proso millet.
3.3 Materials and Methods

The seeds of Colorado proso millet were provided by Bunge North America (St. Louis, MO).

3.3.1 Germination Process

Samples were shipped from St. Louis, MO to the University of Guelph where they were stored in a -20°C freezer prior to germination. Samples were germinated at 3 temperatures (20°C, 25°C, and 30°C) and 3 times (48 h, 72 h, and 96 h) for a total of 9 treatments. Whole grain proso millet was included in the experiments as a control. For preparation of each treatment, 500 g of sample were removed from the freezer, weighed and left at 22°C for an hour. Samples were sterilized with 2% (v/v) H₂O₂ by soaking in the solution. After 15 minutes, the grains were thoroughly rinsed with distilled water to remove all H₂O₂ residue. The seeds were then steeped in a 1:1 ratio of seeds to distilled water for 6 hours at 10°C. The millets were drained and spread onto synthetic mesh screens that were disinfected using 70% ethanol. The screens were held at 20°C, 25°C and 30°C in a SANYO Environmental Test Chamber (MLR-351H) at a constant 90% relative humidity. The seeds were hydrated using water every 6-8 hours, or as needed to ensure seeds were kept moist. They were removed from the incubator after 48 h, 72 h and 96 h.

3.3.2 Sample Preparation

After 48 h, 72 h and 96 h, the samples were placed in a -80°C freezer to end the sprouting process. They were then freeze dried for 48 hours to inactivate any enzyme activity within the grain. Using a method of abrasion, the sprouts were removed from the grains. About 15 g of sprouted millet were placed into a large sifter and shaken until the roots and shoots had fallen off. They were milled using a Smart Grind Stainless Steel Coffee Bean Grinder (Black and
Decker, USA) for 1 minute to achieve a particle size of approximately 2.0 mm. The samples were then put into individual canisters in a -20°C freezer.

3.3.3 Proximate Analysis

Nutritional analysis was conducted on all germinated millet samples and the control. Protein content (AACC 46-30.01), fat content (AACC 30-10.11) and ash content (AACC 08-01.01) were all analyzed using standard AACC methods (AACC International, 1999). Moisture content was analyzed using a moisture balance (MB 45 OHAUS) where the standard method for flour was applied (180°C for 2 minutes). Protein, fat, ash, and moisture content were added together and subtracted from 100 to obtain the carbohydrate content.

3.3.4 Determination of α-amylase Activity

Amylazyme-Red tablets by Megazyme (Megazyme International, Ireland) were used for the Amylazyme method (AACC 22-05.01), which measures α-amylase activity in cereals. For each sample, 1.00 g of germinated flour was weighed and added to 20 mL of sodium maleate buffer (100 mM, pH 6.0). The slurry was stirred for 15 minutes and then an aliquot was centrifuged at 1,500 g for 10 minutes. An amount of 0.2 mL of the supernatant was diluted 25-fold using the same buffer. Aliquots of 0.5 mL of the diluted supernatant were put into test tubes and incubated for 5 minutes in 40°C water bath. An Amylazyme-Red tablet was added to each test tube and incubated for exactly 10 minutes at 40°C. In order to terminate the reaction, 10 mL of Trizma base solution (2% w/v, pH 8.0) was added to each test tube. A reaction blank that did not contain the addition of an Amylazyme-Red tablet was prepared. Tubes were vortexed and then filtered and measured against the reaction blank at 510 nm (Cary 1-Bio UV-visible spectrophotometer, Ontario, Canada). Alpha-amylase was determined using a standard curve.
relating to the activity of pure malted barley in order to convert absorbance to Ceralpha units of activity. Ceralpha units per gram of flour was used as the unit of measurement.

3.3.5 Free Sugar Content

One gram of each sample was weighed into separate 100 mL volumetric flasks and water was added to the 100 mL mark. The Millipore filtration system (EMD Millipore Corporation, USA) was attached on top of a 1000 mL Erlenmeyer flask. The sample was poured through the filtration system while the vacuum was turned on and filtered through glass fibre filter paper. Two test tubes were used and 0.2 mL of the sample was added to each; one tube was for glucose determination and the second for sucrose determination. For glucose determination, 0.2 mL of sodium acetate buffer (100 mM, pH 4.5) was added to the first test tube. For sucrose determination, 0.2 mL of an enzyme, prepared by adding 1.67 mg invertase to 100 mL of sodium acetate buffer (100 mM, pH 4.5), was added to the second test tube. Three glucose standards were made using 0.1 mL glucose standard solution and 0.3 mL distilled water. Two reaction blanks were made up of 0.2 mL of sodium acetate buffer (100 mM, pH 4.5) and 0.2 mL distilled water. All test tubes were incubated at 50°C for 20 minutes. Following the addition of 3 mL of glucose oxidase-peroxidase reagent (Megazyme International, Ireland) to each test tube, prepared samples were incubated at 50°C for another 20 minutes. For each sample, absorbance was measured against a reaction blank using a spectrophotometer set at 510 nm (Cary 1-Bio UV-visible spectrophotometer, Ontario, Canada). Absorbance and weight of the sample were added to a Megazyme data calculator in order to determine glucose and sucrose levels.

3.3.6 Total Starch

A Megazyme (Megazyme International, Ireland) kit was used to determine total starch in malted flour. 100 mg of each sample was weighed into test tubes. After addition of 0.2 mL of
80% ethanol the tubes were vortexed. This was followed by the addition of 3 mL of thermostable α-amylase to each test tube which were then incubated in a boiling water bath for 10 minutes. During this time, samples were removed from the bath and vortexed every 2 minutes. At the completion of incubation, 4 mL of sodium acetate buffer (200 mM, pH 4.5) and 0.1 mL of amylloglucosidase (Megazyme International, Ireland) were added to the tube and the tubes were incubated at 50°C for 30 minutes. The solution from each tube was put into individual 100 mL volumetric flasks and distilled water was added to adjust the volume. An aliquot (about 40-50 mL) from each tube was centrifuged at 3000 rpm for 10 minutes and 0.1 mL of each supernatant was removed from the original test tube and added to clean test tubes. The blank used 0.1 mL of distilled water. To each tube, 3 mL of glucose oxidase-peroxidase reagent (Megazyme International, Ireland) was added, including the blank and the tubes were incubated at 50°C for 20 minutes. The absorbance was read on a spectrophotometer set at 510 nm (Cary 1-Bio UV-visible spectrophotometer, Ontario, Canada) against a reaction blank. The weight of samples and absorbances were included in a Megazyme data calculator for total starch determination.

3.3.7 In Vitro Starch Digestibility

The procedure for starch digestibility is based on the methods used by Englyst et al. (1996). For each sample, a 10% slurry of millet flour containing 750 mg of starch was cooked for 10 min with constant stirring with a magnetic stirrer. The mixture was removed from the heat and 7 glass beads were added to the flask. After addition of 10 mL of sodium acetate buffer (0.1 M, pH 5.2) the flask was covered with parafilm and placed into a shaking water bath at 37°C for 5 minutes. After 5 minutes, the flask was removed, 5 mL of enzyme was added and the flask was immediately returned into the water bath. The enzyme was made up of pancreatin (Sigma-Aldrich Co., USA), invertase (Sigma-Aldrich Co., USA) and amylloglucosidase (Megazyme
International, Ireland). After 20 minutes of incubated shaking, 0.1 mL of the slurry was pipetted into a micro-centrifuge tube (Eppendorf, Canada) containing 0.8 mL of 80% ethanol. This was continued every 20 minutes for 2 hours. The micro-centrifuge tubes were stored in a -20°C freezer.

To prepare samples for glucose determination, tubes were removed from the freezer and microcentrifuged at 4900 rpm. After the addition of 3 mL of glucose oxidase-peroxidase reagent (Megazyme International, Ireland) to 0.4 mL of the samples, test tubes were incubated at 50°C for 20 minutes. The absorbance was read on a spectrophotometer at 510 nm (Cary 1-Bio UV-visible spectrophotometer, Ontario, Canada) against a reaction blank. The amount of glucose released was multiplied by 0.9 to convert to starch.

Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistance starch (RS) measurements were obtained, according to Englyst, Kingman, & Cummings (1992). The calculations used were:

\[ \text{RDS} = \text{glucose released at }20\text{ min} \times 0.9 \] [equation 1]

\[ \text{SDS} = (\text{glucose released at }120\text{ min} - \text{glucose released at }20\text{ min}) \times 0.9 \] [equation 2]

\[ \text{RS} = \text{total starch} - (\text{RDS} + \text{SDS}) \] [equation 3]

Goñi, Garcia-Alonso, & Saura-Calixto (1997) established an equation to measure hydrolysis curves:

\[ C - C_\infty (1 - e^{-kt}) \] [equation 4]
$C$ is the concentration at time $t$, $C_\infty$ is concentration of starch at equilibrium (120 minutes), $t$ is the time, and $k$ is the kinetic constant. The hydrolysis index (HI) was found by dividing the area under the hydrolysis curve (AUC) of the sample by that of a reference sample, usually white bread at a value of 100 (Goñi et al., 1997). The equation used for the calculation of the AUC was:

$$\text{Area Under Curve} = C_\infty (t_f - t_o) - (C_\infty/k) \left[1 - e^{-k(t_f-t_o)}\right] \tag{equation 5}$$

The equation used for obtaining the hydrolysis index was:

$$\text{HI} = \frac{\text{AUC of Sample}}{100 \times \text{(AUC of White Bread)}} \tag{equation 6}$$

The eGI of the samples were calculated from the equation by Granfeldt, Björck, Drews, & Tovar (1992):

$$\text{eGI} = 8.198 + 0.862(\text{HI}) \tag{equation 7}$$

3.3.8 Pasting Properties

The pasting properties of germinated flour were obtained using Rapid Visco™ Analyser (RVA-4, Newport Scientific, Warriewood, Australia). The software used was Thermocline for Windows (TCW3). To measure pasting properties, 3.0 g of flour was mixed with 25.0 g of distilled water, following correction of the flour sample to 14% moisture. The slurry was inserted into the instrument with a paddle attached and heated from 50°C to 95°C for 23 minutes at a shear rate of 160 rpm. From the resulting pasting curve, pasting temperature, peak viscosity and final viscosity of the slurry was taken.
3.3.9 Statistical Analysis

All tests were completed in duplicate. SAS v. 9.2 statistical package (Raleigh, North Carolina) was used for the analysis of all data. For all measured parameters, ANOVA and Tukey’s honestly significant difference tests were performed to determine significant differences among treatments at $\alpha=0.05$.

3.4 Results and Discussion

3.4.1 Changes to macronutrients during germination

Germination has been shown to improve the nutritive value of millet so more changes to macronutrients, as measured during proximate analysis, were expected to occur as germination time increased. Table 3.1 shows the differences seen in the proximate analysis done on all samples. It is important to note that samples 30°C/72 h and 30°C/96 h moulded within the incubator and therefore could not be included in the analysis.

Table 3.1 Proximate analysis (%) and standard errors (dry basis) of whole grain millet and seven\(^1\) germinated sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.2a±0.05</td>
<td>3.8ab±0.10</td>
<td>2.1b±0.19</td>
<td>82.9a±0.04</td>
</tr>
<tr>
<td>20°C/48 h</td>
<td>11.2a±0.11</td>
<td>4.1a±0.02</td>
<td>3.5a±0.03</td>
<td>81.2d±0.10</td>
</tr>
<tr>
<td>20°C/72 h</td>
<td>10.9ab±0.04</td>
<td>3.7ab±0.07</td>
<td>3.1a±0.10</td>
<td>82.3b±0.02</td>
</tr>
<tr>
<td>20°C/96 h</td>
<td>10.8bc±0.03</td>
<td>4.1a±0.07</td>
<td>3.3a±0.01</td>
<td>81.8c±0.12</td>
</tr>
<tr>
<td>25°C/48 h</td>
<td>10.9ab±0.07</td>
<td>4.1a±0.12</td>
<td>2.3b±0.14</td>
<td>82.6ab±0.19</td>
</tr>
<tr>
<td>25°C/72 h</td>
<td>10.4d±0.00</td>
<td>3.9ab±0.02</td>
<td>3.1a±0.10</td>
<td>82.7ab±0.12</td>
</tr>
<tr>
<td>25°C/96 h</td>
<td>10.3d±0.05</td>
<td>3.6b±0.02</td>
<td>3.2a±0.09</td>
<td>82.9a±0.03</td>
</tr>
<tr>
<td>30°C/48 h</td>
<td>10.4cd±0.08</td>
<td>3.9ab±0.01</td>
<td>3.0a±0.06</td>
<td>82.7ab±0.12</td>
</tr>
</tbody>
</table>

\(^1\)Samples 30°C/72 h and 30°C/96 h were not included for analysis
\(^2\)All means in the same column with the same letters are not significantly different ($p<0.05$), n=2

Protein content in whole grain millet began with 11.2±0.05%, which is slightly lower than numbers cited by others. Kalinova & Moudry (2006) compared many cultivars of proso
millet and found the protein content ranged from 12.1-13.6%. Others have reported proso millet to contain 12.5% protein (FAO 1995), which is within the range observed by Kalinova & Moudry (2006) and higher than what was observed in the current research. These germinated samples slightly decreased in protein content, ranging from 10.3-11.2% (Table 3.1).

Many studies of millet show that protein increases as germination time increases, as a result of dry matter loss (Mbithi-Mwikya, Van Camp, Yiru, & Huyghebaert, 2000; Sade et al., 2009). However, this study showed protein content slightly decreased or stayed relatively similar as germination time increased within the same temperature. This was also seen in millet studies done by Choudhury, Das, & Baroova (2011) and Malleshi & Desikachar (1986) where protein significantly decreased from 11.7% to 9.7% and 8.2% to 6.8%, respectively as germination time increased. This could be attributed to removal of roots and shoots from sprouted products. Roots and shoots are known to be rich in nitrogenous compounds. This is due to breakdown of storage proteins during germination and subsequent translocation of breakdown of products, such as free α-amino nitrogen from the kernel to the roots and shoots (Pelembe, Dewar, & Taylor, 2002). Germination activates enzymes responsible for breaking down proteins and carbohydrates into simpler forms. Proteases may have been responsible for some of the protein breakdown into amino acids (Elkhalifa & Bernhardt, 2010).

As shown in Table 3.1, fat content of whole grain millet was 3.8±0.09%. This is consistent with the fat content of 3.5% reported by the FAO (1995). Decreases in fat content were expected as germination rate increased as this has been observed in previous research. Ocheme & Chinma (2008) found fat content significantly decreased in pearl millet from 4.6% in whole grain pearl millet to 3.0% in germinated millet at 32°C for 48 h. This loss was attributed to the action of lipolytic enzymes during the germination process. With the temperature used in
the current study, fat content stayed very consistent. However, it can be seen that samples germinated at 25°C for 96 h did have the lowest fat content at 3.6±0.02%. This could be due to a transfer of nutrients needed in the growing embryo or lipolytic enzyme activity breaking down triglycerides (Malleshi & Desikachar, 1986; Ocheme & Chinma, 2008).

Ash content gives a rough estimation of the minerals in a specific product. The ash content in the whole grain sample was 2.1±0.19% (Table 3.1). This is lower than the ash content of 3.3% found by Kulkarni, Naik, & Katarki (2012) but closer to the range observed by Ravindran (1991) who found that many cultivars of proso millet had ash contents of 2.1-2.8%. For most germinated samples, ash content differed significantly from the whole grain millet. The exception was 25°C/48 h where the ash content was very similar to the whole grain sample. For all other samples, the ash content increased significantly from the control sample but did not differ amongst the other germinated treatments. Others have observed similar results where ash content significantly increased from the control sample and did not vary significantly with germination time (De Ruiz & Bressani, 1990; Malleshi & Desikachar, 1986).

The total carbohydrate content ranged from 81.2-82.9% (Table 3.1). Bagdi et al. (2011) reported the total carbohydrate of whole grain proso millet to be 80.1%, which is similar to the values observed in the current study. The carbohydrate content of the whole grain sample was 82.9±0.04%. The germinated samples stayed relatively consistent in terms of carbohydrate content. The relatively constant total carbohydrate content could be due to the increase of free sugars that occurs because of the germination process.
3.4.2 Alpha-amylase activity and germination rate

An increase in α-amylase activity was seen as the time of germination increased (Table 3.2). A similar trend can be seen in a previous study investigating germinated corn (Helland et al., 2002). Also, as the temperature increased, a higher α-amylase activity was observed. Varadaraj & Horigane (1998) came to the same conclusion with sprouted finger millet.

Table 3.2: The α-amylase activity (units/g flour), glucose content (g/100 g flour) and sucrose content (g/100 g flour) of whole grain millet and seven germinated samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>α-amylase (units/g flour)</th>
<th>Glucose (g/100 g flour)</th>
<th>Sucrose (g/100 g flour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Undetected</td>
<td>0.10f±0.00</td>
<td>0.36b±0.01</td>
</tr>
<tr>
<td>20°C /48 h</td>
<td>156.4f±0.69</td>
<td>0.20e±0.00</td>
<td>0.03d±0.01</td>
</tr>
<tr>
<td>20°C /72 h</td>
<td>353.2e±0.89</td>
<td>0.36d±0.01</td>
<td>0.01d±0.00</td>
</tr>
<tr>
<td>20°C /96 h</td>
<td>620.9d±0.59</td>
<td>0.49c±0.01</td>
<td>Undetected</td>
</tr>
<tr>
<td>25°C /48 h</td>
<td>696.4c±0.10</td>
<td>0.48c±0.01</td>
<td>Undetected</td>
</tr>
<tr>
<td>25°C /72 h</td>
<td>1269.9b±0.54</td>
<td>0.92b±0.00</td>
<td>0.26bc±0.03</td>
</tr>
<tr>
<td>25°C /96 h</td>
<td>2009.0a±4.43</td>
<td>1.56a±0.02</td>
<td>1.29a±0.05</td>
</tr>
<tr>
<td>30°C /48 h</td>
<td>1269.5b±0.49</td>
<td>0.93b±0.02</td>
<td>0.16cd±0.06</td>
</tr>
</tbody>
</table>

1Samples 30°C/72 h and 30°C/96 h were not included for analysis
2All means in the same column with the same letters are not significantly different (p<0.05), n=2

Ninety-six hours of germination produced the greatest amount of α-amylase activity for each temperature. For the 20°C treatments, values started at 156±0.69 units/g flour for 48 h and increased to 621±0.59 units/g flour at the end of 96 h. The largest increase in enzyme activity was found in 25°C/96 h at 2009±4.43 units/g flour. However, the α-amylase activity after only 48 h at 25°C was 696±0.10 units/g flour. Therefore, a higher temperature can produce more α-amylase at a faster rate. This explains why the 30°C treatment led to such a high activity of 1269±0.49 units/g flour after only 48 h of germination time. All of the treatments were significantly different from one another (p<0.05) except for 25°C/72 h and 30°C/48 h. Overall,
the greatest increases in α-amylase activity were seen in the 25°C treatments. Higher enzyme activity may have been seen in the 30°C/72 h and 30°C/96 h if they had not moulded. Muoria, Bechtel, & Linden (1998) found that germination temperature above 22°C was ideal for greater α-amylase levels in sorghum and pearl millet. This helps explain why samples germinated at 25°C and 30°C had higher α-amylase activities compared to those germinated at 20°C.

Table 3.3: Proximate analysis (%) and moisture content (%) of whole grain millet and seven germinated samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.6±0.06²</td>
</tr>
<tr>
<td>20°C/ 48 h</td>
<td>7.3±0.04</td>
</tr>
<tr>
<td>20°C/72 h</td>
<td>8.6±0.05</td>
</tr>
<tr>
<td>20°C/96 h</td>
<td>11.2±0.14</td>
</tr>
<tr>
<td>25°C/48 h</td>
<td>13.0±0.08</td>
</tr>
<tr>
<td>25°C/72 h</td>
<td>9.9±0.08</td>
</tr>
<tr>
<td>25°C/96 h</td>
<td>8.0±0.09</td>
</tr>
<tr>
<td>30°C/48 h</td>
<td>8.5±0.07</td>
</tr>
</tbody>
</table>

¹Samples 30°C/72 h and 30°C/96 h were not included for analysis
²All means in the same column with the same letters are not significantly different (p<0.05), n=2

The differences observed among enzyme activity can be attributed to the moisture uptake capacity of the grains and the radicle length of the sprout. All the treatments underwent a 6 hour soaking period at 10°C. The moisture content of the whole grain seed was 9.6%. After the steeping period, the moisture increased to 36.3%. Soaking the grains prior to germination is needed to break seed dormancy. The equilibrium moisture content of millet is 30-35% and this moisture was needed in order to initiate the germination process (Malleshi & Desikachar, 1986). Swami, Thakor, & Gurav (2013) found that an increase of temperature during soaking can increase the moisture content of the grain. This leads to a higher moisture uptake. Based on this,
the treatments subjected to higher temperatures may have had a greater ability for maintaining this high moisture content and germinated at a faster rate.

Table 3.3 shows the moisture content of the samples. The samples kept in 25°C chamber had the highest moisture content with 13.0±0.08% moisture. This could be because the temperature was higher than for other samples and the grains were better able to hold onto the moisture. At this temperature, as germination time increased, a decrease in moisture content was observed. Once the germination process began, less water was needed for the grains to grow and moisture content decreased. The samples subjected to the 30°C chamber were more susceptible to mould growth and less moisture was applied to combat this. It should be noted that during germination, water was sprayed onto the samples to maintain hydration. The amount used was not controlled for and in future studies, this amount should be standardized.

A reflection of the rate of germination is the radicle length of the grains. As the temperature of germination increased, the radicle length also increased (Table 3.4). Malleshi & Desikachar (1986) found that four days of germination provided the maximum amount of α-amylase to be activated.

<table>
<thead>
<tr>
<th>Table 3.4: Radicle length (cm) of germinated proso millet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (ºC)</td>
</tr>
<tr>
<td>Time (h)</td>
</tr>
<tr>
<td>48</td>
</tr>
<tr>
<td>72</td>
</tr>
<tr>
<td>96</td>
</tr>
</tbody>
</table>
From these results, it can be seen that the temperature of germination is an important factor for sprouting and the synthesis of α-amylase within the grain. Higher temperature allows for a faster rate of germination, as indicated by radicle length. Alpha-amylase content was highest in those samples with a faster germination rate. Increased temperature may also lead to improved water-holding capacity, which helps the grain better retain the moisture to promote germination.

3.4.3 Free sugar content

Glucose content within the seed increased as germination time and temperature increased (Table 3.2). This has also been observed in previous studies investigating the germination of wheat (Suhasini, Muralikrishna, & Malleshi, 1997) and of malted finger, pearl and foxtail millet (Malleshi, Desikachar, & Tharanathan, 1986). Sucrose content in whole grain millet was high because it is the main disaccharide found in this grain. Suhasini et al. (1997) found that as time of germination increased the sucrose content in millet also increased, however that was not consistently observed in the current research (Table 3.2).

The glucose content was highest after 96 hours of germination for both 20°C and 25°C treatments. Whole grain millet only contained 0.1±0.00 g/100 g of flour. For samples malted at 20°C, there was a moderate rise in glucose content from 0.2±0.00 g/100 g of flour after 48 h to 0.49±0.01 g/100 g of flour at the end of 96 h. Those treatments kept at 25°C saw larger increases of glucose as time increased, with a maximum glucose content of 1.56±0.02 g/100 g of flour after 96 h of germinating. The treatments were significantly different from each other (p<0.05), except the 30°C/48 h and 25°C/72 h treatments.
The glucose content of sprouted millet is related to the rate of germination. Those samples with a faster rate of germination were able to produce higher glucose contents in millet. Glucose content is also related to α-amylase activity (r=0.994, p<0.0001). This means α-amylase activity is directly correlated to glucose found within the grain in this study. Amylases work to hydrolyze α-1,4 linkages in starch polymers which then release products, including glucose, maltose and α-limit dextrins (Muralikrishna & Nirmala, 2005). Glucose content was highest in those samples with higher α-amylase activity.

The sucrose content of whole grain control millet was 0.36±0.01 g/100 g of flour. This number reduced among the 20°C treatments. It went from 0.029±0.01 g/100 g of flour in 48 h to undetectable after 96 h. The opposite was seen among the 25°C treatments. While the 48 h germinated samples had no sucrose content, this reached a maximum 96 h of germination. The 30°C/48 h sample also had a reduced sucrose content of 0.16±0.06 g/100 g of flour compared to the initial sucrose content in whole grain millet.

The sucrose that was initially in the whole grain millet was quickly reduced by the second day of germination in all three temperature treatments. This could be due to cellular respiration as the seed begins germinating. The sucrose would be broken down to glucose to be used as energy needed in the seed for further metabolism (Edelman, Shibko, & Keys, 1959). Sucrose is used as a transporter within the seed and takes carbon and energy from storage reserves to the growing embryo. For this reason, sucrose is synthesized to continue as a transporter. Nomura et al. (1969) found that the scutellum of rice seeds had the highest concentration of sucrose (4.1-8.3 mg per 100 scutella) out of all the free sugars, while much smaller amounts of glucose and fructose were present. The scutellum absorbs the glucose from the endosperm, where starch breakdown is occurring and converts it to sucrose. A specific set of
enzymes are required in order for sucrose to be synthesized from glucose. These include hexokinase, phosphoglucoisomerase, phosphoglucomutase, UDP-glucose pyrophosphorylase, sucrose synthetase and UDP-ATP-kinase which are found in scutellum of germinating grains (Edelman et al., 1959). This could explain the increase of sucrose content after 72 hours at 25°C, as sucrose continues to be synthesized and broken down during this process.

3.4.4 Pasting properties and Starch breakdown

Table 3.5 shows the differences found from different sprouting conditions on the RVA profiles of proso millet. An increase in sprouting time decreased the peak viscosity, peak time and final viscosity of the flour samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak Viscosity (cP)</th>
<th>Peak Time (min)</th>
<th>Final Viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>755b±12.50</td>
<td>9.0a±0.10</td>
<td>1555a±7.50</td>
</tr>
<tr>
<td>20°C/48 h</td>
<td>883a±1.00</td>
<td>8.1b±0.20</td>
<td>964b±16.00</td>
</tr>
<tr>
<td>20°C/72 h</td>
<td>536c±2.50</td>
<td>7.2±c0.04</td>
<td>331c±0.50</td>
</tr>
<tr>
<td>20°C/96 h</td>
<td>198d±4.50</td>
<td>6.5d±0.02</td>
<td>73d±0.00</td>
</tr>
<tr>
<td>25°C/48 h</td>
<td>143e±2.00</td>
<td>6.3de±0.10</td>
<td>72de±2.50</td>
</tr>
<tr>
<td>25°C/72 h</td>
<td>55fg±0.50</td>
<td>6.1de±0.10</td>
<td>31ef±2.50</td>
</tr>
<tr>
<td>25°C/96 h</td>
<td>21g±5.00</td>
<td>6.0e±0.10</td>
<td>6.0f±5.00</td>
</tr>
<tr>
<td>30°C/48 h</td>
<td>66f±6.00</td>
<td>6.1de±0.10</td>
<td>35def±9.50</td>
</tr>
</tbody>
</table>

1Samples 30°C/72 h and 30°C/96 h were not included for analysis
2All means in the same column with the same letters are not significantly different (p<0.05), n=2

Peak viscosity can indicate the water binding capacity of flour. The peak viscosity decreased rapidly as germination time increased, with no gelatinization shown in sample 25°C/96 h. The lowest peak viscosity was seen in sample 25°C/96 h at 21±5.00 cP. Since peak viscosity reduced as germination time increased, it can be concluded that endosperm
modification by α-amylase was occurring at faster rates. Therefore, starch loses the ability to bind water and swell to form a gel.

Final viscosity is a good indicator of a product's ability to form a gel after cooking and cooling. These values also behaved in a similar manner as peak viscosity, with increased germination time and temperature leading to a lower final viscosity. The raw millet flour had a final viscosity of 1555±7.50 cP. The samples germinated at 20°C exhibited a gradual decline, with 20°C/48 h decreasing to 964±16.00 cP and lowering further to 331±0.50 cP and 73±0.00 cP after 72 h and 96 h, respectively. The 25°C samples were drastically reduced from the control sample, ranging from 72±2.50 to 6.0±5.00 cP. 30°C/48 h was also significantly lower at 35±9.50 cP.

Charoenthaikij et al. (2009) germinated brown rice for 3 different times to look at the effect on physicochemical properties. Peak viscosity and final viscosity all decreased as the length of germination time increased. Low numbers for viscosities were also encountered at their longest germination time. The decrease in pasting capacity in this study can be attributed to the α-amylase activity in the grains. The samples with low peak and final viscosities were also the same samples with higher amounts of α-amylase activity. Zarnkow, Mauch, Back, Arendt, & Kreisz (2007) used scanning electron microscopy to monitor the malting process of proso millet and found pinholes in the starch granules just after the steeping process. After one day, the pinholes increased in diameter. Two days of sprouting resulted in large holes in the starch structure, which made it look like a sponge. This is due to starch hydrolysis by α-amylase. According to Macgregor & Balance (1980), α-amylase attacks the starch surface and then forms tunnels to get to the inside of the granule. Hydrolysis of the starch then occurs from inside of the starch granule (Zarnkow et al., 2007).
3.4.5 Factors affecting starch digestibility of millet

The results of in vitro starch digestibility are presented in Table 3.6. The rapidly digestible starch (RDS) ranged from 18.1-34.7% in these samples. The percentages increased as germination time increased for each temperature condition. The slowly digestible starch (SDS) varied between 30.0-42.4%. The resistant starch numbers ranged from 35.2-43.7%. eGI values were found to increase as time of germination increased. The lowest value was 44.8 and increased to 60.8. The 25°C/96 h sample was found to have the highest RDS and eGI, along with the lowest SDS and RS.

<table>
<thead>
<tr>
<th>Sample</th>
<th>RDS (%)</th>
<th>SDS (%)</th>
<th>RS (%)</th>
<th>eGI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.0±0.38</td>
<td>38.4±0.64</td>
<td>41.7±1.00</td>
<td>46.5±0.70</td>
</tr>
<tr>
<td>20°C/48 h</td>
<td>18.1±0.44</td>
<td>42.2±1.18</td>
<td>39.8±1.62</td>
<td>44.8±0.92</td>
</tr>
<tr>
<td>20°C/72 h</td>
<td>22.9±0.35</td>
<td>33.5±1.81</td>
<td>43.7±2.17</td>
<td>48.7±1.10</td>
</tr>
<tr>
<td>20°C/96 h</td>
<td>24.9±1.02</td>
<td>33.7±1.98</td>
<td>41.4±0.95</td>
<td>51.2±0.48</td>
</tr>
<tr>
<td>25°C/48 h</td>
<td>24.5±0.30</td>
<td>33.9±0.85</td>
<td>41.7±1.15</td>
<td>50.8±0.68</td>
</tr>
<tr>
<td>25°C/72 h</td>
<td>31.4±0.56</td>
<td>30.5±1.11</td>
<td>38.1±1.67</td>
<td>57.4±1.62</td>
</tr>
<tr>
<td>25°C/96 h</td>
<td>34.7±0.54</td>
<td>30.0±0.62</td>
<td>35.2±0.38</td>
<td>60.8±0.40</td>
</tr>
<tr>
<td>30°C/48 h</td>
<td>30.9±1.40</td>
<td>30.4±0.13</td>
<td>38.8±0.72</td>
<td>56.7±1.02</td>
</tr>
</tbody>
</table>

1Samples 30°C/72 h and 30°C/96 h were not included for analysis
2Rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS), eGI (expected glycemic index)
3All means in the same column with the same letters are not significantly different (p<0.05), n=2

Rapidly digestible starch (RDS) is the starch that is easily broken down within the first 20 minutes of digestion. The more RDS there is in a food product, the greater the ability to influence glycemic response. Germination increased the amount of RDS in proso millet. SDS is digested completely in the small intestine; however it is done so at a slower rate than RDS. Germination reduced the amount of SDS in the product. RS is the type of starch that passes
through the small intestine and into the large intestine, where it can undergo hydrolysis (Englyst & Hudson, 1996). Major fluctuations in RS didn’t occur during germination.

eGI is used as an estimation of the glycemic index of a food product. Germination increased eGI values, as expected. Starch is being hydrolyzed during this process and this can make it more susceptible to digestion. A value of 55 or less is considered a low GI food. Three of the samples became medium GI foods because of germination. The glucose values of the samples increased dramatically as germination time increased, as a result of starch hydrolysis, making it readily available to be absorbed. It was hypothesized that the eGI values would increase rapidly because of this breakdown. Chungcharoen et al. (2014) found similar results when brown rice was germinated. Although the germinated samples had a higher glucose value, the effect on eGI was not apparent. They attributed this to the increased dietary fibre in the germinated samples as it has been reported to help control digestibility.

Resistant starch is the starch fraction that is resistant to digestion in the small intestine. The RS content can affect the glycemic response to a food. There are five different types of resistant starches that can be found in cereals. Starch that is embedded in a structure that prevents enzyme digestion (RS\(_1\)), starch with structural architecture that limits degradation (RS\(_2\)), retrograded starch (RS\(_3\)), structurally modified starch (RS\(_4\)), and lipid-amylose starch (RS\(_5\)) (Fuentes-Zaragoza, Riquelme-Navarrete, Sanchez-Zapata, & Perez-Alvarez, 2010; Haub, Hubach, Al-tamimi, Ornelas, & Seib, 2010; Jiang, 2010). In the current study, the RS content was not significantly affected by germination. There was moderate reduction in the RS content but this amount still could have helped to reduce the starch hydrolysis and eGI of these samples. Bravo et al. (1998) found that sprouted and cooked Indian legumes significantly increased in digestible starches, decreased in resistant starch and rose in eGI values compared to the raw
legume. This was similar to the results presented in Table 3.6. The samples in this study were also sprouted and then cooked before eGI was tested. Some of the starch in these samples may have retrograded after cooling and made unavailable for hydrolysis.

Starch-protein interactions can also have an effect on starch hydrolysis. Proso millet starch granules are considered small, where A type are >10 µm, B type are <10 µm, and C type are <2.5 µm (Zarnkow et al., 2007). Smaller granules are more susceptible to hydrolysis compared to larger granules. However, the presence of proteins over the surface of starch can limit starch hydrolysis. Proteins work to block the adsorption sites on the granule (Singh, 2010). In proso millet, starch granules are embedded within a cluster structure of protein bodies. Even after germination, Zarnkow et al. (2007) saw the protein bodies were still intact and the starch granules still arranged in a cluster form. This reduced the amount of starch hydrolysis occurring within the granules, leading to a lower glycemic response.

3.5 Conclusion

In conclusion, germination changed the physical properties of proso millet. It changed the nutritional properties slightly, however the nutritive value was maintained. Alpha-amylase activity increased, thereby degrading starch granules and leading to increased free sugars. The peak and final viscosities also decreased as germination time increased. The starch digestibility of proso millet increased as germination time increased, however the highest eGI value was still in the range for a medium GI food product. Germination of proso millet did not create a superior product. However, germination has long been used to create specific food products, as the flavour and taste profile changes. The increased amount of natural sugars present can improve palatability (Inyang & Zakari, 2008). The low viscosity of germinated millet can also lead to
producing weaning products that are easy to swallow and consume (Helland et al., 2002; Marero et al., 1988).

CHAPTER 4: DECORTICATION AND GERMINATION OF PROSO MILLET AND ITS INFLUENCE ON SENSORY EVALUATION AND PHENOLIC CONTENT

4.1 Abstract

Despite the nutritional benefits associated with whole grain millet such as high fibre and antioxidants levels, consumers find the taste of millet to be bitter and unappealing. Decortication and germination are natural pre-processing techniques that have been used as a means of improving the sensory properties of grains. The aim of this study was to employ these techniques and conduct sensory testing to find differences in flavour and taste. Four samples were tested: proso millet germinated at 25°C for 48 h and 96 h, a decorticated sample and a whole grain millet control sample. Alpha-amylase activity was not observed in the decorticated and whole grain samples, but increased in the germinated samples. Alpha-amylase activity and glucose content were directly correlated. Free sugar content was lowest in the decorticated sample and highest in the 96 h germinated sample. Descriptive analysis was conducted on millet porridge samples and six attributes were found. The germinated samples rated highest for sweetness, however this was not enough to mask the bitter taste, which also increased. The decorticated sample imparted a very mild flavour profile. Phenolic content was measured and decorticated millet contained the lowest level. Phenolic content increased in the germinated samples and this could have contributed to the increase in bitter taste.
4.2 Introduction

Millet is an important cereal crop in African and Asian regions of the world. Production around the globe reached 32 million tonnes in 2007, with India and Nigeria being the top producers (Saleh, Zhang, Chen, & Shen, 2013). Nutritionally, it is a good source of protein, fat, fibre, many micronutrients (Léder 2004). Millet is also high in phenolic compounds, many of which are phenolic acids. Generally, ferulic, \( p \)-coumaric and cinnamic acids have been found to be the most abundant within millet (Dykes & Rooney, 2006). These phenolic acids are found within the pericarp, testa, aleurone layer and endosperm of all millet species in both free and bound form (Ragaee, Seetharaman, & Abdel-Aal, 2012).

Current studies involving the effect of germination on phenolic compounds in grains are contradictory, with phenolic contents shown to both increase and decrease with germination (Nelson, Stojanovska, Vasiljevic, & Mathai, 2013). The amount of phenolic acids in whole grain millet is high and there are both advantages and disadvantages to their presence in millet. The advantage of high phenolic content in millet is the contribution to a higher antioxidant level. Phenolics are able to act as antioxidants because of their ability to donate electrons and prevent oxidation in foods (Subba Rao & Muralikrishna, 2002). They help prevent free radical formation in the body and can also prevent undesirable changes in flavour (Pushparaj & Urooj, 2014; Subba Rao & Muralikrishna, 2002). The disadvantage to high phenolic content is that they are associated with the negative sensory properties of millet and can directly contribute to undesirable tastes and aromas (Ho 1992). Off flavours, such as bitterness and astringency, can be
a result of high levels of phenolic acids within the millet grain (Sosulski, Krygier, & Hogge, 1982). Therefore, decreasing the levels of phenolic acids may contribute positively to the sensory properties of millet based products. To date, there have been no published studies looking at the effect of germination on phenolic compounds and sensory properties of millet grains.

Germination has been found to increase certain nutritional and bioactive properties of grains, as well as to improve their sensory properties. Many enzymes are activated during this process, including amylases which are responsible for the breakdown of starch (Malleshi, Desikachar, & Tharanathan, 1986). During germination, an increase in free sugars occurs as amylolysis breaks down starch polymers into sugars. Some of this sugar is used for metabolic processes happening within the grain, however, much of it remains within the grain. The increased free sugar content could alter the sensory profile of millet by masking inherent off flavours, including bitterness and astringency.

Decortication is another pre-processing technique used to improve the sensory qualities of grains. It involves removing the pericarp and germ in order to isolate the endosperm. Desikachar (1981) found that pearling, or decortication, of sorghum improved consumer acceptability. It is not clear how decortication will affect sensory properties of millet.

The aim of this study was to determine differences in sensory attributes and phenolic content of millet after decortication or germination. This study focused on the pre-processing of proso millet. Descriptive analysis was employed to uncover sensory differences among whole grain, decorticated and germinated millet samples, using a trained panel. Phenolic content was measured.
4.3 Materials and Methods

Bunge North America (St. Louis, MO) provided Colorado proso millet grains for testing.

4.3.1 Germination Process

Two and a half kilograms of proso millet was used for each of the treatments. Prior to germination, millet samples were disinfected in a 20% v/v ethanol solution for five minutes and rinsed thoroughly with distilled water. For germination, the grains were soaked in a 1:1 ratio of seeds to distilled water for six hours at 10°C. Synthetic mesh screens were disinfected with 70% ethanol and the grains were spread along them in a single layer. They were held in a 25°C incubator at 90% humidity for 48 h and 96 h. These samples will be referred to as 25°C/48 h and 25°C/96 h, respectively throughout the remainder of the chapter. Distilled water was sprayed onto the grains to keep them hydrated, every 6-8 hours. They were removed from the incubator and oven dried at 50°C for 14 hours. After drying, the grains were vacuum sealed and kept in a -20°C freezer until next use. Prior to testing, millets were removed from the freezer and a method of abrasion was used to remove the sprouts from millet. To do this, approximately 15 g of sprouted millet was placed into a large sifter and shaken until the roots and shoots had fallen off. The roots and shoots were then discarded and only millet grains were used for testing.

4.3.2 Decortication Process

The decortication process developed by Anderson (2014) was used to decorticate millet. A Satake TM05 laboratory mill (Satake, Houston, Texas) was used to dry mill Colorado Proso millet. Millet was transferred to the unit and using an abrasive stone, the pericarp and germ were removed from the grain. The pericarp and germ travelled through a slit screen in the chamber and were discarded. The endosperm was collected and used in further testing.
4.3.3 Alpha-Amylase Activity

The α-amylase activity of whole grain, germinated and decorticated samples was determined by the Amylazyme-Red tablet (Megazyme International Ireland Ltd.) specifically designed to measure cereal α-amylase activity (AACC 22-05.01). For each sample, 1.00 g flour was stirred with sodium maleate buffer (100mM pH 6.0) for 15 min and centrifuged at 1,500 g for 10 min. 0.2mL of the supernatant was diluted with 10 mL sodium maleate buffer and 0.5 mL of diluted aliquots were incubated with Amylazyme Red tablet for exactly 10 min at 40˚C. The reaction was terminated by adding 10 mL of Trizma base solution (2% w/v, pH 8.5) and then filtered. Absorbance of the filtrate was measured against a reaction blank at 510 nm (Cary 1-Bio UV-visible spectrophotometer, Ontario, Canada) and α-amylase activity was determined by reference to the appropriate standard curve for purified barely malt to convert absorbance to Ceralpha Units of activity. Alpha-amylase activity was reported as Ceralpha Units per gram of flour. All tests were performed in duplicates.

4.3.4 Free Sugars

Free glucose and sucrose contents of whole grain, germinated and decorticated samples were measured. One gram of sample was weighed into a 100 mL volumetric flask and distilled water was added. The Millipore filtration system (EMD Millipore Corporation, USA) was attached to a 1 L Erlenmeyer flask with glass fibre filter paper. The sample was poured through the filtration system with the vacuum on. 0.2 mL of sample was added to two test tubes. 0.2 mL of sodium acetate buffer (100 mM, pH 4.5) was added to the first test tube for glucose determination. An enzyme was prepared by adding 1.67 mg invertase to 100 mL of sodium acetate buffer (100 mM, pH 4.5). 0.2 mL of this enzyme was added to the second test tube for sucrose determination. Glucose standards were used in this experiment and were made up of 0.1
mL glucose standard solution and 0.3 mL distilled water. The reaction blanks were made using 0.2 mL of sodium acetate buffer (100 mM, pH 4.5) and 0.2 mL of distilled water. All test tubes were incubated for 20 minutes at 50°C. 3 mL of glucose oxidase-peroxidase reagent (Megazyme International, Ireland) was added to each test tube and they were incubated at the same temperature for another 20 minutes. Absorbance of each sample was measured against a reaction blank at 510 nm (Cary 1-Bio UV-visible spectrophotometer, Ontario, Canada). Absorbance and weight of sample were added to a Megazyme data calculator in order to determine glucose and sucrose levels.

4.3.6 Porridge Sample Preparation

Sensory testing of porridge made from millet samples was completed. The samples were whole grain millet, decorticated millet, and the 25°C/48 h and 25°C/96 h germinated millet. Porridge was made from a millet to water ratio of 2:5 for all samples except for the 25°C/96 h which will be discussed in the next paragraph. For each sample, 85 g of millet were put into a blender and the contents ground in 10 second bursts. This was repeated four times, with the contents shaken each time to ensure millet was ground well. The millet flour was put into a saucepan and the measured water was added. The contents were brought to a boil, covered, and the temperature was set to a medium heat. A timer of 10 minutes was started and the porridge was stirred every two minutes. After cooking, 15 g of porridge were spooned into 10 styrofoam cups labelled with random three-digit codes. Preparation of porridge always began an hour prior to the panel beginning. The prepared cups were kept at 50°C in a water bath to maintain porridge temperature.

All samples were prepared using the same technique aside from the 25°C/96 h germinated sample. This sample lost much of its gelatinizing power and therefore less water was
used. A ratio of 1:2 of millet to water was used instead. This was to ensure consistency in appearance for the purposes of the panel.

### 4.3.5 Descriptive Analysis

Descriptive analysis to characterize the sensory properties of the millet grains was conducted at the University of Guelph. Ethics was obtained and approved by the University of Guelph Research Ethics Board (REB#11AU004). Nine experienced panellists were recruited for this panel from Guelph, ON. They were given a consent form to sign, listing ingredients and potential allergens. The panel was trained for 10 sessions. The first session consisted of attribute generation. They were told to list any flavours and textures present in the four samples. The next three sessions involved choosing references that fit the flavour profile of the porridge and rating them on the scale for intensity. Table 4.1 lists the references chosen and how they were prepared.

#### Table 4.1: References used for each attribute generated and its intensity

<table>
<thead>
<tr>
<th>Attribute</th>
<th>References</th>
</tr>
</thead>
</table>
| Sweet      | Water with dissolved sucrose solution  
Low: 0.5% sucrose, rated as 1 on the scale  
High: 1% sucrose, rated as 9 |
| Bitter     | Water with caffeine solution  
0.03% caffeine, rated as 14 |
| Green      | Alfalfa sprouts  
3 grams of sprouts, rated as 15 |
| Branny     | Wheat germ cooked in 1 1/3 cup water  
1/3 cup wheat germ, rated as 10 |
| Astringent | Water and tannic acid solution  
0.01% tannic acid, rated as 8 |
| Starchy    | 1 cup water with corn starch dissolved  
2 tbsp of corn starch, rated as 15 |

During the training, the panellists practiced rating the samples based on these flavours and textures using references on line scales. During training and testing, a slice of Golden
Delicious® apple and water were provided to cleanse the palate. Samples were given randomized three digit codes and presented in random order with a two minute break between each sample. The panellists evaluated each of the attributes on a 15 cm line scale with anchors (Table 4.2). This was done under red lighting to mask differences in appearance. All sensory data was collected using Compusense Five © 2008 (Compusense, Guelph, Canada) software.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Definition</th>
<th>Rating Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet</td>
<td>The taste stimulated on the tongue by sucrose and other sugars</td>
<td>0=Not Sweet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15=Very Sweet</td>
</tr>
<tr>
<td>Bitter</td>
<td>The taste stimulated on the tongue by caffeine</td>
<td>0=Not Bitter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15=Very Bitter</td>
</tr>
<tr>
<td>Green</td>
<td>A vegetation aromatic associated with bean sprouts</td>
<td>0=Not Green</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15=Very Green</td>
</tr>
<tr>
<td>Branny</td>
<td>A grainy, dusty aromatic associated with fibre rich foods</td>
<td>0=Not Branny</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15=Very Branny</td>
</tr>
<tr>
<td>Starchy</td>
<td>Aromatics associated with wheat starch</td>
<td>0=Not Starchy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15=Very Starchy</td>
</tr>
<tr>
<td>Astringent</td>
<td>A sensation produced in the mouth that leaves a dry feeling</td>
<td>0=Not Astringent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15=Very Astringent</td>
</tr>
</tbody>
</table>

4.3.7 Analysis of Phenolic Content

Total phenolic content of free and bound phenolic acids was determined using the modified Foline Ciocalteu method (Beta et al., 2004). For extraction of the phenolics, 0.3 g was extracted using 5 mL of an 80:20 ratio of MeOH to H2O. The samples were shaken at 350 rpm for 30 min using a shaker (New Brunswick Scientific G24 Environmental incubator shaker, Edison, NJ). The samples were centrifuged (Thermo Scientific, Sorvall RTI, Waltham, MA) at 10,000 rpm (w15,000 g) for 10 min. The supernatant was then decanted. This was repeated a second time and the supernatants were combined. A portion of the combined supernatants was evaporated under nitrogen and reconstituted to 1 mL with deionized water. This constituted the free phenolic acids in the flour. The remaining pellet was washed with 10 mL of hexane before 5
mL of 2 M NaOH was added. Nitrogen was added to the samples before being shaken for 1 h at 350 rpm. Samples were stored in a dark environment for approximately 20 h. The contents were then acidified to a pH of 2 using 2 M HCl. Using 1:1 ethyl ether and ethyl acetate, the phenolics were extracted three times. The organic layers were separated each time and pooled together. The organic solvents were evaporated to dryness under nitrogen and the residue was reconstituted to 2 mL using deionized water. This constituted the bound fraction of the phenolic content. For total phenolic acid content determination, 0.25 mL of the free or bound phenolic acid extracts was combined with 0.25 mL of freshly diluted (1:1) Foline Ciocalteu reagent. The mixture was allowed to sit for 5 min before 0.5 mL sodium carbonate and 4 mL of water were added. Samples were vortexed and placed in darkness for 30 min. Samples were centrifuged (Thermo Scientific Sorvall RTI, Waltham, MA) before readings were taken at 725 nm using a spectrophotometer (Cary 1-Bio UV-visible spectrophotometer, Ontario, Canada). Means and standard errors of duplicates are reported as ferulic acid equivalents on a flour dry weight basis.

4.3.8 Statistical Analysis

All tests, except sensory testing were conducted in duplicate. The descriptive analysis was conducted in four replicates. ANOVA and Tukey’s honestly significant difference tests were performed on collected data to determine significant differences among treatments (p<0.05) of decorticated millet, whole grain millet, 25°C/48 h germinated millet, and 25°C/96 h germinated millet. Correlation analysis was used to determine the relationship between variables (p<0.05). These tests were done using SAS v. 9.2 statistical package (SAS Institute, Raleigh, North Carolina).
4.4 Results and Discussion

4.4.1 Alpha-amylase activity and its effect of free sugars

Alpha-amylase activity in Ceralpha Units per gram of flour are shown in Table 4.3.

Alpha-amylase activity in the decorticated sample was low, with only 3.3±0.30 units/g flour. Within the whole grain sample, no amylase activity was observed. However, α-amylase activity levels increased upon germination with the highest activity observed for the longest germination time. Similar results were observed in Section 3.4.2 of this thesis.

Table 4.3: Alpha-amylase activity and standard errors in 4 flour samples of decorticated millet, whole grain millet, and germinated at 25°C for 48 h and 96 h

<table>
<thead>
<tr>
<th>Sample</th>
<th>α-amylase Activity (units/g flour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Grain</td>
<td>0.0c1</td>
</tr>
<tr>
<td>Decorticated</td>
<td>3.3c±0.30</td>
</tr>
<tr>
<td>25°C/48 h</td>
<td>336.2b±2.29</td>
</tr>
<tr>
<td>25°C/96 h</td>
<td>879.7a±2.61</td>
</tr>
</tbody>
</table>

1All means in the same row with the same letters are not significantly different (p<0.05), n=2

Alpha-amylase activity provokes the breakdown of starch. Therefore, glucose was measured to show how much free sugar is produced after amylolysis occurs. The glucose content in whole grain millet was 0.12±0.01 g/100 g of flour (Figure 4.1). The decorticated samples had a lower glucose content at 0.05±0.00 g/100 g of flour. Dharmaraj & Malleshi (2011) found that glucose decreased from 0.39 g/100 g of flour to 0.24 g/100 g of flour after decortication of finger millet. The glucose content increased as germination time increased. After 48 h of germination, glucose increased to 0.61±0.00 g/100 g of flour. Germination for 96 h produced the highest amount of glucose with 1.8±0.01 g/100 g of flour.
Sucrose content was also measured as it is the main sugar found in millet. The whole grain sample contained 0.38±0.008 g/100 g of flour (Figure 4.2). After decortication, the content decreased to 0.2±0.01 g/100 g of flour. This reduction has also been observed by Dharmaraj & Malleshi (2011) who found that sucrose content decreased from 0.23 g/100 g of flour to 0.06 g/100 g of flour after decortication of finger millet. Sucrose and glucose sit in the aleurone layer of grains (Aoki et al., 2006) and decortication can remove some of this layer during pre-processing. This could explain the reduction of sugars after decortication.

Sprouting also altered sucrose content. Germination for 48 h led to a slight decrease of sucrose to 0.35±0.03 g/100 g of flour in comparison to the whole grain sample. This could be attributed to cellular respiration occurring within the grain. Germination for 96 h produced 1.0±0.01 g/100 g of flour. Sucrose may have been synthesized to work as a transporter within the cell. It works by taking carbon and energy from the storage reserves of the seed and transporting it to the growing embryo (Nomura, Kono, & Akazawa, 1969).
Correlation analysis was performed to assess the relationship between \( \alpha \)-amylase activity and glucose. The correlation coefficient was 0.996 (\( \text{p}<0.0001 \)). This shows that there is a relationship between these two variables. The \( \alpha \)-amylase activity produced as a result of germination is directly correlated with the amount of glucose found within the seed.

![Sucrose content (g/100 g) in 4 flour samples of decorticated millet, whole grain millet, and germinated millet after 48 h and 96 h of sprouting (n=2).](image)

**4.4.2 Taste and flavour of germinated millet porridge**

The results of descriptive analysis assessing the taste and flavour of porridge are presented in Table 4.4. Germination had a significant impact on the attributes determined by the participants. They generated six attributes that were found in varying degrees in all four samples. Since the glucose content increased in the germinated grains, it was hypothesized that sweetness would increase. The participants found the sweetness of the samples to increase significantly as germination time increased. This high sweetness was hoped to reduce and help mask the off flavours associated with millet, including bitter. However, bitterness also increased as germination time increased.
Table 4.4: Means and standard errors of the six flavour attributes found within the four millet porridge samples

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Whole Grain</th>
<th>Decorticated</th>
<th>25°C/48 h Germination</th>
<th>25°C/96 h Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet</td>
<td>5.09±0.80</td>
<td>1.86±0.91</td>
<td>9.45±0.67</td>
<td>12.62±0.81</td>
</tr>
<tr>
<td>Bitter</td>
<td>5.35±1.23</td>
<td>1.03±0.74</td>
<td>9.46±0.72</td>
<td>11.20±2.05</td>
</tr>
<tr>
<td>Green</td>
<td>4.91±1.27</td>
<td>0.57±0.40</td>
<td>9.18±0.95</td>
<td>11.75±0.92</td>
</tr>
<tr>
<td>Branny</td>
<td>6.92±0.59</td>
<td>1.15±0.81</td>
<td>10.03±0.74</td>
<td>12.18±0.74</td>
</tr>
<tr>
<td>Starchy</td>
<td>8.79±1.27</td>
<td>12.43±0.52</td>
<td>4.33±0.61</td>
<td>1.76±0.93</td>
</tr>
<tr>
<td>Astringent</td>
<td>6.57±0.96</td>
<td>1.61±1.00</td>
<td>9.85±0.76</td>
<td>11.61±0.91</td>
</tr>
</tbody>
</table>

1 All means in the same row with the same letters are not significantly different (p<0.05), n=36
2 All data input on 15cm line scale (0cm = lowest intensity and 15cm = highest intensity

Kebakile, Rooney, Kock, & Taylor (2008) found similar flavour and taste attributes from a sensory test of sorghum porridge. Attributes generated for sorghum porridge included bitter, astringent and cereal (starchy) attributes from the samples. They also perceived a branny aroma. Nantanga (2008) also ran a trained panel using pearl millet porridge. The panel described the sample as having a bitter taste and a sweet, fruity aroma. Unlike the current study, a sweet taste was not perceived.

Within the millet porridges evaluated, sweet and bitter were the two tastes perceived in these samples. Sweetness perception increased as germination time increased (Table 3.4). This increase in sweetness can be attributed to the increased α-amylase activity and glucose content. The highest level of glucose content was found in the 25°C/96 h germinated sample. Therefore, it makes sense that the 96 h germinated sample was rated as the sweetest. Bitter taste also increased with germination. The increased sweetness occurring due to germination was not successful in masking the bitter taste of millet. Germination appears to have enhanced the bitter taste. This may have been due to protein degradation occurring during this process. Partial protein digestion by proteolytic enzymes can produce bitter peptides. These bitter peptides are
known to impart a bitter taste that decreases the sensory quality of a food (Maehashi & Huang, 2009).

The three flavours found in these samples were green, branny, and starchy. The green flavour was a vegetation aroma that was perceived by the panellists. Both the green flavour and the branny flavour increased with germination time. The starchy flavour decreased as germination time increased. After 96 h of germination, the mean starchy flavour was rated very low on the 15 cm line scale, meaning it was hardly perceived by the panellists.

Astringent was perceived in the porridge as a mouthfeel. It was defined by the panel as the sensation in the mouth that leaves a dry feeling. Astringency increased when germination time increased. Whole grain porridge was rated at 6.57±0.96 by the panellists. The rating increased to 9.85±0.76 in the 25°C/48 h germinated sample. The porridge made with 25°C/96 h germinated millet was rated highest for astringency at 11.61±0.91 on the 15 cm line scale.

The decorticated porridge sample was found to have low levels of all tastes and flavours, except starch flavour. Dharmaraj & Malleshi (2011) used decorticated finger millet to make porridge and found it to have a bland taste with a mild millet flavour. The low ratings for the decorticated sample in this study confirm these previous results. The high starch flavour can be attributed to the higher levels of starch found within decorticated millet compared to whole grain millet. Bora (2014) showed that the total starch value of whole grain millet is 64.5% for proso millet with an increase in total starch after decortication to 78.4%. Bran can also dilute starch in whole grain products. West (2012) conducted a trained panel on whole grain pasta and found those who rated branny samples as low on the scale, tended to also rate the samples as high in
starch. The opposite was also seen in his study where those samples rated as low in starch, were rated high in branny flavour.

### 4.4.3 Phenolic content and its contribution to flavour and taste

This study focused on total free and bound phenolic content. The results are presented in Table 4.5.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Free Phenolic Content (ug/g)</th>
<th>Bound Phenolic Content (ug/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Grain</td>
<td>778.9b±70.45</td>
<td>1531.5b±70.34</td>
</tr>
<tr>
<td>Decorticated</td>
<td>719.2b±27.70</td>
<td>113.0c±15.49</td>
</tr>
<tr>
<td>25°C/48 h Germination</td>
<td>3415.9a±120.00</td>
<td>1788.6b±0.75</td>
</tr>
<tr>
<td>25°C/96 h Germination</td>
<td>3826.0a±76.00</td>
<td>2911.3a±83.15</td>
</tr>
</tbody>
</table>

*aAll means in the same column with the same letters are not significantly different (p<0.05), n=2*

The whole grain sample had 778.9±70.45 ug/g of free and 1531.5±70.34 ug/g of bound phenols. After decortication, the free phenolics decreased to 719.2±27.70 ug/g and bound reduced to 113.0±15.49 ug/g. The biggest changes occurred after germination. The free and bound phenolics increased in the 25°C/48 h germinated sample to 3415.9±120.00 ug/g and 1788.6±0.75 ug/g, respectively. The free phenolics content in the 25°C/96 h germinated sample was 3826.0±76.00 ug/g, which was not a significant increase. However, the bound phenolics in the 25°C/96 h germinated sample significantly increased to 2911.3±83.15 ug/g.

Maillard, Soum, Boivin, & Berset (1996) malted 5 different varieties of barley. They found that the total phenolic compounds in each variety significantly increased after germination. Yang, Basu & Ooraikul (2001) germinated wheat grain for up to 192 h. They tested for phenolic compounds and found ferulic acid and vanillic acid decreased until 120 h of germination, when
they gradually increased and reached a peak. This was attributed to the long steeping hours of 24 and 48 h. Leeching of these compounds was happening during this time and 48 h had considerably less compounds as there was more time for leeching to occur. The samples in the current study were only steeped for 6 h and some leeching would have occurred but it would have been minimal in comparison. Kilning, or drying, has also been found to increase the phenolic acid content in germinated grains (Lu et al., 2007; Maillard & Berset, 1995). The samples in this study were dried for 14 hours and this could have increased the total phenolic content.

The increase of phenolic content in the germinated products could be due to the solubilization during steeping and dry matter loss (Glennie, 1983). It could be attributed to major components being hydrolyzed and phenolic biosynthesis occurring in the grain (Yang, Basu & Ooraikul, 2001). Phenylalanine ammonia lyase (PAL) has been shown to be the most important enzyme involved in phenolic biosynthesis (Maillard & Berset, 1995). This enzyme could have been activated during which would explain the rapid increase of phenolic acids after germination started.

Sprouts and seedlings generally contain more phenolic compounds compared to a full grown, mature seed. This is for protection against abiotic stressors. Phenolic acids are able to provide resistance against predators, parasites and pathogens. They work as a natural pesticide. Since the mature grain is being re-sprouted, similar metabolic processes could be occurring in order to protect the seed as it grows (Drewnowski & Gomez-Carneros, 2000). This could also be a factor in the increase of phenolic compounds after germination.
Kobue-Lekalake, Taylor, & de Kock (2007) used sorghum varieties of different phenolic contents to show how they contributed to flavour and taste. Those cultivars that had a higher phenolic acid content were also rated higher in bitterness and astringency by a trained panel. According to Drewnowski & Gomez-Carneros (2000), phenolic compounds have been shown to contribute to bitterness and astringency of a product. This is consistent with the results of the current study. Those samples with higher phenolic compounds were rated stronger for off-flavours. Also, the decorticated samples had a major decrease in total phenolic content. It also had the mildest flavour and taste profile of all the samples in this study. Therefore, some of the negative properties that the panellists tasted after germination could be attributed to the increased amount of phenolics produced.

4.6 Conclusion

Germination can increase the amount of natural sugars present in millet, however off-flavours were more apparent because of the high phenolic content. Decortication proved to be a better method for improving the sensory attributes in proso millet porridge. Although the phenolics negatively affected the flavour of the product, it possesses a high level of antioxidants. It may be worth investigating how marketing germinated proso millet as a natural health product can affect the consumers’ perception and acceptability.
CHAPTER 5: CONCLUSIONS AND FUTURE RECOMMENDATIONS

Recently, millet has increased in importance in North America because of its gluten free nature and low glycemic index. Celiac disease and type II diabetes mellitus are growing in prevalence and there has been increasing interest in exploring new grains for this population. It is highly nutritious and rich in protein, fat and many minerals. Changing environments are changing the agriculture industry and the need for sustainable crops is growing. Millet is a drought resistant crop with a short growing season that is resistant to pests and disease. The main focus of this research is to gain insight into millet and its functionality to increase utilization in food products.

The objective of this research was to naturally process proso millet and observe the effects it had on the physicochemical and sensory properties. Chapter 3 of this research germinated proso millet under three different temperatures of 20, 25 and 30°C for three different times of 24, 72 and 96 h. Germination has been used for many centuries to activate hydrolytic enzymes to modify the structure of major components of a grain and improve the nutritional value. Proximate analysis showed that germination maintained the nutritive value of millet. The enzyme, α-amylase was activated during germination and increased as time and temperature increased. Alpha-amylase breaks down starch polymers into sugars of different chain lengths. Glucose content also increased as germination time and temperature increased, as it was highly correlated with α-amylase activity. During germination, α-amylase attacks the surface of starch to break it down. It loses its capacity to bind water and therefore, peak viscosity drastically decreased in those samples with higher α-amylase activity. Final viscosity also reduced in the same manner as peak viscosity. The eGI values increased as the time and temperature of germination increased with values ranging from 44.8 to 60.8. Whole grain proso millet is a low
GI food and its germination continued to increase its value on the index, with three samples becoming medium GI foods. This increase was expected as the breakdown of starch into sugars makes them more available.

The taste of millet is a major limitation to its use in the North American food industry. Millets are a rich source of phenolic acids, which have been known to contribute to off flavours and aromas in foods. Decortication and germination were employed as an approach to improving the sensory properties of millet by reducing phenolic content in Chapter 4. Proso millet was germinated at 25°C for 48 h and 96 h. Decorticated millet was also used as a sample. Alpha-amylase activity was not observed in whole grain and decorticated samples. It increased in germinated millet with the highest value found in the 96 h sample. Free sugar content was lowest in decorticated millet and the highest in the 96 h germinated sample. This increase in sugars was expected to help mask the bitter taste of millet. Descriptive analysis was conducted and the panellists found six taste and flavour attributes associated with millet porridge. The free sugar increase was perceived as the germinated samples were rated highest for sweetness. However, this sweetness was not able to mask the bitterness of the samples, as the bitter taste increased. The decorticated sample was found to be very mild in flavour, with a significantly lower phenolic content. The phenolic content increased in the germinated samples and this could have contributed to the increase in the perceived bitter taste. The use of decortication improved the negative sensory properties by reducing the phenolics present.

Future recommendations to continue this research include:

- In vivo testing for glycemic index for proso millet used in a food matrix
• In vitro eGI methodology was used to estimate the effect these products would have on the blood glucose levels of a human. However, in vivo studies would give the most accurate record of how germinated millet is processed within the body. A food matrix common to North American consumers should be used for testing.

• Consumer testing for processed millet porridge
  • Although germinated porridge was rated high for negative sensory properties, these were adjusted based on the scale. Consumer testing would better dictate whether this bitterness affected their liking and whether it could be a marketable product as a functional food.

• Optimizing the germination process for proso millet
  • Response surface methodology could be used in order to determine optimal conditions for the germination of proso millet. This could be done for many desirable responses, such as optimal nutrition or taste.

• Exploring other drying methods
  • Oven drying could have led to an increase in phenolic content in the germinated samples. Benchtop tasting was done on samples before oven drying and very little bitterness was perceived. Freeze drying may be a better option as it may lead to less of an effect on taste.
References


Jiang, H. (2010). Resistant-starch formation in high-amylose maize starch. Digital Repository @ Iowa State University.


APPENDICES

Appendix A: Ethics for Sensory Evaluation

University of Guelph Research Ethics Board (REB)

FACULTY AND GRADUATE

Application to Involve Human Participants in Research

Please refer to the University of Guelph Research Ethics Guidelines, found at http://www.uoguelph.ca/research/forms_policies_procedures/human_participants.shtml before completing and submitting this application. If you have questions about this form, please contact the Research Ethics Coordinator, Sandra Auld at ext. 56606, or reb@uoguelph.ca.

Send this form and all accompanying material by email, as attachments, to reb@uoguelph.ca. One hard copy of the signed signature page should be forwarded to the Research Ethics Coordinator, Office of Research, University of Guelph, 437 University Centre, Guelph, ON, N1G 2W1.

If you want to change a previously approved protocol, please complete the “Change Request” form, available at http://www.uoguelph.ca/research/forms_policies_procedures/human_participants.shtml.

Date: 2012-09-18 (yyyy-mm-dd) (For OR use only) Protocol#:

SECTION A – GENERAL INFORMATION

1. Title of the Research Project: Sensory perception of millet products (formerly millet porridge)
2. **Investigator Information**

<table>
<thead>
<tr>
<th>Name &amp; position</th>
<th>Dept./Address</th>
<th>Phone No.</th>
<th>E-Mail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faculty with Principal Responsibility*</td>
<td>Koushik Seetharaman</td>
<td>Food Science</td>
<td>52204</td>
</tr>
<tr>
<td></td>
<td>Associate Professor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faculty:</td>
<td>Lisa Duizer</td>
<td>Food Science</td>
<td>53410</td>
</tr>
<tr>
<td>Co-Investigator(s)</td>
<td>Assistant Professor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Student: Investigator(s)</td>
<td>Abir Sarker</td>
<td>Food Science</td>
<td>56869</td>
</tr>
<tr>
<td></td>
<td>Matt McSweeney</td>
<td>Food Science</td>
<td>56869</td>
</tr>
<tr>
<td>Other:</td>
<td>Investigator(s)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* must be advisor of any student investigators.

3. **Proposed Date**

   a) of commencement: October 31, 2012  
   b) of completion: Sept 30, 2014

Note: The commencement date should be the date the researcher expects to actually begin interacting with human participants (including recruitment). The completion date should be the date that the researcher expects that interaction with human participants, including any feedback or follow-up, will be complete.

4. **Indicate the location(s) where the research will be conducted:**

   University of Guelph

   Other (please specify site): Sensory evaluation laboratory (FS146) and Guelph Food Technology Centre Bake Lab
5. Other Research Ethics Board Approval

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
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<tbody>
<tr>
<td>a) Is this a multi-centred study?</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>b) Has any other institutional Ethics Board approved this project?</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>c) If Yes, please provide the following information:</td>
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<tr>
<td></td>
<td>Title of the project approved elsewhere:</td>
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<td></td>
<td>Name of the Other Institution:</td>
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<td>Name of the Other Board:</td>
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<td>Date of the Decision:</td>
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<td></td>
<td>A contact name and phone number for the other Board:</td>
<td></td>
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<td></td>
<td>OR</td>
<td></td>
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<tr>
<td></td>
<td>A copy of the clearance certificate / approval</td>
<td></td>
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<tr>
<td>d) Will any other Research Ethics Board be asked for approval?</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>If Yes, please specify:</td>
<td></td>
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</tbody>
</table>

6. Level of the Project

- Faculty Research [ ]
- PhD Thesis X
- Masters Thesis X
- Honours Thesis [ ]
- Class Project [ ]
- Internship [ ]
- Practicum [ ]
- Other (please specify): 

7. Funding of the Project
a) Is this project currently funded?  
Yes X No □

b) Period of Funding: April 1 2011 to Sept 2014

b) Agency or Sponsor (funded or applied for)

CIHR:   
NSERC:   
SSHRC:   
Other (please specify): IDRC

Note: Please specify the complete title of the funding source. For example, “NSERC Discovery Grant”.

NOTE: If the funding source changes, or if a previously unfunded project receives funding, you must submit a Change Form to the Research Ethics Coordinator.

8. Conflict of Interest

a) Will the researcher(s), members of the research team, and/or their partners or immediate family members:

i) Receive any personal benefits (for example a financial benefit such as remuneration, intellectual property rights, rights of employment, consultancies, board membership, share ownership, stock options etc.) as a result of or connected to this study? Yes □ No X

ii) If Yes, please describe the benefits below. (Do not include conference and travel expense coverage, possible academic promotion, or other benefits which are integral to the general conduct of research.)

b) Describe any restrictions regarding access to or disclosure of information (during or at the end of the study) that the sponsor has placed on the investigator(s).
No restrictions are in place

c) Discuss the possibility of commercialization of the research findings.

There are no plans to commercialize these products

SECTION B – SUMMARY OF THE PROPOSED RESEARCH

9. Rationale

Describe the purpose and background rationale for the proposed project, as well as the hypotheses/is/research questions to be examined.

The proposed project looks at the physicochemical and nutritional properties of small millets (both sprouted and unsprouted) in different product matrices. The project will also look at the sensory properties and consumer acceptance of different products made with millets and/or millets blended with wheat or rice. The hypothesis driving this project is that millets will have low glycemic response irrespective of the product matrix when compared with similar wheat or rice based products.

10. Methodology

Describe sequentially, and in detail, all procedures in which the research participants will be involved (e.g., paper and pencil tasks, interviews, surveys, questionnaires, physical assessments, physiological tests, time requirements etc.)

*Note: Attach a copy of all questionnaire(s), interview guides or other test instruments. These should be on University of Guelph letterhead if they are intended for public dispersal.*

Individuals from the Food Science department will be invited to participate in a taste panel of millet porridge and cookies. This panel will involve their liking of the porridge as well as perception of flavours and textures of the
porridge and cookies compared to traditional wheat based products. The porridge will be made using pearled millets or millet flour boiled in water with either salt or sugar. The cookies will have similar formulation as traditional wheat based cookies with improvements in process and/or formulation to make the product more acceptable. All products will be manufactured in the Guelph Food Technology Centre bake lab in the Food Science building and in the Sensory Evaluation Facility which is located in the Food Science building (part of the Human Nutraceutical Research Unit). Both facilities are food grade facilities and all food preparation will be done following GMP guidelines and using commercially available products.

Three different types of testing will be conducted on the products.

**Consumer testing:** Participants will be asked to look at and eat each product and to answer questions regarding their liking of the appearance, flavor, texture and overall liking (using 9-point hedonic scales). A copy of the questionnaire for each product type is attached in Appendix 1.

**Trained descriptive analysis:** A trained panel will be used to characterize the millet products. During the training sessions, a trained researcher will ask participants their acceptance of appearance (color, viscosity), aroma, taste, texture, mouthfeel, and residual mouth coating of the millet products. The trained panel will characterize how millet and millet processing affect the overall taste and quality of the product and discuss what they like and dislike about each product. At this stage, the attributes have not been decided because this is the first stage of panel training, therefore it is not possible to show the questionnaire that will be used. Once the attributes are determined and agreed upon by the panel, they will be trained to discriminate the between the samples based on those attributes by tasting each product and providing a response on a 15cm line scale.

**Preferred Attribute Elicitation:** Consumers will be recruited to characterize the millet products using a method entitled Preferred Attribute Elicitation (PAE). There are six steps to this method:

1. Panelists will be asked their liking of flavour and overall liking of each product type evaluated by the panelists on a 9-point hedonic scale. After the evaluation of liking, panelists will be asked to write in the space provided what attributes they like or dislike about the product (Appendix 2).
2. The researcher will then initiate a round table discussion and ask the panelists to say the attributes they wrote down aloud. The attributes will be written on sticky notes and placed on a white board.
3. The panelists will then be asked to group the attributes into categories they find appropriate.
4. 7-point scales are then generated for the attribute categories and panelist will be asked to assign anchor descriptor terms for intensity of each attribute. Panelists are then asked to group synonymous and antonymous attributes into a single scale. If panelists feel that the generated scale does not encompass all of the important attributes that are encouraged to make an additional scale.
5. Panelists are then asked to rank the attribute scales according to their importance in driving liking. If panelists feel that certain attribute scales are equally important, then they can hold the same rank in order of importance.
6. After a short break panelists are presented with fresh product samples and will be asked to evaluate each attribute on the 7-point scale with the anchor terms they selected.
All testing will be conducted in the Sensory Evaluation Facility, which is located in the Food Science building (part of the Human Nutraceutical Research Unit). Participants will taste the products in individual tasting booths and they will input all of their data into Compusensefive (specialist software for collection of sensory data).

11. **Experience**

   What is your experience with this kind of research?

   Drs. Seetharaman and Duizer have been involved with sensory testing as part of other research projects in the Department of Food Science. Both Matt McSweeney and Abir Sarkir have completed the F00D*3700 Sensory Evaluation of Food undergraduate course and both have participated in sensory testing in the past.

12. **Participants**

   Describe the number of participants and important characteristics (such as age, gender, location, affiliation, etc.)

   For consumer testing, Twenty-five individuals will be recruited from the Department of Food Science faculty and graduate students.

   For the trained panel, 10 individuals will be trained.

   For the PAE, 30 participants will be recruited from the Department of Food Science faculty and graduate students.

13. **Recruitment**

   a) Describe how and from what sources the participants will be recruited, including any relationship between the investigator(s) and participant(s) (e.g., instructor-student; manager-employee).

   *Note: Attach a copy of any poster(s), advertisement(s) or letter(s) to be used for recruitment.*
For consumer testing, an email will be sent to the department list-serve requesting assistance with the project. A copy of the email is attached in Appendix 3.

For the trained panel, the email shown in Appendix 4 will be sent to individuals who have previously taken part in trained panel evaluations and have agreed to be involved with other trained panel evaluations.

For the PAE an email will be sent to the department list-serve requesting assistance with the project. (Appendix 5).

b) How and where will you contact these participants?

Individuals will be contacted via email.

c) Time required of participants:

1/2 hour on 1 occasion for consumer testing, 10 sessions of 1 hour each for trained panel evaluations and 2-1 hour sessions for PAE

d) Are participants proficient in the language in which the survey is being conducted? X ☐

If not, is translation available? ☐

14. Compensation

a) Will participants receive compensation for participation? Yes No
i) Financial X
   X
ii) Non-financial X

b) If Yes to either i) or ii) above, please provide details.

<table>
<thead>
<tr>
<th>Individuals for the consumer panel will be reimbursed $5.00.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals for the trained panel will be paid $15.00 for each session they attend.</td>
</tr>
<tr>
<td>Individuals for the PAE will be paid $15.00 for each session they attend.</td>
</tr>
</tbody>
</table>

c) If participants choose to withdraw, how will you deal with compensation?

| If a participant withdraws upon reading the information sheet and refusing to take part in the study, they will still be reimbursed. |
SECTION C – DESCRIPTION OF THE RISKS AND BENEFITS OF THE PROPOSED RESEARCH

15. **Possible Risks**

   a) Indicate if the participants might experience any of the following risks:  
      Yes  No

      i) Physical risk (including any bodily contact or administration of any substance)?  
         X

      ii) Psychological risks (including feeling demeaned, embarrassed worried or upset)?  
          X

      iii) Social risks (including possible loss of status, privacy and/or reputation)?  
          X

      iv) Is there any deception involved?  
          X

      v) Are any possible risks to participants greater than those the participants might encounter in their everyday life?  
          X

   b) If you answered Yes to any of points i) through v) above, please explain the risk.

   Participants will be consuming food, which means that there is the possibility of choking or having an allergic reaction to the product. This is, however, no greater than the risks that would occur with everyday life.

   c) Describe how the risks will be managed (including an explanation as to why alternative approaches could not be used).

   With regard to the slight risk of an allergic reaction, individuals will be asked if they have any known allergies to ingredients present in the cereal products. If they do, they will not take part in the study.

16. **Possible Benefits**
Discuss any potential direct benefits to the participants from their involvement in the project. Comment on the (potential) benefits to the scientific community/society that would justify involvement of participants in this study.

There will not be any direct benefits to the participants during the course of their involvement in the project. However, in the larger context, if the results of the broader research are positive, then we will be able to highlight the benefits of small millets as healthy in the context of glycemic response.

SECTION D – THE INFORMED CONSENT PROCESS

17. The Consent Process

a) Describe the process that the investigator(s) will be using to obtain informed consent, including a description of who will be obtaining the informed consent. If there will be no written consent form, explain why.

When participants arrive for the study they will be greeted by an individual involved with the study. This individual will be fully knowledgeable about the purpose of the study as well as the methodology that will be used so that if questions are asked, they will be able to answer them. The participants will be asked to read an information sheet and all of their questions will be answered at that time. They will fill in a questionnaire related to food allergies/sensitivities. If they indicate that they are allergic to any of the listed ingredients, they will asked to not take part in the study. If they do not have allergies, they will sign a consent form. A copy of the information sheet and consent form is included in Appendix 6.

For information about the required elements in the letter of information and the consent form, please refer to “Instructions for the Preparing Information and Consent Letters” and the sample consent form available at http://www.uoguelph.ca/research/forms_policies_procedures/human_participants.shtml.

Note: Attach a copy of the Letter of Information (if applicable), the Consent Form (if applicable), the content of any telephone script (if applicable) and any other material which will be used in the informed consent process. If the document will be made public, please ensure that it is on University of Guelph letterhead.
b) Will the information provided to the participants be complete and accurate? Yes X No □

If no, please describe the nature and extent of the deception involved. Include how and when the deception will be revealed, and describe the specialized training of the person who will administer this feedback. It is recommended that participants have the opportunity to sign a second consent form, following debriefing when the deception is revealed, to ensure a fully informed consent.

*Note: Attach a copy of the debriefing feedback and, if necessary, a copy of the second consent form on University of Guelph letterhead.*

18. **Consent by an authorized party**

If the participants are minors or for other reasons are not competent to consent, describe the proposed alternate source of consent, including any permission / information letter to be provided to the person(s) providing the alternate consent.

All participants will be above the age of consent and competent to consent.

19. **Alternatives to prior individual consent**

If obtaining individual participant consent prior to starting the research project is not appropriate for this research, please explain and provide details for a proposed alternative consent process.
20. **Participant feedback**

Explain what feedback/information will be provided to the participants after participation in the project. (For example, a more complete description of the purpose of the research, or access to the results of the research).

*Note: Please provide a copy of the written information, if applicable.*

At the completion of the tasting session, if individuals would like to know more about the products that they tasted, they can ask questions of the primary researcher. All questions asked will be answered.

21. **Participant withdrawal**

a) Describe how the participants will be informed of their right to withdraw from the project. Outline the procedures that will be followed to allow the participants to exercise this right.

Participants will be informed via the information sheet that they can withdraw from the study if they are not comfortable consuming the products. Additionally, they will be informed that they can leave the tasting room at any time if they do not feel comfortable consuming the products.

b) Indicate what will be done with the participant’s data and any consequences for the participant of withdrawing from the study.

The data will be collected using specialist sensory evaluation data collection software. If an incomplete dataset is collected, the data will be deleted and not used in the data analysis.
c) If the participants will not have the right to withdraw from the project, please explain.
SECTION E – CONFIDENTIALITY

22. Ensuring confidentiality

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Will all participants be anonymous?</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>b) Will all data be treated as confidential?</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

Please note the difference: Participants’ identity/data will be confidential if an assigned ID code or number is used, but it will not be anonymous. Anonymous data cannot be traced back to an individual participant.

c) Describe the procedures to be used to ensure anonymity of participants and/or confidentiality of data both during the conduct of the research and in the release of its findings.

> After the participants have signed the consent form, they will be provided with a number which they will use to log into the data collection system. This number will not be recorded in such a way to trace the data back to a participant. After the data have been collected, only the student and faculty members involved with the project will have access to the data for analysis. At no time will individual data be reported. All data will be averaged and standard deviations will be calculated.

> d) Explain how written records, video/audio tapes and questionnaires will be secured, and provide details of their final disposal or storage.

> All data and questionnaires will be stored on a computer in the sensory facility. This computer is password protected and the room is locked. Once the data have been analyzed, the data files will be stored on the computer for 1 year and then deleted from the computer.

> e) If participant anonymity or confidentiality is not appropriate to this research project, explain, providing details of how all participants will be advised of the fact that data will not be anonymous or confidential.
SECTION F – MONITORING ONGOING RESEARCH

23. **Annual Review and Adverse Events**

   a) Minimum protocol review requires the completion of a “Renewal/Completed Status Report” at least annually. Indicate whether any additional monitoring or review would be appropriate for this project.

   *Note: It is the investigator’s responsibility to notify the REB using the “Renewal/Completed Status Report” when the project is completed, or if it is cancelled. The form is available at [http://www.uoguelph.ca/research/forms_policies_procedures/human_participants.shtml](http://www.uoguelph.ca/research/forms_policies_procedures/human_participants.shtml).*

   b) **Adverse events** (unanticipated negative consequences or results affecting participants) must be reported to the Research Ethics Board and the Research Ethics Coordinator as soon as possible.
24. Additional Information

(Use an additional page if more space is required to complete any sections of the form, or if there is any other information relevant to the project that you wish to provide to the Research Ethics Board.)

SECTION G – SIGNATURES

Responsible Faculty Assurance:

I, ____________________________ [PLEASE PRINT] have the ultimate responsibility for the conduct of the study described in this application including my responsibilities as an advisor to any students involved in this project. I have read and am responsible for the content of this application. If any changes are made in the above arrangements of procedures, or adverse events are observed, I will bring these to the attention of the Research Ethics Coordinator.

______________________________
Signature

______________________________
Date
Appendix 1: Consumer questionnaire

Porridge questionnaire:

In front of you is a sample of millet. The sample is labeled with a 3 digit code.

Please look at sample 123

1. How do you like or dislike the appearance of this sample?

   Dislike  Dislike  Dislike  Dislike  Neither  Like  Like  Like  Like

   Extremely  Very much  Moderately  Slightly  like nor dislike  Slightly  Moderately  Very much  Extremely

Now please taste sample 123

2. How much do you like or dislike the flavor of this sample?

   Dislike  Dislike  Dislike  Dislike  Neither  Like  Like  Like  Like

   Extremely  Very much  Moderately  Slightly  like nor dislike  Slightly  Moderately  Very much  Extremely

3. How much do you like or dislike the texture of this sample?

   Dislike  Dislike  Dislike  Dislike  Neither  Like  Like  Like  Like

   Extremely  Very much  Moderately  Slightly  like nor dislike  Slightly  Moderately  Very much  Extremely

4. The texture of this product is

   Not thick  Just right  Too thick
5. After swallowing the sample, is there a taste left in your mouth?
□ Yes □ No (go to question 6)

If yes,

What do you like about this taste?
____________________________________________________________________

What do you dislike about this taste?
____________________________________________________________________

6. Overall, how much do you like or dislike this product?

□ Dislike □ Dislike □ Dislike □ Dislike □ Neither □ Like □ Like □ Like

Extremely Very much Moderately Slightly like nor dislike Slightly Moderately Very much Extremely

7. How likely are you to consume this product?

□ □ □ □ □ □ □ □

Extremely Unlikely neither unlikely nor likely Likely

Thank you for your time
Cookie questionnaire:

In front of you is a sample of a millet cookie. The sample is labeled with a 3 digit code.

Please look at sample 123

1. How do you like or dislike the appearance of this sample?

   □ Dislike □ Dislike □ Dislike □ Dislike □ Neither □ Like □ Like □ Like □ Like

   Extremely □ Very much □ Moderately □ Slightly □ like nor dislike □ Slightly □ Moderately □ Very much □ Extremely

Now please taste sample 123

2. How much do you like or dislike the flavor of this sample?

   □ Dislike □ Dislike □ Dislike □ Dislike □ Neither □ Like □ Like □ Like □ Like

   Extremely □ Very much □ Moderately □ Slightly □ like nor dislike □ Slightly □ Moderately □ Very much □ Extremely

3. How much do you like or dislike the texture of this sample?

   □ Dislike □ Dislike □ Dislike □ Dislike □ Neither □ Like □ Like □ Like □ Like

   Extremely □ Very much □ Moderately □ Slightly □ like nor dislike □ Slightly □ Moderately □ Very much □ Extremely

4. After swallowing the sample, is there a taste left in your mouth?

   □ Yes □ No (go to question 5)
If yes,

What do you like about this taste?

___________________________________________________________________

What do you dislike about this taste?

___________________________________________________________________

5. **Overall**, how much do you like or dislike this product?

Dislike  Dislike  Dislike  Dislike  Neither  Like  Like  Like  Like
Extremely  Very much  Moderately  Slightly  like nor dislike  Slightly  Moderately  Very much  Extremely

6. How likely are you to **consume** this product?

Extremely  Unlikely  neither unlikely  nor likely  Likely

Thank you for your time
Appendix 2: PAE Questionnaire
PAE Questionnaire

In front of you is a sample of millet. The sample is labeled with a 3 digit code.

Please eat sample 123

How much do you like or dislike this product?

☐ Dislike ☐ Dislike ☐ Dislike ☐ Dislike ☐ Neither ☐ Like ☐ Like ☐ Like ☐ Like
Extremely Very much Moderately Slightly like nor dislike Slightly Moderately Very much Extremely

What attributes do you like/dislike about sample 123? Please write below.


Please eat sample 124

How much do you like or dislike this product?

☐ Dislike ☐ Dislike ☐ Dislike ☐ Dislike ☐ Neither ☐ Like ☐ Like ☐ Like ☐ Like
Extremely Very much Moderately Slightly like nor dislike Slightly Moderately Very much Extremely

What attributes do you like/dislike about sample 124? Please write below.


Please eat sample 125

How much do you like or dislike this product?

☐ Dislike ☐ Dislike ☐ Dislike ☐ Dislike ☐ Neither ☐ Like ☐ Like ☐ Like ☐ Like
Extremely Very much Moderately Slightly like nor dislike Slightly Moderately Very much Extremely
What attributes do you like/dislike about sample 125? Please write below.

Thank you for your time!
Appendix 3: Recruitment Announcement For Consumer Panel
We need your opinion!

We are looking for people who would like to try millet porridge and cookies and us what you think about these products.

We will be running the study in the sensory evaluation lab located in the Human Nutraceutical Research Unit (Food Science room 146) on ________________ (date and time to be confirmed).

Testing will take one hour.

If you would like to sign up or if you would like more information, contact Lisa Duizer (ext 53410 or email lduizer@uoguelph.ca) during work hours.
Appendix 4: Recruitment advertisement for Trained Panel
Hello,

In the past you have helped us with our research by being a trained panelist and you have indicated that you would be interested in being contacted for future studies.

We will be running a study on millet porridge and cookies in the sensory evaluation lab located in the Human Nutraceutical Research Unit (Food Science room 146) on ______________________(dates and times to be confirmed).

Testing will take one hour each day.

If you would like to sign up or if you would like more information, contact Lisa Duizer (ext 53410 or email lduizer@uoguelph.ca) during work hours.
Appendix 5: Recruitment advertisement for PAE
We need your opinion!

We are looking for people who would like to try millet porridge and cookies and tell us what you think about these products.

We will be running the study in the sensory evaluation lab located in the Human Nutraceutical Research Unit (Food Science room 146) on _________________(date and time to be confirmed).

Testing will take one hour and will be for 2 consecutive days.

If you would like to sign up or if you would like more information, contact Lisa Duizer (ext 53410 or email lduizer@uoguelph.ca) during work hours.
Appendix 6: Information sheet and consent form
CONSENT TO PARTICIPATE IN RESEARCH

Consumer testing of food products

You are asked to participate in a research study conducted by Koushik Seetharaman, from the Department of Food Science at the University of Guelph.

If you have any questions or concerns about the research, please feel free to contact Koushik Seetharaman: Faculty member in the Department of Food Science. Phone: 519-824-4120 ext 52240.

PURPOSE OF THE STUDY

The purpose of this study is to determine your response to millet porridge and cookies.

PROCEDURES

Before signing this consent form, you will be provided with a questionnaire to complete regarding food allergies and sensitivities.

In this study, you will be eating food products which contain the following ingredients:

Millet, flour, sugar, salt, baking powder, shortening (sunflower based), eggs
Additionally, you will be cleansing your palate with a saltine cracker and water.

If you know that any of these products/ingredients are likely to cause you discomfort or you are allergic to them, please do not take part in this study.

You will be eating millet porridge and cookies and answering some questions about your liking of this porridge and cookies.

Please note: Testing times will differ based on the test being completed and only the relevant testing time will be included in the consent form.

(For consumer testing) - Testing should take ½ hour of your time.

(For trained panel testing) – You will be required to attend ten (10) – 1 hour sessions.

(For PAE) – You will be required to attend two (2) – 1 hour sessions.
POTENTIAL RISKS AND DISCOMFORTS

If you do not have any allergies/discomfort with any of the listed ingredients, there are no known risks to being involved with this study.

POTENTIAL BENEFITS TO PARTICIPANTS AND/OR TO SOCIETY

Information collected by this study will help us to have a better understanding of what you like and don’t like about millet products. This knowledge will allow for the development of nutritionally beneficial products which are deemed acceptable by consumers.

PAYMENT FOR PARTICIPATION

You will receive a cash reimbursement of $XXX.

Please note: Amounts will differ based on the test being completed and only the relevant testing time will be included in the consent form.

(For consumer testing) – $5.00.

(For trained panel testing) – $15.00 for each session attended.

(For PAE) – $15.00 for each session attended.

CONFIDENTIALITY

Every effort will be made to ensure confidentiality of any identifying information that is obtained in connection with this study. All data which is collected will be stored on a password protected computer in a locked room. The data will be analyzed to calculate mean scores and standard deviations. All data collection is confidential. There is no way to track your panellist number back to you.

PARTICIPATION AND WITHDRAWAL
You can choose whether to be in this study or not. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind. You may exercise the option of removing your data from the study. You may also refuse to answer any questions you don’t want to answer and still remain in the study. The investigator may withdraw you from this research if circumstances arise that warrant doing so.

RIGHTS OF RESEARCH PARTICIPANTS

You may withdraw your consent at any time and discontinue participation without penalty. You are not waiving any legal claims, rights or remedies because of your participation in this research study. This study has been reviewed and received ethics clearance through the University of Guelph Research Ethics Board. If you have questions regarding your rights as a research participant, contact:

Research Ethics Coordinator
University of Guelph
437 University Centre
Guelph, ON N1G 2W1

Telephone: (519) 824-4120, ext. 56606
E-mail: sauld@uoguelph.ca
Fax: (519) 821-5236
I have filled in the screening questionnaire for allergies and I have read the ingredient listing for the products that I will be trying. I am not allergic or sensitive to any of the listed items.

I have read the information provided for the study “Consumer Testing of Food Products” as described herein.

My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

______________________________________
Name of Participant (please print)

______________________________________  ____________
Signature of Participant                    Date
SCREENING QUESTIONNAIRE

Do you have any food allergies?

☐ Yes  ☐ No

If yes, please list what those allergies are:

____________________________________________________________________

____________________________________________________________________

Do you have any food sensitivities?

☐ Yes  ☐ No

If yes, please list what those sensitivities are:

____________________________________________________________________

____________________________________________________________________
In this study, you will be consuming products which contain the following ingredients

*Millet, flour, sugar, salt, baking powder, shortening (sunflower based), eggs*

*Crackers*

Do you have any allergies or intolerance to millet?

☐ Yes  ☐ No

Do you have any allergies or intolerance to wheat based products?

☐ Yes  ☐ No
Appendix B: Anovas

B.1 ANOVA tables for Chapter 3

Table B.1.1: ANOVA table for proximate analysis parameters from Chapter 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Source</th>
<th>DF</th>
<th>ANOVA SS</th>
<th>F Value</th>
<th>Pr&gt;F</th>
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<td>Protein</td>
<td>sample</td>
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<td>1.96893834</td>
<td>35.53</td>
<td>&lt;0.0001</td>
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<td>0.06</td>
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<td>error</td>
<td>7</td>
<td>0.0554092</td>
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<td></td>
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<td>2.02482736</td>
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<td></td>
</tr>
<tr>
<td>Fat</td>
<td>sample</td>
<td>7</td>
<td>0.54797303</td>
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<td>0.0067</td>
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<td>15</td>
<td>0.61742743</td>
<td></td>
<td></td>
</tr>
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<td>Ash</td>
<td>sample</td>
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<td>3.11039544</td>
<td>25.54</td>
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<td>3.28379134</td>
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<td></td>
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<td>Moisture</td>
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<td></td>
<td></td>
</tr>
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<td></td>
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Table B.1.2: ANOVA table for alpha amylase and free sugars from Chapter 3

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<th>Parameter</th>
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<th>ANOVA SS</th>
<th>F Value</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha amylase activity</td>
<td>sample</td>
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<td>6415957.339</td>
<td>1076679</td>
<td>&lt;.0001</td>
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<td>error</td>
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<td>5.959</td>
<td></td>
<td></td>
</tr>
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<td></td>
<td>total</td>
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<td>6415963.380</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>sample</td>
<td>7</td>
<td>3.21699117</td>
<td>2036.28</td>
<td>&lt;.0001</td>
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<td>error</td>
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<td>0.00157983</td>
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<td>total</td>
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<td>Sucrose</td>
<td>sample</td>
<td>7</td>
<td>2.45447576</td>
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<td>error</td>
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<td>0.01396656</td>
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<td>total</td>
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Table B.1.3: ANOVA table for pasting properties from Chapter 3

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<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Viscosity</td>
<td>sample</td>
<td>7</td>
<td>1643627.750</td>
<td>3315.44</td>
<td>&lt;.0001</td>
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<td>error</td>
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</tr>
<tr>
<td></td>
<td>total</td>
<td>15</td>
<td>1644125.750</td>
<td></td>
<td></td>
</tr>
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<td>Final Viscosity</td>
<td>sample</td>
<td>7</td>
<td>4587398.438</td>
<td>6178.83</td>
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<td>Peak Time</td>
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<td>16.974700000</td>
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<td>error</td>
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<td>0.078100000</td>
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<td>total</td>
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Table B.1.4: ANOVA table for in vitro starch digestibility from Chapter 3

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<th>Pr&gt;F</th>
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</thead>
<tbody>
<tr>
<td>RDS</td>
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<td>481.75</td>
<td>222.94</td>
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<td>error</td>
<td>7</td>
<td>2.16</td>
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</tr>
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<td></td>
<td>total</td>
<td>15</td>
<td>486.87</td>
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</tr>
<tr>
<td>SDS</td>
<td>sample</td>
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<td>253.59</td>
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<td>0.27</td>
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<td>error</td>
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<td>total</td>
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<td>error</td>
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## B.2 ANOVA tables from Chapter 4

Table B.2.1: ANOVA table for alpha amylase activity and free sugar parameters from Chapter 4

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<th>Pr&gt;F</th>
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<td>Alpha amylase activity</td>
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<td>total</td>
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<td>1030662.43</td>
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<td></td>
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<td>Glucose</td>
<td>sample</td>
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<td>total</td>
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Table B.2.2: ANOVA table for descriptive analysis from Chapter 4

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Table B.2.3: ANOVA table for phenolic content from Chapter 4
Appendix C: Interactions

C.1 Interactions in descriptive analysis from Chapter 4

Figure C.1. 1: Sample*Judge interaction for sweet attribute
Figure C.1.2: Sample*Judge interaction for bitter attribute
Figure C.1.3: Sample*Judge interaction for green attribute
Figure C.1.4: Sample*Judge interaction for branny attribute
Figure C.1.5: Sample*Judge interaction for starchy attribute
Figure C.1.6: Sample*Judge interaction for astringent attribute