Dietary fiber content and composition in heritage wheat

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Kostfiberhalt och dess sammansättning i vete som kulturspannmål

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Abstract

Wheat is the main food source in the world. A secured production has however been threatened by environmental changes in combination with the need of producing more food for a growing population. As a result, there has been an increasing interest in finding new crop varieties susceptible to abiotic stress. A growing concern has also been seen among consumers regarding sustainability, local markets and health which has led to an increased interest in heritage cereals due to their claimed health benefits, nutritiousness and better resistance against abiotic stress. Though, these claims lack a proper foundation and more research is needed before any statements can be claimed. The present study aimed to analyze the dietary fiber content and composition of heritage wheat and investigate if potentially differences may be a result of wheat population (genetic background), cultivation location or treatment. Three heritage wheat populations (Dala Lantvete, Ölandsvete and Källunda) and one modern variety (Dacke) were cultivated at two locations in Sweden, within two blocks and with two treatments (with or without fertilizer) in each block. The samples were evaluated regarding dietary fiber content and composition, including arabinoxylan and fructan, which are the two most abundant dietary fibers in wheat. Principal component analysis (PCA) was used for multivariate analysis of the data set, followed with two-way analysis of variance (ANOVA) to find possible differences between samples.

In the PCA the wheat populations were separated in different clusters along PC 2, and fructan was the variable that contributed mostly to this separation. The ANOVA showed that the total dietary content, as well as arabinoxylan content, was mostly influenced by wheat population. Slightly lower contents were found in the heritage wheat populations than in the modern Dacke, with the lowest content in Ölandsvete. Fructan content can both be influenced by genetic background, and be an indication of a stressed plant and was found in the highest content in the modern variety Dacke. However, the differences between the samples were rather small and more research is needed before any general conclusion can be drawn.

Keywords: Heritage cereals, wheat, dietary fiber, fructan, Dacke, Dala Lantvete, Källunda, Ölandsvete

Sammanfattning

Vete anses globalt vara den viktigaste energikällan inom konsumtion. En ökande befolkning i kombination med klimatförändringar hotar dock en säker livsmedelsförsörjning. Detta har tillsammans med ett ökat intresse från befolkningen resulterat i ett ökat engagemang för att hitta nya mer tåliga spannmålssorter. Som följd har ett ökat intresse för äldre odlade kulturspannmålen ökat eftersom de påstås ge mer välsmakande produkter, ha högre nutritionellt värde och ha en bättre motståndskraft mot extremväder Det krävs dock mer forskning inom ämnet eftersom det finns få studier som styrker dessa påståenden. Syftet med denna studie var att undersöka kostfiberhalten och dess sammansättning i olika kulturspannmål av vete för att sedan ta reda på om det finns några skillnader mellan sorter, odlingsplats och behandling (med eller utan kvävegödsling). Tre olika populationer av kulturvetespannmål (Dala Lantvete, Ölandsvete and Källunda) och en modern vetesort (Dacke) odlades på två olika platser i Sverige, uppdelat i två olika block med två olika behandlingar (med eller utan tillsatt kvävegödsling) inom vartdera block. Vete populationerna analyserades på kostfiberhalt och sammansättning, inklusive arabinoxylan samt fruktaner, vilka är de vanligaste förekommande kostfibrerna i vete. Proverna utvärderades med multivariat principalkomponentanalys (PCA) följt av tvåvägs variansanalys (ANOVA).
I PCAn delade de olika vetepopulationerna upp sig i olika grupper, och det var fruktanhalten som bidrog mest till denna uppdelning. ANOVAn påvisade att den totala kostfiberhalten, och även arabinoxylanhalten, påverkades mest av sort med lite lägre halter i kulturpopulationerna än i den moderna sorten Dacke, med allra lägst halt i Ölandsvete. Fruktanhalten påverkades både genetiskt och av yttre stress och återfanns i högst halter i den moderna sorten Dacke. Dock, bör det tilläggas att det endast var små skillnader mellan de olika veteproverna i denna studie. Därmed behövs mer forskning för att kunna bekräfta observerade resultat och dra några generella slutsatser.

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Abbreviations

SLU	Swedish University of Agricultural Sciences
DF	Dietary fiber
SDF	Soluble dietary fiber
IDF	Insoluble dietary fiber
DFtot	Total dietary fiber
Ara	Arabinose
Xyl	Xylose
Man	Mannose
Gal	Galactose
Glc	Glucose
AX	arabinoxylan
N	Fertilizing nitrogen

1. Introduction

An increased interest has been seen among consumers in both sustainability and health-related aspects regarding food production and consumption (Johansson et al. 2021). As the world's population continues to grow along with tangible climate changes there has been an increased demand for finding new sustainable crop varieties to secure future food production. Increased need and interest among consumers, bakeries and farmers have developed into the trend of sustainable and locally produced crops with an increased interest in heritage cereals (Shewry 2018; Wendin et al. 2020). Nevertheless, research in this specific area is required to fully understand and know the potential benefits of cultivating these heritage cereals despite being low-yielding compared to modern varieties (Shewry 2018).

In the Western world countries, cereals are today, a major source of dietary fibers by contributing to about 50% of the total dietary fiber intake, and it is also a staple food in major parts of the world (Escarnot et al. 2010; Verspreet et al. 2015; Prasadi & Joye 2020). The most important crop worldwide is common wheat, *Triticum aestivum L.*, together with rice and maize (Gebruers et al. 2008). Dietary fibers are functionally unavailable carbohydrates, found in cereals, and resistant to human alimentary enzyme activity. They are believed to have part in the beneficial effects of whole grain products on human health (Dhingra et al. 2012; Chibbar et al. 2016; Ciudad-Mulero et al. 2020). However, the whole grain consumption of the society in the Western part of the world is substantially lower than recommended levels, even though the knowledge of health benefits is well known among consumers, scientists and producers (Gouseti et al. 2019; Prasadi & Joye 2020). Even though a substantial decrease in the consumption of bread has been seen, it is still an important food source for most people. A reduced intake of wheat based products such as bread, may pose some risks due to the reduced intake of dietary fibers used as substrate for enteric bacteria which ensure a good health of its host both through the production of health beneficial substances and through promoting the growth of beneficial bacteria preventing harmful bacterial (Yao et al. 2022). Research has shown evidence of dietary fibers helping preventing constipation, reduce obesity, lower the risk of colon cancer, reduce type II diabetes risks, reducing acid refluxes and regulating cholesterol levels by decreasing re-adsorption of bile salt (Morozov et al. 2018; Gouseti et al. 2019; Prasadi & Joye 2020). The soluble dietary fibers (SDF) can be used as energy source for gut bacteria and has also been associated with improved blood glucose levels, prolonged gastric emptying and, reduced glucose absorption/starch degradation in the small intestine and decreased postprandial blood insulin levels (Huffman 2003; Gebruers et al. 2008; Yao et al. 2022). The main function of insoluble dietary fiber (IDF) is to serve as bulking agent due its ability to absorb water, hence resulting in increased fecal mass and decreased intestinal transit time which reduces obesity (Prasadi & Joye 2020). However, the consumer often prefers white bread, typically low in dietary fibers, which result in a low intake. Nevertheless, research have demonstrated potential in increased content of dietary fiber content, without changing quality parameters, with the help of genetic variation in different wheat cultivars. Hence, this could potentially result in a production of healthier bread production using other wheat cultivars (Gouseti et al. 2019). A potentially source of this genetic diversity improving health related traits may be found in different heritage cereals and landraces (Arzani & Ashraf 2017). However, only small differences have been found in research comparing heritage wheats and modern bread wheat regarding the bioactive components. Regarding, dietary fibers, lower values have been found in heritage wheats compared to modern varieties. Though, the differences has remained small (Shewry 2018).

Researchers believe that the first domestication of crop species started 10,000 years ago by humans. Later the wheat domestication eventually resulted in landrace cultivars as the early farmers grew wheat which adapted to the specific conditions and habitats of that farm (Rahman et al. 2020). Today, wheat (*Triticum aestivum*) are considered the most widely grown crop (Tshikunde et al. 2019). Yet, climate changes are affecting wheat yields and are likely to decrease due to an increase in extreme events and warmer climate (Slafer et al. 2021). Hence, yield has been, and are, considered to be the most important trait in designing breeding strategies. Still, this may also unwittingly result in the loss of other shortfalls in composition and less nutritious kernels as these properties has been lost in order to get a higher yield and better pest resistance (Morris & Sands 2006). However, yield outcome is complex to predict as it is a result of several parameters and interaction in different development and growth processes (Shewry 2018; Slafer et al. 2021).

Wheat has thoroughly been analyzed through several studies and different components, such as e.g. starch and dietary fiber (Huang et al. 2022). Regarding dietary fiber content, the main components are arabinoxylans, cellulose, β -glucan, resistant oligosaccharides, arabinogalactan peptides and lignin together with other associated plant substances. (Gebruers et al. 2008). Though, there is a limitation in data available about heritage wheat on dietary fiber content and composition and how it may be affected by environmental conditions (Shewry 2018).

1.1 Aim of the study

The main aim of this study was to investigate how dietary fiber was affected by genetic background and environment in heritage wheat. This was done by analyzing dietary fiber content and composition in three heritage wheat populations, and one modern variety for comparison. All wheats were organically cultivated at two different locations with two different treatments, with or without additional fertilizers.

2. Background

Farmers cultivating heritage cereals often claims that products using heritage cereals are more nutritional and a that they have health benefits (Shewry 2018). However, very little evidence validates this statement but it is logical to expect that genetic differences within different varieties may exist and exhibit differences in composition. It is also known that the composition of the grain is affected by its surroundings and environmental factors as well as the interactions between environmental factors and genetic differences (Shewry 2018). Nevertheless, heritage cereals have the ability to grow in poor soils with less inputs than modern varieties, together with the abilities of having a higher resistance to abiotic and biotic stresses as insects, diseases, drought and extreme temperatures (Arzani & Ashraf 2017). These are crucial trait as it is important to match crop and environment to ensure harvest by resistance during critical periods of the life of the plant. Simulations have showed a loss in grain yield of 5% $°C^{-1}$ at temperature increase across different wheat cropping areas (Slafer et al. 2021).

2.1 Heritage cereals

Heritage cereals, sometimes also called landrace, is a heterogenous crop collection that has locally developed over time by both an evolutionary process and by the farmers selection (Murphy et al. 2005). These varieties differ from the modern varieties as they are not completely homogenous and uniform, instead there can be a mix of different types (Olsson 1997). However, it is unknown how many of these evolutionary mixes that has been existing as very little information can be found. Probably this is a result of several years of reduced interest in combination with years of breeding focusing on improvements only using few chosen varieties (Wiking Leino 2017). The higher yield and easier harvest of modern wheat varieties resulted in a decrease of cultivation of ancient wheat (Kaluthunga & Simsek 2022). Nevertheless the interest in heritage cereals has lately changed and started to increase (Arzani & Ashraf 2017; Johansson et al. 2021).

2.2 Plant breeding and cultivation

Selection of kernels occurred already in the beginning of the domestication process regarding seed dispersal mode, plant architecture, ear and kernel size, seed dormancy, glume reduction and, probably the most important key feature of preventing yield loss, reduced spikelet shattering at maturity. Further on in the history more advanced technology resulted in additional selection attributed to grain carbohydrate content specifically amylopectin, gluten and protein content and mineral content (Morris & Sands 2006; Rahman et al. 2020). The attributes of gluten and amylopectin are especially important as they ensure baking and processing qualities (Morris & Sands 2006). During the late nineteenth hundred the interest of correlations between yield, grain size and germination increased. This resulted in the entrance of new foreign varieties into the Swedish market (Olsson 1997). However, even though those varieties had a higher yield and a stronger straw strength, those varieties lacked the needed winter hardiness together with an inferior baking quality. This problem was partly solved through both using the old heritage cereals collected from various locations, and the new foreign varieties with the aim of creating more uniform and high yielding varieties (Olsson 1997). At the time of post-war in 1950th the cereal yield of the Swedish farmers was characterized with stagnation in contrary to other countries in Europe. Nevertheless, this was a desired result from the Swedish government as there was no ambition of any increase in yield due to sufficient production. Nevertheless, ambitions of increasing farm size, improved efficiency and modernization of the production, using products as fertilizers, existed (Martiin 2015). Though, as a result of reduction in land cultivated, limitation of total production occurred. In combination with the allowance of yield-increasing inputs and the governments interest in reducing the labor force in farming, new improved seeds were used. With greater use of artificial fertilizers the total yield could therefor remain on the same level despite reduced land of cultivation. Wheat was easier than rye to breed and this was probably the reason why wheat had a steeper increase in yield when looking at the bread grains at the time (Martiin 2015). However, a negative correlation between yield and protein content has been shown in most of the new varieties produced by breeding, also known as the "yield dilution phenomenon", despite the breeding focusing on increasing the protein content. This is also believed to be the reason behind the lower contents of minerals in modern varieties (Arzani & Ashraf 2017). As the modern wheat varieties were bred only using a few varieties of the landrace cultivars, the genetic diversity was decreased which accelerated the risks of genetic vulnerability to the adverse conditions of its surroundings. In combinations with rapid changes of environmental conditions and, spontaneous mutations of pathogens and insects, this narrow genetic diversity have caused a severe vulnerability which can and have caused sever crop losses. From 1990 an increase in genetic diversity was nevertheless seen again by introgression of novel materials within the breeding process. This is believed to be the result of breeders experiencing the consequence of narrowing down the genetic diversity of modern breeding. However, the vulnerability still remains as very few varieties are used for the major production (Rahman et al. 2020). Partly, as a result of this in combination with regulations, organically cultivating farmers mostly have to rely on conventionally bred, selected and grown seeds which often include the use of synthetic fertilizer and pesticides which is not allowed in organic cultivation (Murphy et al. 2005).

The global yield of wheat needs to increase from around 3 to 5 tons/ha by 2050 to meet the future demands. Today, the world average wheat yield is 2.9 tons/ha. The differences in yield levels between countries is attributed to various genetic potential of cultivars, agronomic practices and climatic conditions (Tshikunde et al. 2019). However, depending on climate, year, cultivation location and genotype of the grains the results can vary both in yield, nutritional yield, nutrient density and mineral content (Johansson et al. 2021). Modern plant breeding has historically been oriented towards high agronomic yield, disease and pest resistant and easy and consistent to process. Though, this also may have resulted in the proliferation of foods responsible for some moderns dietary problems (Morris & Sands 2006). Due to the diversity of the wheat market and wheats potential uses, breeders needs to have a similarly wide variety in their breeding process (Slafer et al. 2021). However, the wheat grain yield is influenced by several physiological and agronomic traits. Examples of physiological traits are photosynthetic rate, canopy temperature, chlorophyll content and water-soluble carbohydrates; and agronomic traits are total biomass, harvest index, plant height, number of productive tillers, spike length, grain kernels per spike, grain weight and thousand seed weight (Tshikunde et al. 2019; Slafer et al. 2021).

2.3 Wheat

Wheat is one of the most common cereals cultivated and is together with barley, oats and rye the dominating cereal in the Nordic hemisphere (Johansson et al. 2021). These four are all defined as true cereals and belong to the grass family of *Poaceae* (Prasadi & Joye 2020). Nevertheless, wheat grains have been cultivated since around 9000 B.C. and used as food since around 6700 B.C., also known as late Stone Age. Wheat is today, together with rice and maize feeding a large proportion of the world's population (Rahman et al. 2020). Wheat is a collective name of several varieties in the family of *Triticum*, with the variety of Eincorn wheat, *Triticum monococcum* subsp. *Monococcum*, being one of the first found to be used in Sweden more than 5000 years ago (Wiking Leino 2017). However, this variety is far from the most commonly cultivated variety today. The most commonly grown variety today is *Triticum aestivum* which is a result of a crossing between a variety of a emmer wheat, *Triticum turgidum,* and a wild variety of the wild diploid goatgrass, *Aegilops tauschii* (Rahman et al. 2020). As emmer wheat being a allotetraploid specie, hybridized from the diploid *Triticum urartu* and presumably *Aegilop speltoides*, this crossing resulted in a hexaploidy species with genomes from three species (Slafer et al. 2021). The mostly common wheat, or bread wheat, is *Triticum aestivum* subsp. *Aestivum* which is a naked variety where the hulls fall off during threshing. The hulled wheat variety, *Triticum aestivum* subsp. *spelta* is often incorrectly referred of being an old ancient wheat variety where research has shown earlier evidence of the use of bread wheat (Wiking Leino 2017). Nevertheless, wheat can have very different exterior. Hence, wheat can have a huge variety in appearance from being hulled or not, to having white spikes that can be mildly bristled or sometimes even brown colored (Olsson 1997; Bechtel et al. 2009).

There are two different types of wheat, winter and spring type, where the winter types are sown in the autumn, setting flowers the following year. This means that the winter type needs to survive a winter to set flowers. The spring types are sown in the spring, and harvested in the autumn the same year. The spring types are known to hold specific quality attributes as superior baking quality and gluten strength when compared with the winter types (Johansson et al. 2021). Though, winter type wheat generally is assumed to result in a higher yield even though spring wheat cultivars in high yielded environment have similar yields of 12-16 tons/ha. Due to different environment and climate of the world, this does though not mean that these yields are possible all over the world, as for example in the northern hemisphere due to its hard cold winters (Slafer et al. 2021). Heritage wheat varieties have had the historically advantages of having a better winter hardiness, a similar or higher gluten content and a better baking quality (Olsson 1997). Regarding gluten, composed of two proteins of gliadin and glutenin, the overall gluten content is either similar or higher in ancient varieties of wheat. The ratio between gliadin and glutenin does however differ between some ancient and modern varieties as common wheat has a higher glutenin content, hence the ratio being higher in ancient wheat (Zamaratskaia et al. 2021).

2.3.1 Heritage spring wheat

Spring wheat was historically mostly cultivated in areas, such as Bohuslän or in the woods of Småland, where the winter wheat variety struggled to survive the winter. Even though spring wheat characteristics are a long development time and the need of a rich soil containing some lime, it is often found to be cultivated in less rich soils (Wiking Leino 2017). Its tillering ability is much lower than for other bread cereals and hence, demands a greater use of seeds when cultivated. Due to the low ability of tillering it also suffered from a higher stress from weed. Reports from plant breeders in the middle of 20th century describe Swedish heritage spring wheat as having small spikes, long and weak straw and early ripening. The long weak straw of the heritage varieties was one of the reasons to why breeders worked away from these varieties in the search of good quality and high yielding varieties (Olsson 1997). The exterior of the spring wheat had a large variation in appearance, with or without hull and with or without spikelets. According to the plant breeder Åke Åkerman (Wiking Leino 2017), whom studied heritage cereals, the most common varieties were *Dala vårvete* and *Hallands vårvete*. Though, very little about the different varieties is known which also may have resulted in the lack of knowledge about other possible spring wheat varieties that may have existed. However, some varieties as the one from Öland, *Ölandsvete*, was later found along with *Bohuslänskt vårvete* and *Skånskt vårvete* (Wiking Leino 2017). The breeding of spring wheat started for real in the early 2000century (1915) and the result of mixing these spring wheat with winter type wheat, the modern spring wheat varieties where born (Olsson 1997).

2.3.2 Quality aspect of heritage spring wheat

Wheat has a broad spectrum of uses and can be used for bread, pasta, cake, biscuits and noodles or be refined to produce starch, dry gluten or nonfood applications such as ethanol, biodegradable plastics and much more. Hence, the quality of wheat depends on its final use (Slafer et al. 2021). The decisive quality aspect regarding wheat in the baking industry is protein content, specifically high levels of gluten proteins (Wiking Leino 2017). However, there are other basic quality criteria of wheat for food application such as starch content, falling number, minerals, sedimentation values and grain hardness (Muqaddasi et al. 2020). Other grain quality traits are test weight, moisture content, mycotoxins, dough machinability, appearance, germination, particular grain defect limits (fungal, insects damage or sprouted), weed seed limits and other contaminants limits (Finch et al. 2002; Slafer et al. 2021). These latter traits are nevertheless, mainly to ensure a separation between wheat suitable for consumption and those that are not (Slafer et al. 2021). Regarding consumption, consumers prefer the texture and taste of a bread made from white flour, which result in a loss of much of the existing dietary fiber (DF), vitamins and minerals of the grains in the finished product. This since, during the milling process of the grains, the outer parts are removed, where most of those components are located. However, there are differences between wheat varieties in where nutritious components are located. Hence, there is a possibility to increase the nutritious values of a white flour despite the removal of the outer more nutritious part of the kernel without changing the quality parameters preferred by the consumer. One example of this is the possibility of increasing the DF content in a white flour by choosing a variety with more arabinoxylan within the endosperm (Gouseti et al. 2019).

Both heritage varieties and modern varieties of cereals have gone through an evolutionary process of sporadic changes of the genome, rapid alterations due to mutations, hybridization, polyploidization and domestication, which has resulted in profound loss of genes of their progenitors. However, heritage wheat has not undergone breeding to the same extent as modern varieties. Hence, lost genes that may have importance in future wheat improvement, may still exist in the heritage varieties (Rahman et al. 2020). One example of this is a gene thought to have a connection to protein content, NAM-B1, which do not exist in modern wheat varieties. This gene can though be found in more or less all the Nordic bread wheat heritage varieties (Wiking Leino 2017). Heritage cereals has been suggested to be a possible component in the diet of non-celiac wheat sensitive people due to lower immunogenic properties (Carnevali et al. 2014). Triggering factors of wheat allergy or sensitivity can be various wheat proteins, including gluten and non-gluten proteins (Scherf 2019). Still, heritage wheat is found to have a similar or higher gluten content than modern wheat, which is a higher ratio between gliadin and glutenin in ancient wheat compared to modern varieties. Coeliac disease is an autoimmune condition where an immune response appear due to nondigested peptides high in proline, T-cell epitopes, accumulate in the small intestine. The major contributor to this response is mainly the gliadin fractions of gluten. Few studies have fully investigated this, however t-cell stimulatory capacity has been found in some ancient wheats (Zamaratskaia et al. 2021). There is also differences between varieties through different genetic expressions, with the presence of other peptides like the immunoreactive component as the immunodominant 33-mer peptide that only has been found in hexaploidy common wheat and spelt, while not detected in emmer or durum wheat (Scherf 2019).

2.3.3 The wheat kernel

The wheat kernel, together with other cereal grains have a complex structure characterized by different cell layers. However, even though the general structure of the cereals remains largely similar, individual kernels can be significantly diverse in size and composition (Prasadi & Joye 2020). When it comes to chemical composition of common wheat (*Triticum aestivum*) it can also vary a lot. Usually it consists of around 85% carbohydrates, 10-15% protein and 1.2 – 3.9% lipids (Stone & Morell 2009). Within carbohydrates, 63-80% are considered to be starch and 10 - 15.5% are considered DF (Stone & Morell 2009; Shewry & Hey 2015; Chibbar et al. 2016; Wieser et al. 2020). The kernel can, nevertheless, be divided into three major parts, the endosperm which represent 80-85% dry weight (dw) of the kernel, the germ 2-3% and the bran 12-18%. The bran is the other layer that covers both the germ and endosperm (Prasadi & Joye 2020). The germ, also known as embryo, is vital for germination to occur, hence consisting of the highest content of lipids and lipid-soluble vitamins of the kernel for energy and protection of the embryonic axis and scutellum. The energy stored as insoluble nutrients, mainly starch and proteins in the endosperm, will later be used by the developing plant upon germination. The storage component starch, found in the endosperm is composed of two polymers, amylose and amylopectin with a normal ratio of 1:3. As the starch content of the kernel will affect bread quality, such as bread staling, crumb structure and dough rheology the composition will, together with the quality of starch granules, affect the end use of wheat flour (Muqaddasi et al. 2020). The outermost layer of the endosperm, called aleurone layer, is responsible for pigmentation of the kernels and consist of 1 layer in wheat, but can be up to 3 layers of cells, depending on the type of cereal. However, a major part of the aleurone layer is removed during the milling process. It is botanically considered to be part of the endosperm, but it is not included in the refined white flour. The most outer part, the bran, can be divided into several layers, outer pericarp, inner pericarp, testa, and hyaline layer. The outer and inner pericarp are rich in crosslinked polysaccharides such as cellulose, heteroxylans and lignin. Depending on cereal, the testa can be one distinctive or, as for wheat and rye, divided into two layers. The hyaline layer are not evidently visible, and sometimes non-existing, in all cereals but is the maternal tissue that covers the endosperm (Prasadi & Joye 2020).

2.4 Dietary fibres

DF is a component present in different plant material, such as cereal grains, and they are different and distinctive for different grains (Prasadi & Joye 2020). DF are defined by the European union as “carbohydrate polymers with three or more monomeric units (to exclude mono- and disaccharides) which are neither digested nor absorbed in the small intestine” (commission Directive 2008/100/EC, 28 October 2018). This includes cellulose, non-cellulosic polysaccharides such as hemicellulose, gums, pectin substances and mucilage, and the non-carbohydrate component lignin. They are all resistant to degradation by the human alimentary enzymes (Dhingra et al. 2012; Verspreet et al. 2015). As the DF will survive the passage through the stomach and the small intestine, they are considered prebiotics and will selectively be fermented by the colon microbiota beneficial for the host health. This has generally been shown through extensive research regarding the inulin-type fructan (Verspreet et al. 2015). A recommended consumption of DF of at least 25 -35g/day/person has shown strong evidence of being a sufficient intake for protection against the development of dietary related disorders such as cardiovascular diseases and type 2 diabetes (Escarnot et al. 2010; Nordic Council of Ministers 2012). However, DF can largely be divided into IDF and SDF with different properties correspondingly linked to its solubility (Dhingra et al. 2012; Stephen et al. 2017). IDF are mainly present in the plant as structural cell wall components and include cellulose, lignin and water-insoluble hemicellulose. The IDF will mainly serve as bulking agent at consumption resulting in increased fecal mass and decreased intestinal transit time. SDF consist of a variety of oligosaccharides and non-cellulosic polysaccharides, e. g. β-glucan, pectin and water-soluble gums. SDF are highly fermentable which results in an increased production of short chain fatty acids which has proven effect in the management of cardiovascular diseases (Prasadi & Joye 2020). The highly fermentable SDF results also in a lower colonial pH, which means an increase in number and change of the intestinal micro-organisms (Gebruers et al. 2008).

2.4.1 Dietary fiber composition of Wheat kernel

The DF content of the whole wheat grain is ranging between 11.5% and 15.5% including arabinose residues (Ara) (2.1-3.0%), xylose residues (Xyl) (3.6-4.9%), mannose residues (Man) (0.3-0.6%), galactose residues (Gal) (0.3-0.4%), glucose residues (Glc) (2.4-3.7%), uronic acids (UA) (0.4-0.6%), Klason lignin (KL) (0.7-2.0%) and fructan (0.8-1.9%). These are together forming the different DF, with the major part of the nonstarch polysaccharides being arabinoxylan (AX) (5.5%-7.4%), cellulose (1.7%-3.1%), KL, fructan and β-glucan (0.5%-1.%) (Delcour & Hoseney 1986; Andersson et al. 2013). These are all considered to be cell wall components and will differ in distribution within the different tissues of the grain (Delcour & Hoseney 1986; Gebruers et al. 2008). β-Glucans are found in the smallest quantities where only 20% of the 0.51%-0.96% is found in the wheat endosperm cell walls, hence very little exist in white wheat flour (Delcour & Hoseney 1986; Gebruers et al. 2008; Stone & Morell 2009). However, β-glucans will together with AX correspond to the two major DF components of the starchy endosperm, where small amounts of cellulose and glucomannans also are present (Dhingra et al. 2012; Prasadi & Joye 2020). β-Glucans and glucomannans are two out of four categories sometimes referred as hemicellulose with xylans and xyloglucans. Depending on molecular size and structure, these will have different properties depending on if they are soluble or insoluble DF (Dhingra et al. 2012; Prasadi & Joye 2020). The major structural polysaccharide in plants is cellulose which is considered very insoluble as it strongly associates with itself, and it is nondegradable for human enzymes. Cellulose is mostly concentrated to the structural parts of the plant, such as straw, fodder, and hulls. Though, smaller amounts are found in the kernel, around 2%, whereas very small amounts of 0.3% or less are found in the cereal endosperm tissues (Delcour & Hoseney 1986; Stone & Morell 2009). AX represent 60-70% of the starchy endosperm cell walls, hence represent quantitatively the most abundant DF and it represent around 2% of the starchy endosperm (Gebruers et al. 2008). Nevertheless, regarding the entire kernel, AX is considered the most abundant hemicellulose in wheat (Andersson et al. 2013). One third to one fourth of the AX is considered to be extractable. The unextractable AX have a high water-holding capacity and will together with the extractable AX greatly affect the functionality of wheat products within the biotechnological processes e.g. bread production (Delcour & Hoseney 1986). In the characteristic thick walls of the aleurone layer, the proportions of AX and β-glucans remain the same as in the endosperm. Though, the AX is highly crosslinked through diferulic acid and esterified. In the outer layer of the kernels, the pericarp cell wall composition is characterized by highly branched AX, similar to the composition found in straw (Prasadi & Joye 2020). However, the highest content of AX are found in the outer peripheral layers of the kernel, with the highest levels of 40% in the outer pericarp (Gebruers et al. 2008). In the pericarp glucuronic acid residues and Gal residues are also observed which can be found accompanied by a higher content of ferulic and diferulic acid residues (Prasadi & Joye 2020).

2.4.2 Fructan

Fructan is found as a storage carbohydrate in grasses of cool and temperate regions and are believed to protect the plant cells against abiotic stress. Regarding consumption, it is considered a DF (Verspreet et al. 2015; Chibbar et al. 2016). Wheat are considered to be the most important source of fructan in the western diet (Verspreet et al. 2015). Fructan are carbohydrates, mainly or exclusively consisting of fructose, and defined based on the linkage position of the fructose residues (McCleary & Blakeney 1999; Stone & Morell 2009). The core molecule consists of fructose added to a sucrose molecule and depending on the position of the added fructose molecule, three different trisaccharide's can be formed. These three are additionally the basis of the synthesis of five different types of fructan; inulin-type, levan-type, neolevan-type, neo-inulin-type and graminan-type fructan (Verspreet et al. 2015). Specifically the inulin-fructan type have well documented evidence of having health promoting effects, for example, an antioxidant activity believed to be protective against lipopolysaccharide induced damage of the mucosa tissue in the human body (Verspreet et al. 2015). The highest fructan levels are, among cereals, found in rye with content ranging between 3.6 and 6.6 %. In wheat, together with its close relatives, the levels are lower varying between 0.7 and 2.9 %. However, there is a large difference in fructan content in different flours depending on sieving as the bran fraction seems to have higher levels of fructan than the endosperm. There is also evidence of higher levels of fructan in immature kernels. Nevertheless, the content of fructan has been shown to have a high heritability, with no strong interaction between environment and genotype resulting in the possibilities of breeding to enhance these concentrations (Verspreet et al. 2015). Due to the high solubility of fructan in hot water, losses of fructan between 30 and 60% can often occur during food processing. Though, the degradation has been shown to be structure dependent. Hence, it is hard to predict the degradation levels even though both heat and enzymatic degradation e. g. in bread making show an decrease in levels, especially with the use of yeast (Verspreet et al. 2015).

2.4.3 Arabinoxylan

AX is the most abundant DF in wheat with the total content of 0.3-0.75% in spring wheat flour (Gebruers et al. 2008). In refined white wheat flour, AX is the only significant DF (Wieser et al. 2020). It consist of a backbone of β-1,4-linked d-xylopyranosyl residues substituted at O-2 and/ or O-3 with α-L-arabinofuranosyl residues, with Ara over Xyl ratios (A/X) of 0.5-0.8 (Gebruers et al. 2008). Nevertheless, four different structural elements can be found as residues of α -L-arabinofuranosyl which can be attached to Xyl residues at the position of O-2 and O-3. The water extractability of AX can be decreased together with an increase of molecular weight due to the formation of bridges between AX chains made by esterified ferulic acid as Ara residues (Prasadi & Joye 2020). AX is a cell wall polysaccharide and can, based on solubility, be divided into water-extractable and water-unextractable which exist in a ratio of 1:3. Through an ester bond with ferulic acid residues, crosslinks is created in between different AX molecules, hence insoluble. The ferulic acid content is higher in the outer layers of the kernel compared to the starchy endosperm (Wieser et al. 2020). In the outer pericarp AX is more heavily substituted and the aleurone layer differ by the outer pericarp by a low Ara/Xyl ratio (Barron et al. 2007). Nevertheless, 60-70% of the starchy endosperm cell wall consist of AX (Gebruers et al. 2008). Both the extractable and the unextractable AX have the ability to absorb water, up to 20 times more than its own weight, which results in a highly viscous texture. However, the unextractable AX consider to have a negative impact in bread production due to interference with gluten network (Wieser et al. 2020). Nevertheless, a higher content of AX in wheat has shown a slower digestion when consumed possibly due to the increased viscosity (Gouseti et al. 2019).

2.4.4 Lignin

Lignin are complex low molecular weight phenolic polymers present in the cell wall structure of plants and work as a DF constituent whereas it has proven prolonged fermentation time by the gut bacteria (Bunzel et al. 2011). Hence, it is a non-polysaccharide compound, but has demonstrated greater resistance than any other naturally occurring polymer (Dhingra et al. 2012). Lignin works as a glue between the cellulose and the hemicellulose matrix. Though, depending on plant origin, its branched network structure can vary. Very little is known about cereal lignin, except for the existence of ferulate cross-links to carbohydrates (Niemi et al. 2013). When it comes to the role in gastrointestinal fermentation very little is known. However, some studies indicated that lignin is the precursor of the mammalian lignans enterolactone and enterodiol. Lignin has hence been suggested to have health beneficial properties. It has also been association with a decreased risk of cancer and vascular disease, possible by adsorbing carcinogenic compounds and deliver phenolic or antioxidant compounds to the gastrointestinal tract (Niemi et al. 2013).

2.4.5 Uronic acids

The most common UA in plants are D-galacturonic and D-glucuronic acids, and they can be found as hemicellulose, pectin, gums or as alginate in plants.

Hemicellulose is found in the plant cell wall and is a branched polymer of hexose and pentose sugars. This can either be found as acidic or neutral, as where the acidic one has a larger number of UA. In the human body, hemicellulose is partly degraded by microorganisms in the colon resulting in the production of volatile fatty acids. It also has the property of, together with other IDF decreasing the intestinal transit time and slow down the starch hydrolysis (Huffman 2003). In the peripheral layers, especially in the pericarp, AX also contains UA residues (Gebruers et al. 2008).

2.4.6 Cellulose

Cellulose is a cell wall polysaccharide, formed by Glc molecules linked together with β-linkages in position 1 and 4, with 6,000 Glc units in the primary cell wall and up to 14,000 in the secondary cell wall (Stone & Morell 2009).This results in a symmetrical flat structure, almost enzymatically indigestible. Due to the flat structure, cellulose structure can easily attach to each other, strongly linked by hydrogen bonds between layers which result in a hard, water-insoluble material (Cho et al. 1997). Cellulose is the major structural polysaccharide in plants and it is considered very insoluble as it strongly associates with itself, and it is nondegradable for human enzymes. Cellulose is mostly concentrated to the structural parts of the plant, with highest concentrations of 30% in the secondary walls of the pericarp (Delcour & Hoseney 1986; Stone & Morell 2009). Cellulose is not digestible and fermented by commensal microorganisms in the large intestine (Stone & Morell 2009).

3. Materials & Method

3.1 Material

Wheat kernels from three different evolutionary mixes (Dala Lantvete, Ölandsvete and Källunda) and one modern variety (Dacke), in this work referred to as wheat populations, from two different locations (Ekhaga and Krusenberg, Uppsala, Sweden), were provided by the Department of Crop Production Ecology at the Swedish University of Agricultural Sciences. All wheats were grown in four blocks, of which two (block 1 and 4) were selected for this study, with two different treatments (with or without fertilization of 100 kg/ha N). Before analysis all samples were milled in a centrifugal mill (Retsch ZM200, Hann, Germany) with a 0.5 mm sieve at 18000 rpm.

3.1.1 Dacke

Dacke is a spring wheat variety commonly used in organic cultivation as bread wheat and is in this paper considered to be a modern variety even though it has been on the marked for some time. Dacke has a high protein content of around 13.1% and strong long straws. Though, the variety results in a relative low yield, but with a high relative seed number (Hagman & Halling 2020). According to the market, it is a stable variety with good baking properties due to the potentially high protein content and stable falling number (*Vårvete Dacke* 2022). Field trials have also shown the variety to resist better against yellow rust but allows more weed to grow within the fields despite its length (Hagman et al. 2016).

3.1.2 Dala Lantvete

Dala Lantvete is a spring wheat, also known as Dala vårvete. Commercially, it is marketed as a good source of minerals such as iron and magnesium, and vitamin B9 (folate). During late 1900, cultivation occurred from the southern parts of Dalarna up to Malung and some parts of Hälsingland. The cultivation occurred at soils less suitable for winter wheat. The crop was described as having slender plants with bearded red spikes, with poorly yields of small red kernels that easily fell to the ground by the wind. The variety struggled with resistance to yellow rust but became, due to its ability of mature early, an important parent for future breeding with new varieties. However, today the variety of Dala Lantvete are characterized as not being an especially early variety, having a long straw and being difficult to harvest (Wiking Leino 2017).

3.1.3 Ölandsvete

Ölandsvete is a variety that can be used as both a winter wheat and a spring wheat. Still, historically it has mostly been used for cultivation as winter wheat, but changed in present time. In literature, this variety is sometimes incorrectly being called Spelt from Öland. However, the spike differs from Spelt since the kernels are naked. The variety is believed to have a good baking quality with a high gluten content. When cultivated, it has a relatively short straw and are poorly tillering. The spikes are pretty straggly but lacks the longer beard (Wiking Leino 2017).

3.1.4 Källunda

Källunda is a spring wheat cultivated and preserved at the farm of Källunda, Sweden. According to the farmer at Källunda, Magnus Nyman[1], it is a mixture of around 20 wheat cultivars and has been cultivated for 6 years at the farm and have adapted well to the location.

3.2 Methods

All samples were stored at -18°C in plastic containers before analysis. A total of 32 representative samples were analyzed in duplicates, and all results are reported as mean values on a dry matter (DM) basis. DM was determined after drying at 105°C for 16h. The DF content and composition (sugar residues, UA and Klason lignin) was determined with the Uppsala method, and fructan was analyzed separately (described below).

3.2.1 Dietary fibre analysis

The DF analysis was performed according to the Uppsala method (Theander et al., 1995) (AOAC Method 994.139), including analysis of different DF components, such as neutral sugar residues, Klason lignin and UA. In the analysis, starch and free sugars were removed using thermostable α-amylase and amyloglycosidase in acetate buffer. 80% ethanol was used for precipitation of soluble DF followed by hydrolyzation of all DFs using sulfuric acid (72%). The released neutral sugars were quantified as alditol acetates, using gas-liquid chromatography. Klason lignin was determined gravimetrically as ash-free acid-insoluble material, while UA residues were analyzed calorimetrically as acid hydrolysate. The DF content was defined as amylase resistant polysaccharides, UA and Klason lignin (AOAC-NMKL Method, No. 162 1998). The AX content was calculated from Xyl, Ara and Gal residues, assuming that the Ara to Xyl ratio was 0.69 in AX (Loosveld *et al.*, 1997). The ratio of Ara and Xyl was calculated by dividing Ara content with Xyl content. Further where the relative content of the sugar residues calculated by dividing individual sugar residues named R-sugar residue. The total DF (DFtot) was calculated by adding the analyzed DF from the Uppsala method to the fructan content.

[1] Magnus Nyman, farmer at Källunda farm, 2022

3.2.2 Fructan analysis

The fructan analysis was performed with the Megazyme kit K-FRUC (Megazyme, Bray, Ireland, 2020) according to (McCleary et al. 1997) (AOAC method 999.03), with some modifications. The modifications included pre-treatment with α-galactosidase for removal of galactosyl-sucrose oligosaccharides. The extraction step was also modified using 100 mg of sample accurately weighed into glass tubes and heated for 20 minutes in 80°C with 10 ml of deionized water added. The filtration step was replaced with centrifugation, where 1 ml of the extract was centrifuged for 15 minutes at 15000 rpm and the supernatant was then used for analysis.

3.2.3 Analysis of basic quality parameters

Water content, protein content, gluten content, starch content, specific weight, ergosterol content and falling number was analyzed by Agrilab (Uppsala, Sweden) using near infrared transmission (NIR transmittance) (FOSS Infratec 1241, Hilleroed, Denmark). The thousand kernel weight (TKW) was measured by counting and weighing hundred kernels and multiply the weight by ten.

3.2.4 Statistical analysis

Statistical analyses were conducted using two statistical approaches. Overall data structure and relationship between measured variables were evaluated using principal component analysis and the calculation of Pearson´s correlation coefficient (PCA) (SIMCA 17, Sartorius Stedim Data Analytics AB, Germany). Analysis of variance (ANOVA), General linear model, was performed using Minitab®19 for analyzing possible effects of wheat population, location and treatment, as well as interactions between the factors, on all analyzed parameters. Tukey´s pairwise comparison was used to find possible significant differences between wheat populations. Only significant interactions were included into the final models. Values are reported as mean values for wheat populations, locations and treatments, respectively, and P-values <0.05 are considered significant. It was not possible to compare different blocks with each other due to that every block of each location is unique and not possible to analyze in ANOVA. Blocks were therefor treated as replicates in the analysis.

4. Results

The different evolutionary mixes and the modern variety was called wheat population in this work. The reason for this is that the evolutionary mixes may be different when cultivated at other locations and may therefore have different properties.

4.1 PCA

The PCA plots (Figures 1 and 2) illustrates the relationship between the analyzed parameters of the different wheat populations. A total of 56.1% of the variance was explained by PC1 and PC2, with 43.8% explained by PC1 and 12.3% by PC2. The further the variables were located from the center of the plot, the greater impact they have on the variance. Parameters analyzed by using NIR, had less impact on the variance as they were closer to the center of the plot. However, parameters that was found further out from the plot center, for example starch, protein and DF content, had a greater impact on the variance of the data. These parameters also showed a positive correlation with other parameters if they were grouped nearby each other. Thus, protein content correlated with relative arabinose (Rara) and gluten. Sugar residues was though, positioned in opposite side of the plot center and therefor they had a negative correlation to protein and its nearby parameters. Total sugar residues showed instead a positive correlation to the individual sugar residues Xyl, Ara and Glc, as well as to DFtot, DF and AX (Figure 1). Relative uronic acids (RUA), fructan and UA was also located further out from the plot center and showed a negative correlation with the parameters positioned further out on the opposite side as the relative xylose (Rxyl), relative galactose (Rgal) and Gal (Figure 1).

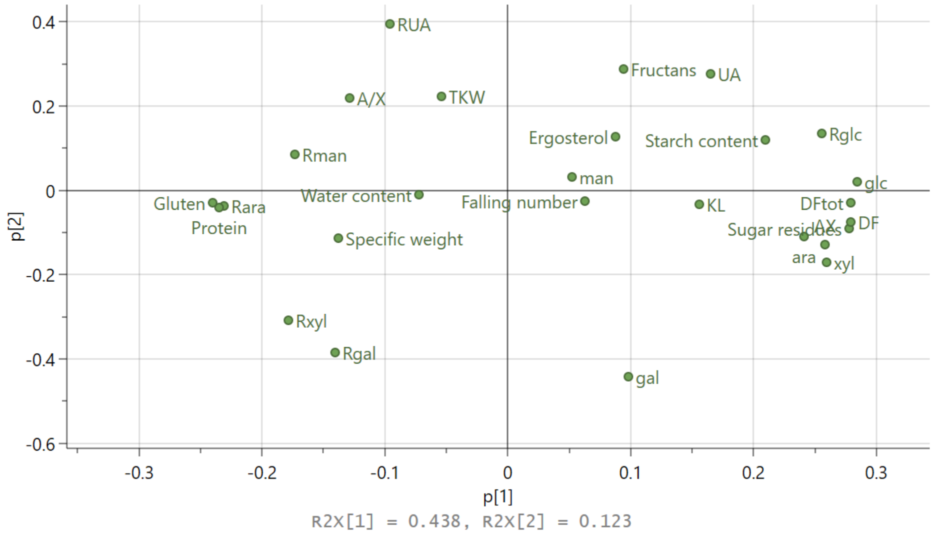


Figure 1. *Loading plot of PCA showing correlation of analyzed parameters. Within these two principle components, 56.1% of the variance is explained*

The score plot (Figure 2), shows the relation between different samples, and a pattern, grouped from the upper part to the lower part, may be seen regarding the different wheat populations, except for a few samples. However, Dacke and Dala Lantvete were positioned furthest out from the center and therefor contributes most to the variance. They were most different since they were positioned in the upper part respectively the lower part of the plot. Källunda and Ölandsvete were more closely positioned in the center of the plot, hence alike each with similar properties.

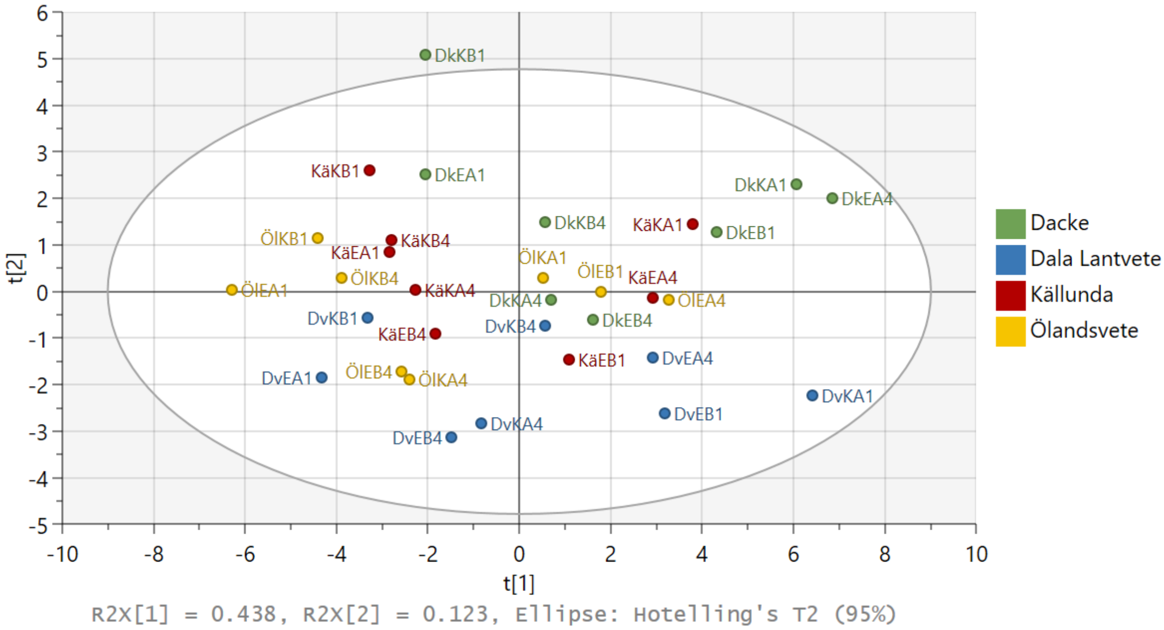
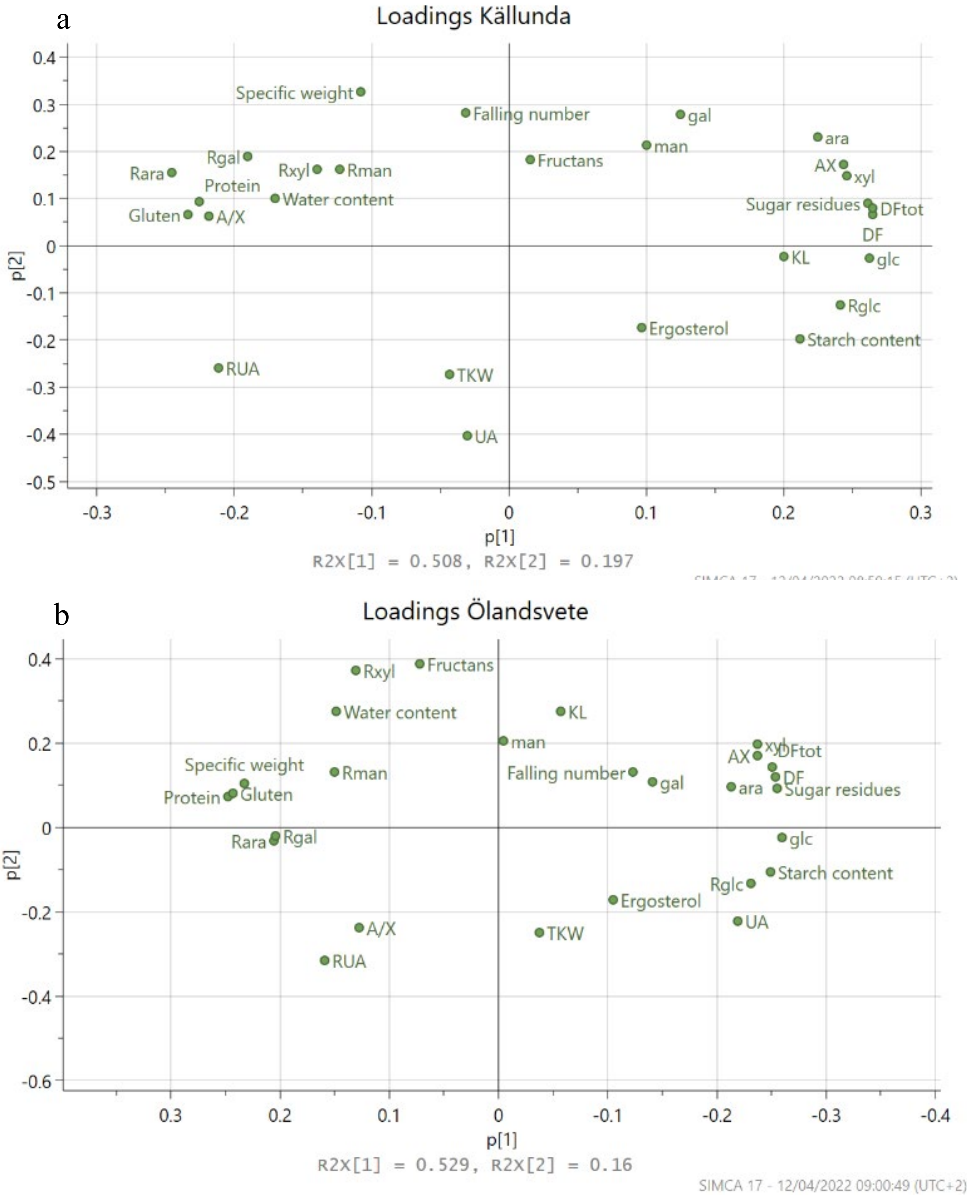


Figure 2. *Score plot of PCA colored according to different wheat populations. Within every color, the same wheat population have different treatment (with or without N addition) and cultivation location (Ekhaga or Krusenberg) and two different blocks (1 and 4) within each location. For abbreviation, see Appendix 1.*

In Figure 3, loading plots were shown for each individual wheat population with around 70% explained variance included. All wheat populations showed similar patterns with gluten, protein and Rara strongly correlated to each other. These parameters correlated negatively to total sugar residues, DFtot, content of the individual sugar residues Ara, Glc and Xyl, as well as AX content. By visually comparing the loading plot (Figure 1) and score plot (Figure 2), Dacke seems to have a correlation with the parameter of fructan content, since they were both found in the upper part of the plots. There was a significant difference in fructan content between wheat populations with Dacke having the highest content (Tables 1 and 2). The fructan content in Dacke seems to correlate to the total sugar residues, DF, DFtot, AX, Xyl and Ara (Figure 3).



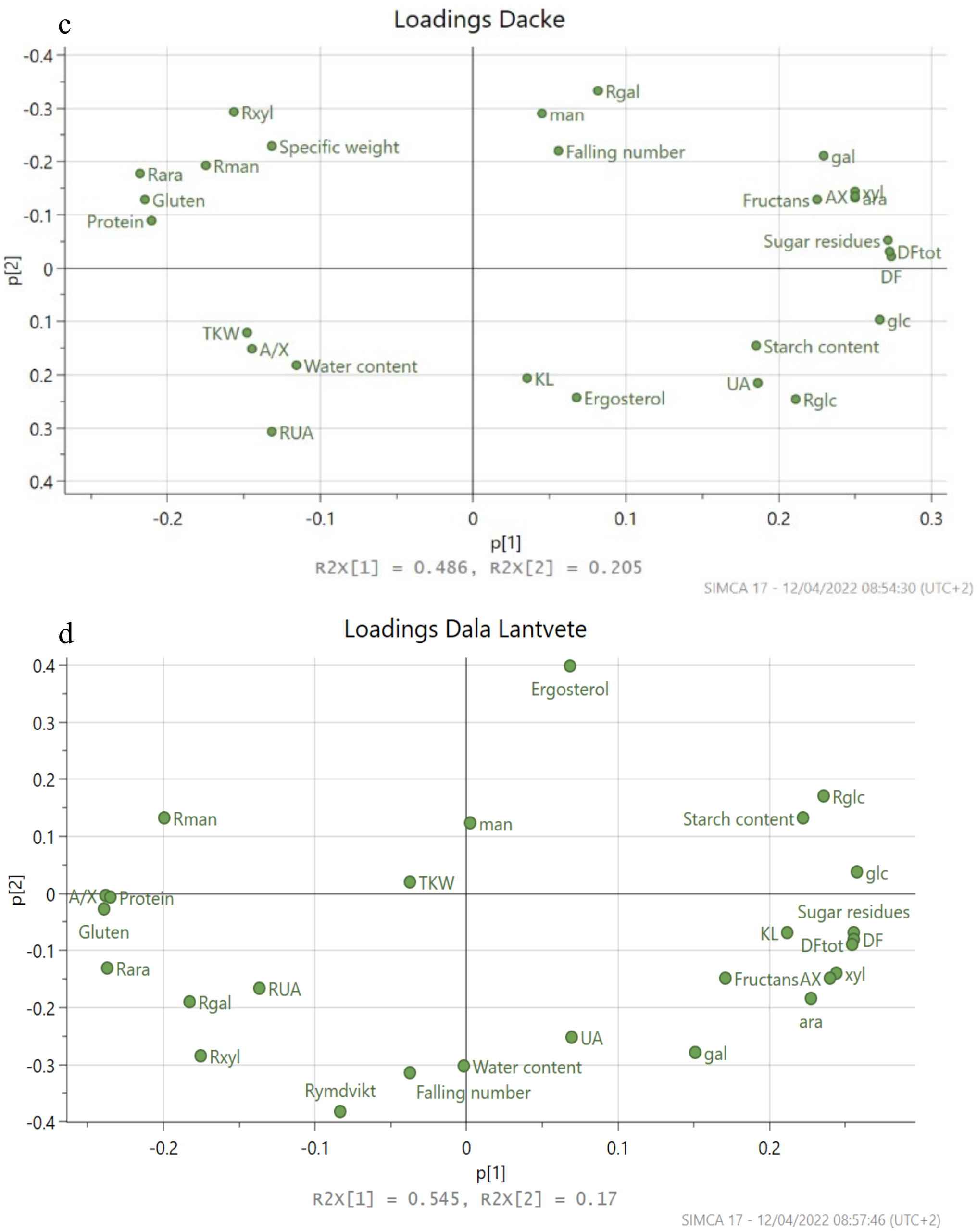


Figure 3. *Loading plots from Principal component analysis, showing the correlation between components within the different wheat populations (a) Källunda, b) Ölandsvete, c) Dacke, d) Dala Lantvete*

4.2 Effect of wheat population, cultivation location and treatment on dietary fibre content and composition

4.2.1 Dietary fibre content and composition

ANOVA was used to analyze if there were any significant differences between wheat populations, locations of cultivation or treatment (Table 2). DFtot significantly differed between wheat populations with the highest content in Dacke (12.8 %) and lowest in Ölandsvete (11.8 %) (Tables 1 and 2 and Figure 4). No significant difference was observed between Dala Lantvete (12.3%) and Källunda (12.1%). The results also showed that there was an interaction between location and treatment, which indicates that DFtot is affected differently by treatment at different locations (Table 1). The similar results were found for DF content and total sugar residues, with significant differences between wheat populations and for the interaction between location and treatment (Tables 1 and 2).

Table 1. *Analysis of variance. P-values for significant effects of wheat population and location, and treatment, as well as interactions for measured parameters*

	Wheat population	Location	Treatment	Location * Treatment	Location * Variety	Treatment* Variety
Arabinose	0.001	ns	ns[a]	0.010	ns	ns
Xylose	0.011	ns	ns	0.004	ns	ns
Mannose	ns	ns	ns	ns	ns	ns
Galactose	0.000	0.019	ns	ns	ns	ns
Glucose	ns	ns	ns	ns	ns	ns
Total sugar residues	0.014	ns	ns	0.010	ns	ns
Klason lignin	ns	ns	ns	ns	ns	ns
Uronic acids	0.000	ns	ns	ns	ns	ns
Dietary fibers	0.009	ns	ns	0.012	ns	ns
Fructan	0.000	ns	ns	ns	ns	ns
DFtot	0.006	ns	ns	0.011	ns	ns
AX	0.003	ns	ns	0.005	ns	ns
A/X	ns	ns	0.004	0.022	ns	ns
Rarabinose	ns	ns	0.026	ns	ns	ns
Rxylose	ns	ns	ns	ns	ns	ns
Rmannose	ns	ns	ns	ns	ns	ns
Rgalactose	0.000	ns	ns	ns	ns	ns
Rglucose	ns	ns	ns	ns	ns	ns
RUA	0.017	ns	ns	0.000	ns	ns

[a] *= not significant*

Table 2. *Content (% DM) of dietary fiber constituents for different wheat populations, locations and treatments, including individual sugar residues, Klason lignin, uronic acids, total sugar*

residues, dietary fibers, fructans, total dietary fibers(including fructans) (DFtot), arabinoxylan (AX), relative values of all sugar residues (Rsugar) and uronic acids (RUA) and ratio of arabinose:xylose (A/X). Different letters in same row of each category (vertical division) show significant difference between values. Data are presented as mean values

	Wheat population				Location		Treatment	
	Dacke	Dala Lantvete	Källunda	Ölandsvete	Ekhaga	Krusenberg	Without N	With N
Arabinose	2.68[a]	2.63[ab]	2.52[bc]	2.50[c]	2.61[a]	2.56[a]	2.59[a]	2.58[a]
Xylose	4.14[a]	4.11[a]	3.96[ab]	3.90[b]	4.08[a]	3.98[a]	4.06[a]	4.00[a]
Mannose	0.35[a]	0.34[a]	0.35[a]	0.33[a]	0.34[a]	0.35[a]	0.34[a]	0.34[a]
Galactose	0.34[a]	0.38[b]	0.35[b]	0.35[b]	0.36[a]	0.35[b]	0.36[a]	0.36[a]
Glucose	2.98[a]	2.86[a]	2.77[a]	2.66[a]	2.83[a]	2.80[a]	2.87[a]	2.76[a]
Total sugar residues	10.5[a]	10.3[ab]	10.0[ab]	9.8[b]	10.2[a]	10.0[a]	10.2[a]	10.0[a]
Klason lignin	0.85[a]	0.83[a]	0.81[a]	0.80[a]	0.84[a]	0.81[a]	0.84[a]	0.80[a]
Uronic acids	0.47[a]	0.43[b]	0.43[b]	0.42[b]	0.44[a]	0.44[a]	0.44[a]	0.44[a]
Dietary fibers	11.8[a]	11.6[ab]	11.2[ab]	11.0[b]	11.5[a]	11.3[a]	11.5[a]	11.3[a]
Fructan	0.94[a]	0.73[b]	0.88[c]	0.82[d]	0.85[a]	0.84[a]	0.86[a]	0.83[a]
DFtot	12.8[a]	12.3[ab]	12.1[ab]	11.8[b]	12.4[a]	12.1[a]	12.4[a]	12.1[a]
AX	6.58[a]	6.48[ab]	6.25[bc]	6.16[c]	6.44[a]	6.29[a]	6.40[a]	6.33[a]
A/X	0.65[a]	0.64[a]	0.64[a]	0.64[a]	0.64[a]	0.64[a]	0.64[a]	0.65[b]
Rarabinose	24.5[a]	24.5[a]	24.3[a]	24.7[a]	24.5[a]	24.4[a]	24.3[a]	24.7[b]
Rxylose	37.8[a]	38.3[a]	38.2[a]	38.4[a]	38.2[a]	38.0[a]	38.1[a]	38.2[a]
Rmannose	3.20[a]	3.15[a]	3.41[a]	3.24[a]	3.18[a]	3.32[a]	3.20[a]	3.30[a]
Rgalactose	3.10[a]	3.56[c]	3.37[b]	3.49[bc]	3.42[a]	3.34[a]	3.35[a]	3.41[a]
Rglucose	27.2[a]	26.5[a]	26.6[a]	26.11[a]	26.5[a]	26.7[a]	26.9[a]	26.3[a]
RUA	4.32[a]	4.03[b]	4.15[ab]	4.14[ab]	4.14[a]	4.18[a]	4.15[a]	4.17[a]

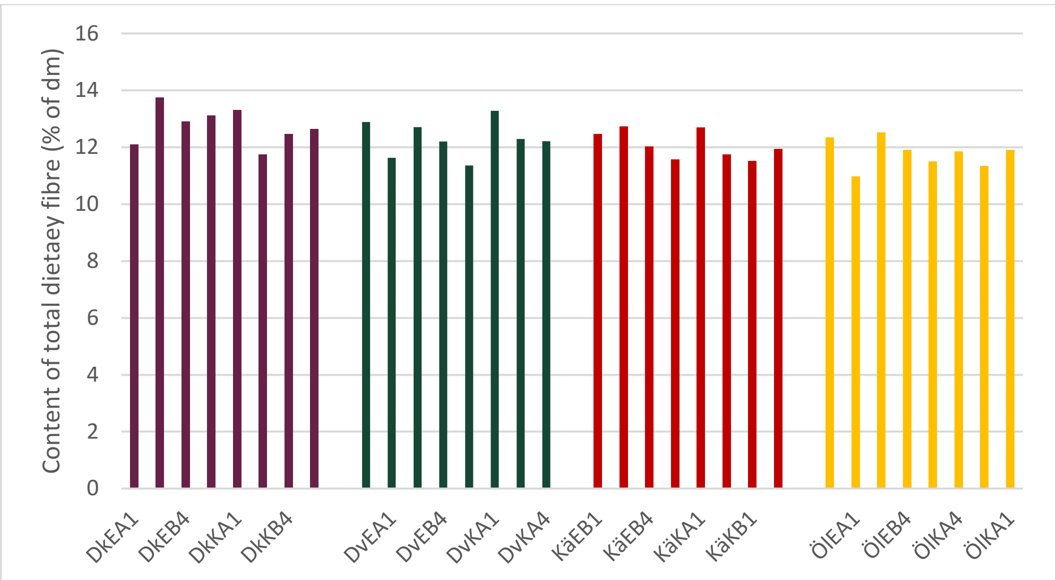


Figure 4.*Total dietary fiber in different wheat populations. Dark red = Dacke, green = Dala Lantvete, light red = Källunda and yellow = Ölandsvete. For other abbreviations, see appendix 1*

The sugar residues Ara, Xyl and Gal did all show significant differences regarding wheat populations (Tables 1 and 2). Regarding Ara and Xyl, there was also an interaction between location and treatment. Ara was significantly different between all wheat populations with highest amounts in Dacke (2.68%) followed by Dala Lantvete (2.63%), Källunda (2.52%) and Ölandsvete (2.50%). There was no significant difference for Xyl between Dacke (4.14%) and Dala Lantvete (4.14%). The lowest content was seen in Ölandsvete (3.90%) whereas Källunda was in between with a content of 3.96%. Ara is together with Xyl forming AX and constitutes the largest component of DF in wheat. Also, for AX a significant difference was seen between wheat populations with the highest content in Dacke (6.58%) and lowest in Ölandsvete (6.16%). There was also a significant difference for the interaction between location and treatment (Tables 1 and 2) meaning that the AX content was affected differently by treatment at different locations.

Similar result was observed for the A/X ratio (Tables 1 and 2). For a high A/X ratio, there is more Ara attached to the backbone of Xyl which results in a more insoluble DF (Saulnier et al. 2012). There was a significant difference in A/X ratio between treatments with a higher A/X with addition of N. There was also an interaction between treatment and locations. However, the values for samples with or without treatment (0.64 and 0.65 respectively) was very close to each other which may indicate consistent values and low variation even though there was a significant difference.

Regarding the sugar residue Gal, significant difference was only seen within wheat populations, with the modern Dacke (0.34%) (Table 2) having a lower content then the other wheat populations. Furthermore, the cultivation location also showed significant differences with higher content when cultivated in Ekhaga. There were no significant differences found regarding Man, ranging from 0.33% to 0.35% or for Glc with the range of 2.66% - 2.98%. This meant that there were no significant differences in the cellulose content. The same was found for Klason lignin, with no significant differences between any of the factors. However, the UA showed significant differences between wheat populations, with Dacke having higher levels (0.47%) then the three evolutionary mixes (0.42% - 0.43%). The fructan content varied between 0.73% and 0.94% (Table 2). There was a significant difference between the different wheat populations (Tables 1 and Figure 5) with the highest amount found in Dacke, and the lowest amount in in Dala Lantvete. No significant differences were seen between the cultivation locations or between the different treatments (table 2). Regarding Klason lignin, no significant difference was seen between either variety, location or treatment.

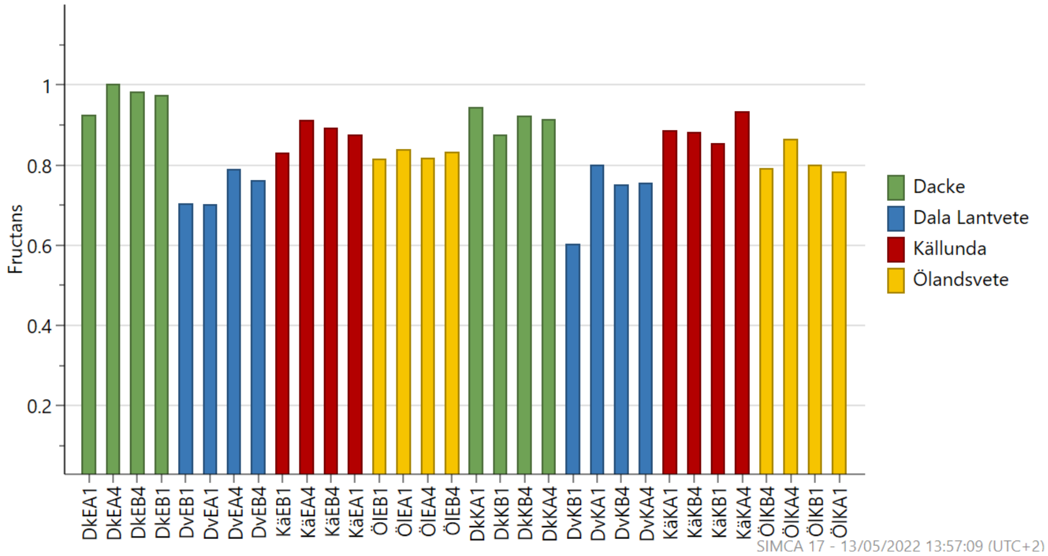


Figure 5*. Fructan content for every sample in different locations, blocks and treatments. For abbreviations see appendix 1.*

By calculating the relative content of each sugar residue (Rsugar) we may get an indication of the composition of the cell wall structure. Rara showed similar pattern as A/X with significant differences between treatments, with a higher relative content of 24.7% if the crop was treated with addition of N than if it was not treated (24.3%) (table 2). However, there were no significant differences for Rxyl, Rman or Rglc, when compared as relative sugars. The relative content of galactose (Rgal) did though show a significant difference between wheat populations with the highest content in Dala Lantvete (3.56%) and lowest in Dacke (3.10%) (table 1). For RUA there was a significant difference between wheat populations (table 1), with highest in the modern variety Dacke (4.32%) and lowest in Ölandsvete (4.14%) (table 1 and 2). There was also a significant difference for interaction between location and treatment (table1).

4.2.2 Basic quality parameters

For other quality parameters, there was no significant difference for water content, protein content, gluten content, starch content or specific weight for any of the factors (Table 3). Though, there was a significant difference for ergosterol content for the interaction between location and treatment (table 3). This can probably be partly explained by that all samples in block 4 at Ekhaga without extra N had higher ergosterol levels than any other samples in this study (Figure 6).

Table 3*. Analysis of variance. P-values for significant effects of wheat population, location and treatment as well as interactions for basic quality parameters*

%	Wheat population	Location	Treatment	Location * Treatment	Location * Wheat population	Treatment* Wheat population

Water content	ns [a]	ns	ns	ns	ns	ns
Protein	ns	ns	ns	ns	ns	ns
Gluten	ns	ns	ns	ns	ns	ns
Starch content	ns	ns	ns	ns	ns	ns
Specific weight	ns	ns	ns	ns	ns	ns
Ergosterol	ns	ns	ns	0.012	ns	ns
Falling number	0.027	0.011	ns	0.017	0.017	ns
TKW	ns	ns	0.012	0.038	ns	ns

[a] = not significant

Table 4*. Content of other parameters for different wheat populations, locations and treatments including water content before analysis (%), protein content (%), gluten content (% of protein), starch content (%), specific weight (g/l), ergosterol (mg/kg DM), falling number (sec), and thousand kernel weight (TKW) (g). Different letters in same row of each category (vertical division) show significant difference between values. Data are presented as mean values*

Average (%DM)	**Wheat populations**				**Location**		**Treatment**	
	Dacke	Dala Lantvete	Källunda	Ölandsvete	Ekhaga	Krusenberg	Without N	With N
Water content	11.3^{a}	11.1^{a}	11.0^{a}	11.3^{a}	11.1^{a}	11.2^{a}	11.3^{a}	11.0^{a}
Protein	14.9^{a}	15.4^{a}	15.0^{a}	15.1^{a}	15.1^{a}	15.2^{a}	14.7^{a}	15.5^{a}
Gluten	32.0^{a}	32.6^{a}	31.8^{a}	32.6^{a}	32.0a	32.5^{a}	31.1^{a}	33.4^{a}
Starch content	63.4^{a}	62.3^{a}	63.2^{a}	63.7^{a}	63.0^{a}	63.3^{a}	63.3^{a}	63.0^{a}
Specific weight	773.7^{a}	770.1^{a}	778.6^{a}	767.5^{a}	773.4^{a}	771.7^{a}	770.3^{a}	774.8^{a}
Ergosterol	10.9^{a}	11.0^{a}	10.9^{a}	10.7^{a}	10.7^{a}	11.1^{a}	11.2^{a}	10.6^{a}
Falling number	214.8^{a}	141.6^{ab}	118.6^{ab}	168.8^{b}	191.0^{a}	130.9^{b}	160.0^{a}	161.9^{a}
TKW	31.9^{a}	31.7^{a}	32.5^{a}	31.8^{a}	32.1^{a}	31.8^{a}	31.3^{a}	32.6^{b}

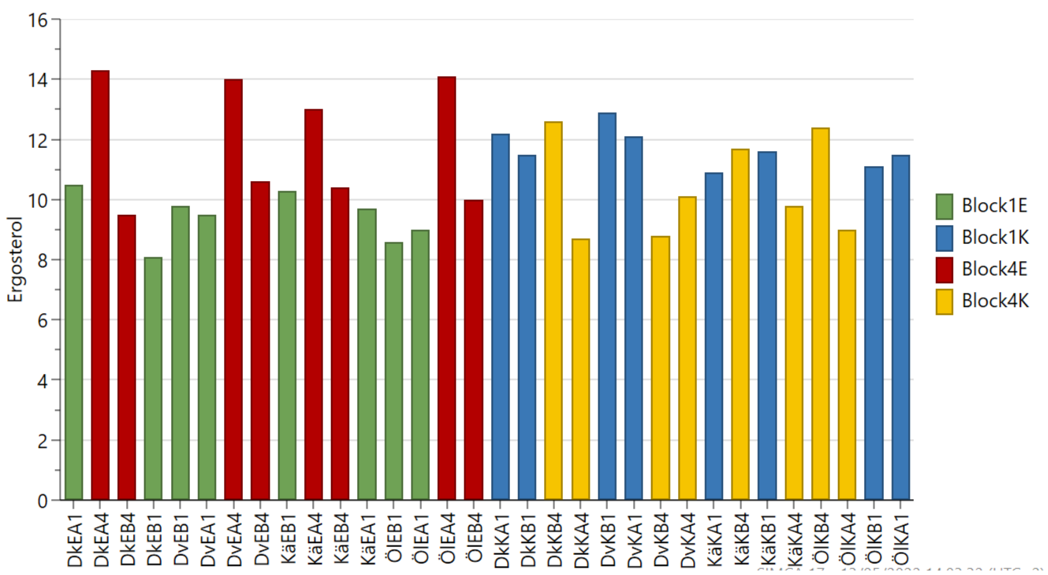


***Figure** 6. Ergosterol content divided by every different wheat population regarding, variety; location; block and treatment. Colored by block and location. Green = Block 1 at Ekhaga, blue = Block 1 at Krusenberg, red = Block 4 at Ekhaga, yellow = Block 4 at Krusenberg. For other abbreviations see appendix 1*

For falling numbers, there were significant differences between wheat populations and location with the highest values for the modern Dacke (214.8 sec) and lowest for Ölandsvete (168.8 sec) (Table 4). Regarding location, Ekhaga showed significantly higher falling number values of 191.0 sec compared to Krusenberg with 130.9 sec. There was also a significant difference for the interaction of location and treatment, probably due to some very high values for Dacke and Ölandsvete at Ekhaga (Figure 7), which may also explain the significant difference for interaction between location and variety as well.

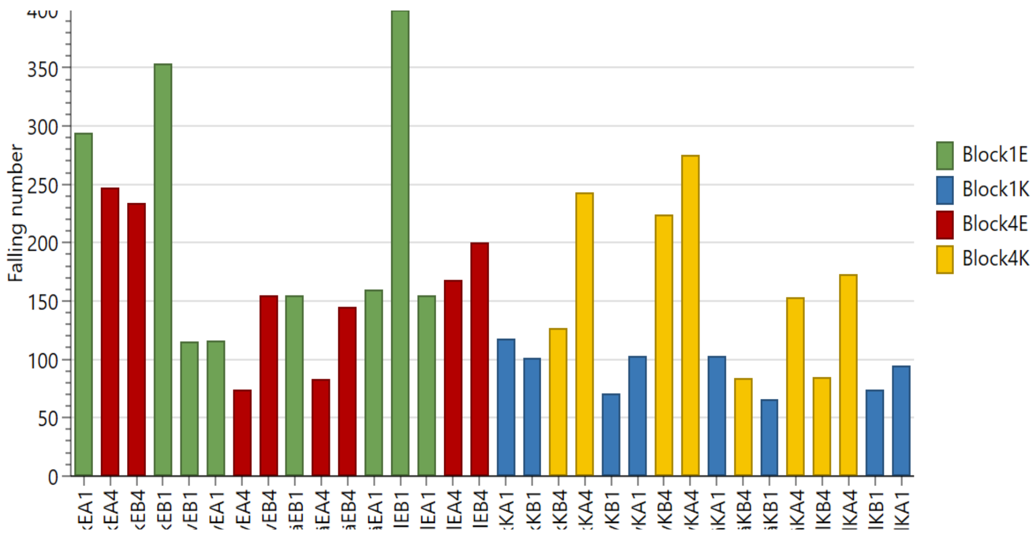


***Figure** 7. Falling number. Colored according to location of cultivation. Green = Block 1 at Ekhaga, blue = Block 1 at Krusenberg, red = Block 4 at Ekhaga, yellow = Block 4 at Krusenberg. For other abbreviations see appendix 1*

For thousand kernel weight (TKW) there was a small but significant difference between treatments (Table 3), with the higher weight for treatment with addition of N (32.6 g) compared to untreated (31.3 g) (table 4). There was also a significant difference for the interaction between location and treatment where Krusenberg showed significant higher TKW if treated with additional N (Table 3 and Figure 8).

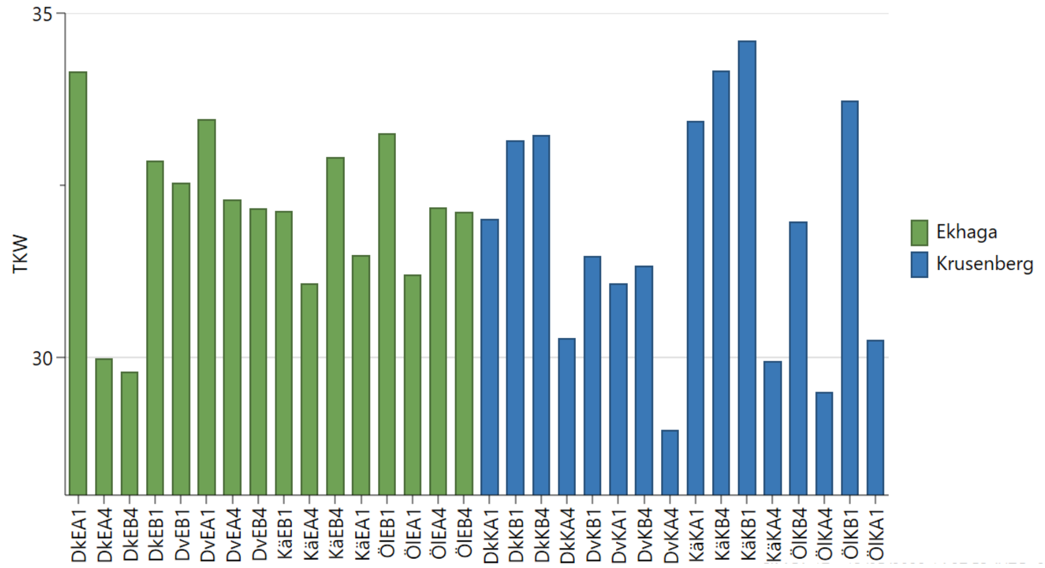


Figure 8. *Thousand kernel weight (TKW). Colored according to location with Ekhaga in the left half (green) and Krusenberg to the right (blue). For abbreviations, see appendix 1*

A positive correlation between Rara and protein content was seen (figure 8), meaning that with a higher protein content, the relative amount of the sugar residue Ara increased. This pattern has been seen in other studies of white flour (Andersson et al. 1994) and does visually have any correlation to different wheat populations in the present study (figure 9).

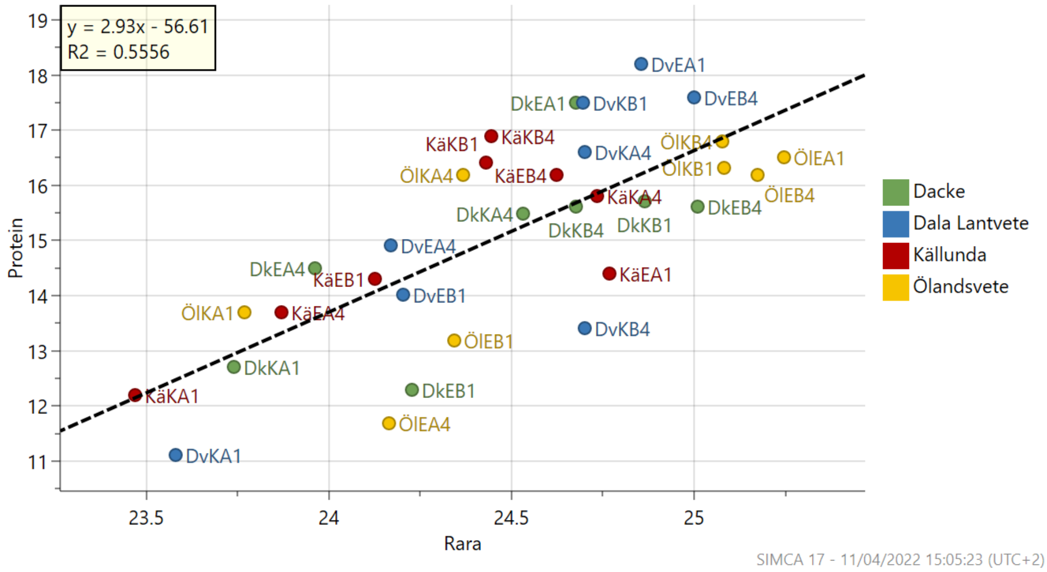


***Figure 9**. Correlation between Protein content and relative content of arabinose (Rara). For abbreviations, see appendix 1*

5. Discussion

The difference between the analyzed wheat populations, one modern and three evolutionary mixes, only tells us the differences specifically when cultivating these at the given locations, hence only these specific wheat populations. However, the ability of adaptation by the evolutionary mixes and its existing genetic diversity of these wheat populations may behave different in other locations. Due to only analyzing wheat kernels from one year, cultivated at two specific locations, similar analyzes may give different results other years and at other locations. We must also keep in mind, the possibility that the evolutionary mixes used in these analyzes may differ severely from those claimed to be the same variety, as there has been reports of confusion of varieties and year of adaption to specific locations, changing the wheat mixes from each other (Wiking Leino 2017). Nevertheless, the wheat populations used in this work showed small but significant differences between the modern variety and the three evolutionary mixes with higher DFtot content in the modern variety Dacke and the lowest in Ölandsvete.

The most abundant DF of wheat, AX, showed differences between wheat populations, following the trend of DF content and fructan content of the different wheat populations with Dacke having the significantly highest content and Ölandsvete the lowest. The significant difference between wheat populations and interaction between location and treatment for the A/X ratio can be questionable to argue if it is of any importance since the values are very similar. Nevertheless, the results indicate that it may be a small variation in the properties of AX depending on addition of fertilizers.

Klason lignin is only found in very small amounts in wheat. However, some Klason lignin is present, but it may be difficult to analyze as the used methodology within this DF analysis is developed for the lignin in wood and have shown not accurately measuring lignin in food products such as cereals (Bunzel et al. 2011). Nevertheless, it is a commonly used method, even though it may not be the most accurately method. The small levels of lignin in wheat also makes it less important in the big context.

The best way to interpret the results of the individual sugar residues from DF is by looking at the relative values of the different sugar residues, since this gives us information of the sugar residues in context with only the other sugar residues. The relative content of UA in Dacke may suggest a higher bran content in this modern variety compared to the other wheat populations (Parker et al. 2005). Ölandsvete showed the lowest UA values. As there is a significant difference between wheat populations, it is also interesting that there is an interaction between location and treatment. This tells us again that the location is very important for the outcome of the cultivation and that it may be crucial for the farmer to know more about specific soils of the cultivation areas in order to achieve a unified harvest with desired quality attributes. Though, at the present this may not be relevant for cultivation of evolutionary mixes as the selling argument more is connected with adaptation to environmental changes and consumers belief of better tasting, healthier food in general and raw materials than a specific DF content. Regarding healthier food, this study does though show lower content of total DF in the heritage wheat populations than in the modern variety Dacke.

The factors treatment and wheat population does not seem to have any interactions with each other. There is also no interaction between location and wheat population, except for the values of falling number. However, most of the high values of falling number are from wheat samples cultivated in the same block (4) at the same location (Ekhaga). A high falling number may be a result of trigged germination of the seeds which can occur by sudden drops in temperature, high humidity or rainfall after the wheat kernel has reach maturity (Liniņa & Ruža 2015). As the results show that something may have affected this specific location, block 4, resulting in higher falling number for these specific wheat samples. Specifically, block 4 was not given additional nitrogen. The lowest falling number of this location was seen in Ölandsvete, except from one specific sample. Though, it is notable that several of the specifically high values was found in the wheat samples of Dacke, which may suggest that this variety have a higher susceptibility against environmental stress. Further proving this possibility is the higher content of fructan found in these specific Dacke wheat populations cultivated in Ekhaga. Fructan levels can be seen as a result of the exposal of abiotic stress. Nevertheless, high fructan levels are also strongly linked to the genetics and strongly inherited (Verspreet et al. 2015). Verspreet et al. (2015) also report a correlation of higher fructan levels with cold temperatures but not affected by the different cultivation locations. Slafer et al (2021) reported that the two major environmental stresses that alter the grain quality and composition is temperature and drought. The ergosterol levels of samples cultivated at Ekhaga without fertilization does however, show higher levels of ergosterol for all wheat populations. Ergosterol indicate the presence of yeast infections and hence, validate a possible additional abiotic stress in this specific location (Tothill et al. 1992).

The positive correlation between Rara and protein content means that a higher protein content result in an increase of the sugar residue Ara. This trend has been seen in other studies of white flour where a di-substituted Xyl residue followed the same pattern. This may indicate an association between AX and protein in wheat, where those constituents have specifically been found in the starchy endosperm cell wall tissue (Saulnier et al. 2012). The results also showed that there was no difference between wheat populations, which may indicate that protein and Rara together correlate to the primary cell wall of the endosperm as there will be more primary cell wall with a larger endosperm. However, more research is needed to understand this correlation.

One aspect, worth mentioning is the differences in appearance of the grains between the different wheat populations. Visually the modern variety excelled as it had a much more uniform appearance compared to the evolutionary mixes. Specifically, Dala Lantvete had a huge variation in size between the different kernels. This makes the values of the analyzed results limited to this study. It may indicate a large variation also within the “variety” which may give very different results, if it was cultivated somewhere else. The differences within a “variety” may also be the reason for the interactions between location and treatment, since they may act and behave differently to additional fertilizers given.

6. Conclusion

This study showed that there was small, but significant differences in DF content between wheat populations. The content of DFtot, including AX, fructan, UA residues and sugar residues, was lower in the evolutionary mixes than in the modern variety Dacke. There were also some differences in the composition of the DF between wheat populations. Though, only A/X ratio and Rara seems to be affected by treatment. No interactions between neither treatment and location nor treatment and wheat populations were observed. Though, the interaction between location and treatment show that the DF content depends on location and how the farmer decides to cultivate the varieties. Furthermore, DF may differ due to genetic difference, for example the ability to synthesize fructan. A lower content was found in the evolutionary mixes, suggesting that those may have a better resistance to environmental changes, as fructan also is a created energy source resulting in combination with abiotic stress. The result in this study is based on very few samples and should be interpreted with caution. More research is needed. The difference between evolutionary mixes and the modern variety Dacke are also very small and may be of less practical importance. Nevertheless, the results show that there are possibilities to affect the DF content and composition by choosing variety and cultivation conditions.

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Popular science summary

Imagine an extremely hot summer with almost non-existing rainfalls. Most people, at least in the Scandinavian countries, would enjoy the warmth, especially with not having to worry about the all so well-known rain and bad weathers. Some people may find it quite wonderful, spending most free days and nights on the beach eating ice cream, while some will find it unbearable and search for every shade possible.

Something that many people probably not think of, is that this heat, no matter how nice it is, also may affect them for a long time even after the summer has passed and the rain has, once again, taken a firm grip of everyday life. I am not talking about a nice sunburn, I am talking about the lack in access of food. How can it be related to the nice weather of the summers you may ask? I will tell you, bear with me!

Every living thing needs water; humans, animals and plants. With hot weathers and few rainfalls, there is less water available. Without enough water, less plants will survive. Not only, has the green areas of the parks been transformed into brown deserts after a hot summer, plants that is vital for our survival such as cereal crops has also been affected. The most important cereal, worldwide, is wheat. Without wheat, a lot of people would go starving as it feeds half of the world's population. For a food lover, the life would also be much more boring since wheat is used for the production of common breads, pasta, noodles, cakes and biscuits together with other valuable nonfood products as ethanol and biodegradable plastics. Well, it is unlikely that all wheat, all over the world, will be lost at the same time. But, with repeated bad weather conditions due to climate changes it is likely that the existing quantities will decrease severely and result in more people suffering from starvation. So what can be done to avoid this from happening? It is hard to change the weather, and much easier to see if it is possible to get more plants to survive or stuff more energy and nutrients into the kernels. However, we do love our bread and would hate to eat less, even though it might be possible to use less as an energy source!

Heritage cereals are old varieties of cereals, such as wheat, which is used to be grown for food. As these old cereals had to survive with no additional possible help from the humans, which today can be done by adding water or missing nutrients to the plants, it had to survive on its own. So why do we not use these anymore? These varieties did not produce very much cereals per square meter. It means that much more land and work was needed for the production of a small piece of bread. New varieties were therefore created, so that more breads and biscuits could be produced from the wheat of one field. But, if the new varieties cannot survive the new extreme weathers created by the environmental changes maybe the older once are better as it at least will be some wheat to produce some bread? Some farmers believe that these old wheats not only survive drought better, but also create better tasting and healthier breads as humans has not forced these kernels to be pumped with quick energy components like starch as with the modern wheat. There is though very little research about heritage cereals, so people do not really know if it is true that heritage wheats are better to eat or not. One thing connected to healthier bread is the dietary fibers. Dietary fibers are compounds, found in different types in the wheat kernel that cannot be used by the body as energy, but it does not make it useless. Dietary fibers creates a flow through our intestines since they are not degraded, and thereby creating movement for the food. In the intestines, the dietary fibers will also be greatly received by the good bacteria, as it is their food, and will repay by produce small components that is good for us and makes sure that we are in good health.

This work was done to find out the dietary fiber content and composition in some heritage wheats cultivated in Sweden; Dala Lantvete, Källunda, Ölandsvete, and one commonly used variety in organic cultivation, Dacke. These were cultivated at two different locations in Sweden, within two different blocks and with two different treatments, with or without nitrogen fertilization. A total of 32 samples were milled and analyzed for total dietary fibers content as well as different types of dietary fibers, such as arabinoxylan, which is the most abundant dietary fiber in wheats, and fructan.

The obtained results indicated that there is a small but existing difference in total dietary fiber content between the wheat samples in this paper. The highest content was found in the modern wheat Dacke and the lowest was found in Ölandsvete. There was also a significant difference found between locations of cultivation and treatments when looking at specific composition of the dietary fibers such as arabinoxylan with addition of nitrogen fertilizers. However, the difference is very small. Otherwise nothing else seems to react to different treatments unless in combination with specific soil conditions. The dietary fiber component fructan is produced in different levels, determined both by genetics and if the plant is stressed or not. The evolutionary mixes of heritage wheat showed lower content of this specific component and possibly meaning that they are less stressed.

So, what do this study tell us? It tells us that these specific wheat samples show a little lower dietary fiber content in the heritage wheats than in the modern variety Dacke grown under these specific conditions. Dacke also showed indication of possible being more stressed, and this without having extremely bad weather, which might indicate less survivability in extreme weathers. More research needs to be done, but this may be the beginning of showing that regarding total dietary fibers, the modern variety Dacke show a little higher content but may result in few breads the year of a hot summer!

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Appendix 1

ID	ID2	Location	Treatment	Variety	Block
DkEA1	E4.Dacke.A1	Ekhaga	A	Dacke	Block1E
DkEA4	E45.Dacke.A1	Ekhaga	A	Dacke	Block4E
DkEB4	E56.Dacke.B1	Ekhaga	B	Dacke	Block4E
DkEB1	E8.Dacke.B1	Ekhaga	B	Dacke	Block1E
DkKA1	K10.Dacke.A1	Krusenberg	A	Dacke	Block1K
DkKB1	K2.Dacke.B1	Krusenberg	B	Dacke	Block1K
DkKB4	K47.Dacke.B1	Krusenberg	B	Dacke	Block4K
DkKA4	K56.Dacke.A1	Krusenberg	A	Dacke	Block4K
DvEB1	E11.DalaLantv.B4	Ekhaga	B	Dala Lantvete	Block1E
DvEA1	E3.DalaLantv.A4	Ekhaga	A	Dala Lantvete	Block1E
DvEA4	E46.DalaLantv.A4	Ekhaga	A	Dala Lantvete	Block4E
DvEB4	E55.DalaLantv.B4	Ekhaga	B	Dala Lantvete	Block4E
DvKB1	K1.DalaLantv.B4	Krusenberg	B	Dala Lantvete	Block1K
DvKA1	K14.DalaLantv.A4	Krusenberg	A	Dala Lantvete	Block1K
DvKB4	K49.DalaLantv. B4	Krusenberg	B	Dala Lantvete	Block4K
DvKA4	K54.DalaLantv.A4	Krusenberg	A	Dala Lantvete	Block4K
KäEB1	E10.Källunda.B6	Ekhaga	B	Källunda	Block1E
KäEA4	E43.Källunda.A6	Ekhaga	A	Källunda	Block4E
KäEB4	E51.Källunda.B6	Ekhaga	B	Källunda	Block4E
KäEA1	E7.Källunda.A6	Ekhaga	A	Källunda	Block1E
KäKA1	K12.Källunda.A6	Krusenberg	A	Källunda	Block1K
KäKB4	K43.Källunda.B6	Krusenberg	B	Källunda	Block4K
KäKB1	K5.Källunda.B6	Krusenberg	B	Källunda	Block1K
KäKA4	K55.Källunda.A6	Krusenberg	A	Källunda	Block4K
ÖlEB1	E14.Ölandsv.B5	Ekhaga	B	Ölandsvete	Block1E
ÖlEA1	E2.Ölandsv.A5	Ekhaga	A	Ölandsvete	Block1E
ÖlEA4	E44.Ölandsv.A5	Ekhaga	A	Ölandsvete	Block4E
ÖlEB4	E54.Ölandsv.B5	Ekhaga	B	Ölandsvete	Block4E
ÖlKB4	K48.Ölandsv.B5	Krusenberg	B	Ölandsvete	Block4K
ÖlKA4	K50.Ölandsv.A5	Krusenberg	A	Ölandsvete	Block4K
ÖlKB1	K6.Ölandsv.B5	Krusenberg	B	Ölandsvete	Block1K
ÖlKA1	K9.Ölandsv.A5	Krusenberg	A	Ölandsvete	Block1K

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