COMPARATIVE STUDY ON HULLED WHEATS: KERNEL, FLOUR, DOUGH QUALITY AND DIETARY FIBER VARIATION

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Title

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

MASTER OF SCIENCE

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ABSTRACT

This study was conducted to evaluate the kernel, flour and dough qualities and dietary fiber content of hulled wheats. Experimental design was separate randomized complete block designs for hulled wheat species with four field replicates. According to the results, significant differences ($p<0.05$) were observed in kernel quality, flour, and dough quality compared to common bread wheat. Einkorn and spelt reported significantly lower insoluble dietary fiber and total dietary fiber content, in contrast emmer had contents with both higher and lower genotypes. Interestingly, few genotypes of hulled wheat had a higher content of low molecular weight soluble dietary fibers (SDF-LM) such as fructo and galacto oligosaccharides. Overall, hulled wheats differed from modern bread wheat in their kernel, flour, baking and nutritional quality. Moreover, due to higher SDF-LM content, hulled wheats would be a potential candidate for breeding and producing health beneficial novel food products.

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DEDICATION

I would like to dedicate this thesis to my parents for their endless love support and encouragement.

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INTRODUCTION

There is an increasing demand for organic and local foods (USDA ERS, 2019) with fewer additives (Kaptan and Kayisoglu, 2015). It was the reason for rediscovery of nutritional quality of whole wheat flour and hulled wheats or ancient wheat. Hulled wheat species are gaining renewed interest in contemporary food design due to several studies that suggested they present a healthier nutritional profile than modern wheats (Lachman et al., 2013, Cooper, 2015). However, there is limited literature about the chemical composition and dietary fiber variation of hulled wheats (Longin et al., 2015).

Hulled wheats are enclosed by tough glumes (husks) on a semi-brittle rachis. Glumes are removed by threshing followed by dehulling. Hulled wheats have been replaced by modern or naked wheat due to the low yield and presence of hull which makes it difficult for processing. Hulled wheats grow excessively tall, thereby becoming susceptible to lodging with significant yield loss (Okuno et al., 2014). Today about 95% of the cultivated wheat worldwide is *Triticum aestivum*, while the remaining 5% accounts for *T. turgidum* susbp. *durum* (Brouns et al., 2013).

Einkorn, emmer, and spelt are the most common hulled wheats. Einkorn (*Triticum monococcum* L. ssp. *monococcum*) is a diploid ($2n=2x=14$) hulled wheat carrying the A genome (Heun et al., 1997). Cultivated emmer wheat (*Triticum dicoccon* Schrank) is a tetraploid wheat with A and B genome and it is close relative of Durum wheat. (Nesbitt and Samuel, 1996). Moreover, it was one of the basic plants in Neolithic agriculture (Abbo et al., 2013). Emmer is a minor crop today, cultivated in isolated, marginal areas (Marconi and Cubadda, 2005; Zaharieva et al., 2010).

Organoleptic properties such as better flavor and aroma was a key reason for the present popularity of emmer and the healthy image of the emmer-based foods. Most of its supposed

nutritional properties have not scientifically proven yet. Spelt is a hexaploid wheat with A, B and D genomes and now cultivated in Europe, Asia, North Africa, and North America (Dvoracek et al., 2002). Spelt has occupied a niche market in North America and Europe in the natural, organic, health and specialty-food markets (Abdel-Aal et al., 2005). It has the potential for making a variety of products including bread, pasta, and breakfast cereals (Bonafaccia et al., 2000).

Studies conducted on nutritional composition of hulled wheats revealed that they are characterized by higher contents of soluble dietary fibers compared to modern bread wheat. However, there are limited number of systematic studies focused on detailed analysis of dietary fiber in hulled wheats (Shewry and Lovegrovea, 2014). The consumption of dietary fiber rich foods has become a growing focus among consumers (Perry and Ying, 2016). It has led to the development of a large and potential market for fiber-rich products and ingredients and in recent years. Therefore, it is a trend to find new sources of dietary fiber that can be used in the food industry (Esteban et al., 2017). The present research was carried out to evaluate the variation in dietary fiber of hulled wheats and to study the kernel, flour, dough, and baking quality of hulled wheats.

LITERATURE REVIEW

Ancient wheat species

Wheat (*Triticum*) crop includes six biological species at diploid, tetraploid and hexaploidy levels (Dvořák, 2001). Most of the wheat species grown today are considered hybrids and they have been subjected to modifications through cross breeding. In contrast, the wheat species that has remained unchanged over hundred years of time are called as ancient wheat. Although modern wheat species have positive properties in relation to yield, little attention has been given to their nutritional value and health benefits. Furthermore, wheat quality has been judged based on its technological function (Valli et al., 2018).

Today, world trend is moving towards healthy food and this laid the foundation for rediscovery of ancient wheat species. There is a renewed interest in ancient wheat in recent years as they have been proposed to have exceptional health benefits (Shewry and Hey, 2015). However, today they are grown in small geographic areas. Ancient wheat species currently have limited uses except as animal feeds, because of the retention of hulls on the grain after threshing. After dehulling, the grains are promoted as being nutritious and healthier than current commercial species (Abdel-Aal et al., 1995). Only a few studies, which were specifically designed to compare the effects of ancient and modern wheat cultivars, have been published. Therefore, further studies are required with multiple genotypes of ancient and modern wheat to evaluate dietary fiber variation and health benefits (Shewry and Hey, 2015). Moreover, common wheat, emmer, spelt and einkorn cultivars were already characterized, these studies are difficult to compare (Shewry et al., 2010, Ward et al., 2008), because the samples were cultivated in different areas and harvest years, fertilized differently and the grains were milled to white or whole meal flours.

The most well-known ancient wheat species are diploid einkorn (*Triticum monococcum*, AA), tetraploid emmer (*Triticum dicoccum*, AABB) and hexaploid spelt (*Triticum spelta*, AABBDD) which is now considered to be the same species as bread wheat (Cooper, 2015). Evolution of different wheat species including emmer, einkorn, spelt, durum and bread wheat is shown in Figure 1.

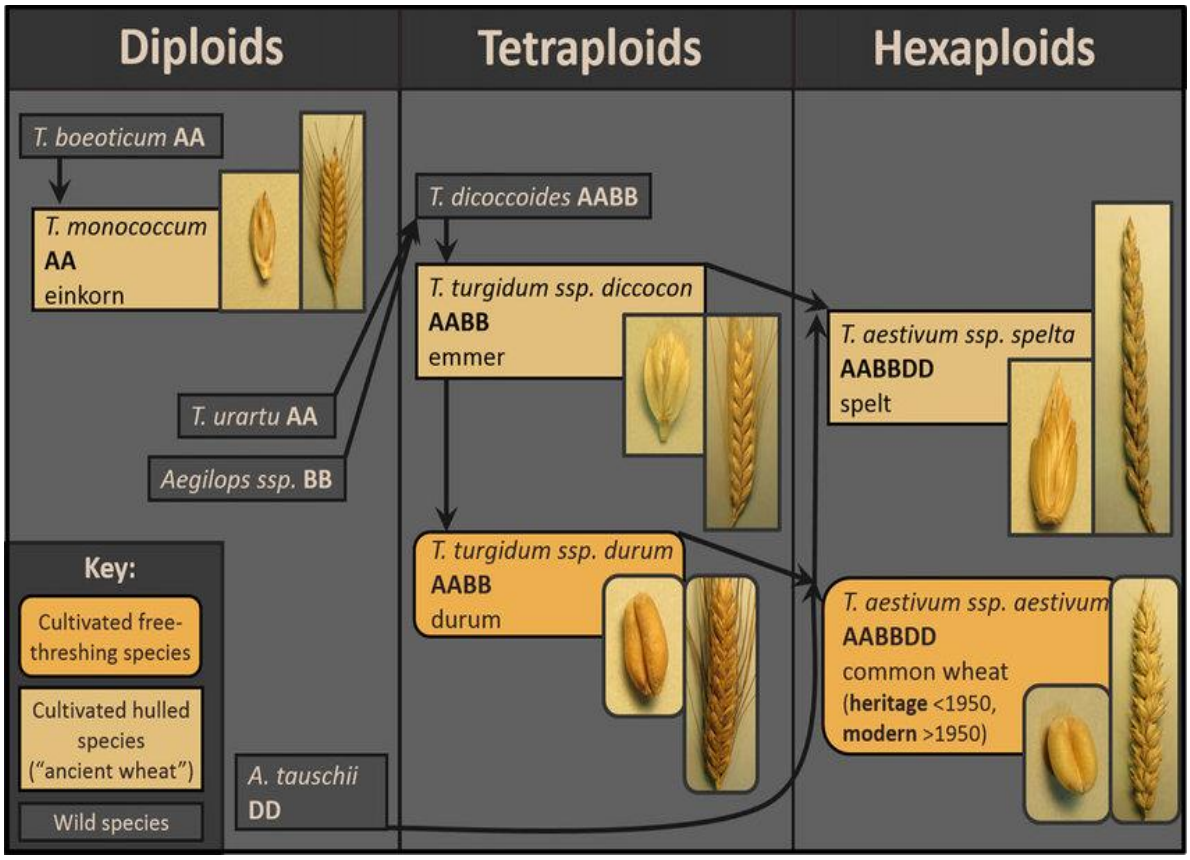


Figure 1: Evolution of different wheat species (Source: Shewry and Hey, 2015).

Einkorn

Einkorn was one of the first crops domesticated around 10,000 years ago in Asia, which was widely cultivated in Neolithic period and later was replaced by tetraploid wheat species (Nesbitt & Samuel, 1996). The yield of einkorn is considerably lower compared to common wheat genotypes (Lùje et al., 2003). Therefore, especially in the United States, einkorn production is limited to evaluations of PI accessions for yield and quality traits and for breeders

to develop genotypes with higher protein content and better disease resistance. However, there is a renewed interest in this crop again due to dietetic and nutritional aspects; but only few studies have investigated the possible applications and health benefits of einkorn (Agnello et al., 2016).

Einkorn is a small- seeded wheat species (Figure 1) with high flour protein and high carotenoid content (Abdel Aal et al., 2002). Einkorn flour is characterized by a higher content of gliadin and glutenin and very lower content of high molecular weight glutenin subunits compared to bread wheat. Several studies suggested that low immune-toxicity of einkorn *in vivo* may depend on gastro-intestinal digestion, as gliadins from einkorn and bread wheat showed similar effects on T-cells after partial peptic tryptic digestion, but that more extensive digestion with gastro-intestinal enzymes resulted in greater breakdown of einkorn gliadins and reduced immune stimulatory properties (Gianfrani et al., 2015). In addition, some studies have focused on the celiac toxicity of einkorn, peptic tryptic digests of gliadins from this species were unable to agglutinate K562(S) cells (type of cells that are commonly used to detect celiac toxicity, if they agglutinate together, considered as toxic), therefore it implies low coeliac toxicity (De Vincenzi et al., 1996).

Another study reported that a peptic tryptic digest of gliadins from einkorn was not toxic to intestinal mucosal cells in an *in vitro* culture system compared to bread wheat (Pizzuti et al., 2006). One detailed study which was conducted to compare two genotypes of einkorn and bread wheat on innate and adaptive immune responses, indicated one genotype of einkorn was unable to active the innate immune pathway which caused for celiac antigenicity (Gianfrani et al., 2012). However, studies about the health benefits of einkorn are controversial.

Emmer

Emmer was domesticated around 8,000 BC and closely associated with Neolithic agriculture (Zaharieva et al., 2010). Emmer wheat has been traditionally used for pasta production in Italy and Egypt (Galterio et al., 2003), soup in Italy, Turkey, and Switzerland, and for beer production in some countries (Papa 1996; Samuel 1996; Cooper 2015). Semolina obtained from emmer wheat has been used for the preparation of Indian traditional foods (Bhuvaneshwari et al., 2005). Additionally, it was also used to prepare baby foods (Zaharieva et al., 2010).

Emmer is well known for its functional compounds such as resistant starch, fiber, carotenoids, and antioxidants (Serpen et al., 2008). Furthermore, it is believed to have potential health benefits such as inhibition of carbohydrate digestive enzymes such as α-amylases and α-glucosidases which are responsible to maintain postprandial glucose homeostasis (Christopher et al., 2018).

Emmer starch was identified as slowly digestible and it could be due to the complexity of the starch structure, high degree of crystallinity and high amylose content (Galterio et al., 2003). Moreover, it is also believed that the gluten structure of emmer differs from that of modern wheat so that people with gluten allergies can safely use it without any adverse effects (Kasarda, 2013). However, further studies are essential to study the health benefits of emmer**.**

Spelt

Spelt is a hexaploid species and it was extensively cultivated in ancient Europe. Spelt wheat is identified as a low-input crop, resistant to pests suitable for growing in soils with limited fertility (Marconi et al., 2002). Spelt wheat gives a good harvest when grown in unfertilized soil and has a greater mineral uptake in comparison with *Triticum aestivum* L. Since

it is pest and disease resistant, spelt is also suitable for organic production (Bonifácia et al., 2000). Cultivation of spelt has declined, but recent interest in use of spelt for healthy foods has led to resurgence in its cultivation. The common way of consuming spelt is as bread and baked products as it is a hexaploid wheat with rheological and technological properties close to those of soft wheat. (Abdel-Aal et al., 1998). Spelt products are available in organic health food outlets as grain, whole grain and white flours, and processed products. Processed products include assorted pasta, cold and hot cereals, and pre-packaged bread, muffin, and pancake mixes.

Spelt has been proposed for inclusion in the diet of patients being treated for health problems such as colitis ulcerosa (Abdel-Aal et al., 2002), neurodermitis (Zieliński et al., 2008), high blood cholesterol (Rozenberg et al., 2003, Zieliński et al., 2008) rheumatoid arthritis, depression and cancer (Abdel-Aal et al., 2002) in alternative medicine. However, spelt is identified as a grain that can cause wheat allergy, gluten enteropathy (Zieliński et al., 2008). Therefore, spelt is not a safe grain for people with celiac disease (Kasarda and D'Ovidio, 1999). The reason is α-type gliadin from spelt and common wheat shared >95% homology (Kasarda and D'Ovidio, 1999, Ruibal-Mendieta et al., 2005).

Figure 2:Hulled and dehulled grains of einkorn emmer and spelt
(Source: https://phys.org/news/2016-01-ancient-nouveau-world-grains-comeback.html)

Organic farming techniques for hulled wheats

Organic farming has become one of the most rapidly developing branches of agriculture. The reason is the growing demand for organic food. Along with the increase of organic cereal acreage, the area of spelt cultivation has also increased because this kind of cereal is grown mostly on organic farms. (Willer & Schaack, 2015). Today the farmers practicing organic agriculture initiate the testing of various genotypes of einkorn and emmer applicable to organic or low-input farming.

There are not enough varieties that have been purposely bred for organic farming over the last few years (Konvalina et al., 2014). Conventional bred and tested varieties which were developed under the organic farming conditions are grown there (Lammerts, 2002). But there are many references from different authors (Wolfe et al., 2008) that have reported low baking quality of bread wheat within organic farming. On the other hand, there are many neglected wheat species such as einkorn, emmer and spelt which have potential to be grown in organic farming and provide high-quality grain (Piergiovanni, 1996).

Kernel quality of hulled wheats

Physical characteristics of a cereal grain can depend upon several factors including variety, ecological and harvest condition. These characteristics are very important parameters in milling and other processing properties and consequently on final product quality (Oručević et al., 2011).

Test weight

The basic factors that affect the test weight of wheat are kernel size, shape and kernel density. Same authors reported that grain virtuousness was positively correlated with grain protein content, and test weight increased linearly with a thousand kernel weight increase

(Oručević et al., 2016). Shape and sphericity of the influence the test weight (Oručević et al., 2013). Test weights of emmer are similar to the test weight of oats (360-440 kg/m^3), while spelt has reported a broader range. (310-465 kg/m^3) in field trails.

Hardness

Baking properties of wheat flour can be affected by hardness of the kernel (Szabo et al., 2016). There are different factors that can determine the hardness of the wheat kernel. Friabilin protein content is one of the most important factors which is used to classify hardness into two classes as high and low (Greuille et al., 2006). It has been identified as a marker protein for grain softness. Friabilin is a lipid binding protein which consist of two units called puroindoline a and b (Martin et al., 2006). When there is a higher friabilin content, hardness value is low and vice versa. Other factors which affect kernel hardness includes genetic factors, environmental factors, lipid, moisture, and pentosan content of wheat grain (Gyimes, 2004; Gyimes et al., 2001).

Hardness is a major factor that can influence the kernel milling properties (Greffeuille et al., 2007 a, b). The hardness of kernels significantly influences energy consumption during milling. The milling of hard wheat consumes more energy when compared to soft wheat (Dziki et al., 2012). Einkorn and spelt has reported a hardness value of 64 and 26 -38 respectively, while hardness value of HRS was 26 (Abdel-Aal et al., 1997).

1000-Kernel weight

1000-Kernel weight depends on the size of the kernel. Big, heavy seeds have a higher proportion of starchy endosperm and smaller amounts of bran and protein rich external pericarp and aleurone layers (Hidalgo and Brandolini, 2008) compared to hard red spring wheat. Einkorn reported to have a lower 1000 - kernel weight than the bread wheat. Several authors (Abdel-Aal et al., 1997; Borghi et al., 1996; Brandolini et al., 2008; Løje et al., 2003) noticed that einkorn

seeds were small and had low kernel weights. 1000-Kernel weight of spring spelt has been reported in between 38.8 - 43.3 g in one study conducted Canada (Abdel-Aal et al., 1997). Emmer grown in different locations reported a 1000-kernel weight in between 44.5 – 46.9 g (Stagnari et al., 2008).

Pasting properties and dough quality of hulled wheats

A study conducted in Italy, reported significant differences among *T. monococcum, T. turgidum* and *T. aestivum* for all the traits considered. Einkorn reported the highest peak viscosity, and final viscosity, while *T. turgidum* exhibited the lowest breakdown and highest setback. Among the *T. monococcum* accessions, peak viscosity averaged 2426 cP, breakdown 765 cP, final viscosity 2788 cP and setback 1126 cP for one study (Brandolini et al., 2008). Furthermore, it was found that kernel weight and starch content were positively correlated to all the pasting parameters of einkorn. Einkorn flour has been identified as a suitable candidate for cookies and biscuits as it has a high peak viscosity (Hidalgo and Brandolini, 2011).

Dough quality of einkorn has been described to be poor in comparison to common wheat and exhibited lower mixograph characteristics (Abdel-Al et al., 1997, Corbellini et al., 1999).

Dough development time of spelt reported to have a range from 1 to 3 min. (Marconi et al., 2002). Long stability time of dough indicates its high tolerance towards the mixing process. The rheological properties of spelt flour can be improved by the addition of common wheat flour (Sobczyk et al., 2017).

Chemical composition and nutritional value of hulled wheats

A study by Marconi and Cubadda (2005) showed that the proximate composition of emmer meal, when compared at the same level of refinement, was similar to that of spelt, durum,

and bread wheats. Proximate composition of hulled wheats and common bread wheat from different studies are summarized in Table 1.

Table 1: Nutritional composition of hulled wheats: einkorn, emmer and spelt

Wheat species	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Dietary Fiber (%)	Reference
Einkorn	1.68 -1.84	16.3-17.5	2.21-2.48	60.9 - 65.8	9.8-10.3	Abdel-Aal, 1995
Emmer	2.3	13.5-19.05	2.01	59.97	7.9	Loje et al., 2003 and Grausgruber et al., 2004
Spelt	2.35	18.2	2.1	61.8	8.7	Abdel-Aal, 1995
Common bread wheat	1.8	13.85	2.14	72.4	12.5	Abdel-Aal, 1995 Davis et al., 1981

Nutritional and technological value of cereals are determined by protein content and composition. Proteins can be divided into water-soluble metabolic proteins (15–25% of total proteins) and water-insoluble, gluten-forming storage proteins (the remaining 75–85%).

High protein contents (17.1% dry weight basis) were found for fifty emmer accessions (Cubadda and Marconi, 1995), while low values (< 10%) were reported for three Italian emmer populations cultivated in three different locations (Galterio et al., 1994). It was reported that three emmer varieties cultivated in the south region of Slovak Republic under organic farming system had a variation of crude protein content from 13.3 – 14.2% (Čurná, Veronika & Lacko-Bartošová, 2017). Spring emmer was found to be higher in protein (14.4%) than winter emmer (11.2%) wheat and common wheat (11.8%) in a study which compared the quality and composition of spring emmer and winter wheat (Giacintucci et al., 2014). Forty-six genotypes of wild emmer grown without soil fertilizers, had a grain protein content on an average of 21%, which is much higher than that of domesticated tetraploid wheats even when grown under optimal conditions (Nevo et al., 1988).

Controversial values (both higher and lower) reported for protein values of 65 accessions of einkorn cultivated in Italy in 2004-2005 had a mean value of 18.1% and seven accessions out of 7 reported to have a more than 20% of protein (Brandolini et al., 2008).

Spelt is reported to have higher protein content than common bread wheat and higher proportion of the aleurone layer in the kernel (Bojňanská and Frančáková 2002). Protein content of five spelt cultivars were reported to range from14.3 to 18.4% (Marconi et al., 1999). Average protein content of three spelt cultivars grown at five different locations were 16.6% on dry weight basis. Protein content of spelt wheats averaged significantly higher than that of common wheats (Ranhothra et al., 1996).

Dietary fiber

Dietary fiber is defined by American Association of Cereal Chemists (AACC 2000) as the edible parts of plant or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber can be classified based on both structure and solubility. Dietary fiber can be classified into two main categories depending on their water solubility as soluble and insoluble dietary fiber.

Insoluble dietary fiber consists mainly of cell wall components (e.g., cellulose, lignin, hemicellulose), while soluble dietary fiber consists of non-cellulosic polysaccharides (e.g., pectin, gums, inulin type fructans, mucilages) (Nair et al., 2010). However, different subcategories can be made further depending on solubility of soluble dietary fiber in 78% ethanol (Table 2).

Soluble Dietary Fiber (SDF)

SDFs bypass the digestion of small intestine and they are readily fermented by the microflora of large intestine and thereby increase stool bulk due to the growth of intestinal and

fecal microflora and their by-products such as gases and short chain fatty acids (Elleuch et al., 2011 and Mudgil & Barak, 2013). Furthermore, SDFs increase total transit time by delaying gastric emptying. In addition, they can slowdown glucose absorption (El Khoury et al., 2012).

Insoluble Dietary Fiber (IDF)

IDFs do not form gels due to their water insolubility and fermentation is severally limited. Most fiber containing foods include approximately one-third soluble and two-third insoluble fiber (Gyimes et al., 2001). IDFs increase fecal bulk and the excretion of bile acids and decrease intestinal transit time providing a laxative effect (Perry & Ying, 2016).

Consumption of IDF slightly affects dietary mineral absorption as mentioned in some studies, in contrast, nondigestible oligosaccharides have been reported to stimulate intestinal microflora to produce vitamins and short chain fatty acids (SCFA) which might promote mineral absorption (Galak et al. 1996, Mussamato & Mancilha et al., 2007).

Table 2: Different dietary fiber fractions in food (https://www. megazyme.com)

Abbreviation	Definition	Examples
HMWDF	High molecular weight dietary fiber (HMWDF = IDF + SDFP)	Cellulose, resistant starch, cereal beta-glucan, guar gum and certain xylans
IDF	Dietary fiber insoluble in water	Cellulose, resistant starch and certain xylans
SDF-HM	Dietary fiber soluble in water and precipitated by 78% ethanol	Cereal beta glucan, guar gum and certain xylans
SDF- LM	Dietary fiber soluble in water and soluble in 78% ethanol. Termed as low molecular weight dietary fiber (LMWDF) or non-digestible oligosaccharides (NDO)	Fructo-oligosaccharides (FOS) Galacto-oligosaccharides (GOS), inulin and resistant maltodextrins (RMD)

Dietary fiber in hulled wheats

The contents of total dietary fiber have been reported as 8.7% in einkorn, 7.9% in emmer, 10.3% in spelt, and 12.3% in bread wheat (Løje et al., 2003). The total dietary fiber content of einkorn ranged from 7.6 - 9.9%, while water-soluble dietary fiber content has been reported in between 0.21 - 1.74% on dry matter basis. The reported vales for total dietary fiber content was considerably lower than modern wheat, but soluble fiber content was higher (Lùje et al., 2003).

Comparative studies of hulled and modern wheat highlighted that the ancient grains are characterized by a higher content of soluble dietary fiber (Hidalgo and Brandolini, 2014). However, there are limited number of detailed studies about soluble fiber fractions from hulled wheat. More studies are essential using wider range of genotypes of hulled and modern wheat species (Shewry,2018).

The average soluble dietary fiber content of emmer is 0.41%, but only small numbers of samples were analyzed in most studies. The dietary fiber values reported for emmer is lower than those reported in the same studies for bread wheat, however the range is wider in some studies (Shewry and Hey, 2015).

Soluble fiber content of spelt has shown a wide variability among cultivars, ranging from 1.2% (*Balmegg*), 2.4% (*Oberkulmer*) and 2.5% on dry weight basis. One study suggested higher content of soluble dietary fiber (1.7-1.8% dry matter basis) in spelt with comparison to the common bread wheat (Lùje et al., 2003).

Components of dietary fiber in hulled wheats compared with modern wheat

There are limited number of systematic studies on detailed composition of dietary fiber in hulled wheats compared to bread wheat, but those that have been carried out have reported little difference (Gerbruers et al., 2008). Studies were carried out to test the components such as β-

glucan, arabinoxylans, cellulose and lignin contents. Marconi et al. (1999) reported 0.92% - 1.27% dry weight of β-glucan in whole meal samples of five spelt samples, while Escarnot et al. (2010) reported the contents of fiber components of whole grain flours of four spelt and three wheat cultivars. These were means of 1.7% - 2.4% cellulose, 5.4% - 7.3% hemicellulose (mainly Arabinoxylans and β-glucan) and 1.3% - 0.7% lignin in spelt and bread wheat, respectively.

Information is also available from the HEALTHGRAIN study, in which ten durum wheat, five spelt, five emmer, and five einkorn samples were analyzed with bread wheat samples. White flour and bran fraction were analyzed for total arabinoxylans and water extractable arabinoxylans of these fractions, and whole meals for β-glucan (Gebruers et al., 2008). Variation of dietary fiber composition and components of ancient and modern wheat species (dry weight basis) from HEALTHGRAIN study are shown in Table 3.

Table 3: Dietary fiber components of ancient and modern wheat species (Shewry & Lovegrovea, 2014).

Species	Genomes	Number of lines	Flour range TOT-AX (%)	Flour mean TOT-AX (%)	Flour range WE-AX (%)	Flour range WE-AX (%)	Bran range TOT-AX (%)	Bran mean TOT-AX (%)	Bran range WE-AX (%)	Bran range WE-AX (%)	Whole meal range Beta glucan	Whole meal mean Beta glucan
Bread wheat	AABBDD	150	1.35-2.75	1.95	0.30-1.40	0.51	12.7-22.1	17.8	0.30-0.85	0.42	0.50-0.95	0.72
Spelt	AABBDD	5	1.60-2.15	1.75	0.30-0.45	0.35	11.1-13.9	12.7	0.30-0.35	0.30	0.55-0.70	0.65
Durum	AABB	10	1.70-2.35	1.95	0.25-0.55	0.40	10.9-13.7	12.0	0.30-0.55	0.40	0.25-0.45	0.35
Emmer	AABB	5	1.40-1.95	1.70	0.15-0.55	0.25	6.1-14.4	8.9	0.20-0.45	0.30	0.40-0.40	0.35
Einkorn	AA	5	1.45-2.35	1.95	0.50-0.65	0.60	9.5-10.4	10.0	0.45-0.65	0.55	0.25-0.35	0.30

(TOT-AX - Total arabinoxylans, WE-AX- Water extractable arabinoxylans)

Value added food products from hulled wheats

Ancient wheat species have become popular as environmentally friendly cereal crops which has stimulated research into their utilization in both traditional and modern food products (Messia et al. 2012). Einkorn, emmer, and spelt are often used as whole grains for salads or soups; while in processing, einkorn and emmer mainly used for pasta products and spelt is used for bakery products (Benincasa et al. 2015).

Bread-making

Einkorn flour has been used in bread making in few studies and reported to have a sticky dough with poor rheological properties, but the existence of cultivars with high bread making quality has been identified (Hidalgo and Brandolini 2011; Brandolini and Hidalgo 2011).

Screening of more than 1000 accessions of einkorn determined that approximately 16% of the total accessions had sodium dodecyl sulfate (SDS) sedimentation values corresponding to the threshold value for bread-making potential (Borghi et al., 1996). In addition, einkorn wheat reported to have a higher peak viscosity and final viscosity than modern wheat in some studies. The differences may be related to the smaller size and different grading of einkorn starch granules as well as to the lower amylose percentage of einkorn flour (Brandolini and Hidalgo, 2011). Moreover, einkorn bread has been reported to have a lighter color than common wheat and durum wheat, it is suggested that einkorn undergoes lower heat damage than modern wheat during baking because low α- and β- amylases limit the degradation of starch (Brandolini and Hidalgo, 2011). As a result, the decreased generation of reducing sugars in the dough limits the Maillard reactions during food processing. Low lipoxygenase activities in einkorn dough also limits the degradation of carotenoids (Hidalgo and Brandolini, 2014).

A comparative study with spelt has shown acceptable sensory scores with significant differences among genotypes (Korczyk Szab´o and Lacko Bartoˇsov´a, 2013), leading to the conclusion that spelt might be a suitable raw material for bread making, but it depends upon the type of spelt cultivar (Korczyk Szab´o and Lacko Bartoˇsov´a, 2015). Spelt genotypes had high crumb elasticity, but low crumb cell homogeneity, which are probably due to its special dough rheological attributes (Callejo et al., 2015). Spelt breads also reported to have less total starch, more resistant starch, and less rapidly digested proteins in comparison to bread made with modern wheat flour (Bonafaccia et al., 2000).

Spelt and emmer has been used in sourdough bread making. It has been reported that they had slightly higher pH values than modern wheat sourdough, but titratable acidity, concentration of free amino acids, and phytase activity were higher than in common wheat sourdough (Coda et al., 2010). Specific volume and crumb of spelt bread has shown resemblance to those of wheat bread. Sensory analysis also revealed that spelt and emmer can be made into acceptable bread products (Coda et al., 2010). However, there are limited number of detailed research studies carried out to evaluate the feasibility of utilizing ancient wheat flour in bread making to gain additional health benefits associated with ancient wheat species.

NEED STATEMENT AND OBJECTIVES

Einkorn, emmer and spelt are hulled wheat species that were consumed by people for centuries before they were replaced by modern bread wheat. Hulled wheats are becoming popular again due to social, cultural, and economic reasons (Bekes et al., 2017). Therefore, exploitation of hulled wheat species has become an important factor to further drive consumer trends as they can satisfy emotionally driven trends of people (Longin and Wurschum, 2016). Bakery products of these species are attracting more attention as niche products with proposed health and nutritional benefits compared to modern wheat bread and other bakery products (Coda et al., 2009). However, only a few studies have been focused on kernel, flour, dough, and baking quality of hulled wheats. Definitive comparisons of these species with modern bread wheat are rare due to the difference in farming systems (Shewry and Hey, 2015).

Furthermore, hulled wheats have become an exclusive and fashionable for the consumers and they are even willing to pay a higher price than any other wheat product (Bekes et al., 2017). Moreover, the necessity of food diversification and directed plant breeding towards improving nutritional quality of crops have led to a renewed interest on these species (Arzani, 2011). Contradictory information has been reported on the variation of dietary fiber in hulled wheats and detailed analysis of fiber components are rare.

Therefore, this study was carried out with following objectives in mind.

Two major objectives as follows.

- To evaluate the kernel, flour, dough and baking quality of hulled wheat species einkorn, emmer and spelt
- To study the variation in dietary fiber fractions in hulled wheat flour and bread

MATERIALS AND METHODS

Materials

All the chemicals and reagents were of analytical grade. All wheat samples were provided by Dr. Richard Horsley in the Department of Plant Sciences, North Dakota State University. Ten genotypes from three different hulled wheat species namely einkorn, emmer and spelt (Figure 3) were grown at Carrington, North Dakota utilizing randomized complete block design with four replicates in 2018. Cultivation data are shown briefly in Table 4. Eight genotypes of hard red spring wheat from the same location were used along with hulled wheats for comparison (Table 5).

Einkorn **Emmer** **Spelt** **Hard Red Spring Wheat**

Figure 3: Einkorn, emmer, spelt and bread wheat used in the study

Table 4: Wheat cultivation data of wheat species

Wheat species	Planted date	Harvested date	Previous crop
Einkorn	May 2	August 8	Cover crops: radish, pea
Emmer	May 2	July 31	Cover crops: radish, pea
Spelt	May 2	August 8	Cover crops: radish, pea
Hard Red Spring	April 30	August 6	Canola

Table 5: Selected ancient and modern wheat genotypes from Carrington farming trail in 2018

Einkorn	Emmer	Spelt	HRS
TM 23	Vernal	CDC Zorba	Sy Ingmar
WB Apline	Lucille	94-288	Barlow
PI 538722	ND common	SK3P	Elgin-ND
	Yaroslav		Linkert
			Glenn
			Rollag
			ND Vitpro
			Lang-MN

Sample preparation

Einkorn, emmer, spelt and hard red spring wheat samples were cleaned by passing through a dockage tester XT5 3/03 (Carter Day International, Minneapolis, MN). Cleaned samples (500 g) were collected into muslin cloth bags. Einkorn, emmer, and spelt samples were dehulled using a laboratory impact dehuller in the pilot plant of NDSU. Hulls were blown off to separate grains from the hulls. Next the samples were milled on a Quadramat JR. laboratory mill (C.W. Brabender Instrument, Inc., South Hackensack, NJ) by removing the sieve to produce whole wheat flour and it was a single stream process. The conditions in the milling laboratory was maintained as follows: temperature (22-23ºC) and relative humidity (65%-68%). All samples were stored in sealed plastic bags at 4 ^{0}C until analysis.

Kernel quality traits

Kernel quality traits such as test weight, 1000-kernel weight, kernel hardness and kernel size distribution were evaluated for all the samples. Test weight of kernels was measured by using AACC Method 55-10.01. 1000-Kernel weight was determined by removing all dockage, shrunken and broken kernels and other foreign material from wheat samples. A mechanical seed counter (Seedburo Equipment Co., Chicago, IL) was used to count ten grams of wheat and the number of kernels in ten grams was converted to 1000-kernel weight. Kernel hardness was

determined using a Single Kernel Characterization System (SKCS) (Ohm et al., 1998). In addition, for hulled wheat species, husked ratio will be calculated as follows.

$$\text{Husked ratio (\%)} = 1 - (W_f/W_i)$$

W_i is the mass of the sample before husking (g) and W_f is the mass of wheat after husking (g) (Minaei et al., 2007).

Proximate composition of whole wheat flour

Moisture content of all the samples was determined using AACCI approved method 44-15A Moisture –Air – Oven Method (AACC International, 1999).Total starch, protein, crude fat and ash contents of whole wheat flour were analyzed by AACCI approved methods of 76-13.01, 46-19.01, 30-25.01 and 08-01.01 respectively. In addition, fatty acid composition was analyzed by gas chromatography (AACC International Method 58-18.01).

Determination of dietary fiber content of ancient and modern wheat flour and bread

Dietary fiber analysis was performed according to the method AOAC 2011.25/32-50.01 using ANKOMTDF automated dietary fiber analyzer (Megazyme, 2020). An exact amount of sample (0.5 g) was weighed in insoluble and soluble dietary fiber bags. An enzyme mixture of pancreatic α-amylase/ amyloglucosidase (2000 U/mL α-amylase/ 136 U/mL AMG) was added into each bag to remove starch, and the samples were incubated for 16 hours at 37 °C. Sorbitol (1 mL; 100 mg/mL) was added to each enzymatically treated sample as an internal standard for SDF - LM analysis. After 16 hours, the samples were hydrolyzed using protease (35 tyrosine U/mL, 30 min, 60 °C) to remove proteins (Figure 5).

Determination of Insoluble Dietary Fiber (IDF) and Low Molecular Weight Soluble Dietary Fiber (SDF-LM)

The enzymatically hydrolyzed samples were filtered, and the filtrations were used for the separation of dietary fiber components that differ by water-solubility (IDF from SDF) and size (SDF-HM and SDF-LM). During the first filtration, IDF was separated from SDF. SDF-HM was precipitated by ethanol, and during the second filtration, SDF-HM was separated from SDF-LM. Filtrate, containing SDF-LM, were used in HPLC analysis. IDF and SDF-HM residues were dried at 105 0C for 90 minutes, cooled and weighed. Ash and residual protein contents were determined from the dried IDF and SDF-HM and reduced from the dietary fiber amount.

HPLC analysis of Low Molecular Weight Soluble Dietary Fiber (SDF-LM)

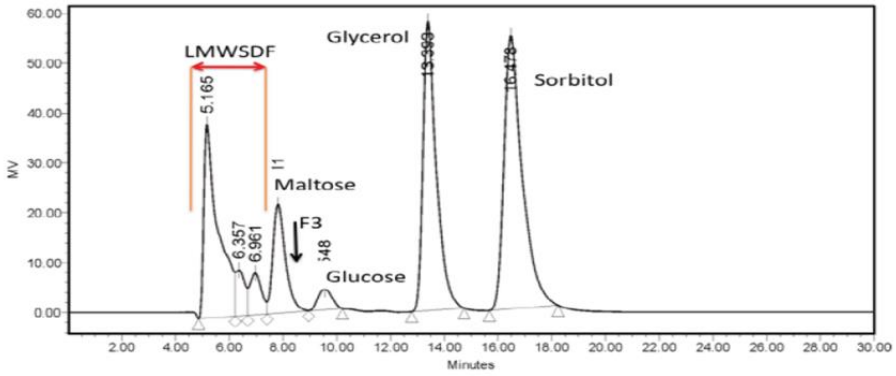


Figure 4: Chromatogram of SDF-LM in a sample on a sugar-Pak column

SDF-LM filtrates were concentrated and the samples were deionized and freeze dried before HPLC analysis. For deionization, Bio-Rad polystyrene columns (Bio-Rad, Hercules, CA, USA) were packed with mixed resin (Megazyme), 4 g of both Amberlite FPA 53 (OH−) and Ambersep 200 (H+)). The column used in HPLC was a Waters Sugar-Pak® (6.5 × 300 mm; Waters Corporation, Milford, MA, USA) at 90 °C and the solvent Na2Ca-EDTA (50 mg/L), with a flow rate of 0.5 mL/min. Refractive index (RI; Waters) was used for the detection (50 °C).

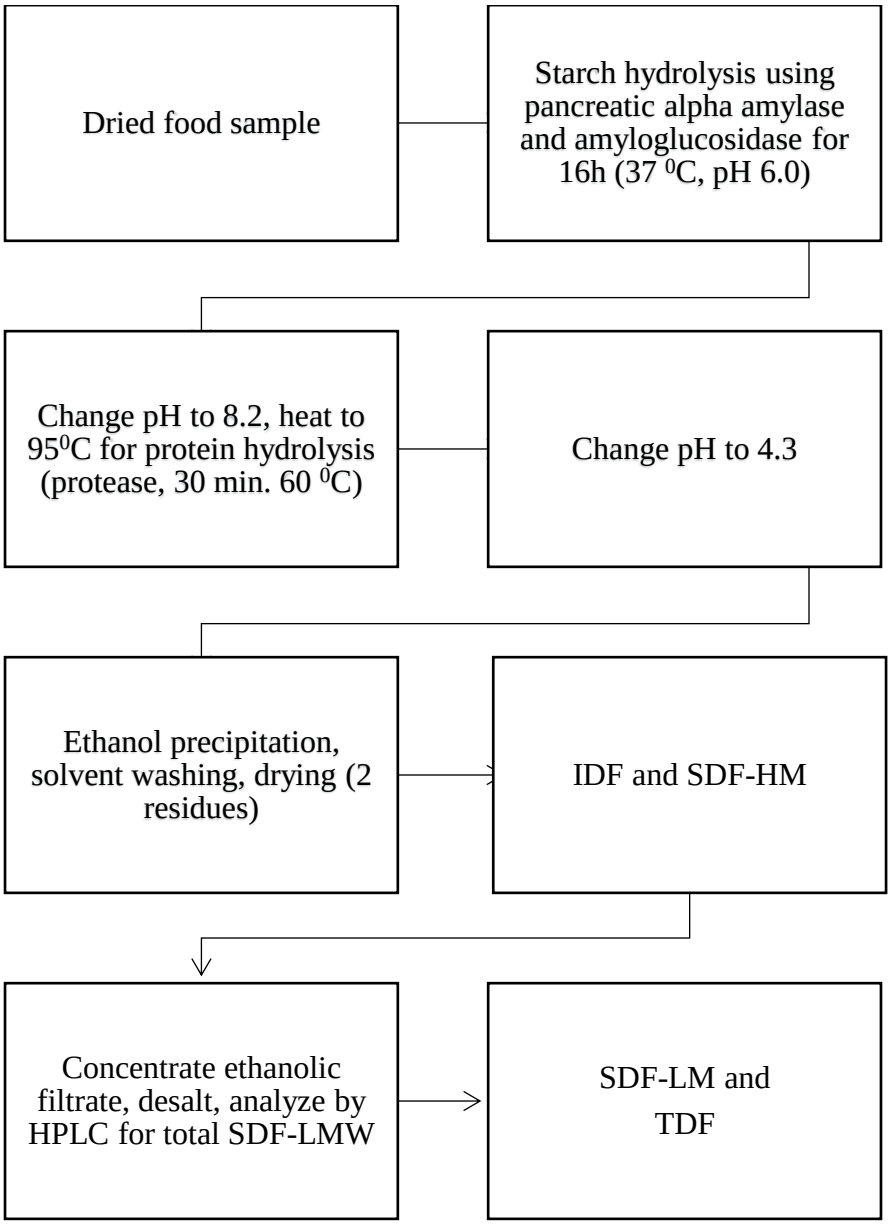


Figure 5:Dietary fiber determination: AOAC method 2011.25/32-50.01

Arabinoxylan content of hulled wheats

Total arabinoxylan levels in whole wheat flour was determined by gas chromatography of alditol acetates. Sugar composition and arabinose to xylose ration was determined according to the method of Mendis and Simsek, (2015).

Localization of arabinoxylan and β glucan in kernels

Kernels (10) of common wheat, einkorn, emmer and spelt were soaked in 10% neutral buffered formalin solution for 24 hours at room temperature to make it easy for fix in sectioning. Then kernels were dehydrated in a series of ethanol solutions as described by Dornez et al., (2011). The kernels were embedded in a paraffin wax blocks and cut into thin transverse sections (4 mm) with a manual rotary microtome (Leica, Heidelberg, Germany). The sections were then transferred onto Super frost charged microscopy slides (ThermoFisher Scientific, Waltham, MA, USA) and dried on a metal heating plate at 40 ^{0}C prior to staining.

Immunolabelling of arabinoxylan

For immunolabeling, sections were pre-incubated for 18 h in Phosphate Buffer Solution (PBS) at pH 7.4 with 0.10% Tween20 (PBST) containing 3.0% bovine serum albumin (BSA) to prevent non-specific binding. The sections were then incubated for 2.0 h with primary antibody solution. For AX, primary antibodies were diluted to 1:50 ratio, in a PBST buffer solution containing1.0% BSA. Appropriate controls were made of sections derived from the same sample blocks by substituting the primary antibody solution by PBST buffer containing 1.0% BSA After incubation, the sections were rinsed thoroughly with PBST solution. Then, they were pre-incubated again for 30 min with PBST buffer solution containing 1.0% BSA.

Subsequently, the sections were incubated for 2.0 h in the dark with fluorescently labeled secondary antibodies, i.e. Alexa Fluor $^{@}$ 555 goat anti-rat IgG. All incubations were performed in moisturized chambers at room temperature. Slides were then rinsed with PBST, PBS and water. Before the sections were completely dry, a drop of ProLong Gold anti-fading reagent and a cover slip were put at the top of the sections and the anti-fading reagent was allowed to react for 24.0 h. Then, fluorescent imaging was done.

β-Glucan by calcofluor staining

The kernel cross sections were stained for 2.0 min with 0.1% (w/v) Acid Fuchsin to stain aleurone proteins followed by 2.0 min with 0.01% (w/v) Calcofluor White, staining β-glucan. After each staining, the sections were rinsed with deionized water and dried. Then, fluorescent imaging was done. In exciting light, plant cell walls stained with Calcofluor appear blue. With Acid Fuchsin, the aleurone protein is stained red and the endosperm protein orange or light brown. Starch is unstained and appears black.

Rheological behavior of dough by farinograph

Dough rheology properties were determined using computerized Farinograph® according to AACCI Approved Method 54-21.02 (C.W. Brabender Instruments Inc., NJ, USA) with a 10 g mixing bowl.

Pasting properties of whole wheat flour

Pasting properties were analyzed according to the method of AACC 76-21.01.

Preparation of whole wheat bread using ancient and modern wheat genotypes

Samples (100% whole wheat flour) were baked according to AACCI Approved Method 10-09.01 with the following modifications: fungal α-amylase (15 SKB) instead of malt dry powder, instant yeast (1.0%) instead of compressed yeast, and the addition of 10 ppm of ammonium phosphate.

Baking qualities were characterized by baking absorption, dough handling properties, bread loaf volume and weight, crumb firmness and oven spring. Baking absorption was determined as the amount of water required for optimum dough baking performance and was expressed as a percent of flour weight on a 14% mb. Loaf volume was determined by rapeseed displacement method (AACCI Approved Method 10-05.01).

In vitro starch digestibility

The Englyst assay was conducted to determine different starch fractions, hydrolysis index and estimated glycemic index (Whitney and Simsek, 2017) with slight modification of the procedure according to (Sopade and Gidley, 2009). The samples were weighed (0.2 g) into 50 mL plastic centrifuge tubes, and the sample weight was adjusted for moisture content to a dry weight basis. The bread samples were incubated at 37°C with an enzyme mix (3.0 mL, amyloglucosidase, invertase, and pancreatin) for 180 min. The glucose concentration in the digesta was measured with an Accu-Check® Performa® glucometer at specific periods and calculations were done as described by Sopade and Gidley, 2009.

The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of the sample by the area obtained for commercial white bread (hydrolysis curve 0–180 min). The eGI of the samples was calculated using the equation described by Ovando-Martınez et al. (2011b).

Statistical analysis

All measurements were carried out at least twice and data were presented as the means with LSD values. Data were subjected to analysis of variance using the 'GLM' procedure in Statistical Analysis System 9.4 (SAS Institute, Cary, NC, USA). A least significant difference (LSD) with a 5% significance level was used to declare differences.

Fields replicates were not used in HRS samples. Therefore, Type III error was used in GLM procedure and LSD estimation was done using harmonized degrees of freedom. Contrast option was used to check the differences within a species. Results of combined replicates (Dietary fiber, crude fat, fatty acid composition) were analyzed separately.

RCBD with 2 factors (Variety and sample (Flour and bread) were used to see difference between flour and bread samples. Differences was considered significant when the probability value *p* is lower than 0.05. Pearson's simple linear correlation coefficient was obtained using the 'CORR' procedure in Statistical Analysis System 9.4 (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Kernel quality traits of hulled wheats

Mean values for the kernel quality traits of hulled wheat and common bread wheat are presented in Tables 6 and 7. Significant differences ($p < 0.05$) were observed in test weight, 1000 kernel weight and hardness index of hulled wheats, compared to HRS. Test weight is used as an indicator of grain quality and as a measure of grain bulk density. It is used to determine the price for wheat during trading. Therefore, test weight was measured with and without hull for einkorn, emmer and spelt. Among hulled wheats, highest test weight was for spelt (94-288) while the lowest is for emmer (Yaroslav). Same trend was followed for test weight with and without hull. However, higher test weights were reported for HRS with compared to einkorn, emmer and spelt.

1000 kernel weight (TKW) is an indicator of wheat milling value. Highest thousand kernel weight values were reported for spelt (44 g), while the lowest was for einkorn (28 g) with compared to HRS (Table 3). Similar values were reported for einkorn in several studies (Abdel-Aal et al., 1997; Borghi et al., 1996; Brandolini et al., 2008; Løje et al., 2003). Thousand kernel weights of these spelt samples seem low when compared with a previous study done with Oberkulmer Rotkorn, and Rouquin grown in Europe (Marconi et al 1999). In Europe, the 1,000 kernel weights were 49–55 g, however, differences were shown due to the diversity of environments. Significant positive correlations were observed in large kernel content and thousand kernel weight, while negative correlations were found medium size kernel content and thousand kernel weight (Table 7). These results indicate that seed size highly affect thousand kernel weight.

Kernel hardness index values of einkorn and spelt had significant differences ($p > 0.05$) compared to HRS (Table 6). No significant differences were observed between emmer and HRS. Einkorn can be categorized as extra soft wheat since hardness index is below 50, while emmer can be categorized as hard wheat similar to common bread wheat. In addition, both soft (CDC Zorba and SK3P) and hard (94-288) genotypes were found in spelt. However, higher hardness values have been reported in one study for einkorn, while similar hardness values for some genotypes of spelt (Abdel- Aal et al., 1997). Hardness is an important factor that determines the tempering level wheat prior to milling of refined flour (Doekes and Belderok, 1976). Harder wheat needs to be tempered and conditioned for a longer period compared to wheat with lower hardness. Additionally, such wheat requires more water to make dough of a proper consistency. Therefore, lower hardness values imply that einkorn and spelt (except 94-288) do not require tempering prior to milling of refined flour. It was reported that SKCS provided the best discriminating measure of genetically different wheat based on hardness (Morris et al., 1999; Chen et al., 2007; Wang et al., 2008; Feiz et al., 2009). Hardness index was significantly and positively correlated with grain protein and farinograph water absorption. Similar results were found in a set of homozygous recombinant inbred wheat lines (Wanjugi et al., 2007b). These results are important to determine the milling parameters for hulled wheats.

According to the results of kernel sizing (Table 7), significant differences were observed in hulled wheats compared to HRS. Einkorn and emmer kernels were 90% medium sized while in HRS, about 60% kernels were medium in size. Interestingly, 80% of kernels were large in one genotype of spelt (SK3P), while other two genotypes of spelt had large kernels in between 35-44%. These results revealed that there could be differences in kernel size among genotypes of spelt grown under same environmental conditions.

Positive correlation was observed for larger size kernels with protein content, 1000-kernel weight, and husked ratio, while negative correlation was observed with crude fat content of whole wheat flour (Table 8). Results of kernel quality traits can be used to design manage and breeding strategies for grain quality improvement. Moreover, they are useful for milling industry.

Table 6: Kernel quality traits of einkorn, emmer, spelt and hard red spring wheat

Wheat species	Genotype	Test weight with hull [a] (lbs/bu)	Test weight without hull (lbs/bu)	Moisture content (%)	Husked ratio [b]	1000 kernel weight (g)
Einkorn	TM 23	28.39	57.89	8.6	0.19	30.9
	WB Apline	28.36	59.63	9.1	0.15	28.8
	PI 538722	30.92	58.17	8.6	0.16	28.0
Emmer	Vernal	37.65	57.00	9.0	0.16	33.8
	Lucille	37.61	57.14	9.1	0.11	34.0
	ND common	38.50	56.19	9.0	0.12	32.9
	Yaroslav	38.37	54.98	8.9	0.13	33.6
Spelt	CDC Zorba	23.65	56.42	8.5	0.33	35.1
	94-288	24.67	60.02	9.1	0.35	35.8
	SK3P	29.86	58.57	8.6	0.29	44.0
HRS	Sy Ingmar	-	63.85	10.3	-	33.9
	Barlow	-	65.01	10.3	-	32.8
	Elgin-ND	-	63.52	10.1	-	33.7
	Linkert	-	64.49	9.6	-	36.6
	Glenn	-	65.68	10.3	-	34.3
	Rollag	-	64.93	9.8	-	34.3
	ND Vitpro	-	65.52	10.0	-	34.4
	Lang-MN	-	65.44	10.8	-	31.5
LSD value (0.05)		3.41	1.01	0.26	0.06	1.42

Mean values are presented in Table 6 (number of field replicates for Hulled wheats = 4)
a, b – Test weight with hull and husked ratio were calculated only for hulled wheats: einkorn, emmer and spelt

Table 7: Kernel quality traits of hulled wheat and modern wheat species

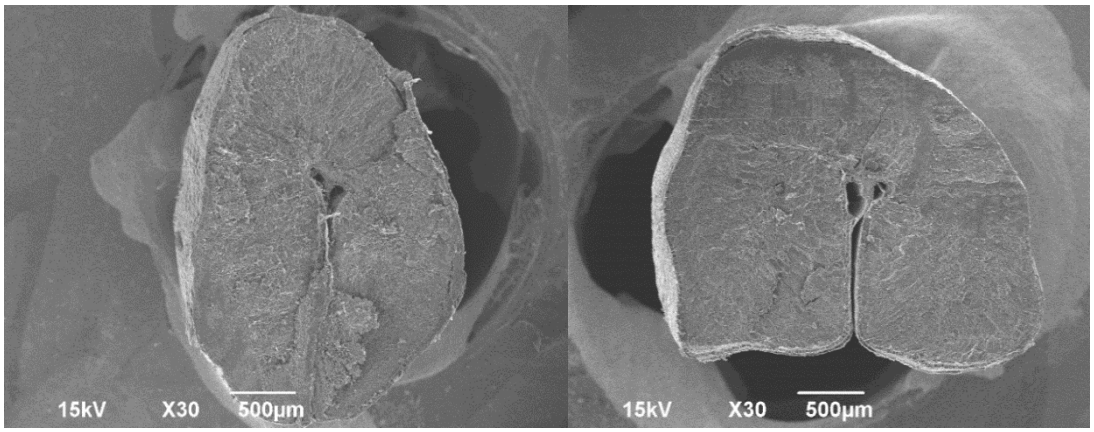
Wheat species	Genotype	Hardness index	Large kernel content (%)	Medium kernel content (%)	Small kernel content (%)
Einkorn	TM 23	1.8	3	92	4
	WB Apline	2.4	2	97	2
	PI 538722	2.5	7	89	3
Emmer	Vernal	73.8	3	91	5
	Lucille	75.6	3	93	3
	ND common	74.4	3	91	6
	Yaroslav	73.8	2	91	6
Spelt	CDC Zorba	24.4	44	54	2
	94-288	56.0	35	64	1
	SK3P	17.8	79	21	0
HRS	Sy Ingmar	68.3	32	67	1
	Barlow	79.8	31	67	1
	Elgin-ND	75.3	34	64	1
	Linkert	66.1	38	61	1
	Glenn	77.4	33	66	1
	Rollag	78.0	37	62	1
	ND Vitpro	73.1	33	66	0
	Lang-MN	80.8	31	67	2
LSD value (0.05)		2.5	3	3	2

Mean values are presented in Table 7 (number of field replicates for Hulled wheats = 4)

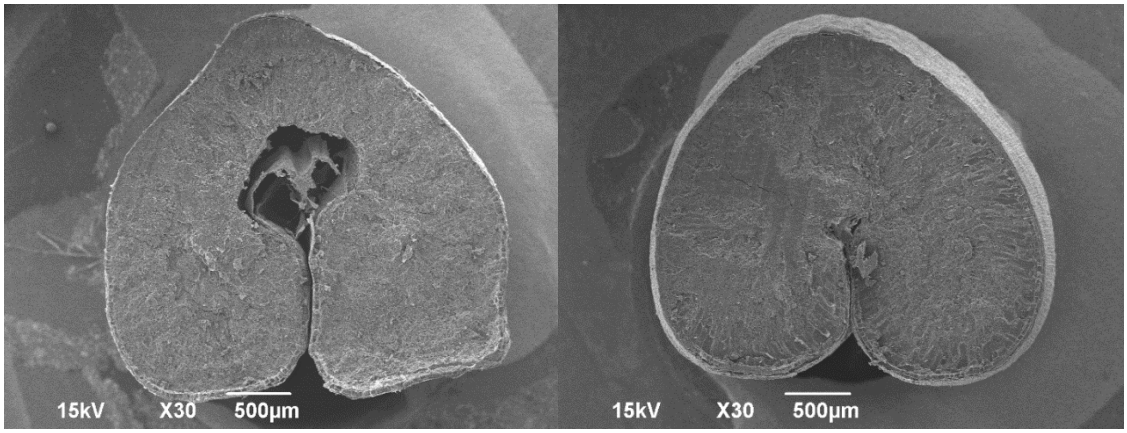
Table 8: Correlations between proximate analysis and kernel quality traits

Factor	Test weight	1000 kernel weight	Hardness index	Large kernel content	Medium kernel content	Small kernel content
Moisture content	0.86 ***	-0.07 NS	0.73 ***	0.20 NS	-0.18 NS	-0.37 NS
Protein content	0.86 ***	0.27 NS	0.44 NS	0.51 *	-0.48 *	-0.65 **
Total starch content	-0.40 NS	-0.04 NS	0.32 NS	-0.51 *	0.49 *	0.61 **
Crude fat content	-0.82 ***	-0.16 NS	-0.62 **	-0.50 *	0.48 *	0.60 **

*** P-value <0.001; ** P-value <0.01; * P-value <0.05; NS: non-significant

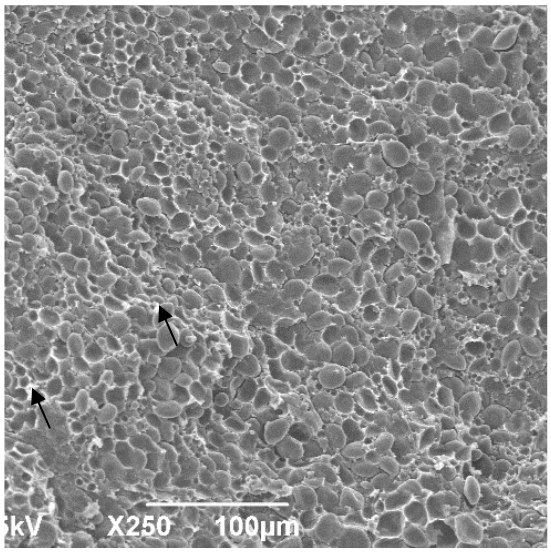


Einkorn (Emmer)

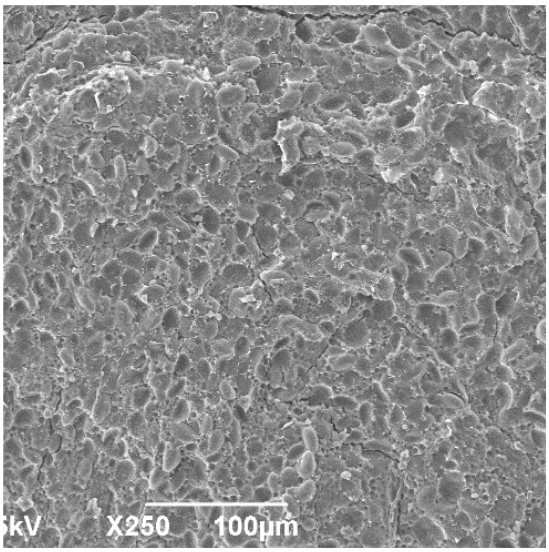


(Spelt) (Hard Red Spring Wheat)

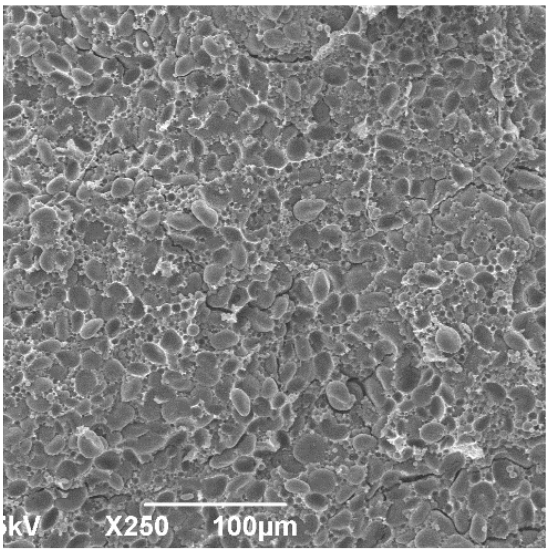
Figure 6: Microstructure of transverse section of wheat kernels.



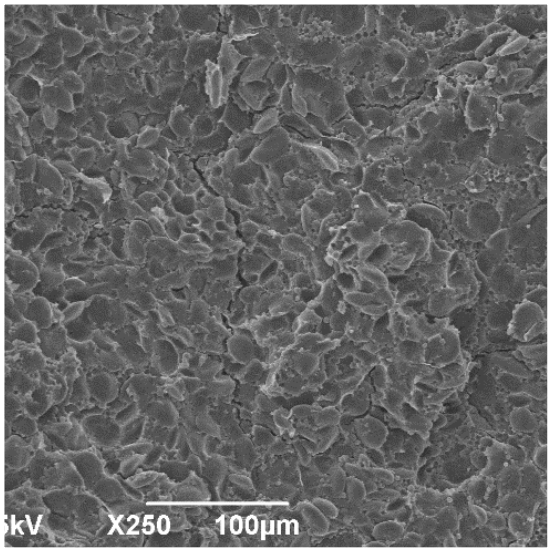
(Einkorn)



(Emmer)

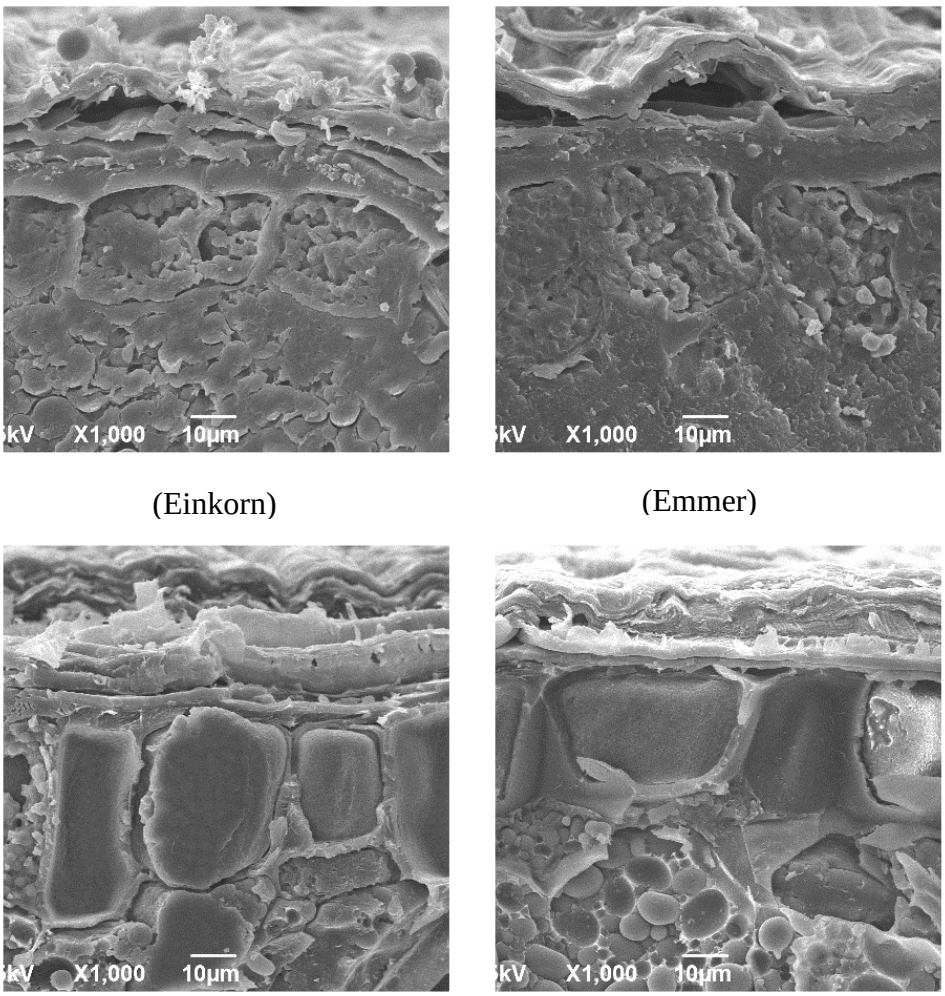


(Spelt)



(Hard Red Spring Wheat)

Figure 7: Microstructure of endosperm of wheat kernels



(Einkorn) (Emmer)

(Spelt) (HRS)

Figure 8:Microstructure of aleurone layer and outer layers

Microstructure of hulled wheat kernels by Scanning Electron Microscopy (SEM)

Microstructure of hulled wheat kernels are important as there were pronounced differences in kernel harness as discussed above under quality traits. Therefore, SEM images of transverse section of kernels, endosperm structure and aleurone layer are shown in Figures 6, 7 and 8, respectively.

Transverse section of einkorn kernel is more unique and elongated, significantly different in shape compared to emmer, spelt and HRS (Figure 6). In contrast, emmer and spelt look similar but not perfectly round (Figure 6). HRS is round in shape.

Shape could be another factor that can affect the packing efficiency of wheat, thus test weight. This could be a possible reason for the differences in test weights among hulled wheats.

Discrete and separated starch granules were observed in einkorn (Figure 7). Furthermore, two separate types of starch granules were clearly seen in the endosperm, large granules were lenticular in shape, while small granules are spherical shape. Size of these granules are not uniform and showed a wide dispersion in size. Moreover, starch granules are loosely packed, less embedded in a partial presence of protein matrix was observed. Therefore, starch protein adhesion is much lower in einkorn and it was evident by presence of small air pockets in the endosperm (indicated by arrows). This was opposed to the features observed in HRS (Figure 7). Aleurone and outer layers of einkorn is shown in Figure 8. Einkorn is characterized by a thinner pericarp and cube shaped aleurone cells. Presence of thinner pericarp, incomplete protein matrix and air pockets would be possible reasons for softness of einkorn kernels.

The endosperm of emmer (Figure 7) showed a densely packed compact starch granules firmly cemented by a protein matrix. Unlike einkorn, large and small starch granules are completely embedded in the protein matrix. Moreover, much more complete protein matrix was observed. Emmer is similar to HRS wheat as it showed an intimate contact between starch and protein. These differences are well manifested in the fracture pattern of wheat grains/endosperm (Morris and Beecher 2012). Aleurone layer is characterized by thick cell walls and cube shaped cell walls (Figure 8). Spherical protein bodies are clearly visualized inside aleurone cells.

Starch granules of spelt wheat were observed to be noticeably less firmly embedded in the internal matrix protein structure than the starches of the harder emmer and HRS wheat varieties, but firmer than einkorn (Figure 8).

Medium soft textured spelt wheat characterized by relatively loose starch granules in the matrix held together by less tenacious starch-protein bonds. More elongated aleurone cells were observed in spelt compared to other species (Figure 8). However, clear sub aleurone layer was not observed as in HRS.

These results suggest that differences in kernel hardness that exist among einkorn, emmer, spelt and HRS are related to the morphological differences in kernel structure. Therefore, the results would be useful in discriminating between hulled wheat and hulless wheat. In addition, these images would be useful in identifying vitreous and non-vitreous kernels.

Proximate analysis of whole wheat flour

The proximate compositions of the einkorn, emmer and spelt whole wheat flour were analyzed and compared to eight genotypes of HRS whole wheat flour samples (Table 9). The moisture content values in whole wheat flour of hulled wheats varied between 8.8% - 9.5% (Table 9), while whole wheat flour of HRS genotypes had values around 10.0% - 10.5%. Moisture content is not a wheat-grade determinant but is important for providing information used for pricing the commodity and is essential information when storing or processing the wheat. Significant differences ($p < 0.05$) were reported in the moisture content of hulled wheat flour with compared to HRS whole wheat flour. Moreover, significant differences ($p < 0.05$) in the moisture content were also observed between genotypes of emmer, spelt and HRS. This could be due to the difference in harvest dates of different wheat species as mentioned in the methodology section.

The ash content ranged from 2.0 - 2.3 % and 2.0 - 2.2 % for hulled wheats and HRS respectively. Significant differences ($p < 0.05$) were observed in between spelt and HRS wheat, but not for einkorn and emmer when compared to HRS. The ash content values in the hulled wheat flour were similar to those previously reported in different studies (Brandolini, 2008: Loje et al., 2003).

The whole wheat flour of hulled wheats showed protein contents between 13.6% - 15.8% (Table 9), while for HRS, protein content was between 15.9% - 18.4% (Table 9). As it was observed, significantly lower ($p < 0.05$) protein contents were found in hulled wheats compared to HRS. Additionally, significant differences ($p < 0.05$) were also observed between genotypes of einkorn emmer and spelt. However, no significant differences were observed between einkorn and emmer.

Lower protein values were observed for hulled wheats compared to modern wheat. However, several studies revealed that protein content in whole wheat flour of ancient wheat are commonly superior to those of the modern counterpart (Abdel-Aal et al., 1995; Loje et al., 2003; Marconi and Cubadda, 2005; Shewry et al., 2013). The difference in protein contents can be due to the differences in fertilization levels. In addition, these differences can be due to agronomic practices, soil fertility, disease control and weed control. According to the correlation coefficients presented in Table 9, the protein content showed a strong positive correlation with the kernel test weight and farinograph water absorption.

Starch content of wheat is related to the yield, but also to the quality properties of different wheat-based foods (Peña-Bautista et al., 2017). Significantly higher ($p< .05$) starch content was observed for emmer, but difference was not significant for einkorn and spelt compared to HRS. The starch content in both hulled wheats and HRS (Tables 9) varied from

61.2% - 66.9% and 60.5% - 65.1%, respectively. The values for einkorn and emmer were similar to those reported in other research studies. Total starch content for einkorn and emmer reported as 62.3% - 65.0% respectively (Abdul Aal et al., 1995, Abdul Aal et al., 1998). Higher values were observed for emmer (66%) when compared to bread wheat while lower values were observed for einkorn and spelt. However, several studies reported either a comparable or even lower starch content of hulled wheats compared with that of bread wheat (Brandolini et al., 2008, Haghayegh and Schoenlechner, 2010). Preliminary work (Abdel-Aal et al., 1999a) revealed lower starch yields from spelt vs. common wheats and higher lipid levels.

Crude fat content is a minor component in wheat grain compared to starch and protein, ranging from 0.57% - 1.44% and 1.25% - 2.53% for common bread wheat and hulled wheats, respectively (Table 9). Hulled wheat had higher crude fat contents than that of common bread wheat, and these findings are consistent with the findings of Hidalgo et al. (2009). Significantly higher ($p < 0.05$) crude fat contents were reported for hulled wheat flour with compared to HRS whole wheat flour. Moreover, significant differences ($p < 0.05$) in the fat content were also observed between genotypes for spelt.

It is believed that genetic predisposition has the biggest influence on lipid content in wheat (Konopka & Rotkiewicz, 2004). Furthermore, lipid content in different genotypes of wheat grown in the same environment varied more than when the same wheat genotype was grown in different environmental conditions (Konopka & Rotkiewicz, 2004). However, results of fat content are important towards nutritional value and storage ability of cereal based foods although values are low.

Fatty acid composition of einkorn, emmer, spelt and HRS is shown in Table 10. Significant differences ($p < .05$) were observed for einkorn, emmer and spelt in the content of

palmitic acid, palmitoleic acid and oleic acid when compared to HRS. Einkorn showed the highest content of palmitoleic acid (0.23%) and oleic acid (27%) among hulled wheats. Interestingly, hulled wheats were low in saturated fatty acids and they were rich in monounsaturated fatty acids compared to HRS. However, poly unsaturated fatty acids such as linoleic and linolenic acids reported to be low in einkorn, emmer, and spelt compared to HRS.

Table 9: Proximate composition of whole wheat flour of hulled wheat species

Species	Genotype	Moisture (%)	Ash (%)	Protein (%)	Total starch (%)	Crude fat (%)
Einkorn	TM 23	9.0	2.2	15.4	62.1	2.3
	WB Apline	9.0	2.2	13.9	62.8	2.5
	PI 538722	9.1	2.1	14.5	61.7	2.1
Emmer	Vernal	9.4	2.3	15.2	64.3	2.0
	Lucille	9.5	2.3	15.0	66.9	2.0
	ND common	9.5	2.1	14.2	66.6	2.0
	Yaroslav	9.3	2.1	13.6	65.8	2.3
Spelt	CDC Zorba	8.8	2.2	14.6	61.2	1.7
	94-288	9.1	2.0	15.1	61.8	1.3
	SK3P	8.9	2.1	15.8	61.9	1.9
HRS	Sy Ingmar	10.5	2.1	18.4	61.4	1.0
	Barlow	10.4	2.1	16.6	65.1	0.7
	Elgin-ND	10.3	2.2	16.9	60.5	1.2
	Linkert	10.0	2.2	17.5	62.9	1.4
	Glenn	10.4	2.2	17.3	64.3	1.3
	Rollag	10.3	2.1	17.6	61.5	1.4
	ND Vitpro	10.4	2.3	17.9	62.0	0.9
	Lang-MN	10.8	2.0	15.9	60.5	0.6
LSD value (0.05)		0.2	0.5	0.6	2.3	0.3

Mean values (%) are presented in Table 6 (number of field replicates for Hulled wheats = 4)
All the values are expressed as dry weight basis

Table 10: Fatty acid composition of hulled wheat and common bread wheat

Wheat species	Genotype	PAL	PAO	MAR	STE	OLE	VAC	LIO	LIN	GON	EDA	HAL	NER
Einkorn	TM 23	15.0	0.2	0.3	1.2	28.0	1.1	54.0	3.6	1.5	0.1	0.1	0.2
	WB Apline	15.4	0.2	0.2	1.0	27.8	1.2	54.7	3.2	1.6	0.1	0.0	0.2
	PI 538722	15.7	0.2	0.2	1.2	27.6	1.1	54.0	3.1	1.4	0.1	0.0	0.2
Emmer	Vernal	17.6	0.2	0.2	1.6	27.1	0.9	53.0	3.4	1.4	0.1	0.1	0.2
	Lucille	17.7	0.2	0.2	1.6	26.4	0.9	53.3	3.4	1.3	0.1	0.1	0.1
	ND common	17.6	0.3	0.2	1.5	24.7	1.0	55.2	3.2	1.3	0.1	0.1	0.1
	Yaroslav	17.6	0.2	0.2	1.3	24.9	1.1	55.9	3.3	1.3	0.1	0.0	0.2
Spelt	CDC Zorba	16.9	0.2	0.2	0.6	25.1	1.1	56.6	2.5	1.2	0.1	0.0	0.1
	94-288	18.7	0.3	0.3	1.2	16.7	1.1	62.5	3.2	0.7	0.1	0.1	0.1
	SK3P	16.9	0.3	0.2	1.3	22.3	1.1	59.4	2.9	0.7	0.1	0.1	0.1
HRS	Sy Ingmar	19.9	0.2	0.2	1.2	16.2	1.0	61.8	3.9	0.7	0.2	0.1	0.1
	Barlow	20.5	0.2	0.2	1.1	12.7	1.0	63.1	4.0	0.7	0.2	0.1	0.2
	Elgin-ND	18.9	0.2	0.2	1.2	15.8	1.0	61.4	3.8	0.7	0.2	0.1	0.1
	Linkert	19.2	0.2	0.2	1.2	14.8	1.0	63.1	3.7	0.6	0.2	0.1	0.2
	Glenn	19.1	0.2	0.2	1.2	15.3	1.0	62.3	3.8	0.7	0.2	0.1	0.2
	Rollag	19.1	0.2	0.2	1.2	15.1	1.0	62.7	3.7	0.6	0.2	0.1	0.2
	ND Vitpro	19.1	0.2	0.2	1.2	15.2	1.0	62.5	3.7	0.7	0.2	0.1	0.2
	Lang-MN	19.1	0.2	0.2	1.2	15.1	1.0	62.6	3.7	0.6	0.2	0.1	0.2
LSD value (0.05)		0.9	0.1	0.0	0.6	1.1	0.1	1.3	0.1	0.1	0.1	0.0	0.1

PAL: Palmitic (16:0), PAO: Palmitoleic (16:1), MAR: Margaric (17:0), STE: Stearic (18:0), OLE: Oleic (18:1), VAC: Vaccenic (18:1), LIO: Linoleic (18:2n6), LIN: Linolenic (18:3n3), GLI: g-Linolenic (C18:3n6), Gonodic (20:1n9), EDA: Eicosadienoic acid (20:2), HAL: Homo-a-linolenic (20:3n3), NER: Nervonic (24:1n9)
Values are expressed as percent of total fat

Table 11: Dietary fiber variation of whole wheat flour of hulled wheat and bread wheat

Species	Genotype	IDF	SDF-HM	SDF-LM	AX	A/X ratio
Einkorn	TM 23	7.5	0.7	4.8	2.9	0.7
	WB Apline	8.6	1.2	5.0	4.1	0.7
	PI 538722	8.9	1.9	6.7	3.2	0.7
Emmer	Vernal	7.8	1.4	5.8	2.3	0.7
	Lucille	12.7	2.6	5.4	2.1	0.7
	ND common	10.2	3.0	5.2	2.3	0.7
	Yaroslav	13.3	3.7	5.2	2.6	0.7
Spelt	CDC Zorba	10.7	0.8	4.1	1.6	0.8
	94-288	11.4	1.6	4.8	2.0	0.6
	SK3P	10.6	1.8	6.1	2.1	0.7
	Sy Ingmar	11.3	4.4	3.1	3.4	0.8
HRS	Barlow	11.6	2.0	4.0	3.6	0.8
	Elgin-ND	13.4	4.2	3.1	2.4	0.8
	Linkert	13.0	4.0	4.3	4.4	0.9
	Glenn	11.3	2.9	4.9	2.9	0.7
	Rollag	13.0	4.1	4.5	2.6	0.7
	ND Vitpro	11.4	1.3	4.7	3.8	0.8
	Lang-MN	11.6	1.1	4.7	2.8	0.8
LSD (0.05)		1.6	1.0	1.1	0.7	0.1

AX- Arabinoxylan A/X = Arabinose/ Xylose

Dietary fiber consists mostly non-starch polysaccharides, such as arabinoxylans and cellulose (Stone and Morell, 2009). Dietary fiber are two categories as insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) (Carson and Edwards, 2009). Soluble dietary fiber can be further divided into two categories depending on their solubility in 78% ethanol. Low molecular weight dietary fiber such as fructo and galacto-olisaccharides are soluble in ethanol (SDF-LM), while high molecular weight dietary fiber such as βglucan and certain xylans are insoluble in 78% ethanol (SDF-HM). Results for einkorn, emmer, spelt and HRS whole wheat flour are shown in Table 11.

In einkorn, IDF, SDF - HM and SDF - LM values ranged from 7.5 - 8.9 %, 0.7 - 1.9 % and 4.8 - 6.7% respectively. In addition, TDF content ranged from 13.0 -17.8 %. Genotypes were

significantly different ($P<0.05$) for TDF, SDF - HM and SDF - LM, but the differences were not significant ($P>0.05$) for IDF (Table 11). IDF content was reported for TM23 genotype of einkorn as 6.9 % (Abdel- al et al., 1995). Nearly similar values were reported in this study for TM23 (7.53%). However, comparable and lower values were reported for TDF content of einkorn (9.3 - 12.88 and 9.8 %) in several studies (Gebruers et al., 2008, Arentz-Hansen et al., 2000, Arzani, 2011).

Interestingly, einkorn genotypes were characterized by higher percentage (33-38% of TDF) of SDF- LM or LMWDF such as raffinose, stachyose, fructo and galacto oligosaccharides compared to HRS (16 - 25 % of TDF) (Table 12).

These results indicate that einkorn can be used as a potential candidate for breeding and as a prebiotic source in novel food products. SDF content for einkorn has been reported to range from 0.2 - 1.7% on dry matter basis (Lùje et al., 2003). However, DF can vary depending on the genotype, growth factors and location. Moreover, it can also vary depending upon the method of analysis. However, dietary fiber definition varies among different governing organizations, U.S. Food and Drug Administration (FDA) currently makes it optional to include non-digestible oligosaccharides of 3 - 9 monomeric units in fiber declared on food labels (U.S. Food and Drug Administration, 2018); while the Codex Alimentarius Commission, excludes these components (Zielinski et al., 2013). Therefore, it is up to each country to decide whether to adopt or modify this definition.

Table 12: Different dietary fiber fractions of einkorn, emmer, spelt and bread wheat

Species	Genotype	IDF (%)	SDF-HM (%)	SDF-LM (%)	DF-HM (%)
Einkorn	TM 23	57.9	5.1	37.0	63.0
	WB Apline	58.4	7.8	33.8	66.2
	PI 538722	50.7	10.7	38.6	61.4
Emmer	Vernal	52.3	9.2	38.5	61.6
	Lucille	61.5	12.4	26.1	73.9
	ND common	55.4	16.2	28.4	71.6
	Yaroslav	59.8	16.8	23.4	76.6
Spelt	CDC Zorba	68.9	4.8	26.3	73.7
	94-288	63.8	9.0	27.2	72.8
	SK3P	57.2	9.8	33.1	66.9
	Sy Ingmar	60.3	23.3	16.5	83.6
HRS	Barlow	65.7	11.4	22.9	77.1
	Elgin-ND	64.5	20.5	15.0	85.0
	Linkert	61.0	18.8	20.3	79.7
	Glenn	59.1	15.2	25.7	74.3
	Rollag	60.3	19.1	20.6	79.4
	ND Vitpro	65.2	7.7	27.1	72.9
	Lang-MN	66.5	6.4	27.1	72.9

Significant differences ($p<.05$) were observed for dietary fiber fractions in emmer compared to HRS. IDF, SDF-HM and SDF-LM values of emmer were 7.8 - 13.8%, 1.3 - 3.7% and 5.2 - 7.9 % respectively. The average soluble dietary fiber content of emmer is reported to be 0.41%, but only small numbers of samples were analyzed in most studies and the range is wider in some studies (Shewry and Hey, 2015).

TDF values ranged from 15.0 - 20.7 % for whole wheat flour of emmer. TDF content of 11.8 - 11.5% reported for whole wheat flour of spring and winter emmer cultivated in Italy (GiacinTucci et al., 2014). Moreover, wider range 7.2 - 12.0 % was reported in another study conducted in Belgium (Gerbuers et al., 2008). In contrast, higher values were reported in this study. The whole meal of three emmer genotypes had a TDF content of about 10 - 12%, mainly

insoluble fractions (85 – 88% of TDF) (Marconi and Cubadda, 2005). However, in this study lower (52- 61%) IDF fraction and, in contrast higher (23 -38%) SDF-LM content fraction was reported (Table 12).

Significant differences ($p < .05$) were observed in all the fractions of spelt compared to HRS. Spelt had an IDF, SDF-HM and SDF-LM content ranging from 10.6 - 11.4 %, 0.8 - 1.8% and 4.1 - 6.1 % respectively. IDF content of spelt has been reported ranging from 8.0 - 8.4 % for genotypes cultivated in Belgium (Gerbuers et al., 2008). Lower values were reported for TDF in the same study. In this study higher values were reported for TDF, ranging from 15.5 - 18.5% for wholegrain flour of spelt. Soluble fiber content of spelt has shown a wide variability among cultivars, ranging from 1.2% (*Balmegg*), 2.4% (*Oberkulmer*) and 2.5% on dry weight basis. One study suggested higher content of soluble dietary fiber (1.7 - 1.8% dry matter basis) in spelt with comparison to the common bread wheat (Lùje et al., 2003).

The IDF, SDF-HM and SDF-LM values of HRS whole wheat flour varied between 11.3 - 1.1 - 4.4 and 3.1 - 4.7 respectively. Higher IDF content of HRS than spelt was attributed to the difference in hemicellulose and cellulose (Escarnot et al., 2010). As mentioned before, hulled wheat study conducted in Europe reported TDF content of 12.1 – 17.5% for hard red spring wheat, but they have used an indirect approach to calculate the dietary fiber content (Gerbuers, 2008). Dietary fiber values highly depend upon the method used. Therefore, it is difficult to compare those values with the values in our study. Due to this reason, dietary fiber values in food composition databases and on food labels do not represent the same group of chemical compounds (Stephen et al., 2013; Westenbrink et al., 2013; Zielinski et al., 2013). Changing definitions and values in food composition databases for dietary fiber for the same foods but determined by different methodology have consequences for research on intake of dietary fiber

and health outcomes, dietary recommendations, and nutritional assessment of "adequacy". However, another study was conducted in Europe using the same procedure, AOCC 2011.25 for different cereals. Wholegrain flour of wheat has shown a variation from 10.4 – 15.6 % for TDF content (Rainakari et al., 2016).

The other methods used to determine DF including Southgate, Uppsala and Prosky underestimate the components such as resistant starch, maltodextrin, inulin and fructo and galacto oligosaccharides (McClearly et al., 2013). AOAC 2011.25 method used in this study has the advantage of being advanced and it also analyzes separate components of DF. In addition, this is the first study this method has been applied to hulled wheats to the best of my knowledge. Therefore, these data are important, and they can be used to update the existing Food Composition Data bases and useful in nutritional labelling purposes.

Arabinoxylans (AX), are non-cellulose component of the wheat cell walls and they are considered a minor component in wheat, but they have significant impact on quality of the wheat flour, impact bran and endosperm separation during milling and can also stabilize dough structure (Courtin and Delcour 2002). AX content and structure can affect the dough consistency and water absorption of flour (Goesaert et al., 2005; Dornez et al., 2008). They might also act to stabilize gas cells during fermentation and baking. AX ratio is another important factor, which indicates the rate of substitution with arabinose and it has an important role in the AX functionality (Goesaert et al, 2005). A/X ratio determines the shape, size and solubility of the polysaccharide. AX content and AX ratio of wholegrain flour of einkorn, emmer, spelt and HRS are shown in Table 11.

The AX of Einkorn wheat was significantly different ($p<0.05$) among genotypes of einkorn and HRS. AX and AX ratio of 2.9 - 4.1 db% and 0.70 - 0.73, respectively. Total AX

content of wholegrain flour of einkorn has been reported to be1.95 % on db (Gebruers et al., 2008). In Healthy grain diversity studies, they have reported a mean value ranging from 1.45 – 2.35 % on dry weight basis for einkorn flour (Shewry and Hey, 2015). However, higher values were reported in our study for all the genotypes of einkorn. Genotypes with higher AX can be used in breeding or in product processing that concern achieving high levels of AX. However, as discussed above, einkorn dough was characterized by a higher sticky property, which could be due to the substitution of AX with a ferulic acid moiety (Koh et al., 2009).

In emmer, significant differences ($p<0.05$) were observed compared to HRS. AX and AX ratio varied from 2.1 - 2.6% and 0.68 – 0.72, respectively. Reported range for AX of emmer wholegrain flour is 1.40 – 1.95%. Therefore, slightly higher values were reported in this study. It could be depending upon genotype, growth factors and analysis method. Furthermore, genotypic differences were found in einkorn and emmer for total AX in this study, suggesting that genotype have an impact on AX content of einkorn and emmer wheat.

Significant differences were not found among the genotypes of spelt, but differences were observed ($p<0.05$) in AX content and ratio compared to genotypes of HRS. AX and AX ratio varied from 1.64 - 2.09% and 0.64 – 0.75, respectively. Similar values were reported for AX in Healthy grain diversity study 1.6 – 2.2% (Shewry and Hey, 2015). Some genotypes of spelt had lower A/X ratio suggesting that they are low in arabinose substitution. Significant differences ($p<0.05$) were observed in A/X ratio among genotypes of spelt, indicating that arabinose substitution depended upon the genotype used in the study. Significant variation in AX substitution among genotypes were also observed by other researchers (Storsley et al., 2003; Simsek and Ohm, 2009).

Pasting properties

Pasting properties of einkorn, emmer, spelt and HRS are presented in Table 13 and Figure 9. Significant differences ($p < 0.001$) were observed in peak viscosity, trough, breakdown, final viscosity and set back of hulled wheats with compared to HRS. The maximum consistency of flour that can be obtained when the flour is heated in excess water is indicated by peak viscosity. All the genotypes of spelt showed higher peak viscosity values when compared to HRS. These results could be due to the water binding capacity of starch at equilibrium point between swelling which caused an increase in viscosity while rupturing and re-alignment cause its reduction (Sanni et al., 2001). Starch granule size can also affect the peak viscosity. Since larger granules would occupy more space within the measuring systems, they are expected to develop more viscous pastes (Singh et al., 2010). There could be larger starch granules in einkorn and spelt with compared to HRS, larger granules swell faster when heated and more of the components will leach out. However, SEM micrographs mentioned above were not supported this assumption.

Peak viscosity values of einkorn were comparable with HRS. Significant genotypic differences were observed in emmer. Highest peak viscosity was for ND common (3341 cP) while the lowest for Lucille (1462 cP) among the genotypes of emmer. However, a study of the rapid viscosity analyzer pasting properties of 65 einkorn accessions of different geographical origin showed that einkorn had higher peak viscosity and final viscosity than *T. turgidum* and *T. aestivum* (Brandolini et al., 2008). Breakdown values also followed the same trend as peak viscosity. The breakdown values are associated with decreased viscosity caused by the disruption of swollen starch granules with continuous heating and agitation (Ragaee and Abdel-Aal, 2006).

It explains why higher breakdown values were obtained for the samples that presented higher maximum viscosity.

During cooling, the re-association of leached starch polymers and the interactions between intact remnant granules, dictate the extent of setback and final viscosity (Jane et al., 1999). Moreover, the reassociation of starch molecules, results in paste structure formation and increased viscosity (Ragaee and Abdel-Aal, 2006). Higher final viscosities were observed for majority of hulled wheat genotypes compared to HRS.

All the genotypes of spelt had comparable low pasting temperatures as HRS Glenn. In contrast, higher or similar pasting temperatures were reported for some genotypes of emmer (Yaroslav and vernal) and one genotype of einkorn (PI 538722) compared to HRS.

Pasting properties provide information valuable for food processing and product development. Initial peak and final viscosity of spelt and final viscosity of einkorn flour implies the suitability of these flours for instant puddings and food formulations requiring little heat during processing such as yogurt, smoothies, and ice cream. Low final and set back viscosities of one genotype of emmer (Lucille) indicates its suitability to use in low water activity products with mixed formulations such as multigrain bread.

Table 13: Pasting properties of einkorn, emmer, spelt

Species	Genotype	Peak 1 (cP)	Trough 1 (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Peak Time (minutes)	Pasting Temperature (°C)
Spelt	CDC Zorba	3189	1952	1237	3147	1195	6	67
	94-288	3761	2145	1616	3503	1358	6	68
	SK3P	3249	1988	1262	3219	1231	6	67
Einkorn	TM 23	2643	1741	902	3147	1406	6	73
	WB Apline	2628	1741	887	3127	1386	6	70
	PI 538722	2432	1733	699	3121	1388	6	81
Emmer	Vernal	2415	1735	680	3066	1331	6	84
	Lucille	1462	411	1051	902	491	5	75
	ND common	3341	2188	1154	3613	1425	6	70
	Yaroslav	2285	1142	1143	2144	1002	6	81
	Sy Ingmar	2141	1323	819	2398	1075	6	69
	Barlow	2463	1511	953	2642	1131	6	85
HRS	Elgin-ND	2687	1728	959	2873	1145	6	86
	Linkert	2732	1744	988	3010	1266	6	87
	Glenn	2578	1359	1219	2237	878	6	70
	Rollag	2702	1813	889	2979	1166	6	86
	ND Vitpro	2852	1742	111	2931	1189	6	78
	Lang-MN	2858	1638	1220	2769	1131	6	70
LSD (0.05)		109	91	90	108	68	0	5

Mean values are presented in Table 11 (number of field replicates for Hulled wheats = 4)

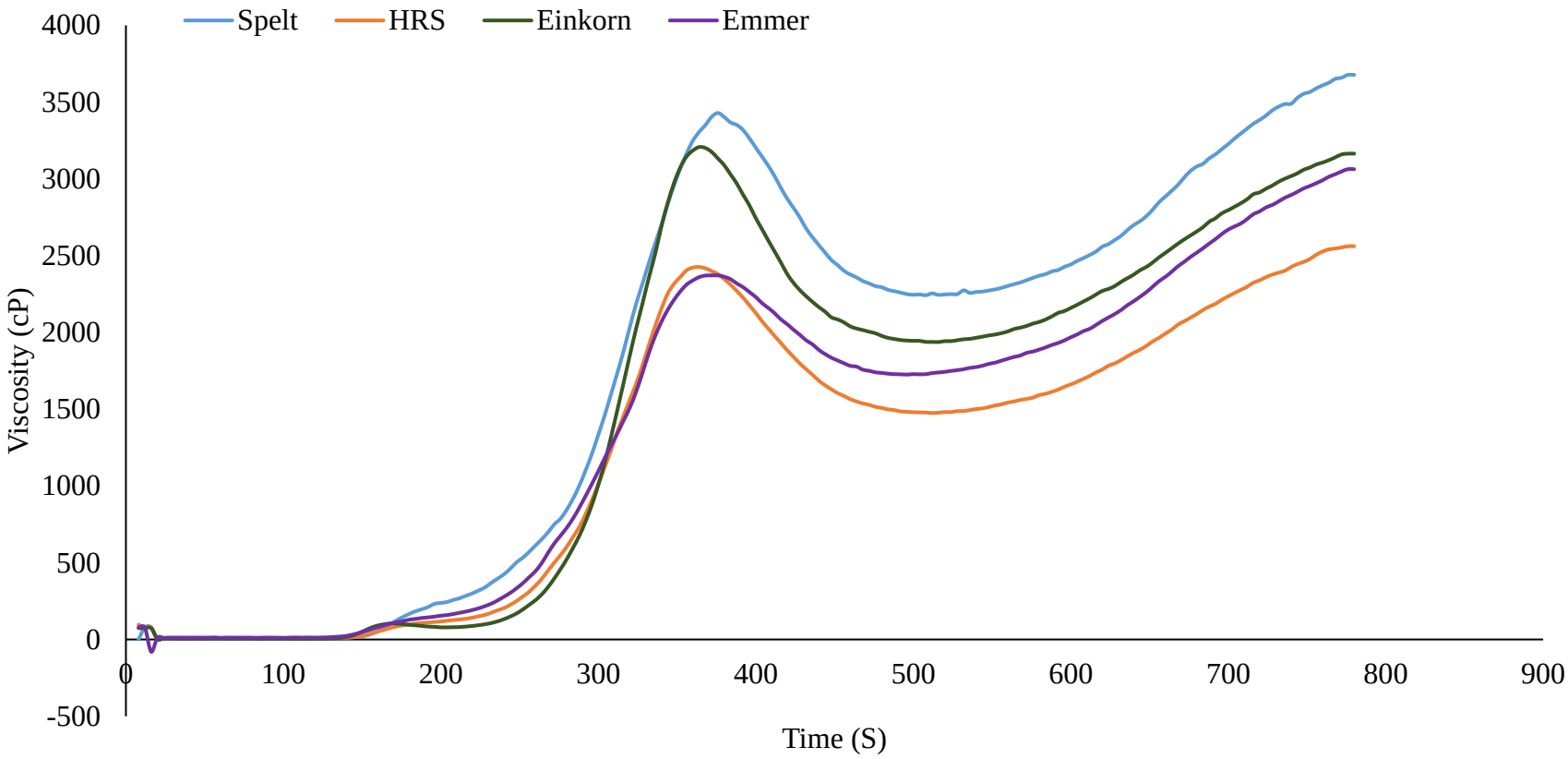


Figure 9: Typical RVA profile of einkorn, emmer, spelt and HRS wheat

Dough and baking quality

Water absorption is one of the most fundamental quality parameters of wheat flour and is the amount of water needed to hydrate flour components to produce a dough with optimum consistency (Bushuk and Békés, 2002). Correct water absorption for flour is critical for production of the best quality bread and flours with high water absorptions often being desired by bakers. Significant differences ($p < 0.001$) were observed in farinograph water absorption of hulled wheats compared to HRS (Table 14). Water absorption was highest for spelt (71.1 - 72.3%) followed by emmer (64.6 - 67.1%) and einkorn (58.7 - 59.7%). However, lower water absorption values (49-59) have been reported for spelt (Sobczyk et al., 2017). It can depend upon the quantity of damage starch present in the flour.

Water absorption is also an indicator of the relative amounts of the components capable to be hydrated (starch, proteins, pentosans) and their specific water binding capacity. Furthermore, it depends upon the number of hydroxyl groups in the fiber structure, which allows more associations with water molecules through hydrogen bonding, thereby increase competition for water with proteins and starch, and influence water distribution in the dough (Rosell et al., 2010; Bock et al., 2013). As discussed previously, lower protein contents were observed for hulled wheats compared to HRS which could be a reason for low water absorption values. Interestingly, strong positive correlation was found with protein content ($r = 0.86$), kernel test weight ($r = 0.88$) and hardness index ($r = 0.63$). In contrast, negative correlation was observed between crude fat content ($r = 0.88$) and water absorption. These water absorption values of einkorn, emmer and spelt dough is effective in predicting the processing behavior of hulled wheat flour and in controlling the quality of food products.

Table 14: Dough quality of hulled wheat and bread wheat evaluated by farinograph

Wheat species	Variety	Absorption (14%)	Peak Time (min)	Stability (min)	MTI	Quality Number
Einkorn	TM 23	51.58	1.8	0.8	100.5	24.3
	WB Apline	52.13	1.8	0.7	82.0	25.3
	PI 538722	53.15	2.0	0.7	66.5	27.5
Emmer	Vernal	54.80	1.7	0.6	155.8	21.0
	Lucille	54.60	1.8	0.6	151.8	21.8
	ND common	55.90	1.8	0.7	127.0	23.5
	Yaroslav	56.73	2.2	0.7	138.8	26.3
Spelt	CDC Zorba	58.40	3.1	1.7	60.7	48.0
	94-288	61.20	5.1	7.8	37.8	87.8
	SK3P	60.33	2.8	1.5	56.0	40.3
	Sy Ingmar	68.65	4.8	3.5	44.0	68.0
	Barlow	68.80	4.5	2.8	58.0	59.0
	Elgin-ND	68.75	4.7	3.4	46.5	66.0
HRS	Linkert	68.50	5.6	6.9	33.0	92.5
	Glenn	68.20	5.6	5.4	37.5	87.5
	Rollag	68.70	3.4	1.8	75.0	48.0
	ND Vitpro	68.25	4.2	3.2	51.5	63.0
	Lang-MN	66.30	4.8	3.9	36.5	79.5
LSD (0.05)		0.8	0.4	0.5	13.6	5.2

Mean values are presented in Table 9 (number of field replicates for Hulled wheats = 4)

Einkorn, emmer and spelt showed significant differences ($p < 0.001$) in dough development time with compared to HRS. The highest dough development time was reported for spelt (genotype 94288) among hulled wheats, this value was comparable to some of the genotypes of HRS (SY Ingmar and Land MN). Positive correlations were observed for dough development time with flour protein content ($r = 0.72$) and kernel test weight ($r = 0.82$).

The peak time will suggest to the baker how much energy it will take to mix dough to optimum consistency. The peak times of the whole wheat samples ranged from 3.35 - 5.60 minutes. In contrast, peak time for einkorn, emmer and einkorn ranging from 1.98 - 1.75, 1.65 -

2.15, 2.80 - 5.13 minutes respectively. The increase in peak time is a result of the competition of water between the protein and bran. Arabinoxylan concentrated in the bran portion of the wheat, can increase dough development time (D'Appolonia and Kunerth, 1984).

The stability and MTI revealed how tolerant the dough is to over-mixing. Significant differences ($p < 0.001$) were observed in dough stability time for einkorn, emmer and spelt with compared to HRS (Table 14). Interestingly, highest dough stability time was reported for one genotype (94288) of spelt among all the wheat species including HRS. Krawczyk et al. (2008) and Cegli_nska (2003) reported that dough stability time of spelt dough fluctuated in a wide range of 3.3 - 12.5 min and 2.9 - 9.2 min respectively. However, all the authors stated that stability time of spelt dough was greater than wheat dough. This contrasts with observation of Bonafaccia et al. (2000) and Marconi et al. (2002).

The mixing tolerance index (MTI) values of einkorn, emmer, and spelt were significantly different from HRS. Higher values were observed for hulled wheats with compared to HRS. However, the low MTI values of some whole wheat flours did not represent better flour quality, but a great interference of the fibers in the consistency of the dough.

After assessment of the dough quality, the flours were baked to determine the end product quality. The baking quality of breads made from whole wheat flours are shown in Table 15. The bake absorption followed a similar trend as the absorption determined using the farinograph. HRS whole wheat flour had significantly higher mean baking water absorption (65.5%) than hulled wheat flour (62.1%). Water is the cheapest ingredient in breadmaking, this difference translates to higher economic return for using HRS wheat flour, provided that the prices of those two wheat classes were the same. However, this improvement is at least partially

offset by the increase in mean mixing time required to achieve optimal dough for HRS wheat flour (3.75 min) versus hulled wheat flour (2.31 min) (Table 15).

Einkorn dough samples were very soft and sticky during kneading. It could be due to the predomination of gliadins in hulled wheats, gliadins are identified as very sticky monomeric plasticizer, while glutenins were recognized as a networking polymeric factor (Abdel-Aal et al., 1997).

Table 15: Baking quality of einkorn, emmer, spelt and HRS wheat

Wheat species	**Variety**	**Bake absorption (14%)**	**Mixing Time (min)**	**Oven spring (cm)**	**Oven volume (cm^3)**	**Loaf weight (g)**	**Specific loaf volume (cm^3/g)**	**Crumb firmness (N)**
Einkorn	TM 23	58.7	1.2	-0.4	323.8	128.8	2.5	11.4
	WB Apline	59.1	1.0	-0.4	330.0	126.9	2.6	11.5
	PI 538722	59.7	1.0	-0.3	312.5	125.3	2.5	12.1
Emmer	Vernal	64.9	1.3	0.3	381.3	134.6	2.8	18.0
	Lucille	64.6	1.3	-0.2	363.	136.3	2.7	18.9
	ND common	65.9	1.4	0.0	380.0	135.8	2.8	15.7
	Yaroslav	67.1	1.3	0.0	378.8	136.7	2.8	17.8
Spelt	CDC Zorba	71.1	2.3	-1.1	490.0	142.5	3.4	6.8
	94-288	72.3	2.3	1.1	702.5	143.7	4.9	1.9
	SK3P	71.6	2.3	-0.5	401.3	145.1	2.8	11.3
	Sy Ingmar	78.8	3.4	-0.1	752.5	143.4	5.3	1.4
	Barlow	78.9	3.3	0.2	722.5	145.4	5.0	0.9
	Elgin-ND	78.9	3.4	0.9	717.5	144.6	5.0	1.6
HRS	Linkert	79.2	3.4	0.6	777.5	147.4	5.3	1.4
	Glenn	80.0	3.8	1.0	752.5	146.6	5.1	1.0
	Rollag	80.0	3.0	-0.9	587.5	146.8	4.0	2.1
	ND Vitpro	78.9	3.4	0.7	707.5	146.4	4.8	1.5
	Lang-MN	78.5	3.5	-0.7	572.5	142.7	4.0	3.8
LSD (0.05)		0.8	0.3	0.5	28.5	6.2	0.2	2.4

Mean values (%) are presented in the Table
Number of field replicates for Hulled wheats = 4

HRS wheat flour loaf volume was significantly higher (777.5 - 572.5 cm^3) than for einkorn (313 -330 cm^3), emmer (364 -383 cm^3), or spelt (401 -703 cm^3). Bread loaf volume can be affected by protein content and quality as well as by other grain traits (Finney et al 1985;

Bruckner et al 2001; Seyer and Gelinas 2009; Gelinas and McKinnon 2011). It is assumed that bread of good quality should be characterized by a volume of at least 400 cm^3 (for of 100 g of flour) (Sobczyk et al., 2017). This limit was exceeded by all the genotypes of spelt. However, bread volume of einkorn and emmer did not reach the 400cm^3 threshold. Therefore, bread volume can be improved by substituting HRS flour in the formulation instead of 100% hulled wheat flour.

Negative oven spring values were observed for all the genotypes einkorn and some genotypes of emmer and spelt. Highest oven spring value (1.08 cm) was for 94288 genotype of spelt across all the wheat species. Moreover, specific loaf volumes of einkorn, emmer and spelt genotypes are low for majority of genotypes (2.52- 3.44) except 94288 of spelt (4.89). Similar trend was observed for loaf weight. Crumb firmness is an indicator that can be used to determine sensory quality of bread. Higher crumb firmness values were observed for einkorn, emmer and spelt compared to HRS. However, 94288 genotype of spelt has a comparable hardness value as HRS. Overall, 94288 was identified as a potential line of spelt that can be released due to its excellent dough and baking quality.

Dough quality and baking quality data of hulled wheat species are important for food industry in order to develop food products to cater the consumer demand for hulled wheat products. Moreover, these data would be useful in breeding to develop new and release improved lines of einkorn, emmer and spelt.

In vitro starch digestibility of hulled wheat bread

Starch is a very important component in human diet as it provides nearly 70-80% of calories consumed by human beings worldwide (Delcour and Hoseney, 2010). Starches in foods are digestible but not all starch fractions are digested in the same way. Nutritionally important

starch fractions were estimated using a modified Englyst assay. The analyzed fractions include rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al., 1992). Values for enzymatically assessed readily digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS) and total starch (TS) contents of whole wheat bread are shown in Table 16.

Table 16: In vitro starch digestibility of hulled wheat and modern wheat bread

Species	Genotype	RDS[a]	SDS	TS	RS	HI	eGI
Einkorn	TM 23	30.4	25.8	58.4	2.2	89.9	85.7
	WB Apline	30.6	26.9	60.0	2.5	89.1	85.0
	PI 538722	28.1	29.2	59.6	2.3	86.8	83.0
Emmer	Vernal	32.0	25.5	60.0	2.5	87.2	83.4
	Lucille	32.2	25.7	60.2	2.4	89.9	85.7
	ND common	31.9	27.7	62.0	2.4	89.7	85.5
	Yaroslav	32.3	25.7	60.1	2.2	89.7	85.5
Spelt	CDC Zorba	30.9	26.7	60.0	2.4	86.6	82.8
	94-288	31.2	26.0	59.7	2.5	88.5	84.5
	SK3P	31.4	26.2	60.0	2.3	87.6	83.7
HRS	Sy Ingmar	32.6	16.4	51.6	2.5	85.4	81.8
	Barlow	31.5	19.7	53.9	2.7	83.5	80.2
	Elgin-ND	31.8	16.1	50.5	2.6	84.8	81.3
	Linkert	32.6	16.5	51.4	2.3	84.1	80.7
	Glenn	32.3	17.8	52.3	2.2	86.4	82.7
	Rollag	34.6	13.8	50.6	2.1	86.5	82.8
	ND Vitpro	33.7	18.0	53.8	2.1	85.5	81.9
	Lang-MN	31.4	18.1	51.6	2.1	81.6	78.5
LSD value (0.05)		1.0	1.7	1.8	0.1	1.3	1.2

Mean values are indicated averaged over four reps for hulled wheats
a: Results are expressed in dry weight basis

RDS is readily and completely hydrolyzed by gut enzymes in human small intestine and it is associated with more rapid elevation of postprandial plasma glucose (Kaur et al., 2015). Furthermore, RDS is mainly comprised of amylopectin. RDS content of einkorn bread was significantly lower ($p < .05$) than HRS (Table 15). In contrast, no significant differences were observed in RDS of emmer and spelt compared to HRS. However, percentage of RDS content of

total starch content was lower for einkorn, emmer and spelt bread (Table 16). Starch digestibility can depend on amylose/ amylopectin ratios and the presence of various antinutrients, such as polyphenols, phytic acid and other antinutrients (Deshpande and Cheryan 1984; Thomson and Yoon 1984).

SDS is digested and absorbed more slowly in the gut and is composed mostly of amylose. Significantly higher SDS fraction was detected in hulled wheats compared to HRS. Significantly higher ($p<0.05$) SDS content was reported in einkorn, emmer and spelt. Mean SDS fraction of the breads followed the order einkorn > spelt > emmer > HRS. Most interestingly, higher percentage (42 - 48%) of SDS (out of total starch) were found in hulled wheat breads Table 17). SDS is very important to exert potential health benefits on human beings as is coupled to a low glycemic response. Therefore, it may be helpful in controlling and preventing hyperglycemia-related diseases.

Table 17: In vitro starch digestibility fractions as a percentage of total starch

Species	Genotype	RDS	SDS	RS
	TM 23	52.0	44.2	3.8
Einkorn	WB Apline	51.0	44.8	4.1
	PI 538722	47.2	49.0	3.9
	Vernal	53.4	42.5	4.1
Emmer	Lucille	53.5	42.6	3.9
	ND common	51.4	44.7	3.9
	Yaroslav	53.7	42.7	3.6
	CDC Zorba	51.5	44.5	4.0
Spelt	94-288	52.3	43.6	4.1
	SK3P	52.4	43.7	3.9
	Sy Ingmar	63.3	31.8	4.9
HRS	Barlow	58.5	36.5	5.0
	Elgin-ND	63.0	31.9	5.2
	Linkert	63.5	32.1	4.4
	Glenn	61.8	34.1	4.1
	Rollag	68.5	27.3	4.2
	ND Vitpro	62.6	33.5	3.9
	Lang-MN	60.9	35.1	4.0

RDS-Rapidly digestible starch, SDS – Slowly digestible starch RS -Resistant starch

Beside these two starch fractions, RS is the starch fraction that cannot be digested in the small intestine, but it passes to the large intestine and fermented in the colon as dietary fiber, which may prevent disease and lead to better colonic health (Englyst et al., 1992). RS content was not significantly ($p > 0.05$) different among wheat species and ranged from 2.0 – 2.6%.

Hydrolysis Index (HI) and estimated Glycemic Index (eGI) are another two important parameters of Englyst assay. HI index expresses the digestibility of the starch in foods in relation to the digestibility of starch in a reference material, namely white bread. It can be used to indicate the amount of starch that is broken down by enzymes in the gut, while GI to the blood glucose response after consumption of a particular food, where the rate and extent of the digestion of starch is reflected in the magnitude and the duration of the glycemic response (Englyst et al. 1992; Brouns et al., 2013). When *in vitro* assay methods are employed, the term is referred to as estimated GI (eGI) and is measured based on the glucose released from the test food compared to the glucose released by the reference food (Ovando-Martínez et al., 2011).

Significantly higher ($p < 0.05$) HI and eGI was reported for hulled wheats compared to HRS. Both HI and GI followed the same trend: emmer > einkorn > spelt > HRS. Although hulled wheat breads are significantly higher in its SDS content, higher eGI and HI cannot be explained by using only these results. Maybe significantly higher HI and eGI could be due to significantly higher total starch content in these breads (Table 14) and may be different ratios in amylose and amylopectin.

Dietary fiber variation in hulled wheat bread

Dietary fiber content of einkorn, emmer, spelt and HRS bread is shown in Table 17. The IDF and SDF- HM content of einkorn breads were not significantly ($P<0.05$) different among their genotypes, but differences were observed in SDF- LM and TDF. IDF content was

varied from 8.1 – 8.4 % for einkorn bread. SDF- HM and SDF - LM content of einkorn was reported as 1.4 -3.7 and 3.8 – 4.7 % respectively. In addition, TDF content varied from 13.3 – 16.7 % among different genotypes.

Most interestingly, significant differences were observed in SDF- LM and SDF-HM contents of einkorn flour and bread. Increased contents of SDF - HM were found in bread, in contrast SDF-LM content has been reduced in bread. Higher SDF-HM contents could be due to the conversion of IDF to SDF - HM due to heat and lower SDF-LM contents could be due to heat damage of fructans. However, differences were not significant in IDF and TDF content of flour and bread.

IDF content of emmer was not significantly different among genotypes, but genotypic differences were observed in SDF- HM, SDF - LM and TDF contents. IDF, SDF - HM, SDF-LM and TDF content of emmer were 7.2 – 7.3%, 1.5 – 2.5%, 3.1 - 5.8%, 12.1 -15.4% on dry weight basis. Significant differences ($p<0.05$) were found in emmer flour and bread for all the four components of dietary fiber. Significant differences were also observed in IDF, SDF-LM and SDF-HM content of emmer and HRS bread. Emmer bread have been analyzed for TDF content and it was reported as 9.9%, which is lower than the values reported in this study.

IDF, SDF-LM, SDF-HM and TDF contents were not significantly different among genotypes of spelt bread, but significant differences ($p< 0.05$) were observed in IDF and TDF contents among HRS and spelt breads. In addition, IDF, SDF-LM and TDF contents were significantly different ($p <0.05$) in spelt flour vs. bread. Lower IDF contents were observed in spelt bread which could be due to heat damage. Spelt bread had a TDF content ranging from 11.6 -13.8 %. Patijin et al. (2018) has reported a TDF content of 11.3% for spelt bread which is

similar to the value reported in this study. IDF, SDF-HM and SDF-LM contents of spelt bread were 7.6 – 8.1, 0.9 – 1.5 and 3.1 – 3.6 % respectively.

In hulled wheat breads compared to flour a slight increase in soluble dietary fiber was observed in some genotypes. It could be due to the solubilization and breakdown of IDF components due to heat. In some genotypes increase in IDF contents were observed, it could be due to the formation of resistant starch during baking (Johansson et al., 1984). There are studies that confirmed the formation of resistant starch during baking and did not change at drying (preparation of samples for dietary fiber analysis), freezing and storage of the bread. Moreover, the resistant starch is not likely to be due to amylose-lipid complexes since enzymes were used in the dietary fiber analysis, would hydrolyze the complexed starch at the high temperature (Holm et al., 1983).

The nutritional data in food composition databases are used by industry, dietitians, researchers, risk assessors and consumers. Therefore, dietary fiber content in these data bases should be updated. In addition, dietary fiber content is also important in product labelling and to study the relationship between dietary fiber and diseases. As a staple product and one of the main sources of dietary fiber in many diets (Johansson et al, 1984), it is important for bread products to be high quality and nutritious. Although many studies conducted for the flour, it is not edible and moreover, limited studies have been done on processed products. There can be differences in dietary fiber content due to processing. For example, in sourdough bread, IDF can be hydrolyzed to SDF due to the action of microorganisms. Therefore, results of this study would be useful in above mentioned aspects.

Table 18: Dietary fiber variation in whole wheat bread of einkorn, emmer, spelt and bread wheat

Wheat species	Genotype	IDF[a]	SDF-HM	SDF-LM	TDF
Einkorn	TM 23	8.1	1.4	3.8	13.3
	WB Apline	8.3	3.7	4.7	16.7
	PI 538722	8.4	2.1	4.0	14.5
Emmer	Vernal	7.3	1.7	3.1	12.1
	Lucille	7.2	2.5	5.8	15.4
	ND common	7.2	1.3	5.7	14.3
	Yaroslav	7.2	1.5	4.3	13.0
Spelt	CDC Zorba	7.6	0.9	3.1	11.6
	94-288	8.0	1.3	3.5	12.8
	SK3P	8.1	1.5	3.6	13.3
HRS	Sy Ingmar	11.2	2.2	2.1	15.5
	Barlow	10.7	1.5	3.3	15.5
	Elgin-ND	11.1	1.5	5.3	17.9
	Linkert	9.8	2.1	4.9	16.7
	Glenn	9.2	1.7	1.7	12.7
	Rollag	8.4	1.9	3.4	13.6
	ND Vitpro	10.0	1.7	2.5	14.2
	Lang-MN	10.1	1.4	2.5	14.0
LSD (P=0.05)		1.6	1.0	1.1	2.3

Mean values (%) are presented in Table 18

Values are expressed in dry matter basis

a: Number of combined replicates for Hulled wheats = 2

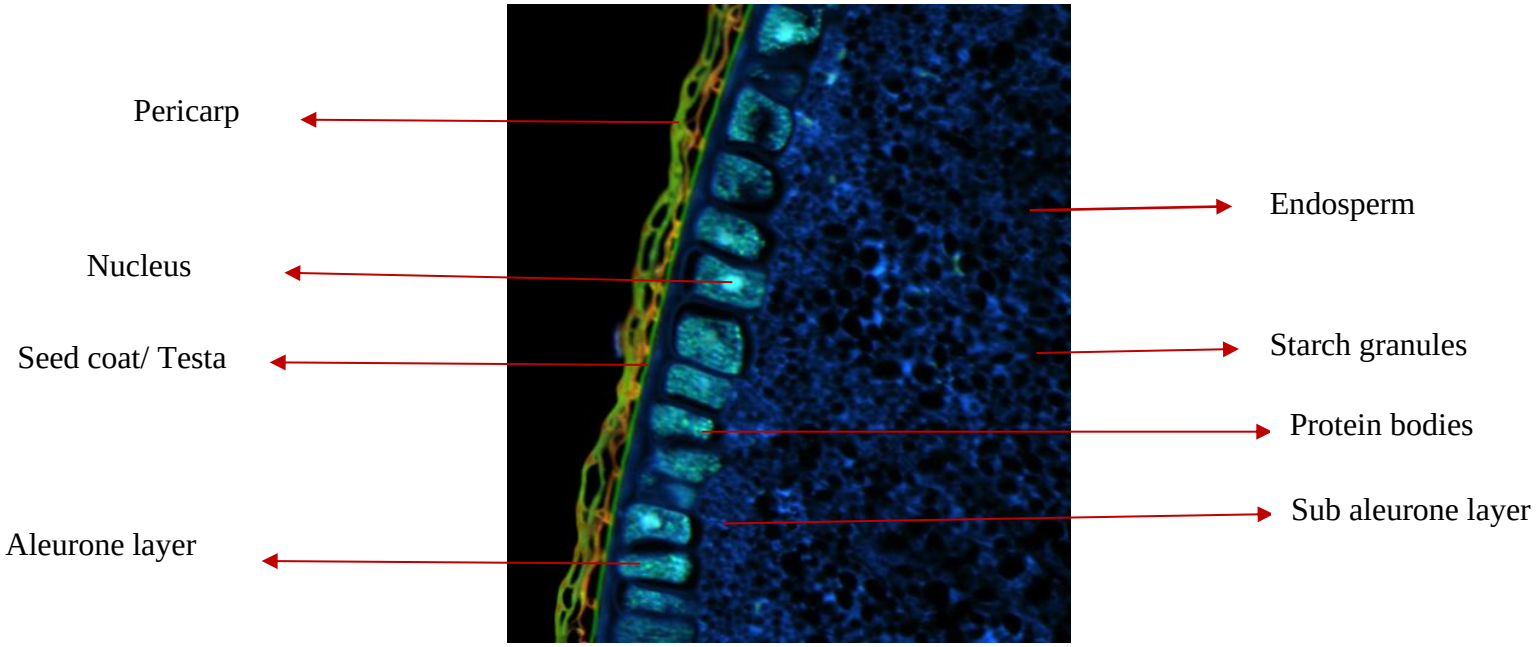
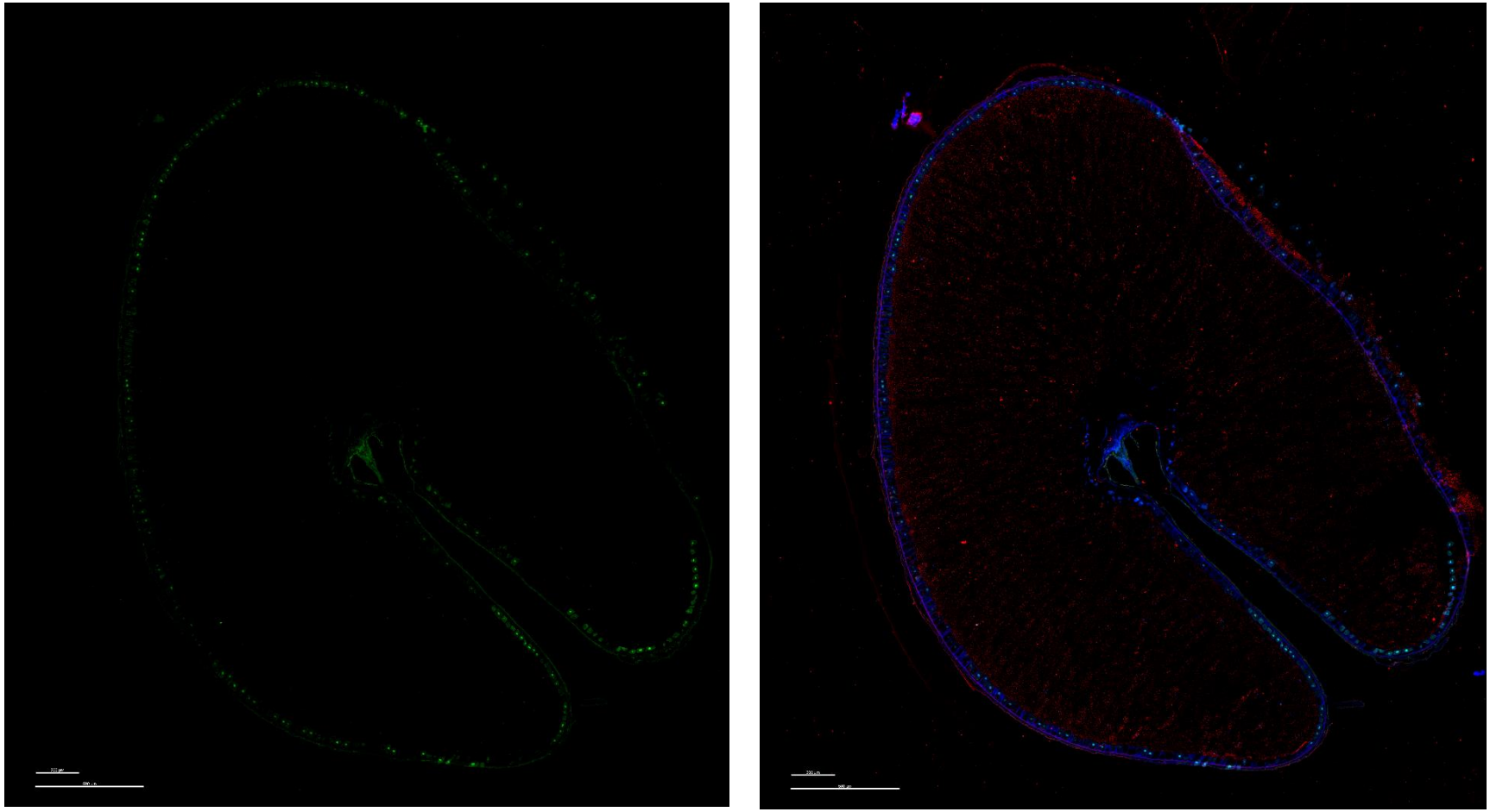
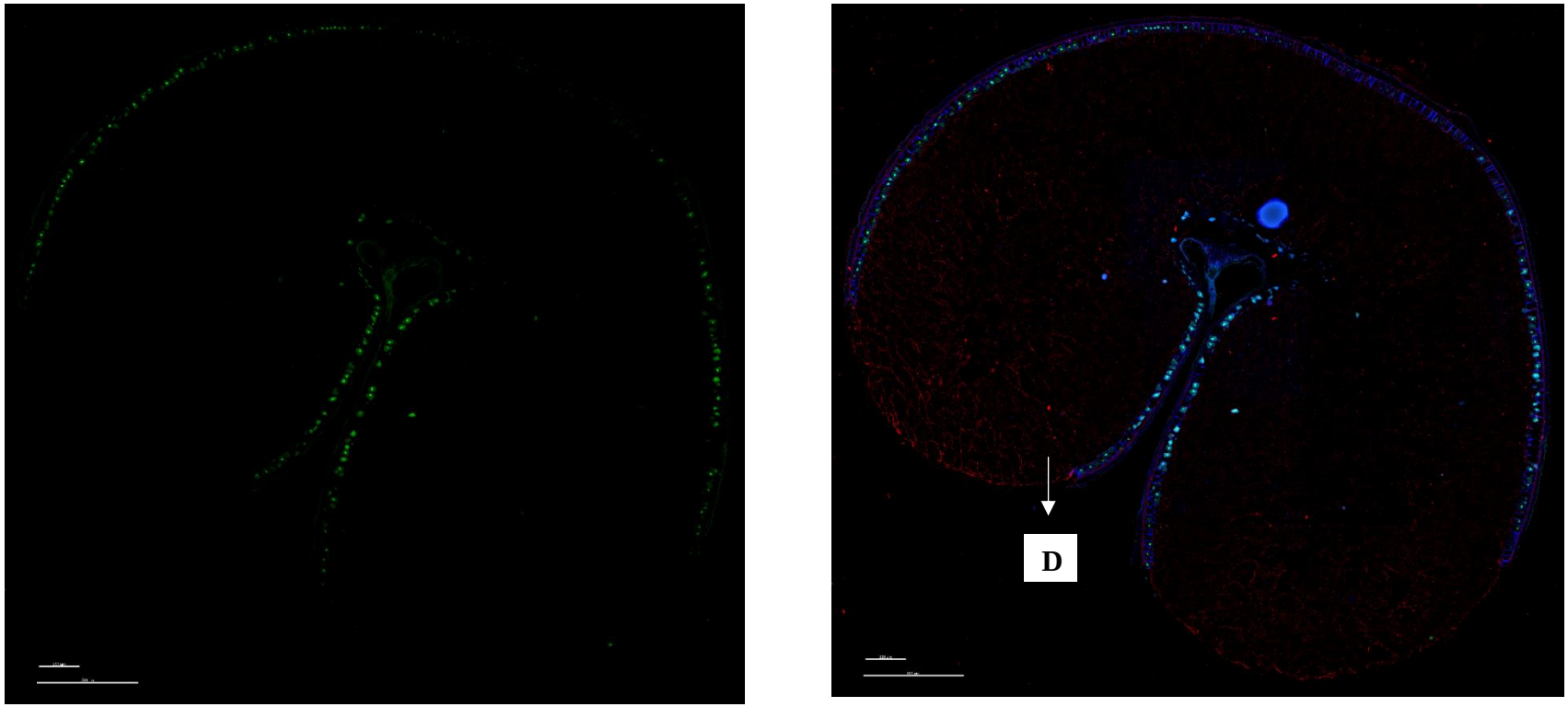


Figure 10: Transverse section of einkorn kernel stained with calcofluor white and acid fuschin



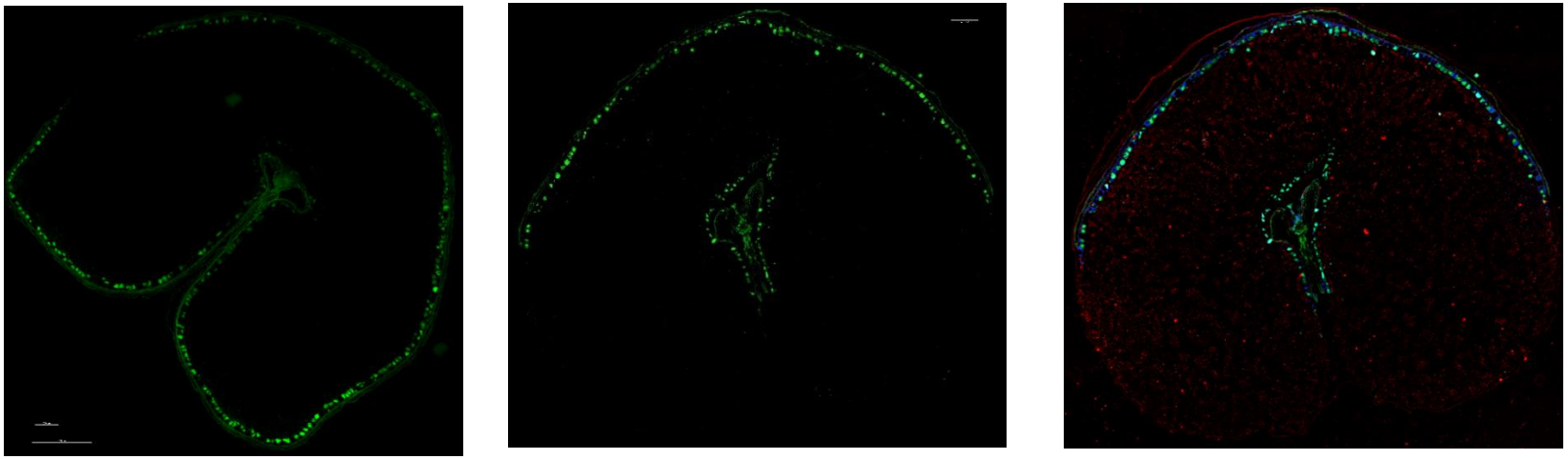
(a) (b)

Figure 11: Cross section of einkorn kernel stained by immunolabelling (a) autofluorescence (b) stained with LM11 monoclonal AX antibody



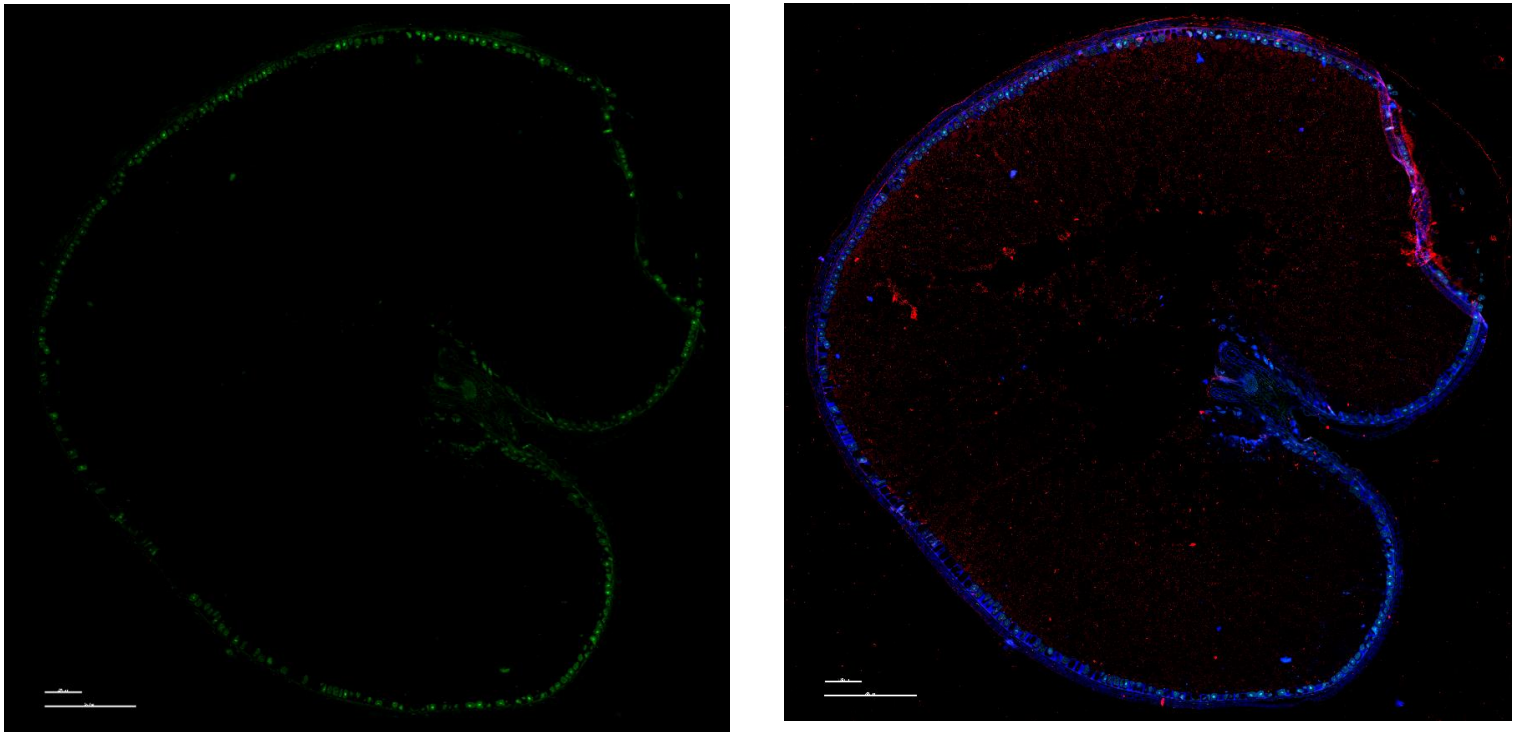
(a) (b)

Figure 12: Cross section of emmer kernel stained by immunolabelling (a) autofluorescence (b) stained with LM11 monoclonal AX antibody



(a) (b) (c)

Figure 13: Cross section of spelt kernel stained by immunolabelling (a) Control (b) autofluorescence (c) stained with LM11 monoclonal AX antibody



(a) (b)

Figure 14: Transverse section of common bread wheat stained by immunolabeling
(a) autofluorescence (b) stained with LM11 monoclonal AX antibody

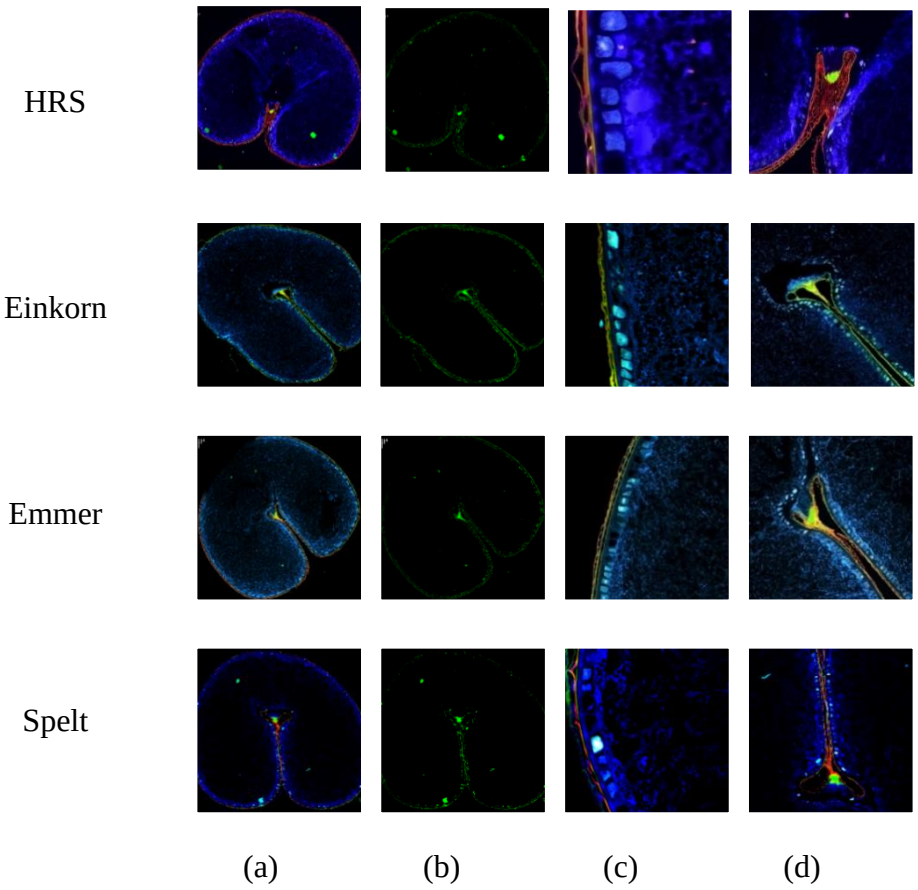


Figure 15: Transverse sections of wheat kernels stained with calcofluor white (a) cross section of the whole kernel (b) autofluorescence (c) Outer kernel layers and central starchy endosperm (d)crease region of kernel cross sections of HRS, einkorn, emmer and spelt stained with Acid Fuchsin - Calcofluor.

Localization of dietary fiber components in hulled wheat kernels

Cell walls are important components of cereal grains and there is a renewed interest in the composition and structure of the cell walls of cereal grains to understand their physiological mechanisms and to use them successfully in various food applications (Dornez et al., 2011).

β-Glucan and arabinoxylans are cereal dietary fibers that are recognized to have positive physiological effects on human health (Broekaert et al., 2011; Gemen et al., 2011; Raninen et al., 2011). The localization of these non-starch cell wall polysaccharides in wheat kernels were examined by microscopic fluorescent analysis of kernel cross-sections. Structural differences in

cell walls of wheat kernel cross-sections were analyzed in outer kernel layers, the central starchy endosperm, and the crease region of the grain kernel. Figure 11 shows a wheat kernel cross-section in which the different layers and zones that were analyzed are indicated. From the exterior to the interior, the outer layers of the grain kernel consist of outer pericarp, inner pericarp (cross and tube cells), seed coat (two cuticle layers and a pigment strand), nucellar epidermis, aleurone layer, and sub-aleurone starchy endosperm.

The pericarp resulted the most variegated structures in terms of thickness among four wheat species (Figure 15 c). HRS wheat showed the thickest pericarp, while thinnest one was observed for einkorn. The thickness of the cell walls can highly affect different operations during processing. For example, einkorn, the integrity of the caryopsis during pearling is not assured by the thinnest structures found in in einkorn wheat, which require careful and mild manipulations to avoid seed breakages. As discussed above einkorn kernels were categorized as extra soft wheat species and thinner pericarp provide a valid reason for the softness of these kernels.

Figure 15 (b) shows the auto-fluorescence of the outer layers of four wheat species, together with the same sections stained with Calcofluor White, which was used to localize the beta-glucan. Auto-fluorescence (Figure 15 (b)) represents the presence of the of aromatic compounds, such as aromatic amino acids, phenolic acids, and lignin. Aleurone cell walls are composed of phenolic acids which are mostly ferulic acid (90%) and coumaric acid (10%) (Antoine et al., 2003). However, auto-fluorescence does not allow the identification of the nature of phenol compounds, that is free or bound (Fulcher, 1982).

The cells of the aleurone (Figure 15 c) appear cube shaped, well separated from each other by cell wall. The cells of the aleurone were organized in one layer for all the wheat species and it contained protein body globoids within its cells. Acid fuchsin was used to stain the

proteins in wheat kernels in red color in order to obtain a better differentiation between cell components. The proteins in aleurone cells were not stained successfully for all the wheat species. All images showed the presence of globular structures in the protein matrix.

Sub aleurone layer can be observed in between aleurone layer and starch endosperm for HRS and spelt, in contrast, clear sub aleurone layer was not observed for einkorn and emmer. The cells of the sub aleurone layer (Figure 15 c) were smaller and more condensed than those detected in the aleurone. They appeared spherical in shape, Moreover, the cells of the sub aleurone layer contained large amounts of small starch granules in black color and they could be developing starchy granules (Figure 15 c - HRS); however, there are clear differences in the size starch granules among different species.

With Calcofluor White, HRS wheat showed the highest intensity of blue, thus confirming the high β-glucan levels found in HRS wheat. Higher intensity was also observed for spelt other than common bread wheat, but β- glucan was localized only in the aleurone and sub aleurone layer, whereas in common bread wheat, it was detected in the endosperm layer, other than aleurone and sub aleurone cell walls. Einkorn and emmer had low intensity of blue; however, β glucan was detected in aleurone, sub aleurone and endosperm cell walls.

Calcofluor White, exhibit selective binding to cell walls and it has been widely used as a fluorescent dye to visualize cell walls in higher plants (Herburger and Holzinger, 2016). However, staining specificity of Calcofluor white has not been resolved (Wysokowskiet al., 2013; Mitra and Loque, 2014). It can bind to the beta linkages in cellulose and other polysaccharides. It indicates the limitations of this method used to visualize β glucan in cereal grain cell walls in this study.

Cell walls in the starchy endosperm and the aleurone layer consist predominantly of arabinoxylans (AX; 70% of wall polysaccharides) and (1/3,1/4)-β-D-glucans (20% of wall polysaccharides) (Antoine et al., 2003; Bacic and Stone, 1981). LM 11 monoclonal primary antibody was used along with a secondary antibody. LM 11 monoclonal primary antibody is a neoglycoprotein (xylopentaose-BSA) which can be used to recognize unsubstituted and relatively low-substituted xylans. Moreover, it can also accommodate more extensive substitution of a xylan backbone and binds strongly to wheat arabinoxylan (McCartney et al., 2005).

Micrographs taken from immunolabelling of arabinoxylans of wheat species are shown in figures 12-15. Autofluorescence of wheat cross sections of einkorn, emmer, spelt and HRS are shown in figure 12 (a), 13 (a), 14 (a) and 15 (a), respectively. The reasons for auto-fluorescence are discussed above in localization of β glucan. Control sample is shown in figure 14 (b), it was used to ensure the specificity of secondary antibody for arabinoxylans. Figure 14 (b) similar to auto-fluorescence images of wheat kernel cross sections, revealed the specificity of secondary antibody.

Arabinoxylans were detected in red color in the micrographs. Most of the areas in pericarp of spelt (figure 14. a) and HRS (figure 15. b) were stained by immunolabelling, in contrast no/low staining was observed in einkorn and emmer. It was also observed that staining of aleurone cell walls are low for einkorn, spelt and HRS, but notable for emmer in some areas of aleurone layer as indicated in Figure 13 (b) letter D, while more distinctive staining pattern was observed for endosperm of einkorn (Figure 12 b). Furthermore, cell walls of endosperm in other wheat species were also stained but not uniform. The difference in staining of arabinoxylans in the different wheat species and tissues can be explained by both the level of arabinoxylans and

their degree of arabinose substitution. Moreover, the LM11 primary antibody requires five consecutive unsubstituted xylose residues. It could be another reason for weaker staining of some wheat tissues by immunolabeling (Barron et al., 2007; Parker et al., 2005; Saulnier et al., 2007). In common wheat, arabinoxylans of the aleurone layer, inner pericarp, testa and nucellar tissue have a low A/X ratio of approximately 0.3–0.4 (Antoine et al., 2003; Bacic and Stone, 1981; Rhodes et al., 2002). Moreover, walls of aleurone cells are heavily esterified (1.8%, w/w) compared to the starchy endosperm (0.04%, w/w) (Bacic and Stone, 1981).

The knowledge on composition of cell walls and their distribution of components such as non-starch polysaccharides have a great impact on milling, baking, brewing, or animal and human nutrition. These results are also useful in developing new cereal lines with contents and compositions. Moreover, manipulating the levels of non-starch polysaccharides in hulled wheat species can be used to improve the dietary fiber contents, thus the nutritional value.

Although cell wall components of mature grains have been largely characterized, the basic understanding of their occurrence and distribution across the endosperm is still lacking. Therefore, findings obtained from localization of non-starch polysaccharides in einkorn, emmer and spelt can be used to help people to understand the importance of wholegrain consumption compared to refined flour since nutritional and functional compounds are also concentrated in the outer layers of the grains. Furthermore, it also helps food technologists to regulate the industrial processes such as milling and pearling in order to save healthy nutrients in the wholegrain flour (Rosa-Sibakovet al., 2015).

SUMMARY AND CONCLUSIONS

This study was conducted to evaluate the kernel, flour dough and baking quality of einkorn, emmer and spelt. In addition, the study evaluated the variation in dietary fiber fractions in hulled wheat flour and bread. Hard red spring wheat genotypes were used to compare the results of hulled wheats.

Significant differences were observed in test weight and grain hardness of hulled wheats which could be explained by the shape of the kernel and microstructure of endosperm. Einkorn was identified as extra soft, in contrast emmer was hard type wheat as common bread wheat. Both medium soft and hard genotypes were observed in spelt.

There were significant differences in chemical composition of hulled wheat compared to bread wheat. Whole wheat flours of einkorn emmer and spelt were characterized by significantly lower protein content and higher fat and starch content (emmer). Furthermore, they had lower content of saturated fatty acids and higher content of mono-unsaturated fatty acids, in contrast, HRS had higher contents of saturated fatty acids and poly unsaturated fatty acids. In addition, hulled wheats reported lower IDF and TDF, but higher fraction of SDF-LM. It implies that hulled wheats would be a potential candidate for producing health beneficial novel food products such as prebiotic enriched foods.

Moreover, one genotype of spelt (94288) was identified with better farinograph dough stability and baking quality which is comparable with common bread wheat. Therefore, it can be a suitable candidate to use in breeding programs to produce hulled wheat cultivars with improved dough and baking quality. Other genotypes of spelt, emmer and einkorn can also be used to produce 100% whole wheat bread with unique flavor and aroma, but with reduced loaf volumes. Hulled wheat breads characterized by significantly higher SDS content, but comparable or higher

eGI values. Imaging of dietary fiber tissues in hulled wheat kernels implied the importance of whole wheat bread/products consumption.

Dietary fiber fractions namely IDF and SDF-HM were significantly different between hulled wheat flour and bread. Hulled wheat breads such as einkorn and spelt were characterized by reduced contents of IDF and elevated levels of SDF-HM.

The results of this study made a detailed comparison between hulled wheats and modern bread wheat grown in North Dakota, which can be used to differentiate einkorn, emmer and spelt from common bread wheat starting from the field to the table.

FUTURE RESEARCH DIRECTIONS

More studies on hulled wheats should be conducted in order to make thorough comparisons between these species and modern wheat. The aspects that need to be addressed in any such comparative study should include genotype and location interactions and other required phenotypic assessments through multiple-year and location trials. In addition, health benefits of hulled wheats should be investigated by using different genotypes of hulled wheats. In this study, *in vitro* starch digestibility of bread was done, but it is better if test can be conducted *in vivo* condition. Moreover, health benefits such as reduced celiac wheat antigenicity of hulled wheat should be investigated. Hulled wheats were characterized by higher soluble fiber content; therefore, it would be useful to conduct a study to evaluate the effect of these fibers in colonic fermentation.

More research work is needed in order to optimize technological processing and formulations of hulled wheat to fit their compositional and morphological characteristics. Thus, hulled wheat might constitute an alternative, which can combine with pseudo-cereals for creating new health-beneficial food products.

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APPENDIX

Table A1: ANOVA table for kernel test weight

Source	DF	Mean Square	F Value	Pr > F
Dependent Variable: KTW KTW				
Variety	17	0.60	1.2	0.0284
REP	3	0.22	0.5	0.708
Error	24	0.48		
Contrast				
Einkorn	2	3.50	7.3	0.0021
Emmer	3	3.94	8.2	0.0004
Spelt	2	11.04	23.0	0.0000
HRS	7	0.63	1.3	0.4412
Einkorn vs Emmer	1	34.24	71.5	0.0000
Einkorn vs Spelt	1	0.30	0.6	0.3641
Einkorn vs HRS	1	144.98	302.6	0.0000
Emmer vs Spelt	1	25.75	53.7	0.0000
Emmer vs HRS	1	284.31	593.4	0.0000
Spelt vs HRS	1	153.31	320.0	0.0000

Table A2: ANOVA table for kernel hardness index

Source	DF	Mean Square	F Value	Pr > F
Dependent Variable: KHI KHI				
Variety	17	1296.60	437.9	0.0000
REP	3	3.10	1.1	0.390
Error	24	2.96		
Contrast				
Einkorn	2	0.61	0.2	0.8009
Emmer	3	2.66	0.9	0.4809
Spelt	2	1627.31	549.6	0.0000
HRS	7	28.48	9.6	0.0000
Einkorn vs Emmer	1	35753.03	12074.6	0.0000
Einkorn vs Spelt	1	5278.79	1782.8	0.0000
Einkorn vs HRS	1	18373.64	6205.2	0.0000
Emmer vs Spelt	1	11108.55	3751.6	0.0000
Emmer vs HRS	1	0.01	0.0	5.6491
Spelt vs HRS	1	6031.80	2037.1	0.0000

Table A3: ANOVA table for kernel moisture content

Source	DF	Mean Square	F Value	Pr > F
Dependent Variable: KMC KMC				
Variety	17	42.17	1346.6	0.0000
REP	3	0.05	1.5	0.233
Error	24	0.03		
Contrast				
Einkorn	2	0.29	9.1	0.0006
Emmer	3	0.02	0.8	0.5415
Spelt	2	0.29	9.4	0.0006
HRS	7	0.14	4.4	0.0039
Einkorn vs Emmer	1	0.33	10.7	0.0012
Einkorn vs Spelt	1	0.02	0.7	0.3180
Einkorn vs HRS	1	6.48	206.8	0.0000
Emmer vs Spelt	1	0.52	16.5	0.0001
Emmer vs HRS	1	4.90	156.4	0.0000
Spelt vs HRS	1	7.00	223.4	0.0000

Table A4: ANOVA table for whole wheat flour protein content

Source	DF	Mean Square	F Value	Pr > F
Dependent Variable: WPC WPC				
Variety	17	2.81	19.1	0.0000
REP	3	0.98	6.6	0.002
Error	24	0.15		
Contrast				
Einkorn	2	2.28	15.5	0.0000
Emmer	3	1.93	13.1	0.0000
Spelt	2	1.55	10.5	0.0003
HRS	7	0.60	4.0	0.0063
Einkorn vs Emmer	1	0.11	0.8	0.3067
Einkorn vs Spelt	1	1.20	8.1	0.0036
Einkorn vs HRS	1	23.04	156.5	0.0000
Emmer vs Spelt	1	2.21	15.0	0.0002
Emmer vs HRS	1	27.41	186.2	0.0000
Spelt vs HRS	1	15.28	103.8	0.0000

Table A5: ANOVA table for Farinograph water absorption of the whole wheat flour

Source	DF	Mean Square	F Value	Pr > F
Dependent Variable: FWA FWA				
Variety	17	64.36	198.8	0.0000
REP	3	0.64	2.0	0.143
Error	24	0.32		
Contrast				
Einkorn	2	2.56	7.9	0.0014
Emmer	3	3.95	12.2	0.0000
Spelt	2	6.73	20.8	0.0000
HRS	7	0.68	2.1	0.1295
Einkorn vs Emmer	1	71.23	220.0	0.0000
Einkorn vs Spelt	1	334.33	1032.5	0.0000
Einkorn vs HRS	1	921.58	2846.2	0.0000
Emmer vs Spelt	1	127.91	395.0	0.0000
Emmer vs HRS	1	637.85	1969.9	0.0000
Spelt vs HRS	1	250.17	772.6	0.0000

Table A6: ANOVA table for Farinograph peak time

Source	DF	Mean Square	F Value	Pr > F
Dependent Variable: FPT FPT				
Variety	17	3.84	66.6	0.0000
REP	3	0.03	0.5	0.673
Error	24	0.06		
Contrast				
Einkorn	2	0.06	1.0	0.3644
Emmer	3	0.18	3.1	0.0463
Spelt	2	6.30	109.3	0.0000
HRS	7	0.52	9.1	0.0000
Einkorn vs Emmer	1	0.00	0.0	2.4491
Einkorn vs Spelt	1	18.64	323.6	0.0000
Einkorn vs HRS	1	26.02	451.6	0.0000
Emmer vs Spelt	1	20.79	360.9	0.0000
Emmer vs HRS	1	27.77	482.1	0.0000
Spelt vs HRS	1	2.83	49.1	0.0000

Table A7: ANOVA table for Farinograph stability

Source	DF	Mean Square	F Value	Pr > F
Dependent Variable: FST FST				
Variety	17	12.70	98.8	0.0000
REP	3	0.14	1.1	0.388
Error	24	0.13		
Contrast				
Einkorn	2	0.03	0.2	0.8058
Emmer	3	0.03	0.2	0.6914
Spelt	2	48.53	377.6	0.0000
HRS	7	2.53	19.7	0.0000
Einkorn vs Emmer	1	0.03	0.2	0.7457
Einkorn vs Spelt	1	49.46	384.8	0.0000
Einkorn vs HRS	1	32.65	254.1	0.0000
Emmer vs Spelt	1	58.53	455.4	0.0000
Emmer vs HRS	1	36.75	285.9	0.0000
Spelt vs HRS	1	0.03	0.2	0.7715

Table A8: ANOVA table for mixing tolerance index

Source	DF	Mean Square	F Value	Pr > F
Dependent Variable: FMT FMT				
Variety	17	4497.30	51.9	0.0000
REP	3	107.48	1.2	0.317
Error	24	86.69		
Contrast				
Einkorn	2	1159.00	13.4	0.0001
Emmer	3	683.73	7.9	0.0006
Spelt	2	537.55	6.2	0.0044
HRS	7	189.50	2.2	0.1143
Einkorn vs Emmer	1	24943.53	287.7	0.0000
Einkorn vs Spelt	1	5612.86	64.7	0.0000
Einkorn vs HRS	1	3552.90	41.0	0.0000
Emmer vs Spelt	1	54011.81	623.0	0.0000
Emmer vs HRS	1	32194.86	371.4	0.0000
Spelt vs HRS	1	0.24	0.0	7.4608

Table A9: ANOVA table for Farinograph Quality Number

Source	DF	Mean Square	F Value	Pr > F
Dependent Variable: FQN FQN				
Variety	17	1311.83	104.3	0.0000
REP	3	6.85	0.5	0.657
Error	24	12.58		
Contrast				
Einkorn	2	11.08	0.9	0.3981
Emmer	3	21.75	1.7	0.2003
Spelt	2	2523.07	200.5	0.0000
HRS	7	225.03	17.9	0.0000
Einkorn vs Emmer	1	44.30	3.5	0.0380
Einkorn vs Spelt	1	6179.47	491.2	0.0000
Einkorn vs HRS	1	6947.96	552.3	0.0000
Emmer vs Spelt	1	8122.20	645.6	0.0000
Emmer vs HRS	1	8379.55	666.0	0.0000
Spelt vs HRS	1	448.56	35.7	0.0000

Table A10: ANOVA table for Arabinoxylan content

Source	DF	Mean Square	F Value	Pr > F
Dependent Variable: AXP AXP				
Variety	17	1.33	6.5	0.0000
REP	3	0.15	0.7	0.555
Error	24	0.20		
Contrast				
Einkorn	2	1.52	7.4	0.0019
Emmer	3	0.19	0.9	0.4732
Spelt	2	0.13	0.6	0.5066
HRS	7	0.45	2.2	0.1086
Einkorn vs Emmer	1	7.50	36.6	0.0000
Einkorn vs Spelt	1	11.86	57.9	0.0000
Einkorn vs HRS	1	0.02	0.1	1.3047
Emmer vs Spelt	1	1.04	5.1	0.0159
Emmer vs HRS	1	3.62	17.7	0.0001
Spelt vs HRS	1	6.59	32.2	0.0000

Table A11: ANOVA table for Arabinoxylan ratio

Source	DF	Mean Square	F Value	Pr > F
Dependent Variable: AXR AXR				
Variety	17	0.0051	1.4	0.4428
REP	3	0.0034	1.0	0.427
Error	24	0.0035		
Contrast				
Einkorn	2	0.0009	0.3	0.7508
Emmer	3	0.0009	0.3	0.7060
Spelt	2	0.0137	3.9	0.0268
HRS	7	0.0023	0.6	0.7810
Einkorn vs Emmer	1	0.0021	0.6	0.3843
Einkorn vs Spelt	1	0.0084	2.4	0.0795
Einkorn vs HRS	1	0.0120	3.4	0.0414
Emmer vs Spelt	1	0.0029	0.8	0.2899
Emmer vs HRS	1	0.0217	6.1	0.0093
Spelt vs HRS	1	0.0325	9.2	0.0023

Table A12: ANOVA table for baking water absorption

Source	DF	Mean Square	F Value	Pr > F
Dependent Variable: BSV BSV				
Variety	17	2.08	77.6	0.0000
REP	3	0.00	0.0	0.991
Error	24	0.03		
Contrast				
Einkorn	2	0.01	0.5	0.6108
Emmer	3	0.02	0.8	0.5378
Spelt	2	4.67	174.1	0.0000
HRS	7	0.26	9.8	0.0000
Einkorn vs Emmer	1	0.37	13.6	0.0004
Einkorn vs Spelt	1	7.58	282.4	0.0000
Einkorn vs HRS	1	18.30	681.5	0.0000
Emmer vs Spelt	1	5.51	205.2	0.0000
Emmer vs HRS	1	15.95	594.0	0.0000
Spelt vs HRS	1	4.37	162.6	0.0000

Table A13: ANOVA table for bread volume

Source	DF	Mean Square	F Value	Pr > F
Dependent Variable: BWA BWA				
Variety	17	105.60	322.4	0.0000
REP	3	0.80	2.4	0.089
Error	24	0.33		
Contrast				
Einkorn	2	0.90	2.8	0.0683
Emmer	3	5.23	16.0	0.0000
Spelt	2	1.28	3.9	0.0256
HRS	7	0.30	0.9	0.6870
Einkorn vs Emmer	1	286.26	873.9	0.0000
Einkorn vs Spelt	1	879.31	2684.6	0.0000
Einkorn vs HRS	1	1455.98	4445.1	0.0000
Emmer vs Spelt	1	231.58	707.0	0.0000
Emmer vs HRS	1	731.64	2233.7	0.0000
Spelt vs HRS	1	214.78	655.7	0.0000