Tritordeum

Evaluation of a new food cereal

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 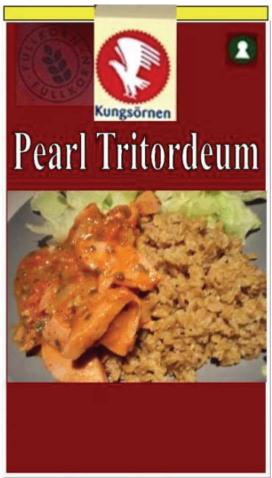

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Abstract

Tritordeum is the result of a cross between a wild barley (*Hordeum chilense)* and durum wheat *(Triticum turgidum)*. Tritordeum have high viscosity and a nice yellow colour. 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 contain 14.3% total dietary fibre (the Uppsala method and fructan) which is more than in wheat, but the β-glucan content was lower than in wheat. Tritordeum contains more arabinoxylan, fructan, Klason Lignin and uronic acid compared to 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

Tritordeum är resultatet av korsning mellan ett vild korn (*Hordeum chilense)* och durumvete *(Triticum turgidum)*. Tritordeum har hög viskositet och en trevlig gul färg. 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 (Uppsalametoden och fruktan) vilket är mer än i vete, medan halten β-glukan visades vara lägre än vete. Tritordeum innehöll också mer arabinoxylan, fruktan, Klason lignin och uronsyra jämfört med vete. Lutein ger Tritordeum sin gula färg. Tritordeum är en ny 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.

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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. It was 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 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 for 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 ***Trit**icum turgidum* (durum wheat) and ***Hordeum** chilense* (a wild barley). Barley have mostly been used as animal feed, only a comparable small amount for malt and food production, 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 quality. Wheat on the other hand is the cereal traditionally used in bread industry due to its high content of gluten protein which is associated with good bread making 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 which is *morphologically, agronomically, chemically and physio-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 with resources from 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 a 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. According to Agrasys the techniques used in the creation of Tritordeum are cross pollination and embryo rescue, both methods included in the so called *classical breeding*. Tritordeum is thus 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 and SLU 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 is to be rich in dietary fibre and hopefully rich in soluble dietary fibre which 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 which is *to evaluate the carbohydrate quality and colour of Tritordeum.*

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.

According to the literature, HT 1608 is shown to have favorable rheological traits. This and the fact that milling was made by Lantmännen in 2006 on the same line are the reasons why this line was selected for the application part. By analyzing this line again also the annual difference could be indicated.

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 the 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 proven 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 bread volume 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 high viscosity (Barcelo, Personal communication).

History - Tritordeum breeding

Already in the beginning of the 70th 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 why breeders have been working in more than 30 years to improve hexaploid Tritordeum to what it is today. Back crossing has been the main tool in the breeding programme (Guedes-Pinto, H. *et al.,* 1996). One after another of the agronomic traits have been improved; studies of hexaploid Tritordeum have shown favourable agronomic traits for biomass, number of spikelets per spike, seed size and protein content (Guedes-Pinto, 1996). Tritordeum have also shown high fertility and good resistance to biotic and abiotic (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 is an important trait for a food crop.

Evaluating Tritordeum

Pre-knowledge of the five Tritordeum lines being evaluated in this thesis (Barcelo, personal communication; 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 covered barley flour was 14.0 %. Analysis showed this line to contained 0.2% β-glucan with an average molecular weight (MW) 0.67×10^6. Corresponding result for barley flour was 2.1 % with average 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 so 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 high lutein content. This line is not included among the five samples evaluated in this thesis, but included in the analyses made by 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 composition

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).

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 α -(1→ 4)- linked D-glycoside residues or a branched molecule (amylopectin) with both α -(1→ 6)- linked D-glycoside residues and α -(1→ 4)- linked D-glycoside residues (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 (Abrahamsson, 2001).

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 purified 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 and pectins), 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/oligomers 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). Fructan is non-digestible carbohydrate and thus part of the dietary fibre.

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

Sugar residues

Sugar residues are units of polysaccharides. They consist mainly of the 5 or 6 carbon monosaccharides glucose, galactose, arabinose, mannose and xylose. 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. Uronic acid residues is another group of dietary fibre component maintained by oxidation of a hydroxyl group in a sugar molecule, e.g. glucose will be forming the uronic acid glucuronic acid.

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 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 thus decrease the risk for coronary heart disease (CHD) (Truswell, 2002). The viscosity of the polysaccharide (1→3, 1→4) - β- glucan is important for the serum cholesterol lowering effect of oat and barley (Bourdon *et al*., 1999; Sundberg *et al*., 1995). 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 enzymatic degradation. The addition of yeast and the oven-baking do not affect the molecule weight of β- glucan (Andersson *et al*., 2004).

Recently EFSA presented scientific opinion of a new health claim for beta-glucans: *"Regular consumption of β -glucans contributes to maintenance of normal blood cholesterol concentrations*" (EFSA, 2009a). This health claim is allowed to be used for food contributing with minimum 3 g β -glucan per day from oats, oat bran, barley, barley bran in foods with 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 in the so called dietary fibre complex 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, 2010d).

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

Phytic acid
In the auluerone 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 (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 of cereals 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 re-

esterified forms to enter the target cells. The metabolism is characterized by oxidation of the hydroxyl group (EFSA, 2009b).

Lutein is the main carotenoid in wheat. Cereal products contain in general about 2 µg lutein per gram eatable part (Brugård Konde *et al*., 2006). In wheat, a measured amount of lutein is 8.4 µg/g (maximum values of 13.4 µg/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 in durum wheat (Atienza *et al*., 2007). Einkorn has 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 high activity of biosynthetic genes may underline the 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 physical 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 normalized 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 cardio vascular diseases (CVD).

Fructo-oligosaccharides, often referred to as prebiotic, 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 whole grain. 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 is 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, 2009). 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, 2010c).

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*., 2003) and to 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/100g 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/100g (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, rolled or similar, but the botanical 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 whole grain per day for women and men respectively (NNR, 2004; SLV, 2010b). 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, 2010a)

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

Materials

The following amount of the Tritordeum lines were sent to Lantmännen in October 2009 (2009a).

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 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 flour fractions (1-6) and 2 bran fractions bran (A) and short bran (B). The different fractions were weighted and the yield was calculated. In 2006 15 kg HT 1608 was milled.

Ash

The five Tritordeum lines and the 8 milling fractions of HT 1608 were analyzed for ash content, all in duplicate.

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 milling 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

Two determinations were made. One the same day as milling and the second 5 days later, 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 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, and 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 mill Tecator (Höganäs, Sweden), and thereafter in 0,5µm ultra centrifuge mill type ZM 1 Retsch (Haan, Germany) (SLU, 2009)

The five samples (double samples) were dispensed in acetate buffer and 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 non-starch polysaccharide residues and Klason lignin. Arabinoxylan was calculated as the content of arabinose and xylose residues. Cellulose is the difference between the amount of glucose residue and β- glucan analyzed by a separate method.

β- Glucan analysis
Megazyme mixed- linkage β- glucan assay kit was used. The method is based on enzymatic degradation of β- 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
Fructan was 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 with diluted acetic acid. The fructan was later hydrolyzed to fructose and glucose with purified fructanase and thereafter measured by the p-hydroxybenzoic acid hydrazide method for reducing sugars (Megazyme, 2008).

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 an impact mill (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, and 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 with a known method (Mínguez-Mosquera *et al*., 1993) within a week.

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 (0.5mm) 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). 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 an 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 cut in the production at Lantmännen (Järna, Sweden). Same method was used as for normal production of pearl grains (Lantmännen, 2009b).

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 oats 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 1.4 mm (both wholegrain and polished grains). Water content was measured (see method for 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 was performed according 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 which was fried for 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 baking tin. Baking at 225ºC for 10 min.

Results and discussion

1000 kernel weight, ash and starch

HT 354 had the largest and HT 361 the smallest seed size (Table 1). 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. 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 (Lantmännen and SLU, 2006)	38.3	NA	NA
Durum wheat (Lantmännen and SLU, 2006)	42.2	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 (Fig 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 occasions of milling, 34.5 and 39.9%.

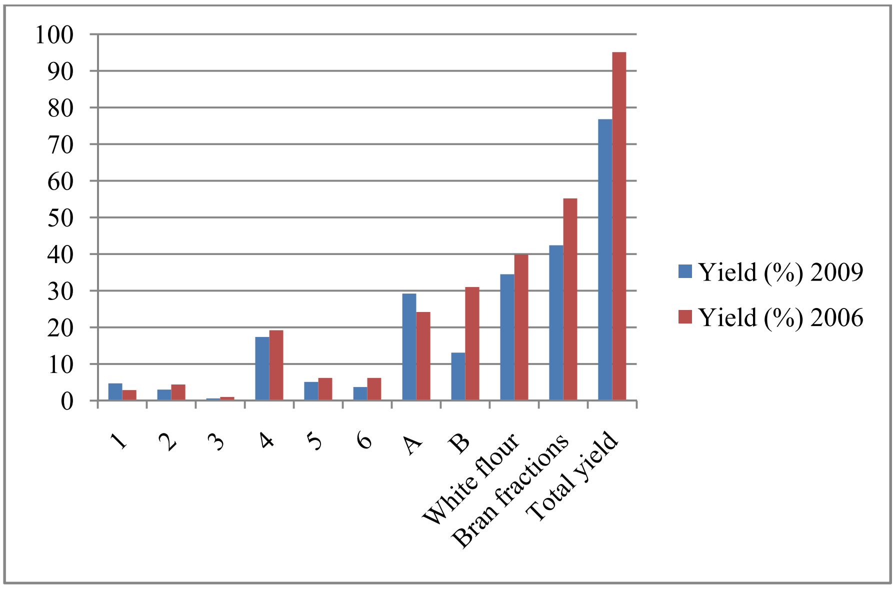


Figure 1. Diagram of milling 2009 (left) compared to 2006 (right).

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 in 2010 may be the higher moistering grade of 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 it is high in the bran fractions (Fig 4). 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 (Fig 2 and Fig 3).

The six fractions B1, B2, B3, C1, C2 and C3 are originating from the following position of the Bühler Mill (Fig3):

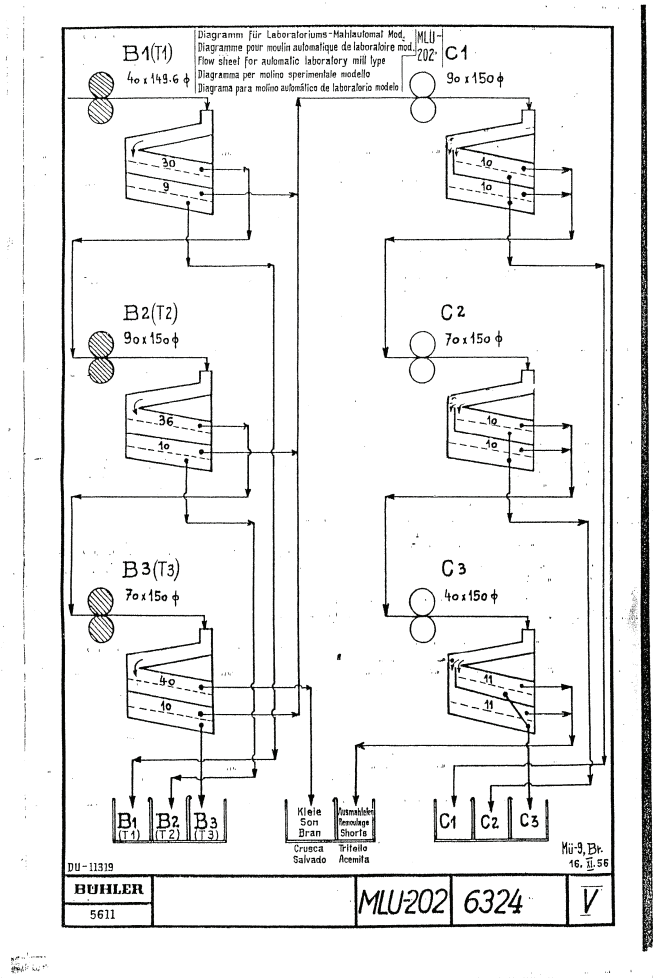


Figure 3: Diagram Bühler mill.

Ash

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

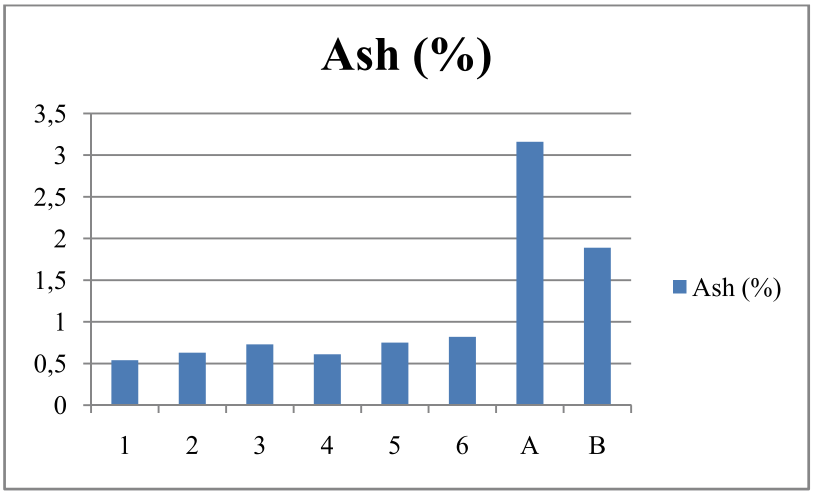


Figure 4. Diagram ash content (fresh weight) in the different fractions of Tritordeum HT 1608 (08/09).

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 (Fig 5). The samples were air dried to increase the storage ability and reduce the risk of mould during storage. The large water loss of the bran fraction (A) showed 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 given. This to make the water reach the centre of the grain and further to equilibrate the yield of the fractions.

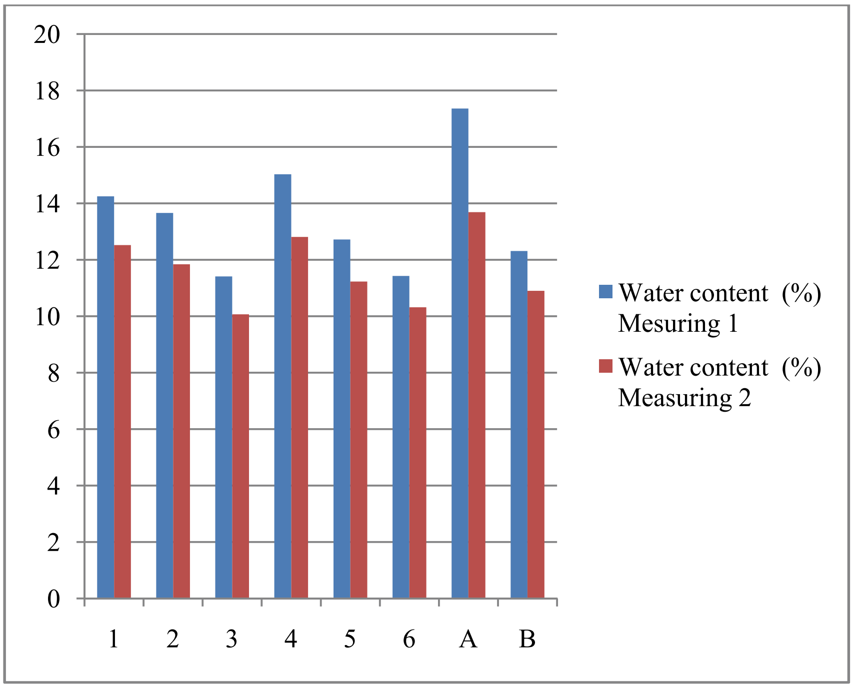


Figure 5. Diagram water content (%) in the different fractions of Tritordeum line HT 1608 (08/09)

Colour analysis

Fractions A (bran) and B (short bran) are darker (low L value) (Fig 6) and more red (high a-value) (Fig 7) than the white flour (fractions 1- 6). The white flour has a more greenish color Fractions 1 to 6 have a stronger yellow color (b-value) (Fig 8) compared to the bran fractions. Fraction 2 have the strongest yellow color and fraction 1 was 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.

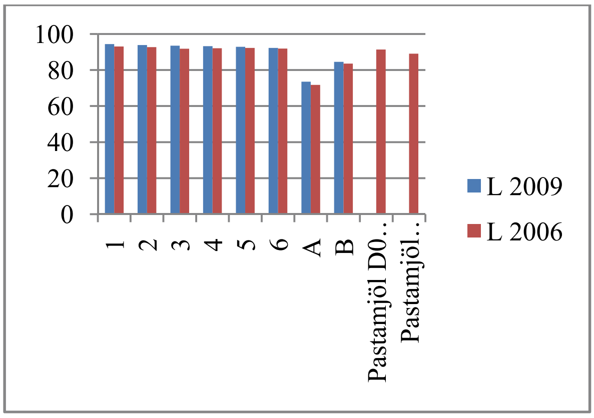


Figure 6: L-value

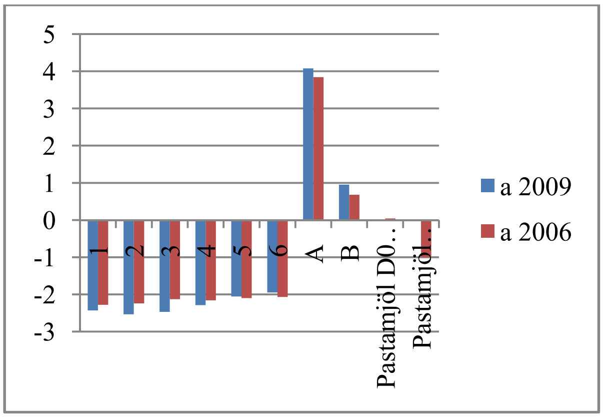


Figure 7: a-value

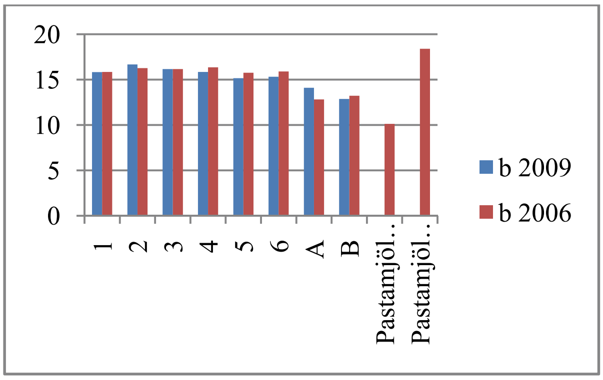


Figure 8: b-value

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 (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 content is higher than in wheat. Agrasys currently is working to explore a large genetic diversity in germ plasma of Tritordeum to produce varieties with improved agronomic value and 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 wheat (Fig 9). This is an explanation to the good viscosity and bread making quality of Tritordeum. Tritordeum do also contain more fructan, Klason Lignin, uronic acid and total dietary fibre compared to wheat.

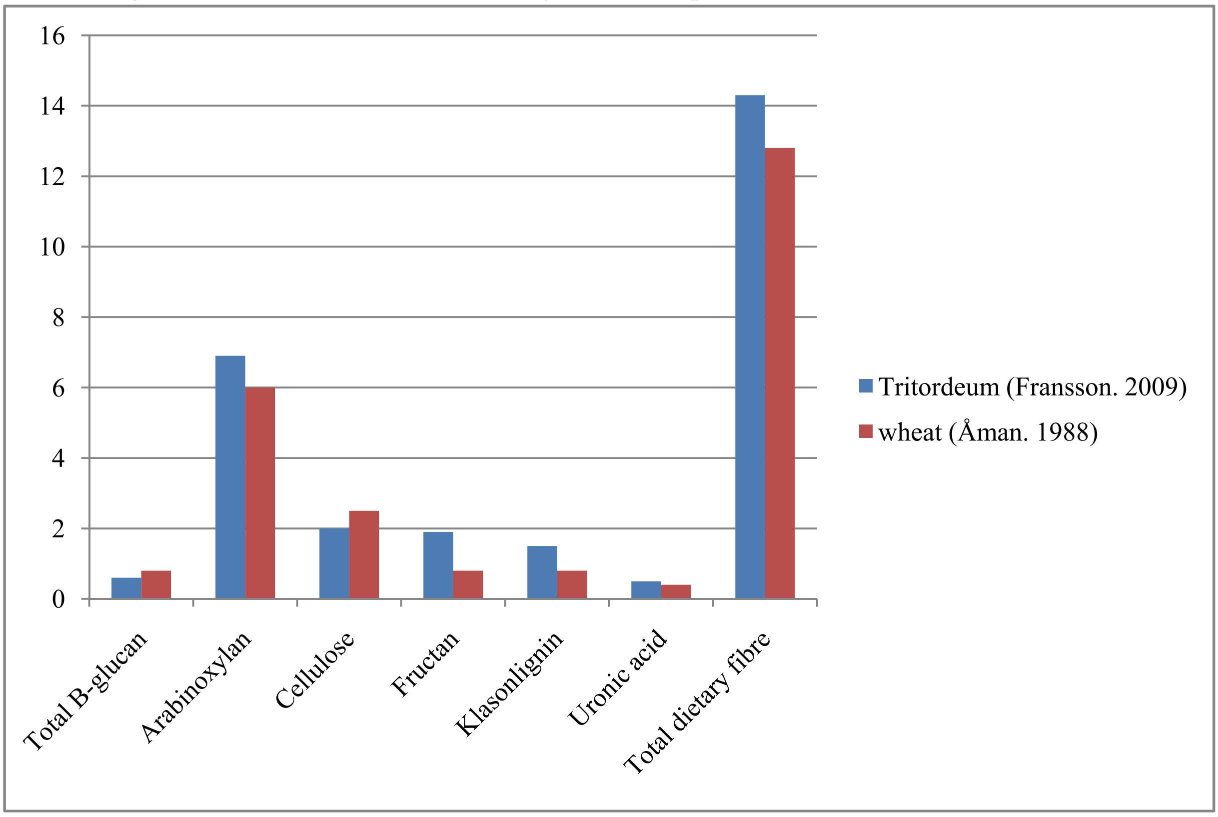


Figure 9. Diagram of total β-glucan, arabinoxylan, cellulose, fructan, uronic acid and total dietary fibre in Tritordeum and wheat (Åman, 1988).

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 whole grain flakes had higher water content (10.9 %) 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.

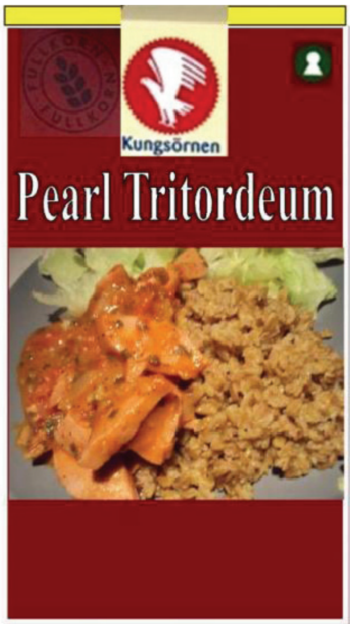
Breakfast cereals of Tritordeum were used to make porridge and pearl Tritordeum.

Porridge
The porridge had a nice yellow colour (Fig 10) and a round and rich taste. The porridge made of cutted grains reminds about fibre enriched oatmeal porridge. The most important values of Tritordeum porridge, as I see it, is the aromatic taste and the yellow colour. 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 10. 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 (Fig 11). The consistence of Tritordeum was similar to pearl wheat while the colour was more similar to pearl barley.

Figure 11: Pearl cereals, from the left Pearl wheat, pearl barley, pearl oat and pearl Tritordeum



Pearl Tritordeum present a nice taste and structure which make it interesting as an alternative to rise. 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 whole grain product and may therefore use the wholegrain symbol.

The yellow stripe on the top of the packaging is the corresponding stripe as on the packaging of already existing pearl grains. The reason to put the yellow colour on the stripe is to get the consumers to associate Tritordeum with the yellow colour. In the future hopefully results from new research can support my expectations of lutein being a functional food as a complement to daily food, with functional properties like reduced incidence of cataract, age-related macular degeneration (AMD), reduced incidence of breast cancer and cardiovascular diseases. According to this other possible application area for Tritordeum whole grain would be “AXA Fibre Eyes”.

Whether Pearl Tritordeum would be a low GI product is a question to think about *if* decision to produce this special product is made.

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 Tritordeum whole grain bread was very tasty with a rich, sweet and aromatic taste and aroma. The whole grain bread was very soft and elastic and the colour was brown almost like syrup bread (See figure 12 & 13).

Figure 12: From the left: wheat flour, Tritordeum white flour, Tritordeum whole grain flour.

Figure 13: left photo: cutting area of wholegrain Tritordeum bread (top), white Tritordeum (centre) and wheat bread (bottom). Right photo: compared size of wheat bread, white Tritordeum and whole grain 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 hand 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 (Fig 14) 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 14: Tritordeum white flour as binding in the spinach and Tritordeum wholegrain flour as breading on fried flatfish.

Collection and compilation of Data

Tempe

<u>First trial:</u>

The mycelia were better developed when more polished material was used. Tritordeum-tempe cookies were not as stabile as Tempe made of pearl barley or pearl oat (Fig 15).

polished 1min polished 2min polished 4min

Fig 15: mycelia of Tempe (Hemesath. 2009)

<u>Second trial:</u>

The mycelia were developed as expected. Tritordeum- breakfast cereal Tempe became too hard and crumbled (Fig 16).

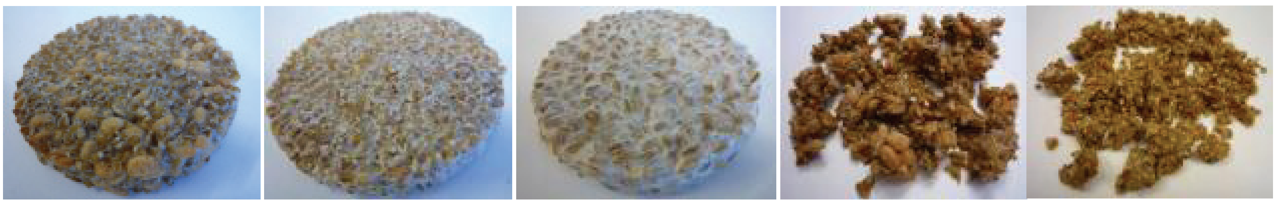


Figure 16. Tempe from the second trial, from left to right: Tempe from cutted grains with puffs; Tempe from cutted grains; Tempe from grains polished 2 min and boiled in broth; Breakfast cereal Tempe from cutted grains with puffs; Breakfast cereal Tempe from cutted grains (Hemesath. 2009).

Lutein

The result indicates that Tritordeum contains in average ≥ 10 times more on lutein than bread wheat and ≥ 7 times more Lutein than durum wheat. The five lines contain 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 (Fig 17).

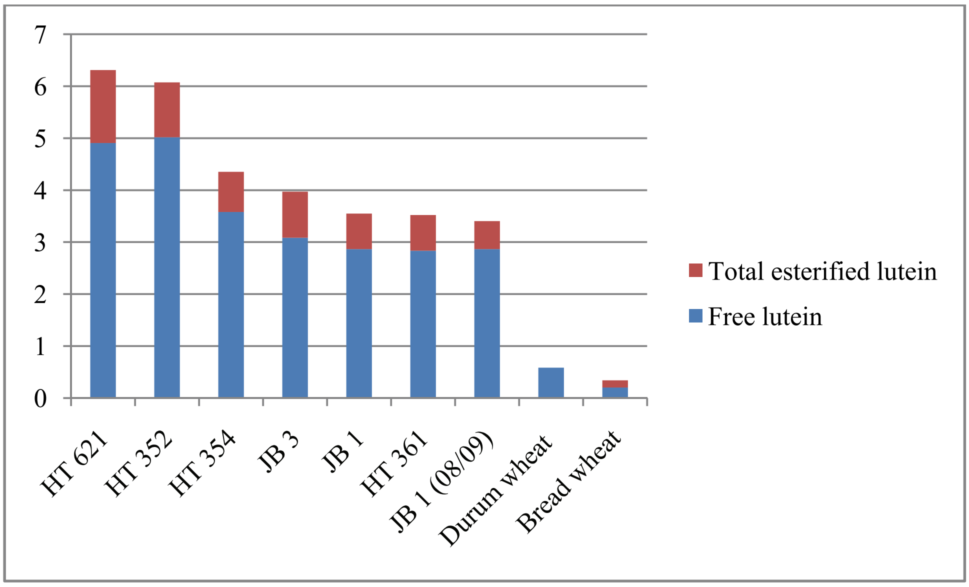


Figure 17: Total esterified and free lutein in Tritordeum lines compared to bread wheat and durum wheat. (Data from IATA, CSIC commissioned by Agrasys).

The Lutein content in Tritordeum is relatively high, but when comparing the result for Tritordeum with bread wheat there seem to be a difference if using Swedish (8µg) or Spanish references (0,3µg) for bread wheat. 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.

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 (Fig 18).

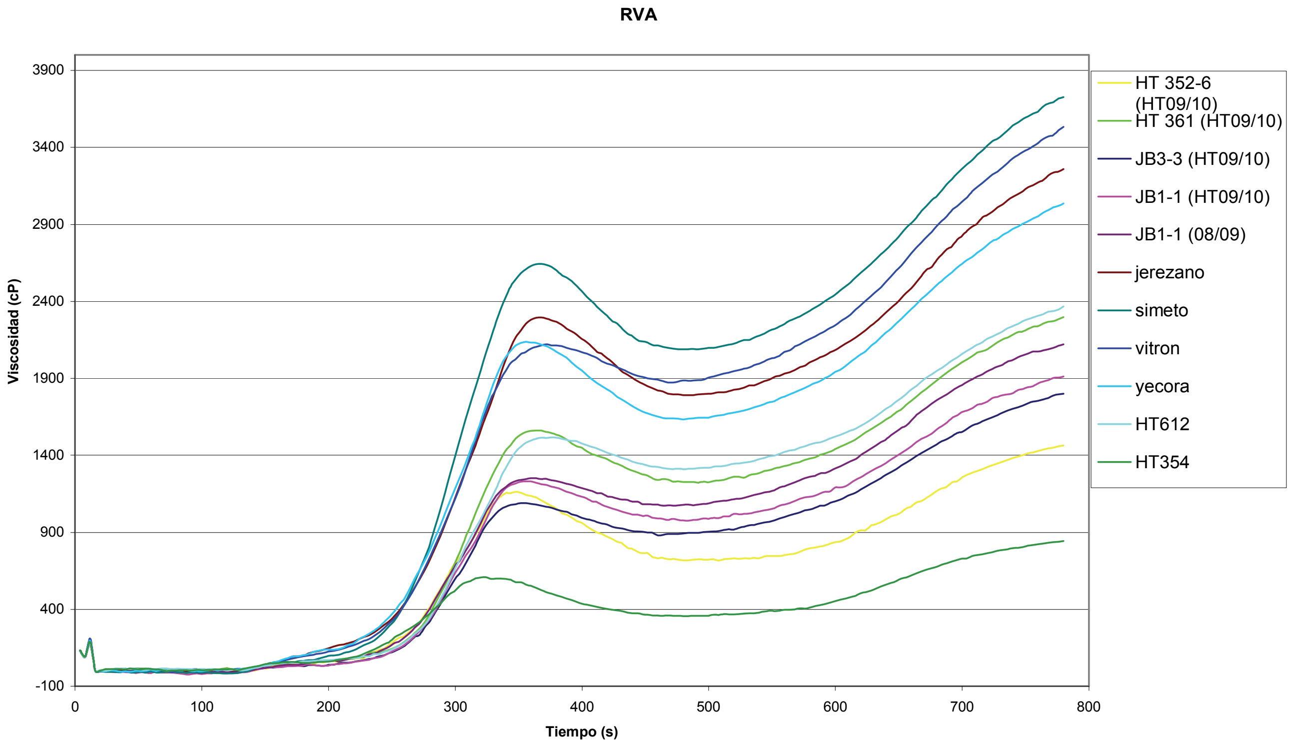


Figure 18: Diagram: Rapid visco-analyzer (RVA) showing the viscosity for Tritordeum lines compared to bread wheats (Jerezano and Yecora) and durum wheats (simeto and vitron) (Data from IATA, CSIC commissioned by Agrasys, 2010)

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 437 had the 2nd biggest grain size 2nd lowest ash content. HT 1608 has small grain 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 2218 (JB3) showed the lowest ash content and medium 1000 kernel weight. HT 1608 had the 2nd highest lutein content and 2nd lowest viscosity. As curiosa this line had the highest amount of phenolics among the Tritordeum lines analyzed by Agrasys (Fig 19).

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 determine 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 it 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

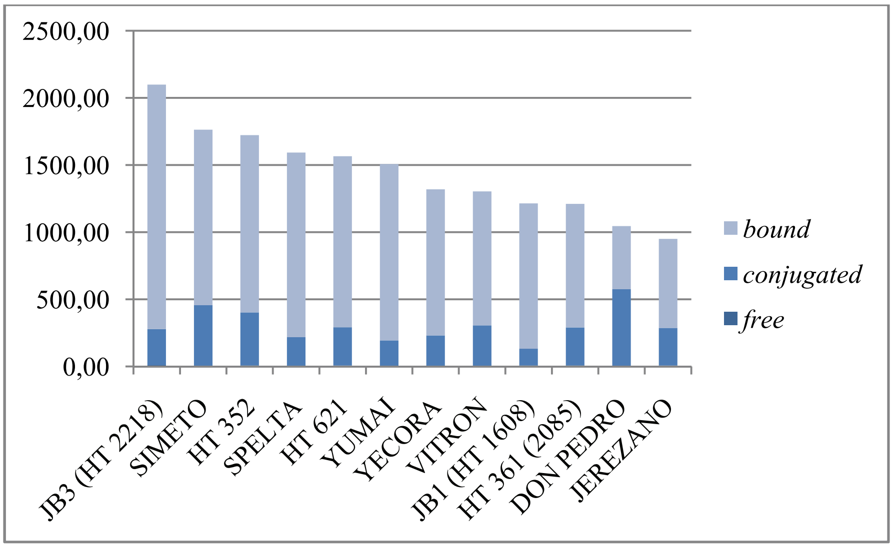


Figure 19: phenolics in Tritordeum lines and wheat. (Agrasys)

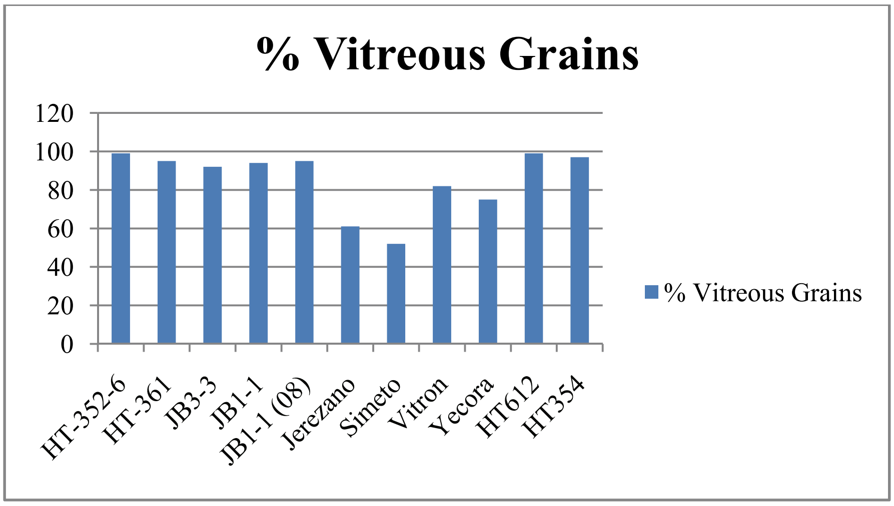


Figure 20: Percent vitreous grains in Tritordeum lines and wheat. (Agrasys)

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