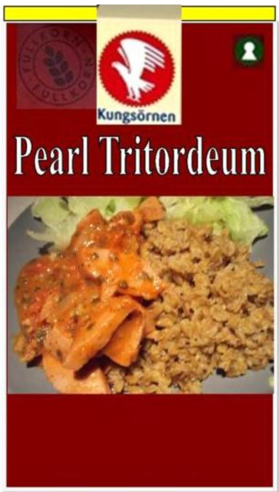
Tritordeum

Evaluation of a new food cereal

Anna Erlandsson

Examensarbete

Institutionen för Livsmedelsvetenskap

Swedish University of Agricultural Sciences
Department of Food Science.

Publikation nr

Uppsala 2010

Tritordeum
Evaluation of a new food cereal

Anna Erlandsson

Supervisors: I. Börjesson & P. Åman
Examiner: R. Andersson

Institutionen för Livsmedelsvetenskap | Publikation nr

Swedish University of Agricultural Sciences | Uppsala 2010
Department of Food Science.

Abstract

The objective of this thesis was to evaluate Tritordeum lines HT 354, HT 361, HT 437, HT 2218 (JB3) and HT 1608 (JB1) for use as a new raw material within the food industry. Analyses made were on *dietary fibre, fructan, ash, water content* and *colour.* Compilation of data from analyses made for Agrasys an Agri-Food company in Barcelona having the commercial rights to Tritordeum, included *Lutein* and *rheological properties*. Further an application part was made on Tritordeum HT 1608 with the purpose to find optimal end use for Tritordeum; breakfast cereals, porridge, pearl Tritordeum, bread and flour for binding and breading were produced in a small scale production.

Tritordeum was shown to content 14.3% total dietary fibre which is more than in wheat, but the β-glucan content was lower than wheat. Lutein gives Tritordeum its *yellow colour.* Tritordeum is a new cereal and possible cereal products from Tritordeum could be Pearl Tritordeum, bread, Tritordeum white flour binding and Tritordeum wholegrain breading.

Key words: Tritordeum, dietary fibre, fructan, lutein, bread, tempe and pearl grain

Sammanfattning

Syftet med den här uppsatsen var att utvärdera Tritordeum linjerna HT 354, HT 361, HT 437, HT 2218 (JB3) och HT 1608 (JB1) som nya råvaror inom livsmedelsindustrin. Utförda analyser var *kostfiber, fruktan, aska, vattenhalt* och *färg.* Sammanställning av data från analyser gjorda för Agrasys det Agro-Livsmedelsföretag i Barcelona som har de kommersiella rättigheterna för Tritordeum inkluderade analyser av *Lutein* och *reologiska egenskaper*. Vidare utfördes en applikationsdel på Tritordeum HT 1608 med syftet att hitta den optimala slutprodukten av Tritordeum; Flingor, gröt, matgryn, bröd samt mjöl för redning och panering tillverkades i en småskalig produktion.

Tritordeum innehöll 14,3% total kostfiber vilket är mer än i vete, medan halten β-glukan visades vara lägre än vete. Lutein ger Tritordeum sin gula färg. Tritordeum är ett nytt spannmål och möjliga produkter utifrån Tritordeum skulle kunna vara matgryn, bröd, vitt redningsmjöl och fullkorns panering.

Nyckelord: Tritordeum, kostfiber, fruktan, lutein, bröd, tempe och matgryn.

Table of contents

Introduction

Background

There will always be a demand from the industry in developing new products with good technological and nutritional traits at aim being both safe and produced in a sustainable way. A huge break trough when the first man-made cereal Triticale a cross between wheat and rye was developed; a human food crop with good grain quality as wheat and drought tolerance and high yield traits like rye. When breeders first created *Tritordeum* (a cross between durum wheat and wild barley) more than 30 years ago, they saw the same opportunities as Triticale, developing a food crop with advantage traits from two cereal species. The name *Tritordeum* has its origin from the names of its parental cereals ***Triti****cum turgidum* (durum wheat) and *H**ordeum** chilense* (wild barley). Barley have mostly been used as animal feed, only a comparable small amount in malting (beer industry), but from a nutritional point of view barley is interesting as a human food crop. It's a healthy cereal rich in dietary fibre, essential amino acids, vitamins and minerals, but of poor technological traits. Wheat on the other hand is the cereal traditionally used in bread industry due to its high content of gluten protein which gives good bread quality. These facts set the interest in Tritordeum due to genetically possibilities having nutritional traits like barley and technological traits similar to wheat. Already in the initial step Tritordeum showed good quality traits like long ears, large and rich grains and high protein content, even though the agronomic yield in the first Tritordeum lines was much lower compared to the parent durum wheat. Ever since then breeders have been working to improve Tritordeum to what it is today, a cereal who is *morphologically, agronomically, chemically and physic-chemically similar to wheat* and has a *yellow colour* and a *rich taste*. Today there are more than 250 primary lines of Tritordeum.

Tritordeum have during many years been bred by the Spanish research council (CSIC) and especially by the group lead by Professor Antonio Martín at the Institute of Sustainable Agriculture (IAS) in Cordoba. Since 2006 Agrasys, a Spanish plant breeding and Agri-Food company with its main office in the scientific park in Barcelona has the commercial rights of Tritordeum. The first line of Tritordeum registered was JB1 (HT1608) in 2008 under the registered trade mark Vivagran®. JB3 (HT 2218) is the Tritordeum line becoming available this year. Together with various partner companies Agrasys is working to incorporate *Tritordeum* as a *novel food ingredient for various cereal foods, e.g. breakfast cereals, breads, cereal bars and cereal cakes*. A quotation of Pilar Barceló, managing director of Agrasys: "*Vivagran® is a novel cereal which gives food companies the possibility to develop new natural products which are innovative and which have valuable nutritional properties, an ideal response to the requirements of today´s consumers and to the current tendencies of the market".*

The company Agrasys was created in 2005 as a "spin-off" from IAS and with Professor Antonio Martín as promoter. Agrasys perform breeding research in Cordoba and food-related research in Barcelona and other research centres. In general Agrasys uses advanced plant breeding and biotechnology techniques to produce novel crop varieties with added value. The techniques used in the creation of Tritordeum are cross pollination and embryo rescue, both methods included in *classical breeding*. Tritordeum is bred in a natural way.

Analyses of Tritordeum have been made by Agrasys and at the Universities of Cordoba and Barcelona to survey the compounds, giving its special traits. The project Tritordeum is a collaboration with Lantmännen as a part of Lantmännens work to evaluate new cereals and simultaneous help Agrasys in evaluating Tritordeum. This thesis is of importance for the future work of Lantmännen and Agrasys as a compilation of the work made of Tritordeum until today. During the years, many distinct lines of Tritordeum have been bred and evaluated. In this thesis five Tritordeum lines from the harvest 08/09 are to be evaluated. The expectation of these Tritordeum lines being rich in dietary fibre and hopefully rich in soluble dietary fibre is the reason for the dietary fibre analysis made in this thesis. Due to the nice yellow colour of Tritordeum which is expected to originate from the carotenoid lutein evaluations were made from analyses made for Agrasys. These hypotheses and the fact that the main group of nutrient in cereals is *carbohydrate* set the limits for this thesis; *in this thesis the carbohydrate quality and colour of Tritordeum will be evaluated.*

Aim

The aim of this thesis was to survey the chemical composition of the Tritordeum lines HT 354, HT 361, HT 437, HT 2218 (JB3) and HT 1608 (JB1), by new analyses and compiling results from analyses made for Agrasys.

Further the line HT 1608 was evaluated by applications; Breakfast cereals (further used in production of pearl Tritordeum and porridge), bread and tempe was produced in a laboratory scale production with the aim to find the ultimate end use of Tritordeum.

Literature review

Tritordeum

Hexaploid Tritordeum (2n= 6x= 42, $AABBH^{Ch}H^{Ch}$) is the amphiploid (a hybrid organism having a diploid set of chromosomes from each parental species) created by hybridization between the durum wheat *Triticum turgidum* (2n = 4x= 28, AABB) and wild barley *Hordeum chilense* (2n = 2x = 14, H^{Ch} H^{Ch}) (Martín and Sanchez, 1981). The female parent *Hordeum chilense* originating in Chile and Argentina is a self pollinated specie with varying properties, both morphologically and biochemically. Tritordeum got the cytoplasm from its female parent (Martín et al. 1998).

Tritordeum is proved to have higher water use efficiency and higher nitrogen uptake possibilities than wheat (Martín et al. 1998). Compared to durum wheat Tritordeum have a higher carotene pigment content which gives a strong yellow colour (Alvarez. 1998 and Atienza et al. 2007). The flour of hexaploid Tritordeum lines has exhibited viscoelastic properties similar to those of medium quality bread wheat (Alvarez and Martín. 1994) and the rheological qualities of Tritordeum show lower values than bread baked with premium wheat, but higher than those of durum wheat (Martín et al. 1998). Tritordeum have a high content of protein and arabinoxylan which both are compounds associated with god viscosity (Barcelo. Personal comunication).

History - Tritordeum breeding

Already in the beginning of the 70^{th} hybrids were obtained between barley (*Hordeum vulgare)* and wheat (*Triticum aestivum*, *T. dicoccum* and *T.* monoccum). The first main problem was the high frequency of sterility (Kruse. 1972). In 1977 the first Tritordeum line was created by *Professor Antonio Martín* and in 1981 he and research colleagues considered Tritordeum as a good starting point of a breeding program to develop the new cereal to a future food crop (Martín and Sanchez. 1981). Even though the new species showed good quality traits, the yield was much lower compared to the parent durum wheat. This is the main reason to why breeders have been working in more than 30 years to improve hexaploid Tritordeum to what it is today. Back crossing have been the main tool in the breeding programme (Guedes-Pinto, H. *et al*. 1996). One after another the agronomic traits have been improved; studies of hexaploid Tritordeum have shown favourable agronomic traits for biomass, number of spikelet by spike, seed size and protein crop (Guedes-Pinto. 1996). Tritordeum have also shown high fertility and good resistance to biotic, abiotic and heat & drought stress (Rubiales et al. 1995 and Martín. 2009). The hardest trait to obtain and because of that also the last trait that was expressed in hexaploid Tritordeum was to obtain free-threshing hexaploid Tritordeum (Gil-Humanes. 2009; Atienza. 2007) which gives the cereal major opportunities as a food crop.

Evaluating Tritordeum

Pre-knowledge of the five Tritordeum lines being analyzed in this thesis (Barcelo. Personal comunication; Agrasys. 2009):

JB 1 (HT 1608): This line have shown good agronomic performance and is expected to have medium to high bread making quality and medium-high content of the antioxidant lutein.

HT 361 (earlier named 2085): This line has shown medium agronomic performance and is expected to be of good bread making quality. Material from the harvest 2006 has shown high viscosity although not due to β-glucan but more likely to arabinoxylan. Analysis made by Lantmännen 2006 showed HT 2085 to have a gluten and dough quality of normal wheat (Lantmännen. 2006). The dietary fibre analysis from the harvest 05/06 (Andersson. 2006) showed a content of 13.6%. The corresponding result for barley flour was 14.0 %. The same analysis showed this line to also contained 0.2% β-glucan with the molecular weight (MW) 0.67×10^6. Corresponding result for barley flour was 2.1 % with MW 1.81×10^6.

JB 3 (2218): This line is the next to be out on the market and has shown very good agronomic performance in first year trial.

HT 354: This is a relatively new elite line of good agronomic performance but of not good bread making quality.

HT 437: This is also a relatively new elite line of good agronomic performance.

HT 621: This line is of medium agronomic performance, tall, and performs well in Northern Europe. More over it has a high lutein content. This line is not included among the five samples evaluated in this thesis, but included in the analyses made for Agrasys. Analysis made by Lantmännen 2006 showed HT 621 to have high protein content (19.5%) but being of low gluten and dough quality (Lantmännen. 2006).

Chemical

Investigation of spring and winter wheat showed average amount of the main components; 83% *carbohydrates* (calculated by difference), 13% *crude protein,* 2.5% *crude fat* and 1.6 % *ash* (Åman. 1988).

Carbohydrate is *starch, sugars and dietary fibre.* Due to EFSA two categories of carbohydrate can be differentiated according to nutritional effects; *Glycaemic carbohydrates* are digested and absorbed in the human small intestine, and *dietary fibre* which are non-digestible carbohydrates passing to the large intestine (EFSA. 2008).

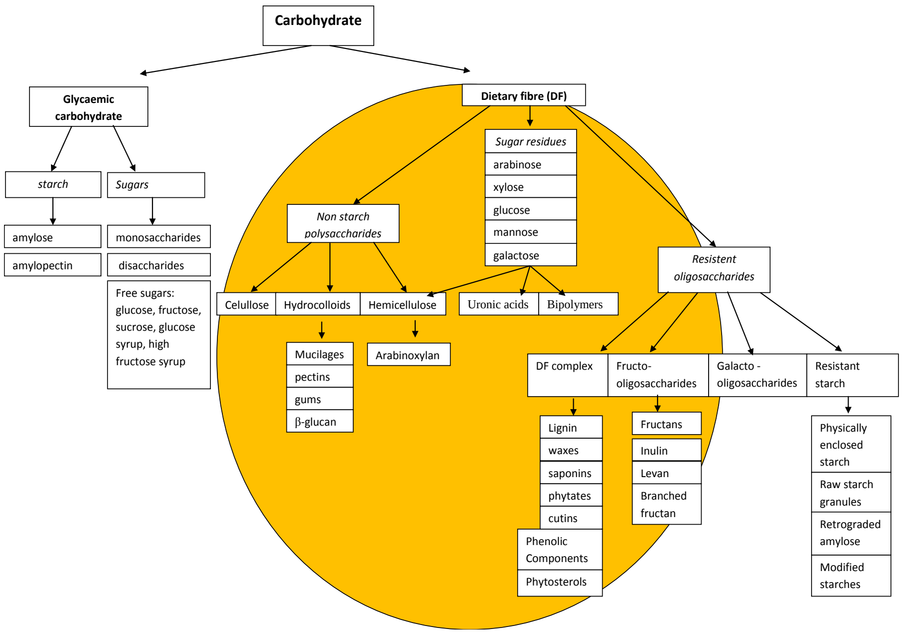


Figure i: The yellow circle is limiting the content in this thesis.

The recommended intake range of carbohydrate is 50-60 energy percent (E %) of the total food intake and the population goal in Sweden is 55 E% (Becker et al 2004; SLV. 2005). *The panel on dietetic products, nutrition and allergies* in the *European Commission* proposes a recommended intake range of 45-60 E% from carbohydrate (EFSA. 2008). In moment of writing the old range is still used for Swedish recommendations.

Starch

Starch is a digestible polysaccharide built of glucose molecules, whether a linear chain forming a helix (amylose) with (1, 4)-α-D-glycoside bonds or a branched molecule (amylopectin) with both (1, 6)-α-D-glycoside and (1, 4)-α-D-glycoside bonds (Abrahamsson *et* al. 2001; Stålberg. 2005). Normally starch from cereals contains 25-30 % amylose, but the proportion of amylose/amylopectin varies from crop to crop.

Sugars

The term "*sugars"* cover *monosaccharides* and *disaccharides*. The term "*added sugars"* includes the free sugars glucose, fructose and sucrose and moreover starch hydrolysates (glucose syrup and high-fructose syrup) and other isolated sugar preparations (EFSA. 2008).

There refined sugars should not exceed 10E % (Becker et al 2004; SLV. 2005; SLV webpage. 2010).

Dietary Fibre

The definition of dietary fibre in the European Union[1]:

For the purposes of this Directive "fibre" means carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories:

- *Edible carbohydrate polymers naturally occurring in the food as consumed;*
- *Edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence;*
- *Edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence.*

The main types of total dietary fibre are non-starch polysaccharides (cellulose, hemicelluloses, pectins, and other hydrocolloids such as gums, mucilages and β-glucans), resistant oligosaccharides (fructo-oligosaccharides, galacto-oligosaccharides) and resistant starch as well as lignin (when analysed with the dietary fibre polysaccharides). Viscous water soluble dietary fibres are for example β-glucan and pectin. Cellulose is not soluble in water and is therefore included in the term insoluble dietary fibre (EFSA. 2008).

For adults the intake of dietary fibre should be 25-30g per day (3g/MJ) (Becker et al 2004; SLV. 2005; SLV webpage. 2010). The panel on dietetic products, nutrition and allergies in the European Commission considers dietary fibre intake of 25 g/day to be adequate for adults (EFSA 2008.

Fructan

Fructan is polymers of fructose molecules and due to differences in the molecular structure they are classified into three main types; Inulin is a group of the linear fructans mainly consisting of (2→1) fructosyl- fructose linkage. Levan is mostly consisted of (2 →6) fructosyl-fructose linkage. Finally the branched group has both (2→1) and (2→6) fructosyl-fructose linkages. (Megazyme. 2008)

---

[1] Regulated by Commission Directive 2008/100/EC (The Commission of the European Communities. 2008)

Sugar residues

Sugar residues are units of polymers in cereals. They mainly consist of the 5 or 6 carbon monosaccharides *glucose, galactose, arabinose, mannose* and x*ylose*. In wheat glucose, xylose and arabinose constitute the largest amount among the sugar residues. (Åman.1988). the sugar residues are important as components in hemicelluloses e.g. arabinoxylan and β-glucan as well as in cellulose.

Arabinoxylan

Arabinoxylan is among the most important dietary fibre polymers in cereals; technologically due to its good viscosity, it strongly affect grain functionality during cereal processing like milling, brewing and bread making and the separation of gluten and starch (Izydorczyk and Dexter. 2007; Gebrunders et al. 2009). Arabinoxylan occurs in water extractable form (WEAX) and water unextractable form (WUAX) and as a result from this arabinoxylan is an important source of both soluble and insoluble dietary fibre. (Gebruers et al. 2009). Both soluble and insoluble dietary fibres have nutritional benefits and due to presence of phenolic moieties (a part or a functional group of a molecule) in the molecular structure of arabinoxylans they may also have some antioxidant traits (Izydorczyk and Dexter. 2007). Arabinoxylans consist of a linear chain backbone of ß-D-xylopyranosyl residues linked through (1→ 4) glycosidic linkages with α-L-arabinofuranosyl residues attached on the O-2 and/or O-3 positions of the xylose residues (Gebruers et al. 2009). This results in four distinct types of structural elements in the molecular structure of arabinoxylans (Izydorczyk and Dexter. 2007).

β- Glucan

Arabinoxylan and β-glucan are the major polymers in the endosperm cell walls of wheat, barley and rye (Saulnier and Quemener. 2009). β- glucan have in cohort and intervention studies been proved to reduce the cholesterol level in plasma (Truswell. 2002; Sundberg and Åman. 2006) and decrease the risk for coronary heart disease (CHD) (Truswell. 2002). The viscosity of the polysaccharide (1→3, 1→4) - β- glucan are important for the serum cholesterol lowering effect of oat and barley (Bourdon et al.. 1999; Sundberg et al.1995). The two factors influencing the viscosity of polysaccharide solutions are molecular weight distribution and concentration (Andersson et al. 2004). To retain the high molecular weight of (1→3, 1→4) - β- glucan it is important to keep the mixing and fermentation time as short as possible when baking to minimize the enzyme degradation. The addition of yeast and the baking do not affect the molecule weight of β- Glucan. (Andersson et al. 2004).

Recently EFSA presented a new health claim for beta-glucans: *"Regular consumption of β - glucans contributes to maintenance of normal blood cholesterol concentrations*" (EFSA (NDA). 2009). This health claim is allowed to be used for food contributing with minimum 3 g β -glucan per day from oat, oat bran, barley, barley bran or minimally processed β –glucan.

Dietary fibre complex

Non-carbohydrate components such as lignin, phenolic components, waxes, saponins, phytates, cutins and phytosterols closely associated with fibre polysaccharides are allowed to be included in the term dietary fibre *if* analyzed together with the carbohydrate. If isolated and added back to food they cannot be considered as dietary fibre (Boros and Åman. 2009).

Bran and germ are rich in phytochemicals (sterols, tocols, folates, phenolic acids and alkyl resorcinol) (Andersson et al. 2007) which may serve as antioxidants (NNR. 2004). The bran and germ are also rich in minerals and vitamins.

Minerals

Cereals are good sources of minerals such as selenium, phosphorous, copper, manganese, iron, potassium, magnesium, zinc, calcium (SLV. 2010).

Vitamins

Vitamins presented in cereals are folates, tocols, thiamine, niacin, riboflavin, B6 and folates. (SLV. 2010).

Phytic acid

In the aulueron e layer of cereals, phosphor is bound in phytic acid. This can lead to complex bindings with metal ions; the absorption of iron, zinc and calcium can be negatively affected (EFSA. 2008), but since wholegrain cereals also contain higher amounts of these minerals the body supply generally is still sufficient (EFSA. 2008). Phytic acid can be degraded by the enzyme phytase (naturally occurring in grains and can be synthesized by lactic acid bacteria). Important factors to attain high available mineral content in whole grain bread are fermentation time and a low pH value, e.g. sour dough fermentation at pH 5. 5 reduce the content of phytic acid in bread made by whole grain wheat with 70% (Mejborn et al. 2008; Poutanen et al. 2009; SLV.2010-01-05).

Antioxidants

The outer coat is rich in phenolic acids with antioxidant traits (ferulic acid, vanilic acid, p-coumaric acid, protocatechulich acid and cafeic acid). The phenolic compounds are biologically active substances (SLV. 2010). Vitamin E (tocols) and carotenoids have also antioxidant properties. (SLV. 2010). Carotenoids are an important compound acting as accessory pigment at the photosynthesis. They are also important at physiological levels in plants and as preventers to photo-oxidative damage. The interaction between nucleus and cytoplasm is important for the seed carotenoid accumulation. (Atienza et al. 2007).

Colour

Lutein and zeaxantin are both inactive pro vitamin A carotenoids included in the group of carotenoids having oxygen in addition to the hydrocarbon chain, so called xanthophylls (Brugård Konde et al. 2006). Lutein is absorbed in the small intestine and zeaxantin is mostly absorbed in the ileum. Both groups of xanthophylls enter the blood stream in its free and reesterified forms to enter the target cells. The metabolism is characterized by oxidation of the hydroxyl group (EFSA. 2009).

Lutein is the main carotenoid in wheat and durum. Cereal products contain in general about 2 µg Lutein per gram eatable part (Brugård Konde et al. 2006). In wheat, measured amounts of lutein is 8.4 µg/g (maximum values of 13.4µg/g)(Abdel-Aal and Young. 2009). Studies have shown that Tritordeum contain 6.6 µg/g, this is about 5 times more lutein compared to durum wheat (Atienza et al. 2007). Einkorn have measured values of 16.8- 33.6 µg/g (Abdel-Aal and Young. 2009).

Lutein is the only carotenoid in Tritordeum responsible for the yellow colour of the grain (Atienza et al. 2007). Lutein may play an important role as antioxidant within the grain. Carotenoids can only be synthesized *de novo* by plants, certain bacteria and fungi, therefore animals need to get them from the diet (Atienza et al. 2007). Due to the high carotene content in Tritordeum, *H. Chilense* is thought to have the strongest influence, but the interactions between both parents do also affect this unique trait of Tritordeum (Alvarez *et al.* 1998). The study by Atienza et al. has shown that Tritordeum have higher esterification degree of Lutein compared to durum wheat which affect carotenoid accumulation and stability. The high levels of lutein esterification together with the activity of biosynthetic genes may underline the explanation of high seed carotenoid content, but further multidisciplinary research is needed (Martín. 2007).

Health effects

Bran and germ of cereals included in the diet in combination with a good life style and physic activity are shown to have health benefits, this due to its content of phytochemicals, vitamins, minerals, antioxidants and unsaturated fatty acids (Kendall et al. 2009). Dietary fibre generally has beneficial properties including decreased intestinal transit time and increased stool bulk, fermentability by colonic micro flora and reduced level of total and/or LDL cholesterol in blood and/or reduced levels of post-prandial blood glucose and/or insulin (Boros and Åman. 2009). Viscous water soluble dietary fibre such as β-glucan and pectin can decrease blood glucose response and lower total LDL-cholesterol by interfering with digestion and absorption of glycaemic carbohydrates and cholesterol and/or bile acids (EFSA. 2008).

According to the report “Diet, nutrition and the prevention of chronic diseases” (WHO. 2003) a high intake of non starch polysaccharides is convincing to decrease the risk of obesity and probable decreasing risk of type 2 diabetes and CVD.

Fructo-oligosaccharides, often referred to as prebiotics, promote micro flora (often bifidobacteria and Lactobacilli) with production of short chain fatty acids such as acetate, propionate and butyrate (EFSA. 2008). These short chain fatty acids decrease the pH of the colonic content which stimulates colonic absorption of minerals like calcium and inhibits formation of potential co-carcinogens from bile acids (EFSA. 2008).

A risk factor to take notice about within cereals is heavy metals, especially when consuming large amounts of wholegrain. Cadmium is one of the heavy metals that we are exposed for in our daily life and cereals and vegetables are the food stuff with possible higher cadmium occurrence. Cadmium does mostly present in the bran fractions and as a result from this whole grain flour contains in general more Cadmium than white flour. The limit for Cadmium in food is 0.05-0.3 mg cadmium per kg food (EFSA). According to calculations made by SLV in Sweden 2009 the average intake of cadmium is below the limits decided by EFSA, also in a diet rich in whole grain (SLV. 2010).

Lutein has been associated with reduced incidence of cataract (Olmedilla et al. 2001), age-related macular degeneration (AMD) (Bone et al. 2001, Atienza et al. 2007), cancer (Michaud et al. 2000; Atienza et al. 2007) and cardiovascular diseases (Osganian et al. 2003). (Abdel-Aal and Young. 2009). Clinical studies have shown that lutein and zeaxantin constitute the macular pigments in the yellow spot (*macula lutea*) of the human retina. The two carotenoids have been suggested to improve visual activity (Olmedilla et al. 203) and severge harmful reactive oxygen species (Handelman. 2001). (Abdel-Aal and Young. 2009). Species rich in lutein have potential to develop grain-based high lutein content functional food which could boost the daily intake of lutein while supplying the nutritional needs (energy, protein, fibre, minerals and vitamins) (Abdel-Aal and Young. 2009). Tritordeum share this opportunity with Einkorn wheat (Atienza et al. 2007).

Carbohydrate quality indicators

Keyhole symbol: For labeling food with the keyhole symbol the minimum content of dietary fibre is 6 g for hard bread, bread mixes, breakfast cereals and müsli, cereal flour, flakes, grains and porridge. For soft bread the minimum content of dietary fibre is 5 g (SLV. 2009). SLV recommends choosing food labeled with keyhole symbol as one of their five consultations for a healthy diet (SLV. 2010).

Wholegrain: Whole grain contains the starchy endosperm, germ and bran; the grain may be milled, squished or similar, but the fractions have to be present in their original proportions for each cereal respectively. Included in the term cereal is wheat, spelt, rye, oat, barley, corn, millet, durra and other sorghum species (translation from SLV. 2009). Based on shown health effects SLV do recommend a daily intake of 75 g whole grain per 10MJ (2400 kcal) which correspond to 70 g and 90 g wholegrain per day for women and men respectively (NNR 2004; SLV.2010). In November 2009 SLV made a reform of the whole grain recommendation. The new recommendation is *"Primarily choose wholegrain when you eat pasta, rise, bread, breakfast cereals and gout*" (SLV. 2010)

GI: Glycaemic index (GI): Low GI product (<55) Glucose is often used as reference (glucose has GI 100). Dietary fibre, resistant starch and starch with a high rate of amylose/amylopectin give low GI, this because Glycaemic carbohydrates inside or from those types of polysaccharides is more difficult for the body to absorb. (SNF. 2009)

Materials

The following amount of the Tritordeum lines were sent by mail (Lantmännen. October 2009).

HT 354: 64g

HT361: 58g

HT 437: 58g

HT 2218: 64g

HT 1608: 1200 kg

All these lines were cultivated in the same field trial in Cordoba 2008-2009 and they have all been treated the same. The five lines were used as material in the analyses made in this thesis. The line HT 1608 was also used in the application part and for some further analyses.

Methods

Literature search

Most of the literature was found in databases in form of reviews. The databases most frequently used were *Web of knowledge, Science Direct* and *Scopus*. Examples of search words were: *Tritordeum, dietary fibre, Tritordeum AND Lutein, Carbohydrate quality, wholegrain, carbohydrate AND health*. Other literature was collected from Healthgrain, the Department of Food Science at SLU, Lantmännen Food R & D and Agrasys webpage.

Analyses

1000 kernel weight

1000 kernel weight was measured of the Tritordeum lines HT 354, HT 361, HT 437, HT 2218 and HT 1608 respectively.
Method: 100 grains were randomly collected by hand to be representing the correct grain size in respective line. The collected grains were afterwards weighed on a *Sartorius LC 6200S* and multiplied with 10.

Milling of Tritordeum HT 1608

28 kg Tritordeum line HT 1608 (08/09) was milled at Nord Mills in Uppsala, using a Bühler Mill MLU-202, Uzwil, Switzerland. The milling gave 8 different fractions; 6 brand flour labeled fraction 1-6 and 2 bran fractions A (bran) and B (short bran). The different fractions were weighted and the yield due to weight before milling was calculated. In 2006 when 15 kg HT 1608 was milled.

Ash

The five Tritordeum lines and the 8 fractions of HT 1608 were analyzed for ash content. Method: Lantmännen quality handbook (Lantmännen. 2005)

2-3 g sample was put into porcelain pot with known weight (double sample was made). Thereafter the samples were left in oven at 600°C over night. Percent ash was calculated according to following formula:

$$Ash\ (\%) = \frac{weight\ of\ sample\ and\ pot\ after\ heating - weight\ of\ pot}{weigh\ of\ only\ sample\ before\ heating} * 100$$

Water content

Water content was measured for the 8 fractions of Tritordeum HT 1608.
Method: Lantmännen quality handbook (Lantmännen. 2008).

10g sample was measured and put into porcelain pot with known weight (double sample was made). Thereafter the samples were left in incubation cupboard at 130°C for 1h. Percent water was calculated in two steps according to following formulas:

$$\text{Weight loss} = \frac{weight\ of\ sample\ and\ pot\ after\ heating - weight\ of\ pot}{weigh\ of\ only\ sample\ before\ heating} * 100$$

Water content (%) = 100 – weight loss

The first determination was made the same day as the milling and the second determination of water content was made 5 days after, just before package in closed plastic bags for storage.

Colour analysis

The eight milling fractions of HT 1608 were measured with Chroma Meter Minolta CR-300, San Diego, USA. Later whole grain flour from Tritordeum lines HT 354, HT 361, HT 437, HT 2218 and HT 1608 were also measured by the same instrument.

2 table spoons (msk) sample was measured and put into a special glass cup belonging to the Minolta measuring instrument (the analyses were made with double samples).

Method: two light sources send light from 45˚ towards the measuring point. The higher L-value the lighter is the sample. The higher a-value the more red is the sample, a negative value means green color. The higher b-value the more yellow is the sample.

Dietary fiber analysis
Total dietary fibre was determined by the Uppsala method as neutral sugar residues (arabinose, xylose, mannose, galactose, glucose), uronic acid residues, and Klason Lignin (Theander et al. 1995).

The grains from Tritordeum lines HT 354, HT 361, HT 437, HT 2218 and HT 1608 were milled in 1µm Gemotec 1090 sample mil, Tecator, Höganäs, Sweden, and thereafter in 0,5µm ultra centrifuge mill type ZM 1 Retsch, Haan, Germany and a (SLU. 2009)

The flour samples (double samples) were put in acetate buffer and were thereafter treated by thermostable α-amylase and amyloglucosidase, this to remove starch from the sample. Later the solubilized dietary fibre was precipitated with Ethanol (80%) while leaving low-molecular weight carbohydrates in solution. After acid hydrolysis of residue using H_2SO_4, neutral polysaccharides residue were determined as alditol acetates by gas-liquid chromatography, uronic acid residues were determined by colorimetry, and Klason lignin was determined gravimetrically as ash-free-acid-insoluble- residue. Thereafter the dietary fibre was calculated as the sum of nonstarch polysaccharide residues and Klason lignin. Arabinoxylan was calculated as the content of arabinose and xylose residus. Cellulose is the difference between the amount of glucose residue and β- glucan analysed by a separate method.

β- glucan analysis- enzymatic method
Megazyme mixed- linkage β- glucan assay kit was used. The method is based on enzymatic degradation of glucans with lichenase and β- glucosidase and the quantification is made by oxidase/peroxidase reagent (Gebruers et al. 2008). The molecular weight distribution of β-glucan was made by the method of Rimsten et al. 2003.

Fructan analysis Enzymatic/Spectrophotometric method.
The samples were extracted with hot water to dissolve fructan. Aliquots of extracts were treated with specific sucrase to hydrolyze sucrose to glucose and fructose. To hydrolyze the starch to glucose a mixture of pure starch degrading enzymes was added. All reducing sugars were reduced to sugar alcohols with alkaline borohydride. Next step was to neutralize the solution and remove excess borohydride; this was made by treatment of diluted acetic acid. The fructan was later hydrolyzed to fructose and glucose with purified fructanase and thereafter measured by the p-hydroxybenzoic acid hydrazide (PAHBAH) method for reducing sugars. (Megazyme. 2008; AOAC Method 999.03; AACC Method 32-32).

Collection and compilation of material

Wholegrain flour from Tritordeum line HT 1608 (08/09).

20 kg Tritordeum line HT 1608 was milled at Nord Mills in Uppsala, using impact miller (Börjesson and Hemesath. 2009). 20 kg wholegrain flour was sent to Unibake.

Tritordeum Tempe (Hemesath. 2009)

First trial

Material:

100g HT- 1608 (2009), polished 1min
100g HT- 1608 (2009), polished 2min
100g HT- 1608 (2009), polished 4min

Production:

Conditioning about 24h
Boiling: 10 minutes (weight after boiling: 215g, 221g, 227g)
Inoculation: 0.2g/100g
Incubation: 20h, 30ºC
Baking: about 15min, 200 ºC

Second trial

Material:

Cutted grains HT- 1608 (2009)
80% cutted grains HT- 1608 (2009), 15% puffs, 5% sugar
HT- 1608 (2009), polished 2min, boiled in vegetable stock

Production:

See first trial, the baking was prolonged to 90 min for the two variants with cutted grains. Baking 105ºC.

Lutein content

Analysis commissioned by Agrasys (Atienza et al. 2007). The analyses were carried out in duplicate. Two durum wheat and two bread wheat samples were used as control samples. 2.0 g Tritordeum flour was used for the analysis. The carotenoids were extracted in acetone (containing 0. 1% BTB). The solvent was evaporated under nitrogen steam and the pigment was dissolved in acetone. The samples were then stored in -30 ° C until high-performance liquid chromatography (HPLC) analysis of carotenoids due to well known method (Mínguez-Mosquera et al. 1993) within a week. HPLC analysis was made to assess the genetic variability of the carotenoids by doing detailed quantitative and qualitative

Lutein and Zeaxantin standards were obtained from mint and red pepper (Mínguez-Mosquera et al. 1993; Mínguez-Mosquera et al. 1992). To identify Lutein, Liquid Chromatography-Mass Spectrophotometry (LC-MS) was used.

Rheology

The grains were milled in Perten 3100 mill, Segeltorp, Sweden. Before analyzing the flour in farnograf and RVA analyses the moisture was regulated to 14%. Method used was ICC Standard No 110/1 (ICC. 1976).

Farinograph: The determinations were made using a Brabender Farinograph, Duisburg, Germany, due to method ICC Standard No 115/1 (ICC. 1972) (Barro. 2009). The recorded load-extension curve is used to assess general quality of flour and its response to improving agents.

RVA (Rapid visco analysis): Data from IATA, CSIC commissioned by Agrasys. The determinations were made by using a *Newport Rapid visco analyzer*. The method used was ICC Standard No 162 (ICC. 1996) Double sample was made.

Tritordeum line HT 1608 (08/09) was used in the application part which includes test production of breakfast cereals and Tempe. The breakfast cereals were cooked as Pearl Tritordeum and Porridge. The production occurred in the factory of Lantmännen in Järna, Sweden. Bread was made from the flour of Tritordeum in a oven normally used for housekeeping.

Applications

Polished grains

The grains were polished 1. 2 or 4 minutes respectively by a Streckel & Schrader, K.G. Hamburg- Wandsbek, Germany.

Cutting

Grains from Tritordeum line HT 1608 were cutted in the production of Lantmännen in Järna, Sweden. Same method was used as for normal production of pearl grains (Lantmännen Cerealia. 2009).

Breakfast Cereals

Steam preparation with Kärcher steam cleaner aggregate, Baden-Würtemberg, Germany, until the grains measured 100° C, this to moisture the grains before rolling. Test steaming on oat were made earlier to see that this steam preparation method should give correct moisturizing (Hemesath. 2009). The grains were rolled by a Bühler DNQB 800, Uzwil, Switzerland, into three different thickness; 0. 60 (both wholegrain and cutted grains), 0.90 (wholegrain) and 1.4mm (both wholegrain and polished grains). Water content was measured (see method water content).

Pearl Tritordeum

1. 4 mm polished whole grain breakfast cereal was cooked as pearl cereals and compared to pearl barley (matkorn), pearl oat (mathavre) and pearl wheat (matvete). About 5 dl water was added to 1 dl grain and boiling occurred due to product description (10 minutes) and the same

time was chosen for pearl Tritordeum. After boiling water was separated from grains and thereafter water content was measured to see water saturating grade.

Porridge

0. 6 mm whole grain breakfast cereals were used to make porridge. 10 cl breakfast cereals, 20 cl water and a pinch of salt was put together and boiled for 3 min.

Binding and breading

Tritordeum white flour was used as binding when making spinach stew: 1 table spoon oil, 2 table spoons flour and 40 cl milk were put together and boiled quickly. Tritordeum wholegrain flour was used as breading on flatfish, frying about 2 min at each side.

Bread

French roll was baked from: Tritordeum white flour (milling fractions 1-6), Tritordeum whole grain flour and wheat flour as reference.

<u>Recipe:</u>

15 cl water

10 g yeast

1 pinch of salt

1 cl oil

About 30 cl flour

Fermented 40 min in the baking tin. Baking at 225ºC in 10 min.

Results and discussion

1000 kernel weight, ash and starch

HT 354 had the largest and HT 361 the smallest seed size. HT 354 had the highest ash and starch content. HT 1608 had the lowest ash content and HT 437 had the lowest content of starch. See table 1. This put HT 354 in the light as a distinct line compared to the other lines.

Table 1. 1000 kernel weight, fresh weight ash content and starch content (%DM).

Sample	1000 kernel weight (g)	Ash(% Fresh weight)	Starch (% DM)
HT 354	44.9	1.79	64.4
HT 361	36.2	1.64	60.8
HT 437	40.5	1.61	60.2
HT 2218	39.0	1.55	61.5
HT 1608	38.0	1.70	63.4
Spring wheat	38.25	NA	NA
Durum wheat	42.15	NA	NA

Milling of Tritordeum line HT 1608 (08/09)

Result from milling Tritordeum HT 1608 showed a total yield of 76.8% which is lower than for 2006 where a yield of 95% was obtained. . see *figure 1*. Wheat have 70-75% yield of white flour, the rest is bran fractions (Hellström). The percent of white flour for Tritordeum was lower than for wheat in both millings, 34.5 and 39. 9% compared to 70-75%.

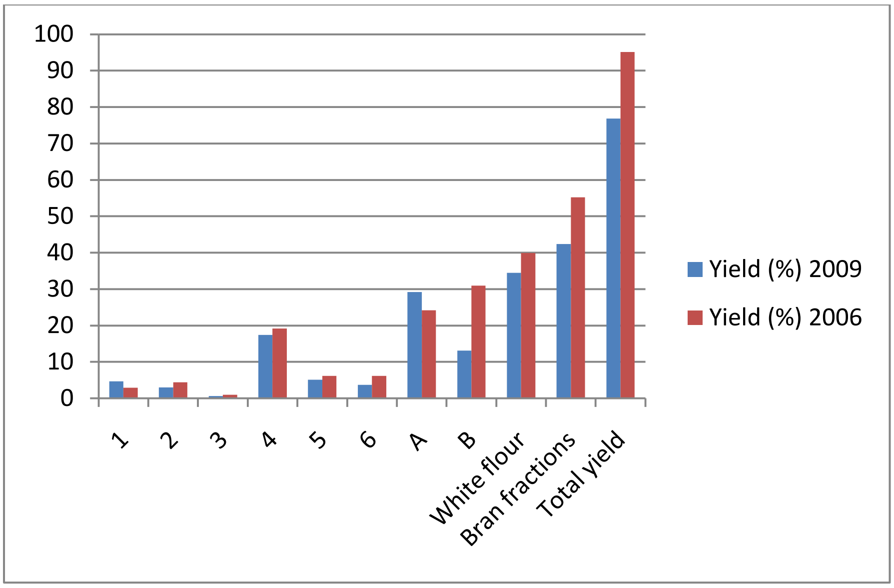


Figure 1. Diagram of milling 2009 compared to 2006.

Figure 2: Photo 1:Bühler Mill. Photo 2: Fraction 1-3. Photo 3: Fractions 4-6. Photo 4: The bag to the left is short bran and the bag to the right is bran fraction.

One possible explanation to why the yield is lower this year may be the higher moistening grade this milling. Almost one kilo was lost as empty milling (tomkörning) and 1.5 kg was lost as dust on the floor, due to mal function of the ventilation during milling. 0.2 kg was lost as rinsed hull. When looking at the results from the flour fractions of HT 1608 you can clearly see that the amount of ach fraction is following the way the Bühler Mill is treating the grains, see figure 3. The first roll is breaking the grain and turns it inside out. Fraction 1 is due to this treatment origin from the center of the grain. The first sieve is separating fraction 2 (continue on the left side of the mill to be milled by groove rolls) and fraction 4 (continue on the right side to be milled by smooth rolls. Due to this, fraction 4 is originating as the second fraction from the grain center (see figure 2). You can clearly see that the ash content is corresponding to the placing in the grain. The fact that bran fractions are rich in minerals is considered even in this thesis.

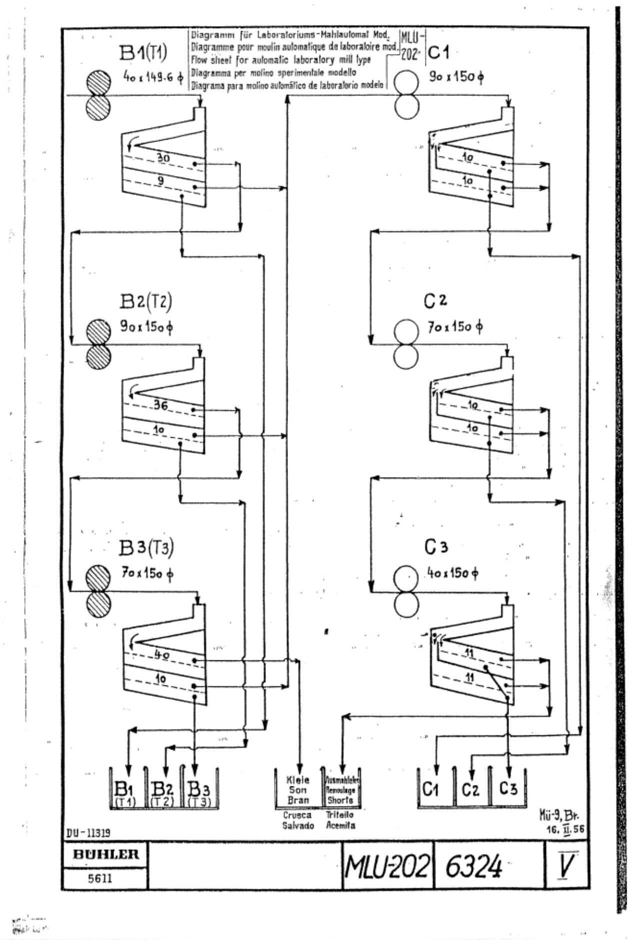


Figure 3: Diagram Bühler mill.

Ash

The bran (A) followed by the short bran (B) have the highest ash content. Among the white flour (1-6) fraction 6, fraction 5 and fraction 3 have the highest ash content (See *figure 4*). This is due to the fact that the minerals are situated in the outer layer of the grain.

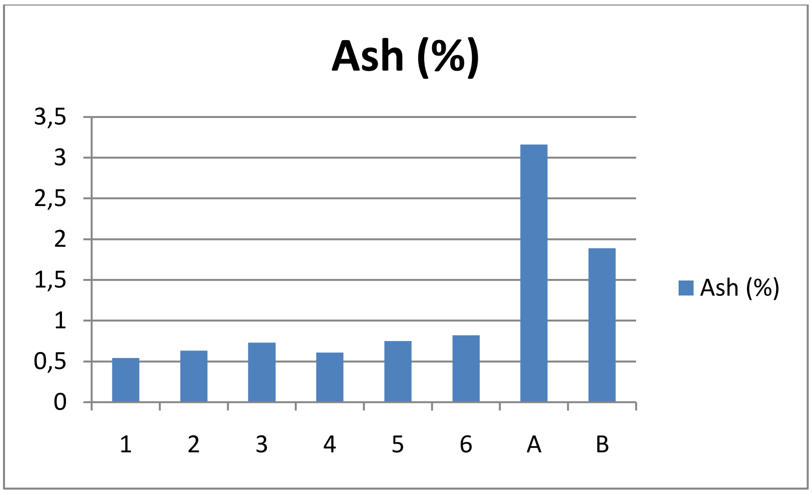


Figure 4. Diagram ash content (fresh weight) in the different fractions of Tritordeum HT 1608 (08/09).

Water content

The highest water content was measured for the bran fraction (A), but also the largest loss of water after 5 days. Fraction 4 contained second most water after 5 days storage (see figure 5). The samples were air dried to longer the storage ability and reduce the risk of mould during storage. The large water loss of the bran fraction (A) shows us that the bran initially contained more water than the other fractions, which also is an explanation to the larger yield. For future work a recommendation of longer moistening time is to give. This to make the water reach the centre of the grain and further to equilibrate the yield of the fractions.

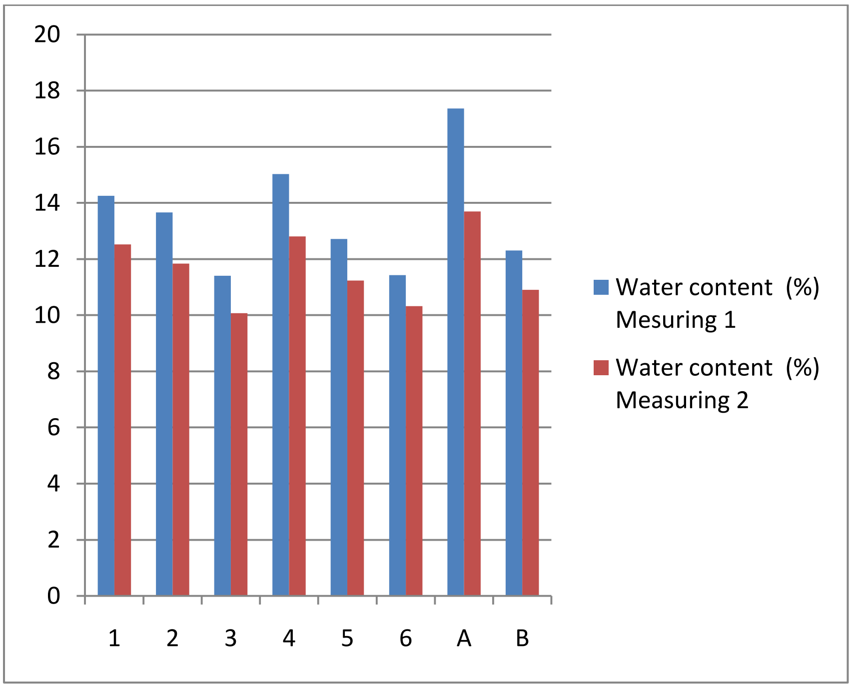


Figure 5. Diagram water content (%) in the different fractions of Tritordeum line HT 1608 (08/09)

Colour analysis

Fractions A (Bran) and B (feed seed) are darker (low L value, see figure 6) and more red (high a-value, see figure 7) than the white flour (fractions 1 to 6). The white flour has a more greenish color Fractions 1 to 6 have a stronger yellow color (b-value, see figure 8) compared to the bran fractions. Fraction 2 have the strongest yellow color and fraction 1 is the lightest, due to it is situated in the centre of the grain it is reasonable. Compared to pasta flour (measured 2006) the white flour (fraction 1-6) of HT 1608 both 2006 and 2009 had higher L value which mean the samples are lighter. According to the a-value, fractions 1-6 were more to the green than pasta flour. Fraction A and B were more to the red compared to pasta flour.

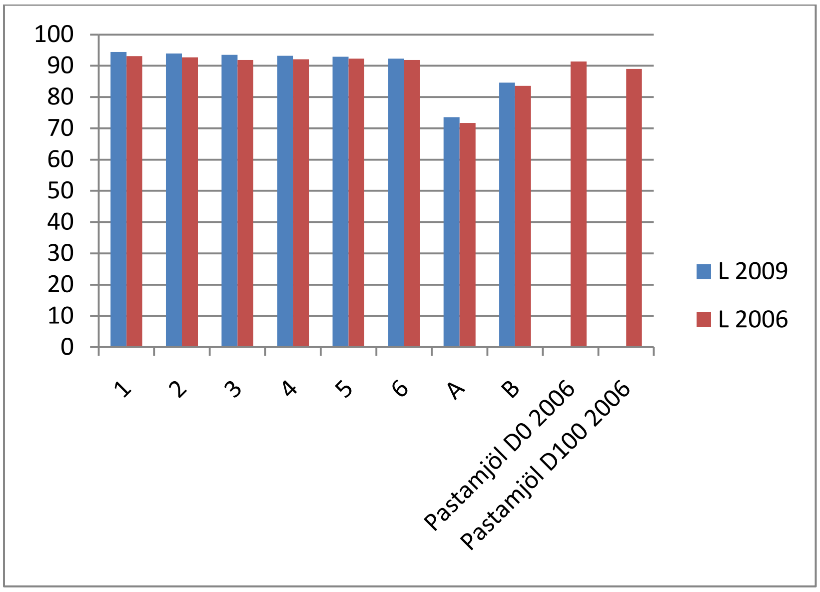


Figure 6: L-value

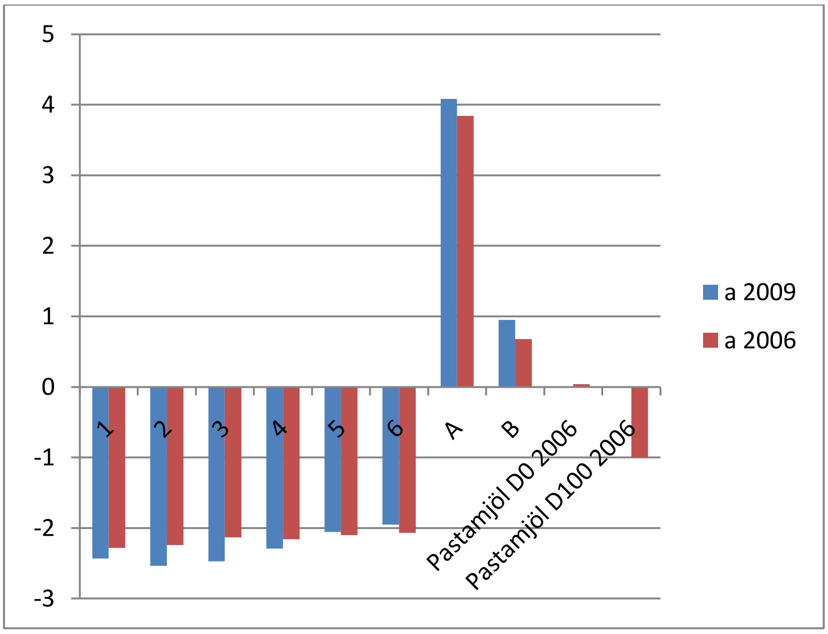


Figure 7: a-value

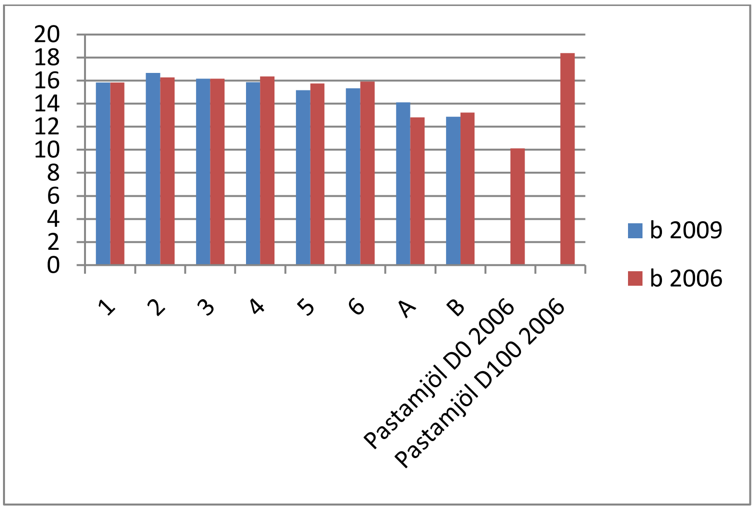


Figure 8: b-value

Due to the Tritordeum lines, HT 2218 was more similar to pasta flour due to the red-green range, but still more to the red than pasta flour. HT 354 is similar to D 0 and HT 1608 is similar to D 100. See figure 9.

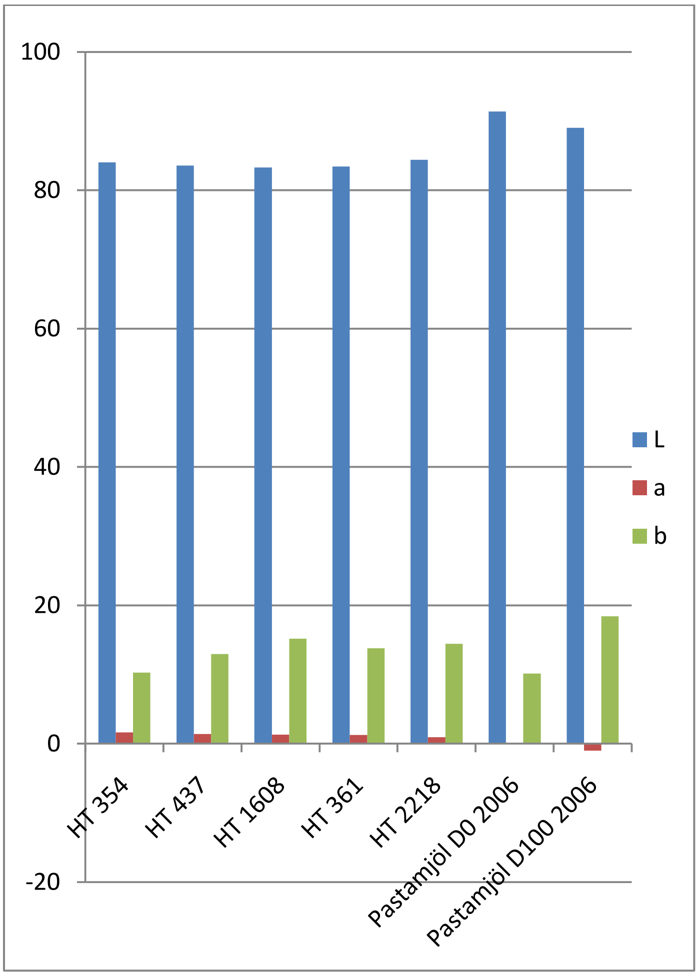


Figure 9: Colour of Tritordeum lines. L=lightness; a= redness; b= yellowness

Dietary fibre

Tritordeum contain more fructan, arabinoxylan, Klason lignin, uronic acid residues and total dietary fibre, than wheat (Åman. 1988). The amount of β-glucan was lower compared to wheat. The content of starch and cellulose are also lower in Tritordeum compared to wheat (see table 2).

Table 2. Content of dietary fibre components and total dietary fibre (% DM) in Tritordeum lines and control barley and wheat (Åman. 1988).

a. Analyzed by the Uppsala method
b. β-glucan measured by enzymatic method
c. Arabinoxylan is calculated as the sum of arabinose and xylose.
d. Cellulose is calculated as the difference of glucose - β-glucan
e. Fructan measured by enzymatic method
f. The sum of the results from the Uppsala method and fructan.

	HT 354	HT 361	HT 437	HT 2218	HT 1608	Tritordeum lines (average)	Control barley	Wheat (Åman. 1988)
Arabinose [a]	2.9	2.7	2.7	2.8	2.8	2.7	2.0	2.2
xylose [a]	4.9	4.3	4.2	4.3	4.2	4.4	4.8	3,5
mannose [a]	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.3
galactose [a]	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.5
glucose [a]	2.7	2.5	2.5	2.5	2.8	2.6	7.9	3.4
total sugar residues [a]	11.3	10.2	10.1	10.4	10.5	10.4	15,4	9.9
Klason lignin [a]	1.4	1.5	1.5	1.5	1.5	1.5	1.9	0.8
Uronic acid residues [a]	0.5	0.5	0.4	0.4	0.5	0.5	0.4	0.4
β-glucan [b]	0.6	0.6	0.6	0.7	0.7	0.6	3.4	0.8
Arabinoxylan [c]	7.8	7.0	6.9	7.1	7.0	7.2	6.6	6.0
Cellulose [d]	2.1	2.1	1.9	1.8	2.1	2.0	5.3	2.5
Fructan [e]	1.3	2.1	1.8	1.8	2.5	1.9	NA	0.8
total dietary fibre [f]	14.5	14.3	13.8	14.1	15.0	14.3	17.6	12.8

Even though the dietary fibre analysis showed that these Tritordeum lines did not contain as high amount of dietary fibre as was expected, the limit is still higher than in wheat. Agrasys currently is working to explore a large genetic diversity in germ plasma of Tritordeum to produce varieties that with improved agronomic yield and further functional properties as a value-added food ingredients, there among expectations in creating a Tritordeum line rich in dietary fibre, hopefully soluble dietary fibre

Tritordeum contains more arabinoxylan than barley, see figure 10. This is an explanation to the good viscosity and bread making quality of Tritordeum. Tritordeum do also contain more uronic acid residues compared to barley.

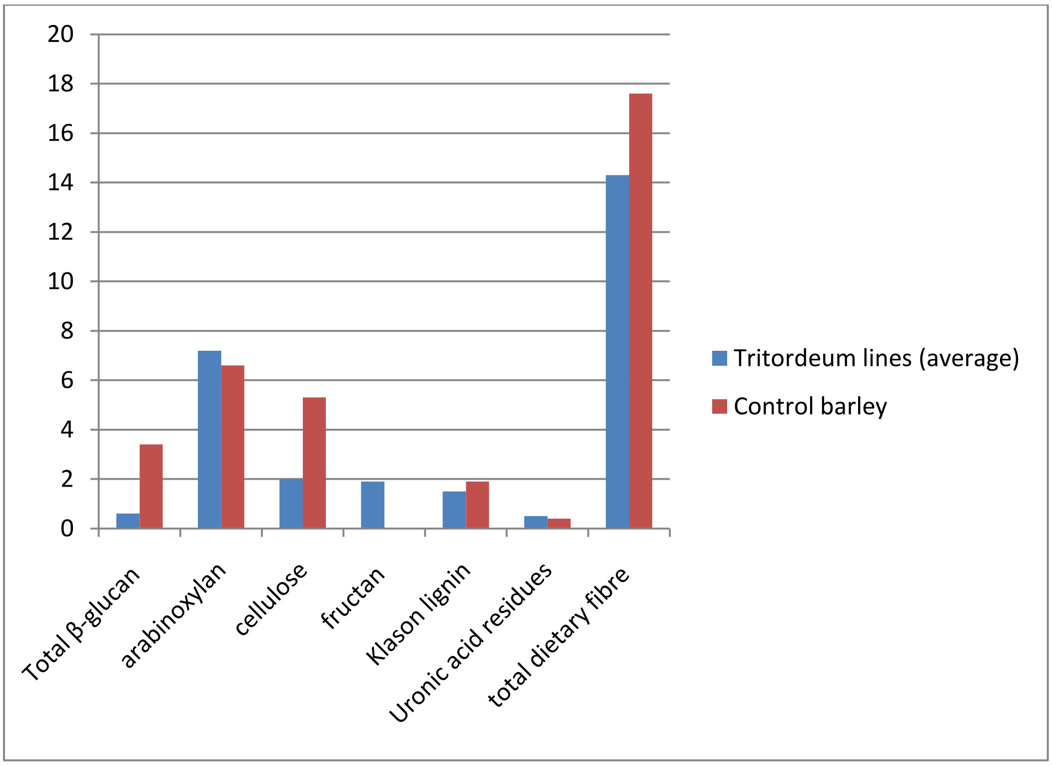


Figure 10. Diagram of total β-glucan, arabinoxylan, cellulose, fructan and total dietary fibre in Tritordeum and control barley.

Application Part

Breakfast Cereals

Breakfast Cereals: The breakfast cereals became yellow and nice and have a nice, round and rich taste. The sample of 0. 6 mm wholegrain breakfast cereal had higher water content (10.92%) compared to the rest of the samples (average 9.7%). One possible reason to this can be larger amount of this sample and because of that a thicker layer when air drying. The same sample did miss an average result of water content because a small amount of one of the double samples was lost by accident.

Breakfast cereals of Tritordeum were used to make porridge and pearl Tritordeum.

Porridge

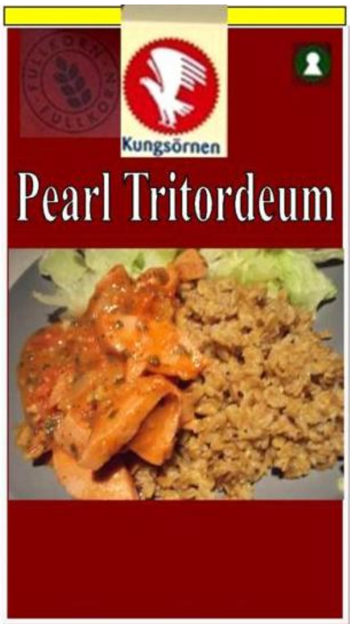
The porridge present a nice yellow colour (see figure 11) and a round and rich taste. The porridge made of cutted grains reminds about fibre enriched oatmeal porridge. The highest more value of Tritordeum porridge, as I see it, is the aromatic taste. Further the knowledge that Tritordeum contain lutein and fructan may increase the consumer interest in this breakfast product as healthy according to antioxidant and prebiotic properties.

Figure 11. Left: porridge made of 0. 6 mm wholegrain. Right: Porridge made of cutted 0. 6 mm wholegrain

Pearl Tritordeum

1. 4 mm polished breakfast cereal was used to make pearl cereal (matgryn). Pearl Tritordeum was compared to existing pearl grains, se figure 12. The consistence of Tritordeum was similar to pearl wheat while the colour was more similar to pearl barley.

Figure 12: Pearl cereals, from the left Pearl wheat, pearl barley, pearl oat and pearl Tritordeum



Pearl Tritordeum is allowed to be labeled with the keyhole symbol due to the fact that the content of dietary fibre is 14.3 % which exceed the minimum level of dietary fibre that is 6% in flakes, grains and breakfast cereals. Pearl Tritordeum is also a wholegrain product and may therefore use the wholegrain symbol. The yellow color chosen is according to the colour of Tritordeum with the sub purpose to be known among the consumers as lutein which as an ingredient in the daily food may have functional properties like reduced incidence of cataract, age-related macular degeneration (AMD), reduced incidence of breast cancer and cardiovascular diseases. Beside these physiological traits Pearl Tritordeum did present a nice taste and structure which make it interesting as an alternative to rise. Whether Pearl Tritordeum would be a low GI product is a question to think about *if* decision to produce this special product is made. This due to the fact that GI measuring is a procedure including a test group of ten healthy persons consuming 10- 50g carbohydrates from the product after an overnight fast and thereafter the blood glucose level will be measured during 2 hours post-prandial in 15-30 minutes interval. The blood samples are thereafter used to plot a blood sugar response curve for two hours period. The area under the curve is calculated as the total rise in blood glucose level after eaten the test food. GI rate (%) is calculated by dividing the area under the curve for the test food with the area under the curve for reference food. Thereafter multiply with 100. White bread or glucose is often used as reference food. The fact that Pearl Tritordeum is a whole grain product rich in dietary fibre the possibility in being a low GI product has reason.

Other possible application area for Tritordeum wholegrain would be "AXA Fibre Eyes".

Bread

The bread baked of Tritordeum white flour was strongly yellow, almost like bread baked with saffron. This bread was softer than wheat bread and also more elastic. The taste was sweeter than wheat bread. The wholegrain bread was very tasty with a rich, sweet and aromatic taste and aroma. The wholegrain bread was very soft and elastic and the colour was brown almost like syrup bread (See figure 13 & 14).

Figure 13: From the left: wheat flour, Tritordeum white flour, Tritordeum wholegrain flour.

Figure 14: left photo: cutting area of wholegrain Tritordeum bread (top), white Tritordeum (centre) and wheat bread (botton). Right photo: compared size of wheat bread, white Tritordeum and wholegrain Tritordeum.

The relation between dough and bread volume is similar when comparing wheat flour and Tritordeum white flour. The explanation to why the bread made of Tritordeum white flour has bigger volume has its answer in that the dough of Tritordeum was stickier and more flour was added to get the dough smooth. As a result from this the wheat bread became stickier than Tritordeum bread, which is pointed as an advantage for Tritordeum. In a try to bake syrup cakes (kolakakor) the result was that the cakes made of Tritordeum became stickier and more buttery than the cakes made of wheat flour when the same amount of flour was added. On the other side Tritordeum cakes presented a really nice taste and an attractive texture due to its stickiness. If more flour would be added probably the structure would be more similar to wheat flour, but this is to be further investigated.

Binding and breading

When using Tritordeum white flour as binding and breading (See figure 15) the fish got a nice yellow colour and both the fish and the spinach had a taste similar or richer than the same food made by wheat.

Figure 15: Tritordeum white flour as binding in the spinach and Tritordeum wholegrain flour as breading on fried flatfish.

Collection and compilation of Data

Tempe

First trial:

The mycelia was better developed when more polished material. Tritordeum- tempe cookies were not as stabile as Tempe made of pearl barley or pearl oat. See figure 16.

polished 1min polished 2min polished 4min

Fig 16: mycelia of Tempe (Hemesath. 2009)

Second trial:

The mycelia was developed

Tritordeum- breakfast cereal Tempe became to hard and crumbled. See figure 17.

Figure 17. Tempe from the second trial, from left to right: Tempe from cutted grains with puffs; Tempe from cutted grains; Tempe from grains polished 2 min and boiled in broth; Breakfast cereal Tempe from cutted grains with puffs; Breakfast cereal Tempe from cutted grains (Hemesath. 2009).

Lutein

The result indicates that Tritordeum contains in average ≥ 10 times more Lutein than bread wheat and ≥ 7 times more Lutein than durum wheat. The five lines contain in average 4.5% lutein and HT 1608 (08/09) 3.4%. Tritordeum contain esterified lutein to a higher grade than bread wheat. Lutein in durum wheat is not esterified at all. See figure 18.

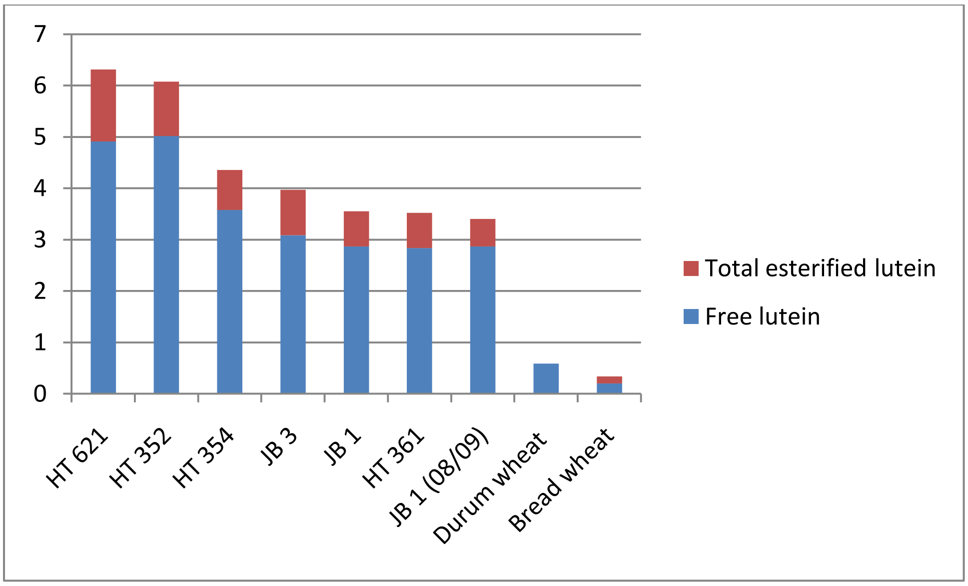


Figure 18: Total esterified and free lutein in Tritordeum lines compared to bread wheat and durum wheat. (Data from IATA, CSIC commissioned by Agrasys).

Even though the Lutein content in Tritordeum is relatively high, still the content in wheat is higher. On the other hand the degree of free and esterified Lutein in Tritordeum is high and because lutein enter the blood stream in its esterified form this is an advantage for Tritordeum. There seem to be a reversed connection with the b-value (yellowness) and the lutein content. These analyses indicate that it is possible to believe that lighter sample (L-value) can have a connection to high lutein content.

New opinion by EFSA about lutein as antioxidant within the 13.1 list, published 1th of October 2009:

2.2. Antioxidant function of lutein (ID 146)

The claimed effect is "promotes the antioxidant function of lutein".

The Panel assumes that the target population is the general population.

The Panel notes that no evidence is provided to establish that having antioxidant activity per se is beneficial to human health.

*The Panel considers that the benefit to human health of the promotion of the antioxidant function of lutein is **unknown**.*

Rheological properties

As shown by RVA analysis the durum wheats (Simeto and Vitron) have the highest final viscosity followed by the bread wheats (Jerezano and Yecora). Among the Tritordeum lines analyzed in this thesis HT 361 has the highest and HT 354 the lowest viscosity, see figure 19.

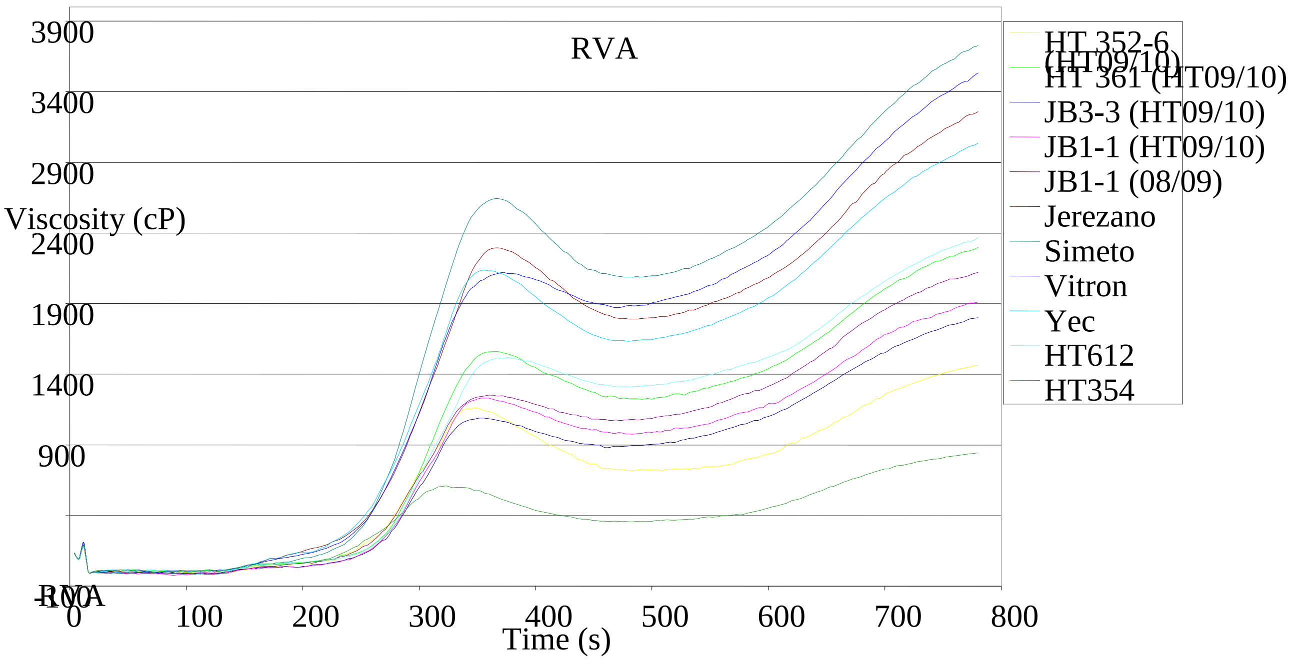


Figure 19: Diagram: Rapid visco-analyzer (RVA) showing the viscosity for Tritordeum lines compared to bread wheats (Jerezano and Yecora) and durum wheats (simeto and vitron) (Data from IATA, CSIC commissioned by Agrasys..2010)

Conclusion

Line HT 354 is differing from the other lines; higher ash content, larger grains and high lutein content, but low viscosity and bad bread making traits.

HT 1608 has small seed size but high ash content. Good viscosity but low amount of phenolics and low lutein content.

HT 361 (earlier 2085) has even smaller seed size, but among these 5 lines the best viscosity. This line has medium ash and low amounts of lutein and phenolics.

HT 437 had the 2nd biggest seed size 2nd lowest ash content.

HT 2218 (JB3) showed colour more similar to pasta flour *than the others*, highest amount of phenolics, but lowest ash content, and medium 1000 kernel weight. Among these 5 lines JB3 has the 2nd highest lutein content and 2nd lowest viscosity.

Due to these analyses there seems to be a connection between high lutein content and low viscosity (RVA analysis).

Agrasys currently work to analyze; raw macronutrients: protein, starch microscope picture of granule size, minerals (Cu, Fe, Mn, Zn, Pb, Cd, Hg, As, Selenium), vitamins (tocols and folates).

Future work with request from Agrasys would be to in a quantitative way measure if colour in flour, dough or baked goods are due to lutein. To measure lipoxygenase (lox) activity, to more in detail study the ratio of amylase/amylopectin in starch, to sketch amino acid profile, to measure soluble sugars or other components related to flavour, and to measure Glycaemic index for specific products. The lines HT 621 and HT 352 are of interest for future evaluations due to high lutein content.

Tritordeum have been used for food production and do not need to be controlled by the novel food regulation due to the fact that breeding practices used for Tritordeum can be regarded to be traditional breeding. Like all cereals Tritordeum have to fulfill the rules in the General Food Law regulation (EC) 178/2002 (The European Parliament and the Council of the European Union. 2002; The General Food Regulations 2004 in the UK) which in short means that Tritordeum don't have evidence in being injurious to health. (Jones, C. 2007).

Tritordeum have until today made a long journey on the breeding stage to become what it is today, but when comes to processing and developing cereal products and finding out new end uses for Tritordeum the journey just have started. Due to international collaboration Tritordeum have enormous opportunities being Triticale´s brother cereal, not as to animal feed but as a cereal food ingredient for human consumption.

Curiosa

Phenolics

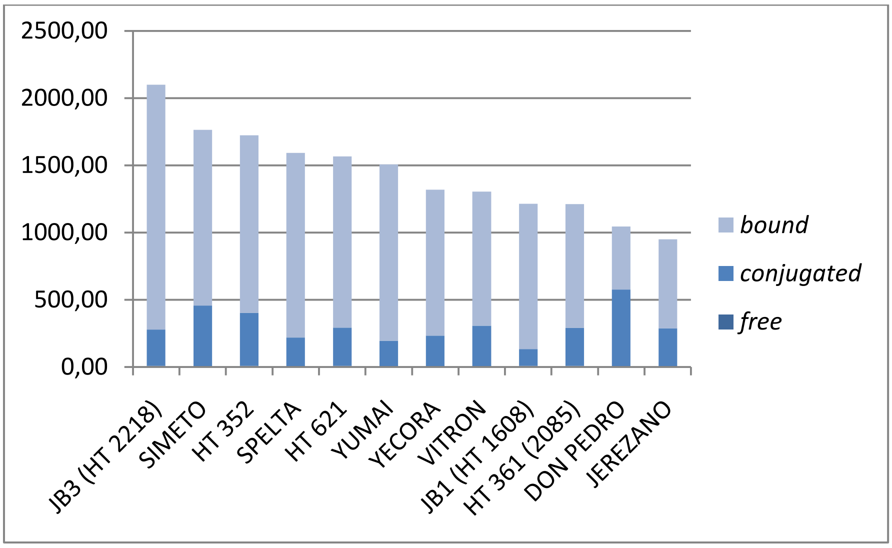


Figure 20: phenolics in Tritordeum lines and wheat.

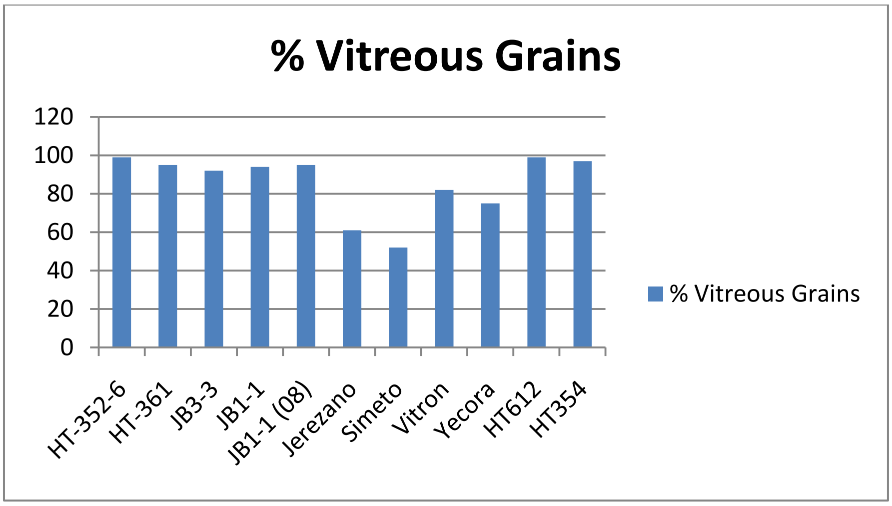


Figure 21: Percent vitreous grains in Tritordeum lines and wheat.

Acknowledgment

Thanks to my supervisors Ingmar Börjesson and Per Åman who have helped me to during the work. I want to give a special thank you to Anna Reumark for supporting and inspiration. Thanks to everybody who has helped me with practical moments: Gunnel Fransson, Silvana Hemesath, Maria Hellström, Anders Karlsson, Vanja Jansson, Csilla Nemeth. Thanks to Susanne Bryngelsson, Hanna Isaksson, Viola Adamsson, Pilar Barcelo and Antony Martín for personal communication. Thank you to my friends and my family who have listened to all my minds about this thesis.

References

AACC Method 32-32.01 Measurement of Total Fructan in Foods by an Enzymatic/Spectrophotometric Method

Abrahamsson, L., Andersson, I., Åberg-Ashan, K., Becker, W., Göranzon, H., Hagren, B., Håglin, L., Jonsson, I., Jonsson, L. and Nilsson, G. Liber AB. 2001. Näringslära för högskolan.

Abdel-Aal. E. S. M. And Young. J.C. 2009. Chapter 8 Carotenoids, HEALTHGRAIN Methods.

Agrasys (www.agrasys.es, 2009-10-01)

Alvarez, J.B. and Martín, L.M. 1994. The amphiploid Hordeum chilense × Triticum aestivum. Evaluation of its grain, flour and dough characteristics, and comparison with those of other related cereals.

Alvarez, J.B., Martín, L.M., Rogers, W.J. and Martín, A. 1995. Relationship between the endosperm storage proteins composition ant the bread making quality in hexaploid Tritordeum.

Alvarez, J.B., Martín, L.M. and Martín, A. 1998. Genetic variation of carotenoid pigment in the ampiploid Hordeum chilense × Triticum turgidum

Alvarez, J.B., Campos, L.A.C., Martín, A., Sillero, J.A. and Martín, L.M. 1999. Genetic analysis of Prolamins synthesized by the H^{ch} genome and their effects on gluten strength in hexaploid Tritordeum.

Andersson, A. A. M., Armö, E., Grangeon, E., Fredriksson, H., Andersson, R. and Åman, P. 2004. Molecular weight and structure units of (1/3, 1/4)-b-glucan in dough and bread made from hull-less barley milling fractions

Andersson, A. 2006. Results of dietary fibre and β-glucan analysis of barley and oat samples. Swedish University of Agricultural Sciences. Department of Food Science.

Andersson, A., Tengblad, S., Karlström, B., Kamal-Eldin, A., Landberg, R., Basu, S., Åman, P. and Vessby, B. 2007. Whole-Grain Foods Do Not Affect Insulin Sensitivity or Markers of Lipid Peroxidation and Inflammation in Healthy, Moderately Overweight Subjects.

AOAC Official Method 999.03 Measurement of Total Fructan in Foods. Enzymatic/Spectrophotometric Method First Action 1999.

Atienza, S.G., Ramírez, C.M., Hernández, P. and Martín, A. 2003. Chromosomal location of genes for carotenoid pigments in Hordeum chilense.

Atienza, S.G., Ballesteros, J., Martín, A. and Hornero-Méndez, D. 2007. Genetic variability of Carotenoid concentration and degree of esterification among Tritordeum (×Tritordeum Ascherson et Graebner) and durum wheat accessions.

Atienza, S.G., Martín, A.C. and Martín, A. 2007. Introgression of wheat chromosome 2D and 5D into Tritordeum leads to free-threshing habit.

Ballesteros, J., Ramirez, M.C., Martínez, C., Barro, F. and Martín, A. 2002. Bread-making quality in hexaploid Tritordeum with substitutions involving chromosome 1D.

Barcelo, P. e-mail.

Barro, F., Barceló, P., Lazzeri, P.A., Shewry, P.R., Martín, A. and Ballesteros, J. 2002. Functional properties and agronomic performance of transgenic Tritordeum expressing High Molecular Weight Glutenin subunit genes 1Ax1 and 1Dx5

Barro, F. 2009. Resultados analíticos de muestras de trigo. Análisis farinografíco; Análisis viscométrico.

Becker, W., Lyhne, N., Pedersen, A.N., Aro, A., Fogelholm, M., Þhórsdottir, I., Alexander, J., Anderssen, S.A., Meltzer, H.M. and Pedersen, J.I. 2004. Nordic Nutrition Recommendations 2004- integrating nutrition and physical activity.

Bone, R. A. Landrum, J. T., Mayne, S. T., Gomez, C. M., Tibor, S. E., and Twaroska, E. E. 2001. Macular pigment in donor eyes with and without AMD: a case control study. Investing Ophthalmol Vis. Sci. 42:235-240.

Boros, D., and Åman, P. 2009. Total dietary fibre. Healthgrain methods: Analysis of Bioactive Components in Small Grain Cereals. Chapter 13.

Börjesson, I. Lantmännen Food R & D. 2009,

Börjesson, I. and Hemesath, S. 2009. Milling at Nord Mills.

CCNFSDU (The Codex Committee on Nutrition and Foods for Special Dietary Uses). 2008. Report of the 30th session**.** http://www.jhnfa.org/co08-11.pdf

EFSA. 2008. Draft scientific opinion. Dietary reference values for carbohydrates and dietary fibre. *The EFSA Journal (2009) xxx, 1-76.*

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of health claims related to beta-glucans and maintenance of normal blood cholesterol concentrations (ID 754, 755, 757, 801, 1465, 2934) and maintenance or achievement of a normal body weight (ID 820, 823) pursuant to Article 13 (1) of Regulation (EC) No 1924/2006 on a request from the European Comission EFSA Journal 2009; 7: 1254.

EFSA. 2009. Safety of use of colouring agents in animal nutrition. Part III: β-apo-8’-carotenal, ethyl ester of β-apo-8’-carotenoic acid, lutein, zeaxanthin and concluding remarks.

Gebruers, K., Courtin, C. M., and Delcour, J. A. 2009. Quantification of arabinoxylans and their degree of branching using gas chromatography. Healthgrain methods: analysis of Bioactive Components in Small Grain Cereals. Chapter 14

Gebruers, K., Dornez, E., Boros, D., Fras', A., Dynkowska, W., Bedo", Z., Rakzegi, M., Delcour, J.A., Courtin, C.M. 2008. Variation in the content of dietary fibre and components thereof in wheats in the HEALTH GRAIN diversity screen. J. gric. Food Chem. 2008, 56, 9740-9749.

Gil-Humanes, J., Pistón, F., Martín, A. and Barro, F. (2009). Comparative genomic analysis and expression of the APETALA2-like genes from barley, wheat, and barley-wheat amphiploid.

Brugård Konde, Å., Staffas, A., Dahl, P., and Becker, W. Rapport 12/96. Karotenoider i livsmedel i Sverige.

Guedes-Pinto Henrique, Norman Darvey, and Valdemar P. 1996. Triticale: today and tomorrow. Carnidec. ISBN 0-7923-4212-7. Dordrecht; Kluwer Academic Publishers, c1996, Engelska xi, 898 p. 56-67 (Tritordeum: Triticale´s new brother cereal).

Hellström, M. Lantmännen Mills, Uppsala. 2009.

Hemesath, S. 2009, Lantmännen food R & D.

Handelman, G. J. 2001. The evolving role of carotenoids in human biochemistry. Nutrition 17: 818-822.

ICC Standard No 110/1. 1976. http://www.icc.or.at/methods3.php 2010-02-04. 04:17pm

ICC STANDARD No. 115/1. 1972. http://www.icc.or.at/methods3.php 2010-02-04. 04:17pm.

ICC Standard No 162. 1996. http://www.icc.or.at/methods3.php 2010-02-04. 04:17pm

Izydorczyk M.S. and J.E. Dexter. 2007. Barley b-glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products–a Review.

Jones, C. Dr. E-mail to Paul Lazzeri sent Monday, June 18, 2007 11:35. Chris.Jones@foodstandards.gsi.gov.uk

Kendall, C.W.C., Esfahani, A. and Jenkins, D.J.A. 2009. The link between dietary fibre and human health

Kruse, A. 1972. Hordeum x Triticum hybrids

Lantmännen. 2005. *Lantmännen quality hand book*. Kvalitetshandbok (K5.400. 6.3). Analysera, dokument 24.

Lantmännen. 2006. Tritordeum PETRI Project; Milling and fractioning. July 2006.

Lantmännen. 2008. *Lantmännen quality hand book*. Kvalitetshandbok (K5.400. 6.3). Analysera, dokument 1

Lantmännen. October 2009. Mail sent to: Börjesson, I., Järna. From Martín, A., Barcelona.

Lantmännen Cerealia. 2009. Process engineer Anders Karlsson, Järna.

León, E., Marín, S., Giménez, M.J., Piston, F., Rodriguez, M., Shewry, P. and Barro, F. 2009. Mixing properties and dough functionality of transgenic lines of a commercial Wheat cultivar expressing the 1Ax1, 1Dx5 and 1Dy10 HMW glutenin subunit genes.

Martín, A. and Sanchez-Monge, E. 1981. Cytology and morphology of the amphiploid Hordeum chilense × Triticum turgidum conv durum.

Martín, A., Alvarez, J.B., Martín, L.M., Barro, F. and Ballesteros, J. (1998). The development of Tritordeum; a novel cereal for food processing.

Martín, A. Agrasys. 2009.

Megazyme. 2008. Fructan assay procedure for the measurement of Fructo-oligosaccharides and fructan polysaccharide. AOAC method 999.03; AACC Method 32.32.

Michaud, D. S., Freskanich, D., Rimm, E. B., Colditz, G. A., Speizer, F. E. willett, W. C., and Giovannucci, E. 2000. Intake of specific carotenoids and risk of lung cancer in two prospective US cohorts. Am. J. Clin. Nutr. 72:990-997.

Mínguez-Mosquera, M. I.; Gandul-Rojas, B.; Gallardo-Guerrero, L. Rapid method of quantification of chlorophylls and carotenoids in virgin olive oil by high-performance liquid chromatography. *J. Agric. Food Chem.* **1992**, *40*, 60-63.

Mínguez-Mosquera, M. I.; Hornero-Méndez, D. Separation and Quantification of the carotenoid pigments in red peppers (*Capsicum Annuum* L.), paprika and oleoresin by reversed-phase HPLC. *J. Agric. Food Chem.* **1993**, *41*. 1616-1620.

Nordic Nutrition recommendations 2004, chapter 6 Food-based dietary Guidlines

Olmedilla, B., Granado, F., Blanco, I., Vaquero, M. and Cajigal, C. 2001. Lutein in patients with cataracts and age-related macular degeneration: a long term supplementation study.

Olmedilla, B., Granado, F., Blanco, I., and Vaquero, M. 2003. Lutein but not α-tocopherol, supplementation improves visual function in patients with age-related cataracts: A two-year double blind placebo controlled pilot study. Nutrition 19:21-24.

Osganian, S. K., Stampfer, M. J., Rimm, E., Spiegelman, D., Manson, J. E., and Willett, W. C. 2003. Dietary carotenoids and risk of coronary heart disease in women. Am. J. Clin. Nutr. 77:1390-1399.

Rimsten, L., Stenberg, T., Andersson, R., Andersson, A., Åman, P. 2003. Extraction of cereal β-glucan and determination of molecular weight using SEC with calcofluor detection. *Cereal Chem.* 2003, 80, 485-490.

Saulnier, L., and Quemener, B. 2009. Enzymatic mapping of arabinoxylan structure. Healthgrain methods: analysis of Bioactive Components in Small Grain Cereals. Chapter 15.

SLU. 2009. Swedish University of Agricultura Sciences. Department of food science.

SLV (nutritional Food administration) Svenska Näringsrekommendationer, fjärde upplagan. 2005.

SLV.2009. LIVSFS 2009:6

SLV hemsida 2010-01-05. http://www.slv.se/sv/grupp1/Mat-och-naring/Kostrad/Vuxna/Fullkorn/

SLV webpage 2010-01-06. http://www.slv.se/sv/grupp1/Risker-med-mat/Metaller/Kadmium/

SLV webpage 2010-01-06. http://www.slv.se/sv/grupp1/Mat-och-naring/Kostrad/Vuxna/Fullkorn/Fullkorn---fordjupning/#kontamin

SLV webpage http://www7.slv.se/livsmedelssok/Sok.aspx 2010-02-04. 06.30 pm.

SLV webpage http://www.slv.se/sv/grupp1/Mat-och-naring/Kostrad/Vuxna/Fullkorn/Fullkorn---fordjupning/ 2010-02-04. 06.45 pm.

SNF. 2009-10-20. http://www.snf.ideon.se/snf/en/rh/GI_en_overview.htm. Glycaemic Index (GI) Criteria/recommendations - overview.

Stålberg, J. 2005. Handout Kolhydrater (Stryer Kap 11).

Sundberg, B. Cholesterol lowering effects of barley fibre flake product.

Theander, O., Åman, P., Westerlund, E., Andersson, R. and Pettersson, D. Journal of AOAC International Vol. 78, No 4, p. 1037- 1039. 1995. Total dietary fibre determined as Neutral Sugar Residues, Uronic Acid Residues, and Klason Lignin (The Uppsala Method): Collaborative study. The Uppsala Method (AOAC 994.13, AACC 32-25).

THE COMMISSION OF THE EUROPEAN COMMUNITIES. COMMISSION DIRECTIVE 2008/100/EC of 28 October 2008. 29.10.2008 EN Official Journal of the European Union L 285/9. Annex II.

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION. Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients
Official Journal L 043 , 14/02/1997 P. 0001 – 0006. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31997R0258:EN:HTML

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION. 2002. REGULATION (EC) No 178/2002 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL Of 28 January2002 laying down the general principles and requirements of food law, establishing the European Food SafetyAuthorityand laying down procedures in matters of food safety. http://eur-lex.europa.eu/pri/en/oj/dat/2002/l_031/l_03120020201en00010024.pdf

Truswell, A.S, European Journal of Clinical Nutrition, 2002, 56, 1-14. Cereal grains and coronary heart disease.

WHO. 2003. 916. DIET, NUTRITION AND THE PREVENTION OF CHRONIC DISEASES

Åman, P. 1988. The variation in Chemical composition of Swedish Wheats.

Åman, P. 2006. Cholesterol-lowering effects of barley dietary fibre in humans: scientific support for a generic health claim.