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Malting Tritordeum

Effect of different malting regimes on the quality of tritordeum for beer brewing.

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Abstract

Cereals for beer brewing have for a long time been dominated by barley (*H.* vulgare) and wheat (*T. aestivum*). Tritordeum, a novel amphiploid hybrid of the South American barley (*H. chilense*) and durum wheat (*T. turgidum*) is considered an interesting alternative. It has high levels of protein and enzyme activity exceeding that of barley. Beer brewing relies heavily on high protein and starch levels as well as sufficient enzymatic activity of the malt applied. This project investigates how different malting regimes influence the malt quality of tritordeum in a beer brewing perspective.

Literature about malting of barley and durum wheat laid the foundation of the two steeping regimes and three germination regimes that were chosen to produce six different tritordeum malts. Wort and grain analysis were performed on the malts and compared to results for commercial malts of tritordeum and barley.

Tritordeum malt with a single air-rest steeping exceeded commercial barley malt in β-Amylase activity as well as free amino nitrogen (FAN) while achieving equal levels of extract after six days of germination. A double air-rest regime did not malt as successfully, and only exceeded the singe air-rest regime in β–Glucanase activity. FAN, extract, acidity and amylase developed with germination time in tritordeum, as reported with barley in literature.

Even though tritordeum malt lacked the high α-Amylase activity present in barley malt, it was still able to saccharify and produce similar extract levels to that of barley malt. Several parameters must be studied further to conclude if tritordeum can compete with barley as a base malt in beer brewing, but it certainly showed promising qualities.

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This project relied heavily on the knowledge already acquired by the private sector on tritordeum as a malt for beer brewing. I am very grateful for Agrasys (ES) and Corte di Rivalta (IT) for allowing me to use their tritordeum malt analysis conducted by Asociación Española de Técnicos de Cerveza y Malta and Centro di Eccelenza per la Ricerca sulla Birra.

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Introduction

Alternatives to traditional malt for beer production are continuously being investigated in order to utilize a variety of cereals and resources (Alfeo et al. 2018). Tritordeum, a cereal novel to the beer industry, has in recent years gained attention due to its beneficial qualities regarding draught resistance and flavour (Tritordeum.com). Tritordeum is a hybrid species (amphiploid) made from cross-breeding of South American barley (*Hordeum chilense*) and durum wheat (*Triticum turgidum*). *H. chilense* is a wild, diploid barley. It is found in Chile and Argentina and is highly polymorphic both morphologically and biochemically. It is known for being a component of cattle feed. Cross-breeding barley and wheat has been remarkably successful with *H. chilense* (Martin et al. 1999). The first tritordeum was obtained by Martin and Chapman at Plant Breeding Institute of Cambridge (UK). After less fertile outcomes of breeding with *H. chilense* and bread wheat (*Triticum aestivum*), durum wheat showed better potential to produce hybrids with higher fertility, biomass, seed size, protein content and number of spikelets. *H. chilense* and wheat cross-breeding has led to hundreds of tritordeum varieties (Martin et al. 1999). Figure 1 shows durum wheat, *H. chilense* and tritordeum grains.

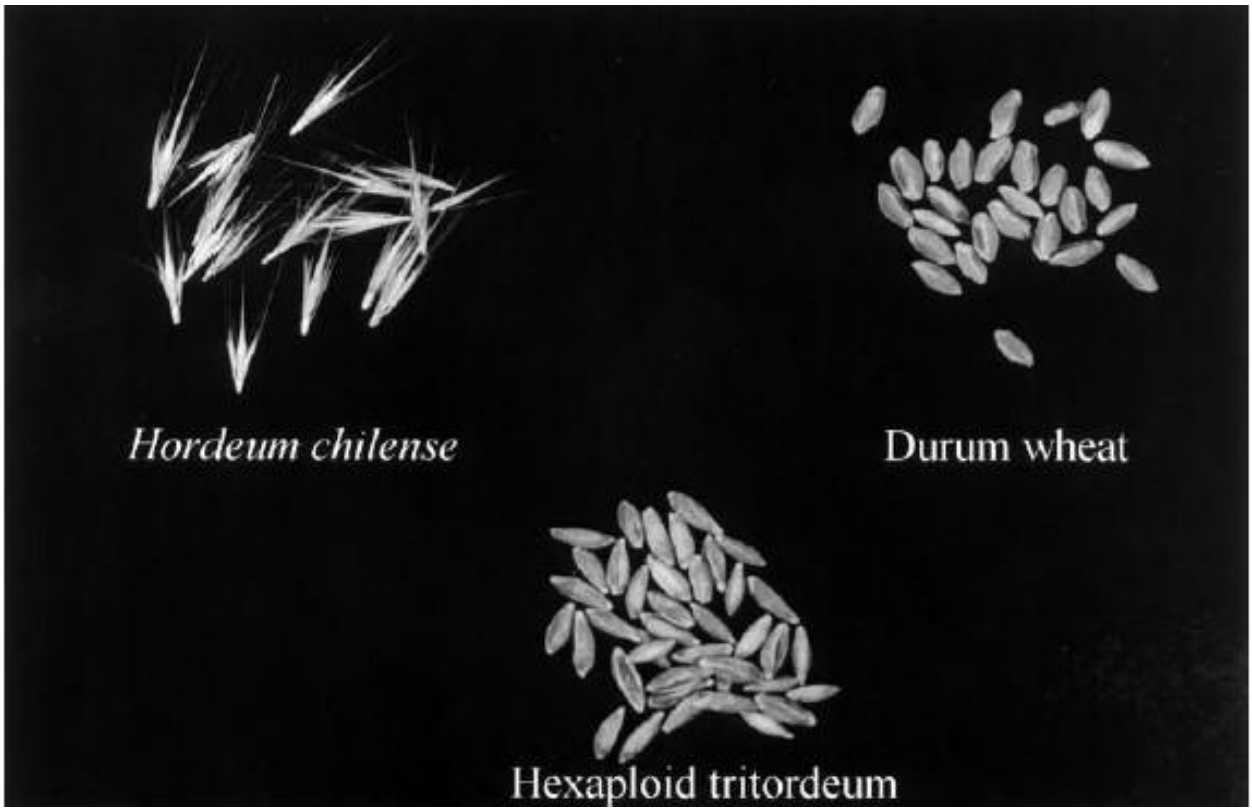

Figure 1 From Martin et al. 1999: Figure 2 depicting grains from the parental cereals *H. chilense* and *T. turgidum* and the cross-bred tritordeum.

The different varieties vary a lot in agronomic traits such as plant height and biomass yield. Tritordeum showed similarities with bread wheat, and they also have in common that they both derive from a cross-breeding of a durum wheat and a wild relative (Martin et al. 1999).

According to Alvarez et al. (1992), tritordeum can also refer to cross-breeding of South American barley and bread wheat. However, in this project only the durum wheat breed will be referred to as tritordeum. Cross-breeding of varieties of barley (*Hordeum vulgare*) and wheat was popular in the 1970s, and papers about tritordeum morphology were published in Cordoba (ES) in the early 1980s (Martin and Sanchez-Monge Laguna. 1980, Martin and Sanchez-Monge Laguna. 1982). They showed promising results due to the high fertility of tritordeum compared to other hybrids of the barley and wheat parent species (Martin and Sanchez-Monge Laguna. 1982). Since then, agronomic traits such as biomass, seed size and protein content have been enhanced through breeding programmes (Erlandsson. 2010 ref: Guedes-Pinto. 1996).

Malt analysis by a few European malsters (Table 1) revealed promising values for beer brewing of tritordeum. Most notable, from a brewing perspective, were the high levels of extract, free amino nitrogen (FAN) and diastatic power. Also, the β-glucan content, which at certain levels can cause difficulties in brewing, was similar to that of barley malt. The Kolbach-index, if high can indicate low malting yield (Jin et al. 2012) and less flavour stability (Kunze. 2014), was higher than barley malt which can prove to be the big challenge when tritordeum is evaluated as a base malt. Bianco et al. (2019) concluded that durum wheat was not suitable for beer brewing due to a Kolbach-index higher than the level recommended in a review (Faltermaier et al. 2014). Certain characteristics of durum wheat has been seen in tritordeum (Martin et al. 1999 ref: Alvarez et al. 1994). However, Martin et al. (1999) reported a micro malting of a mix of tritordeum varieties, which had a Kolbach-index matching a high-quality barley malt. Though *H. chilense* is genetically different from brewing barley, (Hagras et al. 2005), it could be interesting to investigate the mix of characteristics, that make barley suitable for beer brewing, when this barley and durum wheat are cross-bred. Not all barley varieties are relevant for brewing since malting of different varieties have resulted in different qualities of malt (Swanston and Taylor. 1990). A few commercial breweries in Italy, Spain and The Netherlands have already launched beers with tritordeum malt (untappd.com, 2019). However, little information is available about their experience with tritordeum, and no publications on the topic exists as of august 2019.

The impact of malt in beer

Beer is traditionally made from a base of malted cereal, which means that the main source of sugar for yeast fermentation originates from germinated, dried cereal. The process of germinating and drying the cereal is called malting. The malted grains are referred to as malt. *H. vulgare* became popular in the production of beer due to a robust husk and a high enzyme formation, which enhances the production of fermentable sugars in the brewing process (Baik and Ullrich. 2008, Kunze. 2014). Hence, barley malting is the template for explaining malting practices in this project. A study compared six different barley malts in beer production and found that the flavour profile in the final beer, will be heavily influenced by the choice of malt included in the recipe (Bettenhausen et al. 2018). High quality base malt contributes with starch, enzymes for starch breakdown, protein and zinc. Starch consist of carbohydrates such as amylose and amylopectin structures put together to form long chains of sugars. Enzymes such as amylases and dextrinases can hydrolyse these chains of sugars until the end-products are dextrins and mono- and disaccharides (Sissons and MacGregor. 1994). Nitrogen availability in terms of amino acids and protein complexes both enhance vitality of yeast and aroma compounds produced during fermentation and affect visual properties such as foam retention and haze in the beer (Fontana and Buiatti. 2009). Metals like zinc increase yeast vitality with a positive impact on metabolism and thereby fermentation. The degree to which the malt is dried during the process called kilning will also affect the degree of colour contribution to the beer. Maillard reactions of protein residues and sugar will form melanoidin that darkens the malt at high temperatures (Steiner et al. 2012). β-Glucan, derived mainly from the cell walls of the barley endosperm, will form complexes during the process of mashing. This leads to an increase in viscosity which can have a negative effect on further processing (Wang et al. 2004). It is therefore important for a maltster to use barley with low β-glucan levels.

Structure of the kernel

The barley kernel has several crucial features beneficial for beer brewing. The surrounding husk serves as a filtration element during mashing, the endosperm contains the starch and the aleurone layer, provides enzymes during germination. Protein is found in various cell wall structures. In the bottom of the kernel the embryo is located (Fig. 2).

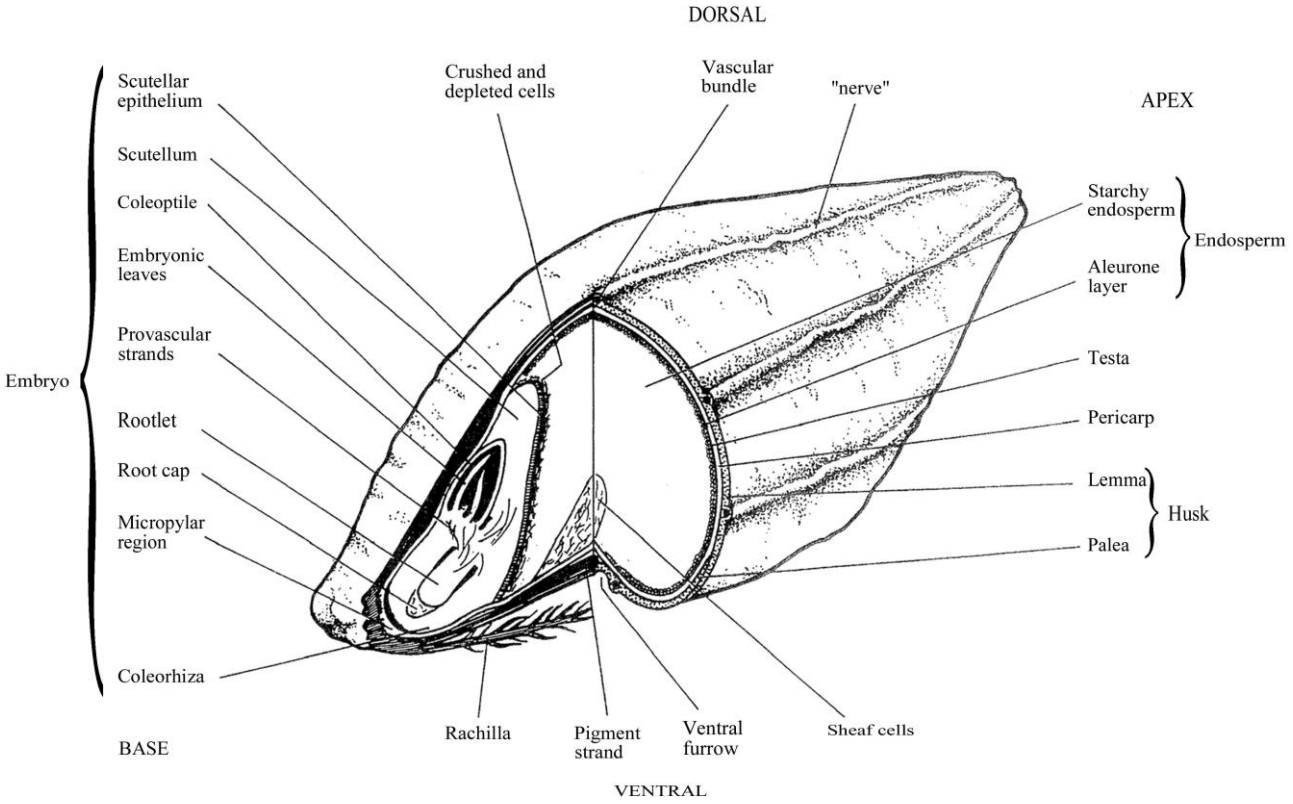

Figure 2: From Briggs (1998): Anatomy of a barley kernel.

Steeping

The kernel can be understood as a pack of nutrients with the ability to germinate a new plant. The purpose of malting is to germinate the plant to a point where enough enzymes for starch breakdown are present and the kernel's nutrients are still mostly intact. To initiate germination kernels are soaked in water (Fig. 4) with the intention of reaching a moisture content of approx. 42 %. This process is called steeping. The water content is also referred to as the degree of steeping. The steeping in malting plants can take place in cylindroconical stainless steel tanks able to process several tons of raw barley. Water uptake happens through the embryo of the kernel, after which it flows to the endosperm of the kernel. When the moisture content reaches 30 % the embryo initiates germination (Kunze. 2014). The first visible sign of this is growth of rootlets which will appear from the end where the embryo is located. Rootlets functions as a transport of nutrients and water from the surroundings to the kernel.

Cozzolino et al. (2013) found a positive correlation between water uptake and certain fatty acid compositions in commercial barley for beer brewing. Especially oleic acid had a positive effect, increasing water uptake, while long saturated chains of fatty acids showed a negative correlation, decreasing water uptake. It should be noted that all barley varieties steeped reached mean moisture percentages (w/w) higher than 44 % after just eight hours of soaking. This must be regarded as acceptable for a barley variety. The coefficient of variation was suspiciously high though for all varieties ranging from 7 to 13 %. As germination starts, the kernel will need air for respiration. Compressed air is applied to the kernels from the bottom of the vessel to secure an even aeration. A typical temperature during steeping is around 15 to 17 ºC, which has proven to be favourable for barley malting (Agu and Palmer. 1997, Rimsten et al. 2002). A study showed that in a temperature interval from 10 to 35 ºC, the loss of solids, such as protein and starch, increased with temperature over a period of 32 hours (Montanuci et al. 2014). However, the rate of hydration increased with increasing temperatures and so it could be argued that a high temperature steeping would be able to take less time if the goal is to reach a certain moisture level in the kernels. The authors argued that the grain tends to break up at temperatures from 30 ºC and up due to the high initial hydration rate, and steeping was not recommended at these temperatures (Montanuci et al. 2014). Also, experiments with high steeping temperatures have resulted in slower filtration rates in the brewhouse (Reeves et al. 1980). Normally, after four to six hours the vessel is drained of water since the kernels need sufficient amounts of air, while the adhesive water on the kernels plus occasional spraying of water on the kernels will be enough to avoid drying out. Reynolds and MacWilliam (1966) found that adequate amylase levels were acquired most effectively if the steeping took a maximum of six hours. Amylase levels are important for the grain modification, which means the starch and protein conversion into smaller residues by enzymes. CO_2 is removed from the bottom of the vessel, where it would otherwise build up and suffocate the kernels. After 24 hours the kernels are soaked again for four hours to increase the moisture content. If kernels lack air during steeping, it can lead to loss of germinated kernels and therefore loss of product (Kunze. 2014). Alternative steeping regimes have been studied and the finding was that a continuous steeping of 24 hours without an air rest reached high quality malt compared to a standard steeping regime including an air rest (Bryce et al. 2010). It is suggested that the finding could help maltsters save time since the continuous steeping method was shorter than a traditional malting regime (Bryce et al. 2010). However, Swanston and Taylor (1990) found that barley of different malting quality had higher hydration and germination rates when steeping regimes included an air rest. The difference in β-glucan levels when two barley varieties

were steeped to a range of different hydration levels varying from 38 to 46 % was investigated by Shaluk et al. (2019). They found that the β-glucan level decreased with increasing hydration of barley. It was proposed that increasing hydrolysis of β-glucan took place as moisture content increased. The chemical composition of water is important to the development of barley roots, where a hard, mineral-rich water was preferred over light, distilled water (Pollock and Pool. 1962).

Germination

Enzymes related to protein and starch breakdown are produced or released by the activity of gibberellic acid released from the embryo to initiate germination. α-Amylases and dextrinases are produced, while β-Amylases and β–Glucanases are already present in the endosperm and aleurone layer (Kunze. 2014). As the degree of steeping becomes acceptable, the kernels are transferred to a germination box. For barley typical moisture content is 42 to 46 % after steeping. They will stay for four to seven days in the germination box depending on the maltster. A study of Polish barley varieties concluded that germination time longer than four days did not increase malt quality in terms of extractivity (Zembold-Gula et al. 2009). Extractivity is connected to modification since it describes the amount of solubilised starch and protein residues in the mash of water and malt called wort. Farzaneh et al. (2017) studied germination time's effect on enzyme activity, β-glucan and starch with increasing germination time in barley. They saw an increase in enzymatic activity and diastatic power, while β-glucan decreased. It must be mentioned that de Sa and Palmer (2004) showed scepticism about using β-glucan as an indicator for modification. They found heterogeneity between level of modification and level of β-glucan. They saw a clear decrease in β-glucan in barley as germination time increased but looking only at modification would not give a clear picture of the β-glucan in the individual grain (de Sa and Palmer. 2004). Diastatic power (DP) is a strong indicator of β-Amylase levels especially, but all protein and starch degrading enzymes contribute to the level of diastatic power (Hu et al. 2014 ref: Evans and Eglinton. 2009, Farzaneh et al. 2017). Malt yield, which is based on the weight before and after malting, decreased with up to 22 % after seven days of germination, and it was suggested that respiration increased during malting (Farzaneh et al. 2017). According to Kunze (2014) this will be the case, and it is stated that 20 % is expected when the loss of water is included in the measurements as it was in the study by Farzaneh et al. (2017). It was not clear from the study how long time steeping and kilning regimes were for the barley studied, as it was just stated that they stopped steeping and kilning when the desired moisture content was reached. The time of these regimes might be of relevance if the experiment should be replicated. An acrospire originating

in the embryo will grow below the husk and upwards to the end opposite of the embryo and rootlets (Fig. 3). The kernel needs the light absorbing acrospire to convert light photons into chemically bound energy. The acrospire growth correlates with the degree of modification of the kernel, which means that the bigger the acrospire, the bigger the loss of nutrients. To have a satisfactory production and release of enzymes, the emergence of the acrospire is inevitable. While the kernels are in the germination box, regularly turning is needed to supply enough air to the kernels (Fig. 4). To limit the energy use by respiration, the temperature is kept at a minimum. However, it still needs to be enough for the enzymatic release to take place. Typically, this temperature is around 10 to 20 ºC (Kunze. 2014).

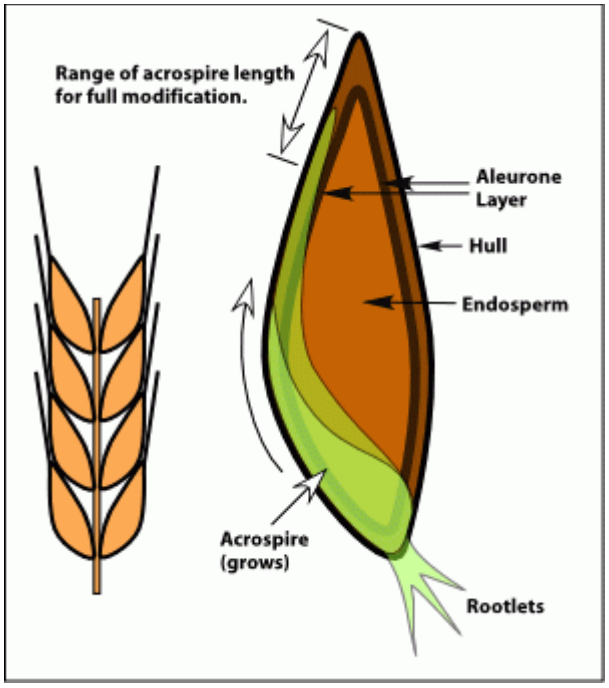

Figure 3 Acrospire growth in barley kernel during germination.
Sketch of barley weed is shown on the left. (howtobrew.com)

Water is sprayed on the kernels during germination to continue water uptake to the desired value, while heavy ventilation is needed to keep the temperature to a maximum of 18 ºC due to the heat developed by respiration (Kunze. 2014).

Bryce et al. (2010) found that with increasing germination time Scottish barley malt increased extract and FAN-levels while lowering wort viscosity. The decrease in viscosity with germination time was also apparent in a study on a Mexican barley, where the viscosity decreased already from the initial germination day to the end after four days (Contreras-Jimenez et al. 2018).

Kilning

To stop germination the kernels are dried with hot air (Fig. 4). Studies successfully used various kilning regimes to malt barley, wheat and durum wheat (Bryce et al. 2010, Alfeo et al. 2018, Farzaneh et al. 2019), but Kunze (2014) described a typical, industrial kilning regime for barley pilsner malt, which will be described in the following. To increase enzyme activity before the enzymes are inactivated at the end of kilning, initial temperatures will typically be between 40 and 60 ºC (Hämäläinen and Reinikainen. 2007). Later the temperature will rise to 80 ºC where enzymes are inactivated which is important if a high starch content is to be secured. In the case of special malts, the temperature might rise above 100 ºC or even 200 for black malts to add roasted flavour (Kunze. 2014). The initial drying at low temperature is called stewing. As water is removed, the growth of the rootlets and acrospire stop.

When the temperature increases to 80+ ºC, Maillard reactions will produce aromatic, colouring compounds, referred to as Maillard products, that both can be positive and negative for the final beer. Put simply, Maillard reactions include carbonyl-groups of reducing sugars and amino acids reacting to form colouring and tasteful melanoidins. Aldehydes of the reaction called Strecker degradation are included in the Maillard products. Compounds deriving from Strecker degradation can decrease the flavour stability causing a stale flavour (Kunze. 2014).

The kilning regimes described by Hämäläinen and Reinikainen (2007) and Kunze (2014) are not definitive to malting, and Alfeo et al. (2018) suggested that kilning at lower temperatures for durum wheat could benefit malt qualities. The study compared malting regimes of durum wheat, where kilning ended at 45 ºC or 70 ºC. Positive effects regarding FAN and levels of enzyme activities was observed for the 45 ºC kilning, while the 70 ºC kilning resulted in malt quality more like that of barley malt regarding Kolbach-index (Alfeo et al. 2018).

In light base malts Maillard reactions are unwanted, due to a flavour destabilising impact. To measure the formation of these products, one can determine the thiobarbituric acid index, abbreviated TBA-index. It indicates the thermal exposure of malt and thereby the degree of Maillard reactions. To limit the amount of Maillard reactions it is important to have low stewing temperature (35 to 50 ºC), low degree of protein modification (<41 %), low degree of steeping and a reduction of oxygen after the third day of germination (Kunze. 2014). TBA is preferred to be below 14 in a congress mash, which is a mash produced from a mashing procedure formulated by the European Brewing Convention

(EBC). There is a correlation in the stability of flavour and the TBA in pale beers. Beer with dark malts are not affected negatively by the Strecker products (Kunze. 2014).

Kilning is also important to limit one of the major off-flavors in beer, dimethylsulfide (DMS). The precursor S-methylmethionine is present in the germinated kernel, and releases DMS during heating. The volatile compound can then be removed at high temperatures leaving little DMS in the final kernel (Kunze. 2014).

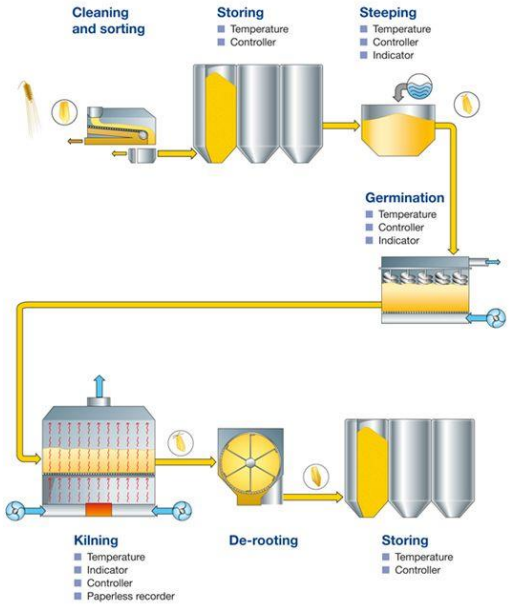

Figure 4 Malting process from intake at the maltster to storage before shipping. (Donau-malz.de)

Mashing

Mashing describes the process during which water and malt are mixed in a certain ratio and heated in intervals of 45 to 80 ºC. The purpose of mashing is to completely degrade insoluble substances, such as starch and certain proteins, into sugars, soluble dextrin and protein residue. The starch derived substances that solubilise are referred to as extract. Most of the extract is produced during mashing by the activity of enzymes acting at their optimum temperatures or in an overlap of optimum temperatures (Kunze. 2014). Prominent enzymes in starch degradation includes α-Amylase, β-Amylase and dextrinases. For the enzymes to gain access to the starch molecule, gelatinisation must happen by heating to approx. 60 ºC. At this temperature, the crystalline structure of starch will deplete and hereby the granules that encapsulate the starch will burst, making the starch accessible to enzymatic catalysed reactions (Kunze. 2014). As the temperature rises above 60 ºC, activity of α-

Amylase will rapidly degrade the large starch residues, amylose and amylopectin, into smaller units. The subsequent decrease in viscosity in called liquefaction.

Following liquefaction is increased β-Amylase activity. The β-Amylase breaks down the smaller, branched units of sugars into maltose. This is a fermentable sugar that traditionally makes up most of the food for the yeast in beer production (Kunze. 2014). Due to the different lengths of amylose and amylopectin, the monosaccharide glucose and the polysaccharide maltotriose are also produced to a smaller degree. Glucose will be preferred by the yeast during fermentation as many monosaccharides are easily digested (Engan. 1972). The amount of glucose in the final wort will be affected by α-Amylase and α-glucosidase activity during germination and mashing (Sissons and MacGregor. 1994).

Temperature and acidity affect the activity level of enzymes, and there are different optimums depending on the enzyme involved. Bertoft et al. (1984) found barley α-Amylase to have optimum temperatures at 65 to 70 ºC, and pH-optimum at pH=5.0 to pH=5.5. The acidity of the mash or wort is mainly dependent on water chemistry and acidity of the malt used (Kunze. 2014). A correlation between mashing temperature and produced carbohydrates was measured by Hu et al. (2014), where e.g. the largest amount of glucose was produced at the interval of 50 to 65 ºC.

Proteinases will catalyse degradation of protein into polypeptides during malting and mashing. This is important to produce amino acids for the yeast metabolism (Steiner et al. 2012). Amino acid content is indicated in the parameter FAN (Free Amino Nitrogen). Amino acids in the wort are an indicator of cytolysis; malt modification. During germination the protein in the kernel is broken down to smaller units by proteinases for the building of new tissues such as rootlets (Kunze. 2014). Besides being crucial for the yeast during fermentation, it also affects the colour formed when heating the malt or wort as it reacts with sugars in Maillard reaction. FAN-levels in high quality brewing wort is expected to be around 200 mg/L (Agu. 2003, Kunze. 2014). However, Bettenhausen et al. (2018) presented specifications of six different barley malts where FAN-levels ranged between 161 mg/L to 204 mg/L.

The proteinases have their optimum at 45 to 50 ºC and a mash-in temperature at that level can be beneficial if the recipe includes low protein adjuncts. If the malt is well modified (successfully malted) there should be no need for high proteinase activity, since the large protein compounds helps foam creation, also called head retention, in the final beer (Kunze. 2014). In a well modified barley

malt, the β-glucan level should not be a concern regarding too high viscosity. If necessary, a low mash-in temperature will increase activity of the β-glucan cleaving enzyme β–Glucanase at 45 ºC.

Tritordeum vs brewing barley

Like wheat and contrary to barley, tritordeum lacks a husk, and the acrospire will therefore be visible from the same end as the rootlets emerge. Figure 5 depicts the two commercial malts analysed in this project, barley and tritordeum. As the husk is regarded as a major filtration aid when brewing with barley, an all-tritordeum beer would raise questions in terms of filterability.

Figure 5 Bestmalz (DE) pilsner malt (left) and Agrasys tritordeum malt (ES) (right). They are referred to as commercial malts in the analysis.

Asociación Española de Técnicos de Cerveza y Malta (AETCM) and Centro di Eccelenza per la Ricerca sulla Birra (CERB) have performed maltings of tritordeum varieties. They both used different malting regimes which varied in temperature, number and duration of steepings and germination days. Nonetheless, the data can be used to discuss tritordeum's qualities in parameters important for beer brewing.

Analysis from 2017 and 2019 conducted at AETCM for Agrasys (ES) (Table 1) used the HT1608 variety. They measured different viscosities for wort of tritordeum malt. The Kolbach-index of tritordeum measured at AETCM was much higher than the recommended value by Kunze (2014). In Kunze (2014) it is stated that Kolbach-index of more than 41 % can be critical to foam and flavour stability. The variety analysed at AETCM was 54 % for the 2019 harvest. In the 2017 harvest it was even 66.27 %. In CERB a variety was measured to be 46.4 %. The high Kolbach-index of tritordeum seems to be the largest disadvantage of using tritordeum as a base malt. The variety tested at AETCM was the same as the one used in this project, and the analysis from 2019 used the same harvest of tritordeum as the one malted in this project. CERB did not inform what variety they malted.

Table 1 Analysis of maltings of tritordeum varieties from AETCM and CERB.

Parameter	Unit	AETCM (Madrid)		CERB (Perugia)
Year of analysis	a	2017	2019	2016
Moisture	%	4.73	5.5	4±0.4
Extract dry/wet	%	83.54/81.5	84.1/82.4	80.9 ±0.8 / 77.7 ±0.7
Viscosity	mPas/s	1.17	1.45	1.7 ±0.03
pH		5.95	5.77	6.03 ±0.08
Colour	EBC	6.75	4.13	6.1 ±0.8
Protein total	%	10.2	12	15.04 ±0.6
Protein soluble	%	6.76	6.5	6.9 ±0.4
Kolbach index	%	66.27	54	46.4 ±1.4
Diastatic power	WK	495	449	456 ±55
Friability	%	72	71	73 ±6
FAN	mg/L	-	-	146 ±15
Nitrogen total (TQ)	N%	-	-	2.53 ±0.09
Nitrogen total (%ss)	%ss	-	-	2.64 ±0.6
Saccharification	min		[10;20]	
Filtration rate	h		<1	

Bianco et al. (2019) found Kolbach-index to be 50.5 % in durum wheat and hence concluded that durum wheat would not make a high quality brewing malt, though it had extract values, enzymatic activity and wort viscosity comparable or even at higher malt quality than barley malt from other studies (Zembold-Gula et al. 2009, Alfeo et al. 2018). Hu et al. (2014) measured Kolbach-index for 13 malted barley varieties and found them to be between 42.9 and 50. The malting of a mix of tritordeum by Martin et al. (1999) showed promising values regarding Kolbach-index (39) and diastatic power (533 WK) especially, however the variety and malting regime of the tritordeum malted was not published. The diastatic power measured at AETCM and CERB were between 449 and 496 WK, where only 1 out of 12 barley malts had as high numbers when measured by Hu et al. (2014).

The conclusion from the malt analysis from CERB was that the tritordeum malt could be used in beer brewing as a base malt, but it would be processed more efficient in the brewing process if barley malt was used as an adjunct due to the lack of husk on tritordeum kernels (Peretti. 2016). A study on enzymatic fingerprinting of arabinoxylan and β-glucan in barley, tritordeum and triticale found that barley and tritordeum shared more similarities in arabinoxylan oligosaccharide structure, which derives from starch (Rakha et al. 2012).

Tritordeum vs brewing adjuncts

Brewing adjuncts such as bread wheat (*Triticum aestivum*), oat (*Avena sativa*) and rye (*Secale cereale*) can be added in beer recipes as a supplement to barley malt. There it can contribute to flavour, foam, mouthfeel etc. (Kunze. 2014). Alfeo et al. (2018) used different malting regimes to produce malts of tritordeum's parent cereal, durum wheat, in a congress mash where it acted as an adjunct to barley malt. The worts were compared to worts where the malted bread wheat was acting as an adjunct to barley malt. They found that wort with durum wheat and barley had lower saccharification time and viscosity compared to wheat-barley wort. Saccharification time is based on a colour reaction between iodine and starch complexes. It indicates if an extensive break down of starch to smaller units of sugar have occurred during mashing. Extract and FAN-levels were even higher than the 100 % barley malt wort. Knowing that durum wheat is more related to tritordeum than the bread wheat it could be proposed that tritordeum malt also exceeds bread wheat malt in saccharification and viscosity. Muñoz-Insa et al. (2011) looked at oat's malting ability and found extract levels were lower than the barley malt and durum wheat measured in other studies (Alfeo et al. 2018, Bianco et al 2019). Tritordeum as a crop was compared to wheats and triticale during water stress (Gallardo and Fereres. 1989, Villegas et al. 2010, Yousfi et al. 2010). Tendencies observed for tritordeum during salinity stress and water stress indicated that tritordeum could be interesting as a drought resistant crop to give high yields in dry environments such as Tunisia. In less dry areas (Spain and Lebanon), triticale and wheat were superior to tritordeum in yield. Aranjuelo et al. (2012) studied wheat, triticale and tritordeum's nitrogen use efficiency during low nitrogen and high nitrogen availability. They concluded that tritordeum showed promising results in the low nitrogen environment, but that more breeding was needed to increase yield and performance in high nitrogen environments. As the focus on draught resistant crops intensifies, due to the increasing global consumption of fresh water and rising global temperatures, tritordeum's potential could reach further than beer brewing.

Aim of the project

This report investigates how different steeping regimes and germination times affect the malt quality of tritordeum. It also brings an overview of the prospects of tritordeum malt in relation to beer brewing by comparison to barley malt.

MATERIAL AND METHODS

Design of sampling

The experiment was designed to explain the germination time's effect on tritordeum malt quality, and if a variation in steeping regime would cause a notable change to the final malt. Applying two different steeping regimes and three different germination times, six different batches of malt were produced. Temperatures were kept constant during steeping and germination to keep the variables at a minimum. The kilning regime was inspired by that of a typical barley pilsner malt (Hämäläinen and Reinikainen. 2007, Kunze. 2014) and by a successful malting of durum wheat by Alfeo et al. (2018). After preliminary maltings with barley and tritordeum it was decided that the steeping regimes would have a duration of 30 hours. One regime would include one air rest of 18 hours in between two 6-hour wet steeps (S1), and the other would include three 6-hour wet steeps and two 6-hour air rests (S2). The 6-hour wet steep is backed up by Reynolds and MacWilliam (1966) who experienced adequate amylase development with maximum six hours of steeping for barley malting. The germination time would vary between four days (96 hours:G1), five days (120 hours:G2) and six days (144 hours:G3). These parameters were based on the water uptake of the kernels and the development of rootlets and acrospire in the trial experiments. The two steeping regimes differed in the duration and amounts of wet steeps and air rests. The single air rest regime was inspired by an industrial malting regime described by Kunze (2014) and a malting of durum wheat of Alfeo et al. (2018). The regime with three wet steeps was inspired by Zembold-Gula et al. (2009), Peretti (2016) and Contreras-Jimenez et al. (2018).

Overview of the regimes can be seen in figure 6 and figure 7.

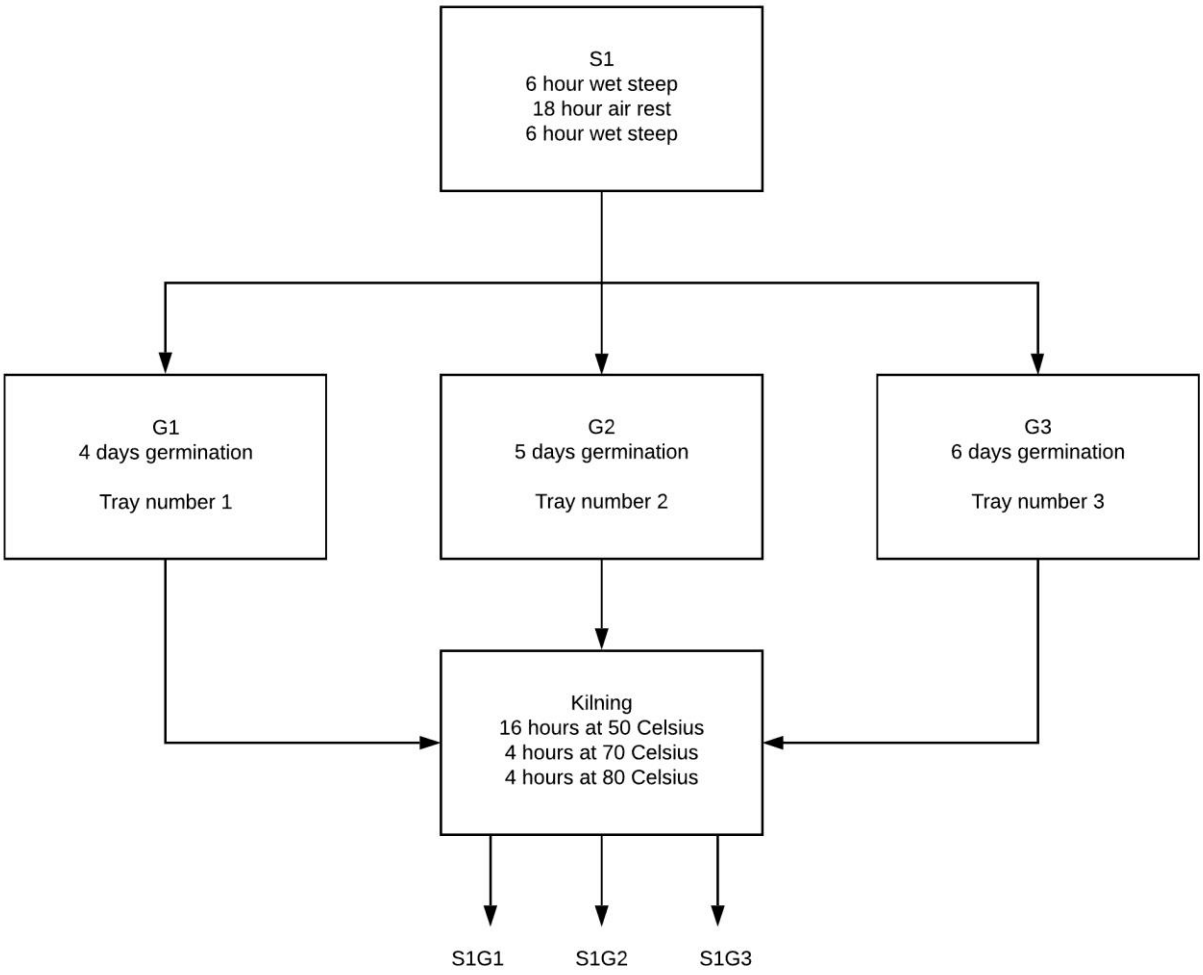

Figure 6 Overview of the malting regimes with the S1-steeping. Temperature during the steeping and germination was 15 °C.

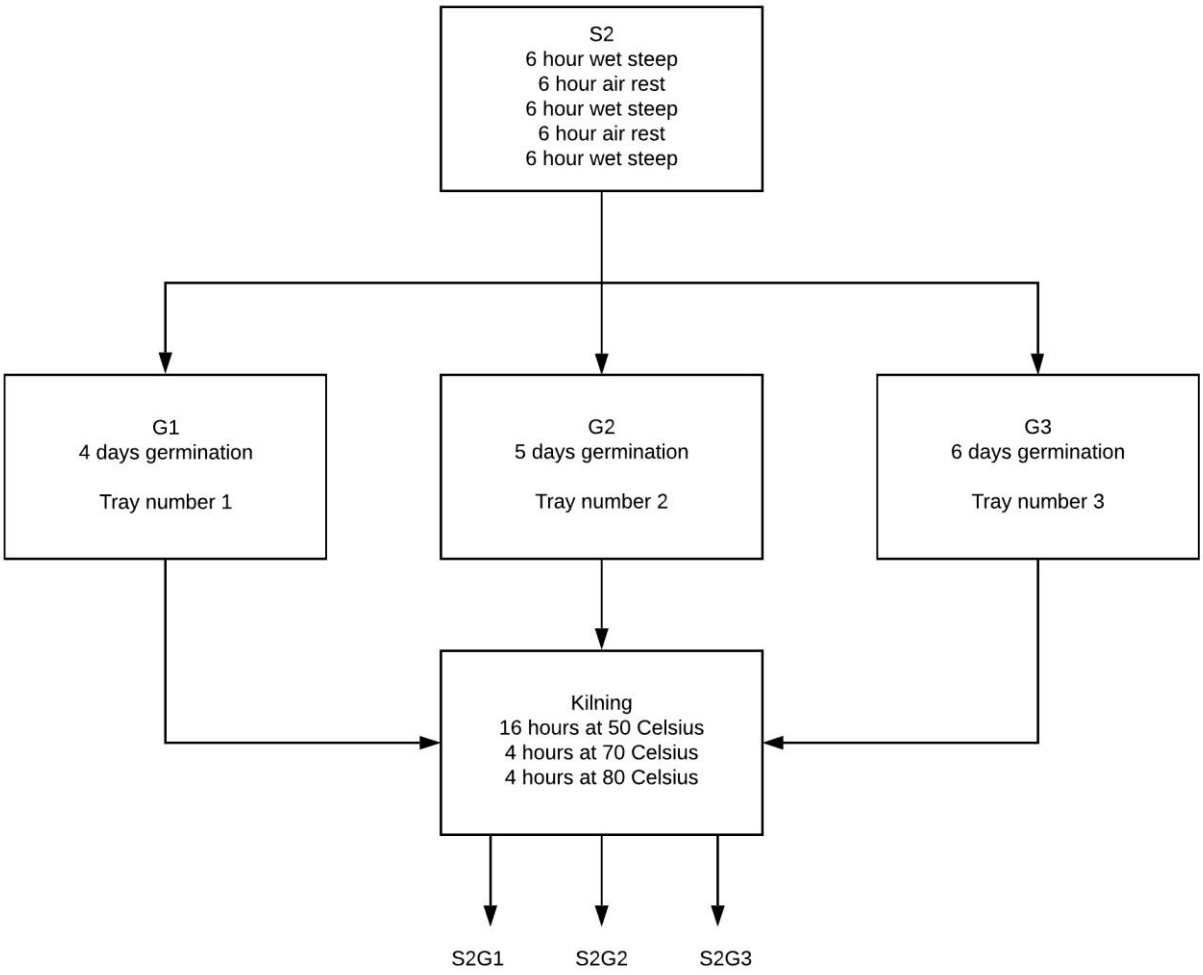

Figure 7 Overview of the malting regimes with the S2-steeping. Temperature during the steeping and germination was 15 °C.

Raw material

The tritordeum kernels malted in this project were of variety HT1608 and were supplied by Agrasys. The raw tritordeum analysed originated from the same shipment. It was harvested in the fall of 2018 and had an expiration in 2021. The tritordeum malt referred to as commercial tritordeum malt was malted by an Agrasys related maltster. The barley malt referred to as commercial barley malt was a Bestmalz pilsner malt. Specifications from Bestmalz (DE) on the Bestmalz pilsner malt can be found in appendix 6. Cracowian tap water was used as steeping water.

Steeping

A polyethylene 5 L bucket was used for steeping (appendix 1). A tap had been attached to the side close to the bottom of the bucket. The bucket was drained by opening this tap and tilting the bucket

towards the tap until water stopped dripping from the tap. To keep the temperature constant during steeping, the bucket was kept in a Q-cell wersja 240 refrigerator, with a set temperature of 15±2 ºC. Before use, the refrigerator was cleaned with soap and water followed by irradiation of ultraviolet light to sterilise the surfaces. The water to kernel ratio (kg) was 2:1.

Germination

After steeping, the bucket was drained of water and the kernels were transferred to three identical polyethylene trays (appendix 2). The trays were placed in a Q-cell wersja 240 refrigerator at 15 ºC. Due to space limitations at the university, another Q-cell refrigerator had to be employed for the S2-steeping and germination. The tray dimensions were 45x31 cm and each tray contained between 780 - 850 g of tritordeum at the beginning of germination. Every 24^{th} hours the trays were sprayed with 30 - 40 mL of tap water to keep the kernels moist. The spraying was done manually with a spray bottle. During the spraying the kernels were turned and mixed by hand.

Kilning

The kilning was conducted over 24 hours. When the desired germination time was reached, kernels from one tray were transferred to a perforated metal plate. The bottom of the plate was covered with a perforated aluminium foil. The plate with kernels was placed in a Memmert oven at 50 ºC. After 16 hours at 50 ºC, the temperature was raised to 70 ºC for four hours. The kilning ended with four hours at 80 ºC. Hereafter the malt was put in a polyethylene container with a screw cap.

Moisture content

During malting the moisture content was measured after each steep, air rest, 24 hours of germination and temperature raise during kilning as well as the end of kilning. The measurements were done by taking samples of five grams from each tray, mixing it, removing surface water with paper towel, and then measuring the moisture in triplicates. Measurements were done with a Radwag MAC50 (d=1mg).

EBC-mashing

The European Brewing Convention (EBC) is the administrative organisation that provides standards for malt, wort and beer analysis. A 1-cube Standard R12 mashing apparatus was used to produce worts according to EBC-analytica method 4.5.1 also referred to as congress mashing. All malts were

analysed in triplicates. A Praca Przerwa Typ WZ-1 scissor mill was used to mill the malt, and the time of milling was three seconds. All equipment used for the analysis listed in table 2 can be found in appendix 3. To compare the tritordeum malts from this project with competitive malts from the industry, a barley pilsner malt from Bestmalz and a tritordeum malt from the maltster associated with Agrasys, were mashed in triplicates. According to the EBC-method both saccharification time and filtration rate were noted.

Malt and congress wort analysis

Table 2 Overview of malt and wort analysis and applied methods.

Type of analysis	Analysis	Specific Method
Analysis of malt	Moisture	-
	Germinative energy	-
	Water sensitivity	MEBAK Band I 2.4.3
	β–Glucanase	Megazyme MBG4
	α-Amylase	Megazyme Ceralpha
	β-Amylase	Megazyme Betamyl-3
Analysis during congress mash	Saccharification time	EBC-Analytica 4.5.1
	Filtration rate	EBC-Analytica 4.5.1
Analysis of congress wort	Acidity	-
	Colour	-
	Extract	-
	Free Amino Nitrogen (FAN)	EBC-Analytica 8.10

Germinative energy was measured after 3 and 5 days for each steeping regime by counting germinated kernels out of 100 kernels from each germination tray. The germinative energy is the percentage of kernels which germinate under normal malting conditions. Water sensitivity of tritordeum was conducted according to the method MEBAK 2.4.3, Band I. According to Essery et al. (1955) it can indicate the germination capacity under excessive amounts of water, where a lack of oxygen can be critical for some grains.

The extract is defined as the sum of all solid material from the malt that dissolves into the liquid phase while mashing. The extract consists of soluble substances such as sugars, dextrins, inorganic substances and certain proteins, as well as insoluble substances including starch, cellulose and high

molecular proteins. In this report, the extract is calculated as the percentage of solubilized kernel residuals from the original amount of cereal added to the mashing.

$$Extract_{\%} = \frac{m_{(aq)}}{m_{(s)}} \times 100\%$$

Where $m_{(aq)}$ is the mass of solubilized grains in the wort and $m_{(s)}$ is the mass of grains forming the base of the mash. $m_{(aq)}$ is calculated from data from an Anton Paar Easydens density meter. Wort colour was measured using a EBC-colorpod, which converts absorbance at 430 nm into the EBC-colour. The acidity of the wort is an important parameter to control since it has an influence on enzyme activity, microbial stability and determines the solubility of proteinaceous substances.
Free amino nitrogen (FAN) analysis of the worts was performed as EBC-Analytica 8.10 method. The FAN method is based on a colour reagent's reaction with the amino group of proteins, peptides and amino acids (Kunze. 2014).

Enzyme assays

All enzyme assays were obtained from Megazyme (IRE) including a list of recommended equipment. In appendix 3 the equipment that was used can be found. All methods were performed in triplicates. For specification of the enzyme kits supplied and used chemicals see appendix 4. For amylase assays a reaction blank was performed for each batch of sample: At first, 3.0 mL of stopping reagent was added to 0.2 mL Betamyl-3 substrate solution or Ceralpha substrate solution (depending on the assay performed) and vigorously stirred. Then 0.2 mL of diluted malt extract was added. For extraction of enzyme: Malt was milled with a scissor mill for 21 seconds. Then, 0.5 g malt flour was weighed into a 50 mL beaker containing 5 mL of Betamyl-3 buffer solution and stirred briefly with a vortex. Afterwards the enzyme was extracted for one hour at room temperature with occasional swirling. The extract was filtered through a filter paper in a funnel. 0.2 mL of the filtrate was added to 4 mL of diluted Betamyl-3 buffer B and mixed to create the extract used for both α -and β-Amylase assay procedure.

β–Glucanase

β–Glucanase activity of the different malts was determined using the MBG4-method (Megazyme, Wiclow, Ireland). A MBG4-unit of activity is defined as the release of 1 μmol/min of 2-chloroe-4-nitrophenol (CNP) from MBG4. The equation below shows the calculation of the MBG4-unit from the absorbance reading.

$$\beta\text{-glucanase activity} = \Delta E_{400} \times 0.145 \times \text{dilution}$$

ΔE_{400} is the absorbance reading subtracted by the blank reading. Dilution is the dilution factor which in this case was 1.

A reagent blank and a malt blank were made to account for the inherent absorbance of malt extract and chemicals. The reagent blank value was obtained by adding 0.9 mL of stopping reagent to a solution of 0.1 mL of pre-equilibrated MBG4 reagent plus 0.5 mL Buffer B. The malt blank value was obtained by adding 0.9 mL of stopping reagent to a solution of 0.1 mL of Buffer B plus 0.5 mL of the specific malt extract.

Extraction of β–Glucanase: 20 grams of malt samples were ground with a scissor mill for 21 seconds. Then, 8 mL of extraction buffer solution B was added to 0.5 g of sample and extracted at room temperature for 15 min with occasional mixing. The samples were filtered through filter paper in a funnel yielding an extract.

Assay procedure: Substrate and extract were pre-incubated at 30 ºC for 3 min together with tubes containing 0.1 mL MBG4 substrate solution. Then 0.5 mL of extract was added to tubes containing 0.1 mL of MBG4 substrate solution and mixed vigorously followed by incubation at 30 ºC for 20 min. At the end of the 20 min incubation period 0.9 mL of stopping reagent was added and stirred vigorously. The absorbance was read at 400 nm.

α–amylase

The α-Amylase activity of the different malts was determined using the Ceralpha method (McCleary et al. 2002). The unit of activity is defined as a Ceralpha unit. One unit of activity is the amount of enzyme, in the presence of excess thermostable α–glucosidase, necessary to release one micromole of p–nitrophenol of BPNPG7 in one minute under defined assay conditions (Megazyme, Wicklow, Ireland). The unit is calculated with absorbance readings obtained from the assay used in the formula below.

$$\alpha\text{-amylase activity} = \Delta E_{400} \times 315.6$$

ΔE_{400} is the absorbance reading subtracted by the blank reading.

According to the standard procedure, a further dilution of the malt extract was made by adding 0.2 mL of the malt extract to 3 mL diluted Ceralpha Buffer A. Afterwards, 0.2 mL of the diluted extract

was added to glass tubes and incubated for 5 minutes at 40 ºC. Ceralpha substrate solution was incubated in the same way. To each tube containing diluted malt extract 0.2 mL of Ceralpha substrate solution was added and the tubes was briefly stirred. Directly afterwards, the tubes were incubated for 10 minutes at 40 ºC. At the end of this incubation period, exactly 3 mL of stopping reagent were added and the tube contents were stirred vigorously. The absorbance of the solutions and reaction blanks was read at 400 nm against distilled water.

β–Amylase

β–Amylase activity of the different malts was determined using the Betamyl–3 method (Megazyme, Wicklow, Ireland) using p–nitrophenyl maltopheptaoside as substrate. One unit of activity is defined as the amount of enzyme, in the presence of excess thermostable α–glucosidase, required to release one micromole of p–nitrophenol from PNP–β–G3 in one minute under the defined assay conditions, and is termed a Betamyl–3 unit. When the absorbance readings are obtained from the assay they can be used in the following formula and the Betamyl-3 unit can be calculated.

$$\beta\text{-}amylase\ activity = \Delta E_{400} \times 19.7$$

ΔE_{400} is the absorbance reading subtracted by the blank reading**.**

Assay procedure: To each sample tube, 0.2 mL of diluted extract was added directly to the bottom of the tube and the tubes were incubated for exactly 5 min at 40 ºC. Betamyl-3 substrate solution was incubated accordingly. To each tube containing extract, 0.2 mL substrate solution was added and stirred. Directly after the tubes were incubated for 10 minutes at 40 ºC. At the end of this incubation period, exactly 3.0 mL of stopping reagent was added and the tube contents were stirred vigorously. The absorbance of the solutions and reaction blanks was read at 400 nm against distilled water.

RESULTS AND DISCUSSION

Water uptake during malting regimes

During the steeping of tritordeum there was a clear increase in moisture content in the kernels (Fig. 8). As Montanuci et al. (2014) observed for barley steeping, the steepest rate of hydration occurs in the initial steeping hours. In figure 8 it seems to be in the initial six hours. The steeping regimes, S1 and S2, did not give different outcomes in final moisture content after 30 hours. This is despite of the

kernels in S2 spending six additional hours in water than the kernels from S1. Final moisture contents after steeping were 37 % for the S1-regime and 38 % for the S2-regime.

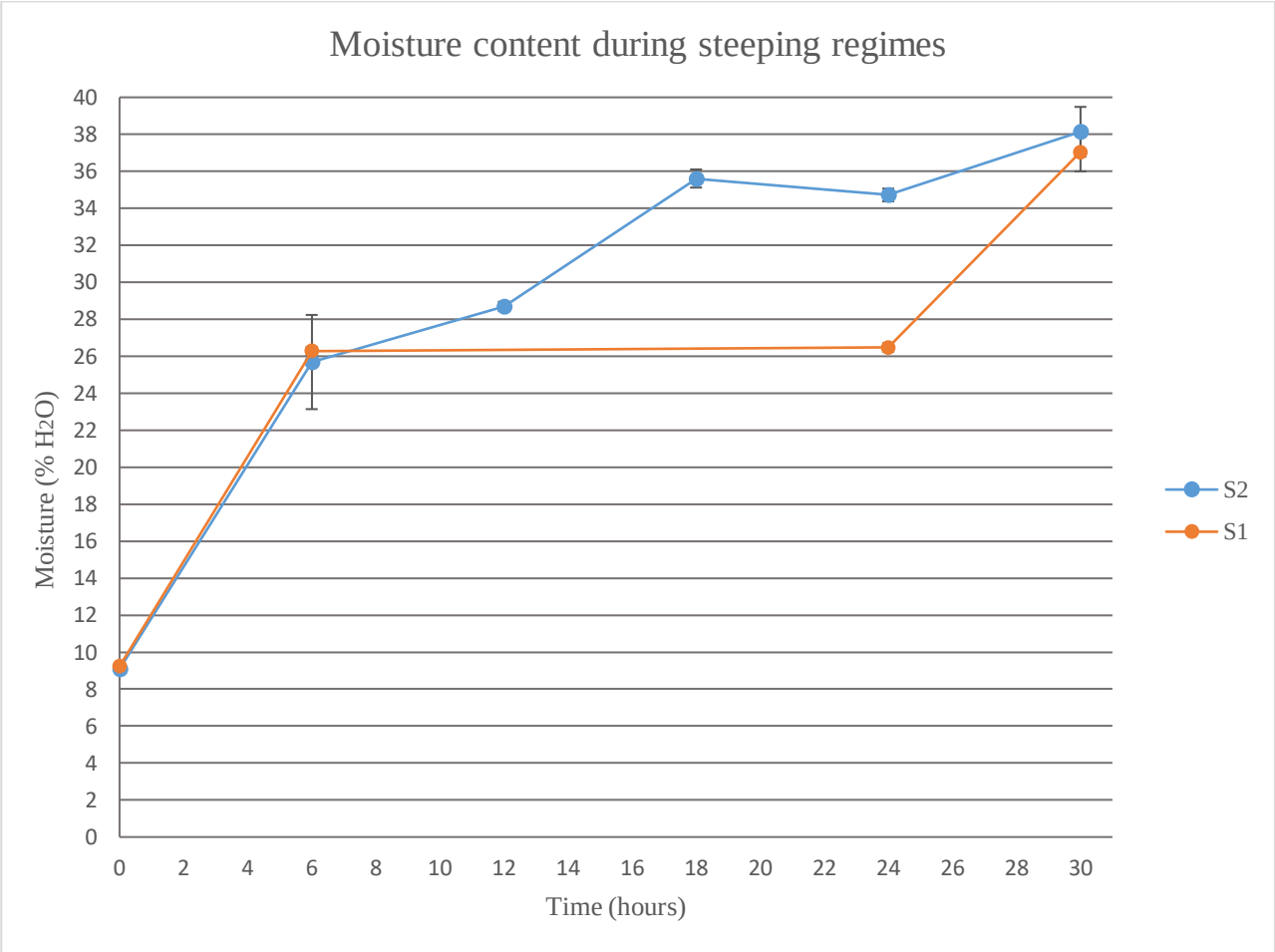

Figure 8 Moisture content in the kernels during steeping. Moisture was measured after each wet steep or air rest were finished, hence the difference in number of data points.

The moisture content of the kernels in the S1-regime had not changed after the 18 hour rest, but then rapidly increased in the following final wet steep. Already after the second wet steep (Time=18h) the S2-regime reached a moisture content not significantly different from the moisture content apparent in the S1-regime after 30 hours. During the first air rest of the S2-regime, an increase in moisture content occurred, while in the next air rest, the moisture content stalled.

Studying the complete malting regimes, the change in moisture content of the kernels is distinguishable (Table 3).

Table 3 Moisture content of tritordeum malt batches. S1 and S2 represent unique steeping regimes, and G1, G2 and G3 germination times. Blue colour indicates measurements during steeping, green colour indicate measurements during germination and red colour indicates measurements during kilning. Grey spaces indicates no meaurements.

%H_2O	S1			S2		
Hours	G1	G2	G3	G1	G2	G3
0	9.23	9.23	9.23	9.08	9.08	9.08
6	26.28	26.28	26.28	25.69	25.69	25.69
12				28.69	28.69	28.69
18				35.61	35.61	35.61
24	26.47	26.47	26.47	34.72	34.72	34.72
30	37.04	37.04	37.04	38.14	38.14	38.14
54	37.64	37.64	37.64	35.41	35.41	35.41
78	37.24	37.24	37.24	34.87	34.87	34.87
102	37.17	37.17	37.17	34.24	34.24	34.24
126	37.18	37.18	37.18	30.30	30.30	30.30
150	6.21	37.00	37.00	6.09	28.81	28.81
174	5.18	6.42	38.18	5.30	6.66	27.06
190	4.40	4.76	6.16	3.85	4.64	6.14
194		3.95	4.66		4.37	4.93
198			3.51			3.79

Kernels with the S1-steeping regime resulted in a stabile level of moist during the germination, but kernels with the S2-steeping regime decreased in moisture during the germination (Fig. 9). The kilning in the end of the regimes evaporated the moisture content down to approx. 4 %. This indicated a minimum moisture content obtainable from kilning tritordeum since the kernels had significantly different levels before kilning.

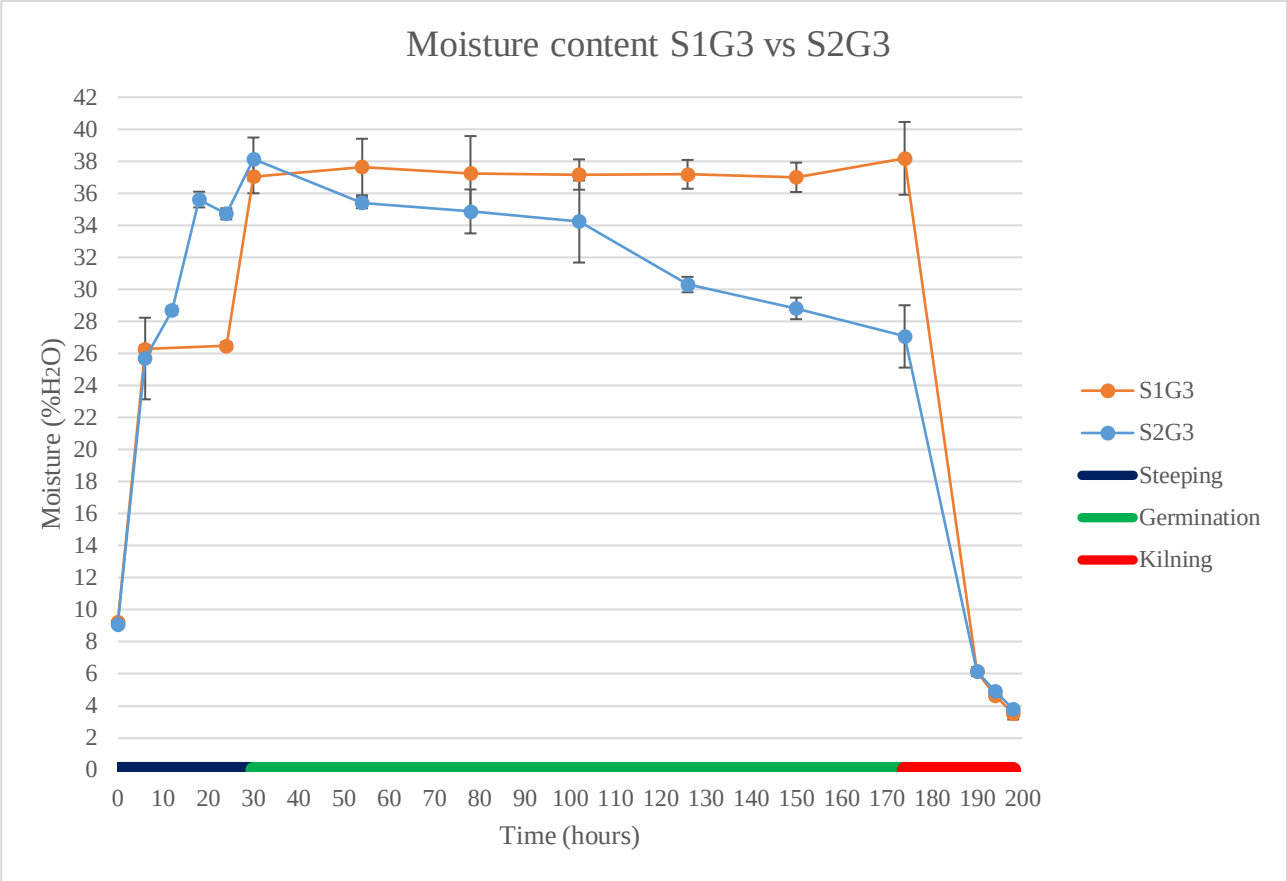

Figure 9 Moisture content in the kernels during S1G3 -and S2G3-malting regimes. Steeping, germination and kilning are indicated in the colours on the x-axis.

A flaw in the collection of data for the moisture content was noted, because the measurements during the first 4 days of germination were from mixed samples from all three trays, whereas the kilning of a batch was done on one specific tray only. This means that the water content got specific for a single tray during kilning, but not during germination. For the fifth germination day, only two trays were left and for the sixth only one tray was left. There was a concern that the kernels in each tray developed slightly different after the S2-steeping. A check of each tray's water content was carried out following the markedly decrease of water in the S2-kernels at the fourth germination day. Here it was found that kernels in the tray that would represent the S2G1-regime had a water content 4 % lower than the trays that would represent the S2G2 -and S2G3-regimes. This could also explain the increase in moisture content for the S1G3 regime on the sixth day of germination, since it was only measured from one tray. The picture of the trays with the assigned malt batches can be found in appendix 2.
But overall it is safe to say that the S2-steeping regime resulted in a germination where the kernels experienced a loss of moisture, whereas the S1-steeping regime had an even level of moisture during the whole germination time.

The commercial tritordeum from Agrasys had a malting regime with two 4-hour steeping steps and an air rest of 20 hours. This was followed by 5 days of germination and then 24 hours of kilning. The kilning temperature increased gradually to 80 ºC. In comparison to the commercial maltster, the moisture content is markedly lower in the batches produced in this project. The moisture content in the commercial tritordeum malt reached a maximum of 43 % during five days of germination where the highest measured moisture reading in this project after the same germination time was 37 %. In figure 10 the moisture content of the commercial malt is compared to the S1G2-malt, which is the most similar regime to the commercial tritordeum malt.

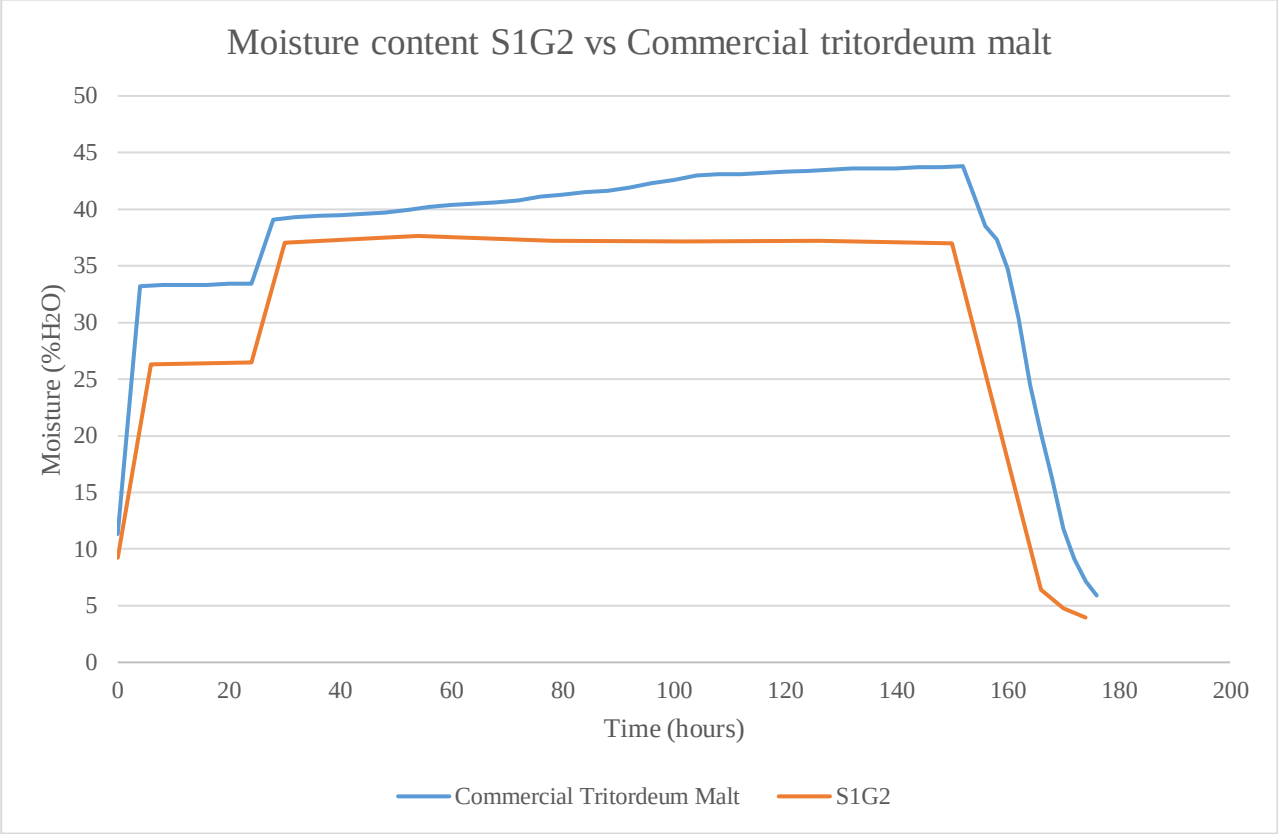

Figure 10 Moisture content of S1G2-malt and the commercial tritordeum malt(Appendix 7). The X-axis does not indicate malting steps since the regimes are not identical.

Why the moisture content of the malts differ could be explained by different malting equipment and settings, variance in measurements due to different methods and equipment for data collection and water availability during germination. At the malting facility, the water sprayed onto the green malt during germination could be in larger amounts than the one applied in this project and the aeration and CO_2-removal during steeping could be more effective, securing a more beneficial environment to the kernels. What effect the difference in malting had on the kernels will be elaborated on in the following sections.

Germinative energy

When the germinative energy was measured after the different steeping regimes, a difference was observed for the tray that would later become batch S2G1 when compared to the trays that would become S2G2 and S2G3. The germinative energy for the S2G1-tray was only 68 % while the other trays were over 90 % Together with the 4 % lower moisture level that was measured in S2G1 on the fourth germination day, this further indicated that the kernels in this tray was developing differently to the other trays that represented the S2G2 and S2G3 batches. The germinative energy for the batches with the S1-steeping varied from 89 to 91 %.

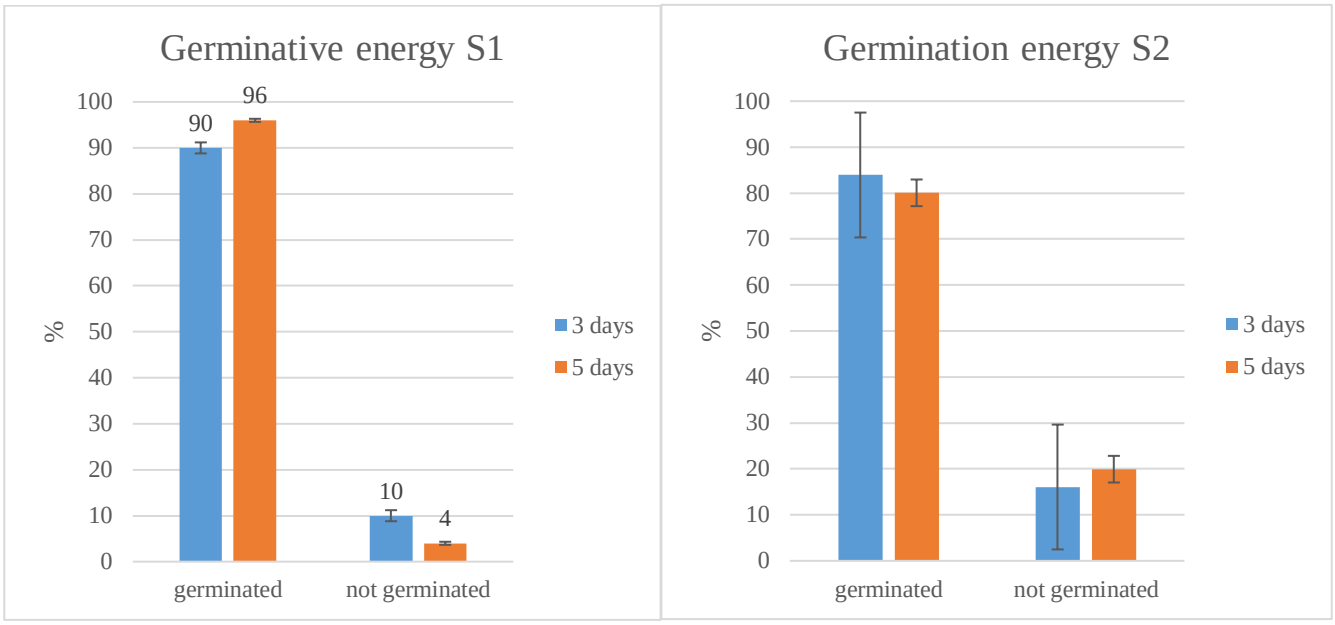

Figure 11 Germinative energy of S1-steeped and S2-steeped tritordeum. Blue colour indicates sample after germination day 3, and orange colour indicates germination day 5.

Water sensitivity of tritordeum kernels was tested according to MEBAK and was found to be 8 % which is defined as very low. This means that tritordeum germinative energy is only affected slightly negatively by an increase in water in its environment. Indirectly it suggests that tritordeum will be able to germinate over relatively long time periods in environments where oxygen is less available (Davidson et al. 1976).

Congress worts

When comparing the saccharification time for the mashes, it indicated that they differed in amylase activity (Table 4).

Table 4 Saccharification time and filtration rate of all congress mashes. "<" means below and ">" more than.

Mashed grain	Saccharification time (Min.)	Filtration rate (h)
Commercial barley malt	<10	<1
Commercial tritordeum malt	<10	<1
Raw tritordeum	>60	>1
S1G1	<10	<1
S1G2	<10	<1
S1G3	<10	<1
S2G1	>60	>1
S2G2	<10	<1
S2G3	<10	<1

All mashes besides the S2G1 and raw tritordeum had a saccharification time below 10 minutes. This corresponded well with the saccharification time noted at AETCM, where it was 10 to 20 minutes. Alfeo et al. (2018) found similar saccharification times for barley malt worts and worts with durum wheat acting as an adjunct. Since the saccharification time is based on a visual inspection of colour formation, it is proposed that the S2G1 had faster saccharification than the raw tritordeum. In figure 12 a picture of S1G3-mash, S2G1-mash and raw tritordeum mash is shown after 60 minutes at 70 ºC. Iodine solution have been added to reveal saccharification in the mashes. If dark colour is formed, starch is present. Dark colour formation indicates that the starch has not been hydrolysed.

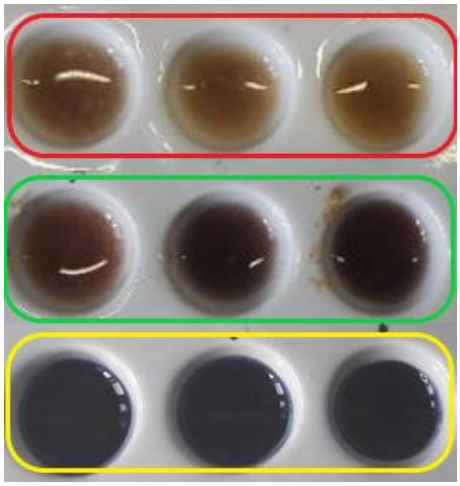

Figure 12: Saccharification test of triplicates of S1G3-mash (red box), S2G1-mash (green box) and raw tritordeum mash (yellow box) in the end of the mashing programme. Dark spots have formed if starch is present.

As seen in the bottom of figure 12, adding iodine solution to raw tritordeum mash made the assay completely black. In the top of the figure the S1G3-mash showed little sign of starch-iodine reaction, and the colour has similarity to what it was before iodine addition. In the middle of the figure, S2G1-mash formed a more transparent dark colour after addition of iodine solution. S2G1-mash did not turn as dark as the raw tritordeum mash, so higher level of saccharification must have taken place in S2G1-wort.

When filtering the mashes to produce the finished congress worts, the filtration rate was noted. Here the raw tritordeum mash and the S2G1-mash also stood out, since they were the only worts with a filtration time over one hour. Hence, they were described as having a slow filtration rate whereas the others were normal. A slow filtration rate can be caused by a high viscosity and low modification, and it was proposed by Reeves (1980) that a slow filtration rate indicates difficulties for brewhouse processing. The mash had to run through a layer of malt residuals and the filter paper, and the more viscous it is, the slower it will move through these media.

Extract

The malt extracts are depicted in figure 13 where the coloured bars are used to show if the worts share the same steeping regime. The S2G1-mash was the only tritordeum malt that was significantly lower in extract of all the tritordeum malts including the commercial tritordeum malt. Raw tritordeum wort

had by far the lowest extract of all the worts which was expected since the malting is expected to modify the kernels. Compared to the second lowest extract, found in the S2G1-wort, raw tritordeum wort only had an extract equivalent of 32.3 % to the S2G1-wort.

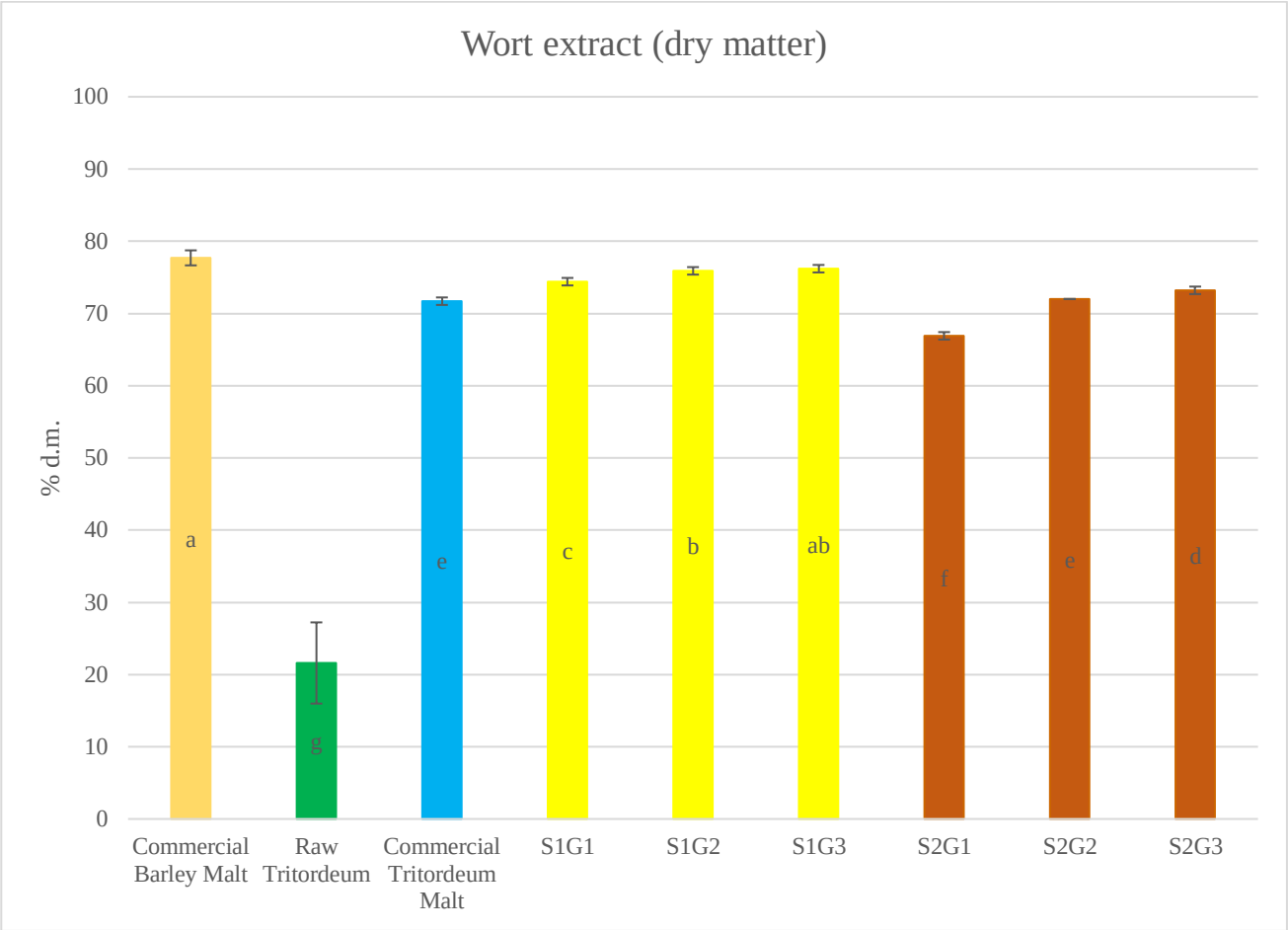

Figure 13 Malt extracts of commercial malts, raw tritordeum and tritordeum malts. The colours indicate unique steeping regimes. Mean values labelled with the same letter are not significantly different ($p<0.05$).

A difference in extract was found between the S1-steeped malts, and the S2-steeped malts, and this could mean that the difference in steeping had influenced the amount of modification in the kernels. The extract level in the S1G3-wort was also the only one that was not significantly different from commercial barley malt. It could advocate for the competitiveness of tritordeum malt when extract is often considered one of the most important parameters in brewing potential for base malts together with enzymatic power (Zembold-Gula et al. 2009, Bryce et al. 2010, Alfeo et al. 2018).

Colour

Comparing the colours of the worts, the lowest absorbance was measured in the raw tritordeum wort and the S2G1-wort (Fig. 14). Again, these two worts were far lower than the other worts, just like in extract, saccharification time and filtration rate. The kilning regime has a big influence on the final colour (Steiner et al. 2012) and since the kilning was different between the commercial malts and the tritordeum malts, it made sense that the there was a gap between these.

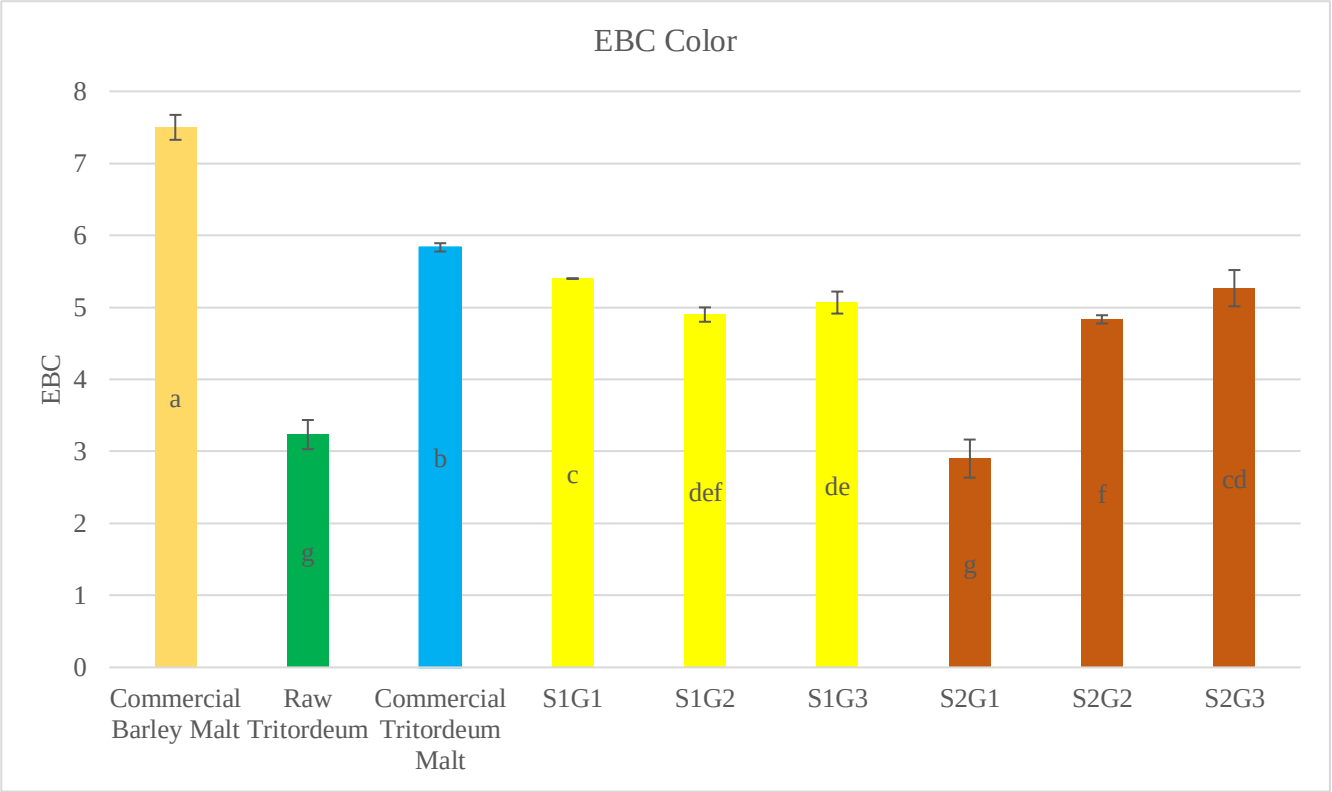

Figure 14 EBC-colour of the congress worts. The colours represent a unique steeping regime. Mean values labelled with the same letter are not significantly different ($p<0.05$).

A trend was visible for the malts with the S2-steeping regime. There was an increase in colour formation correlating with increasing number of germination days, though the most noticeable leap was from four to five germination days. For the worts produced from the S1-steeping regime there was no obvious trend in colour intensity. Why the raw tritordeum and S2G1-wort were not different in colour could be due to low solubility of starch and protein forming a haze in the raw tritordeum wort. The worts from raw tritordeum were hazier than the other worts, and it was decided to filter it through kieselguhr before measuring absorbance. Absorbance reading without kieselguhr filtration for raw tritordeum wort was approx. 25 EBC, and the other worts did not change significantly in EBC

when filtered through kieselguhr. The kieselguhr filtration was done in the ratio of 1 g kieselguhr to 100 mL wort. Pictures of the unfiltered raw tritordeum worts can be found in the appendix 5.

Acidity

The raw tritordeum wort was the least acidic and had higher pH-value. The S2G1-malt had significantly higher pH from all malt worts. The worts produced from malts with the S2-steeping regime were all less acidic than the worts from the S1-steeping regime. Only the S1G2-wort was not different from the commercial tritordeum malt. The S2G2 -and S2G3-worts were the only ones not significantly different from the barley malt wort (Fig. 15). The relatively low acidity (high pH) of the S2G1-wort could be linked to some of the differences in malt extract and free amino nitrogen measured in the S2G1-wort, which was lower than the other S1 -and S2-worts. The commercial tritordeum mean pH-value was 5.78 which aligned perfectly with the value from AETCM of the same harvest (2019) which was 5.77 (Table 1)

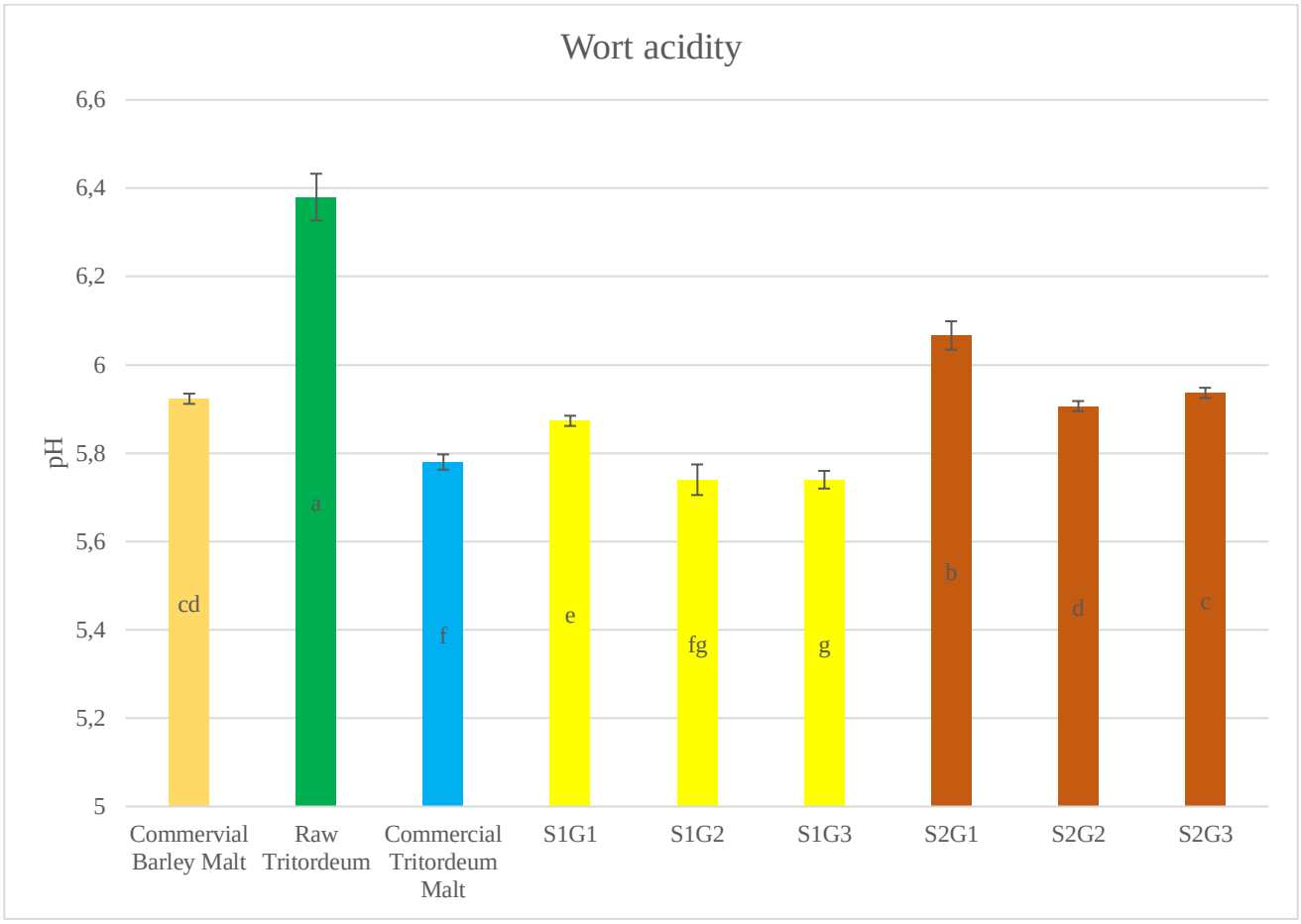

Figure 15 The acidity (pH) of each congress wort. The colour indicates a unique steeping regime. Mean values labelled with the same letter are not significantly different ($p<0.05$).

Free Amino Nitrogen (FAN)

Free amino nitrogen (FAN) was lowest in the raw tritordeum wort, being less than one third of the FAN in commercial tritordeum malt (Fig. 16). Since FAN-levels for the barley malt wort was expected to be around 160 to 200 mg/L (Agu. 2003, Bettenhausen et al. 2018, Kunze. 2014), it was surprising that the mean was 114 mg/L. However, de Sa and Palmer (2004) did measure barley malt to have FAN-levels at 110 to 130 mg/L depending on the variety. The standard deviation was relatively large on the wort triplicates for barley malt, the commercial tritordeum malt, S1G1-malt and S2G2-malt, and a rerun of the assay raised the deviation of barley malt triplicates from 14 to 20 mg/L and decreased the deviation on S1G1-malt from 28.5 to 15.2 mg/L. The FAN-content increased in malt as the germination time increased, just like it was seen in Bryce et al. (2010). However, the S2-regime produced malts with lower FAN in the worts versus the S1-regime. The S2G1-wort had lower FAN than the two other S2-worts and it indicated that the modification was lower. Increasing FAN is a marker of modification in barley malting (Bryce et al. 2010) and the correlation with germination time in S1 -and S2-malts confirmed that it was also the case for tritordeum.

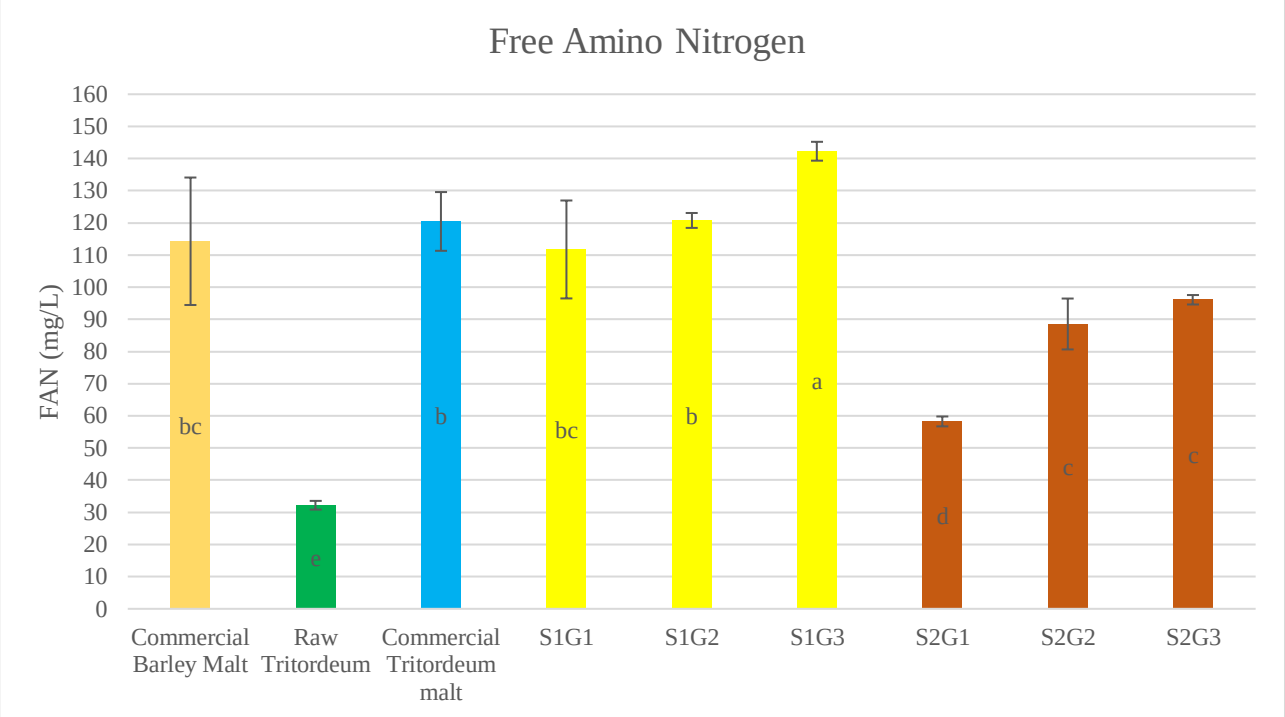

Figure 16 Free Amino Nitrogen (FAN) content of each congress wort. The colours indicate a unique steeping regime. Mean values labelled with the same letter are not significantly different ($p<0.05$).

β–Glucanase

Different patterns in β–Glucanase activity were observed in the different steeping regimes (Fig. 17). The malts with the S1-steeping regime had less activity than the malts with the S2-steeping regime overall. The S1-malts had close to half the amount of activity than the commercial tritordeum malt, but more than twice the amount than raw tritordeum. An increase in activity was measured from the fourth to the fifth day of germination, but not from the fifth to the sixth day. The S2-malts demonstrated an increase in β–Glucanase activity with increasing germination time, though the increase was not significantly different from the fifth to the sixth day of germination. Looking to the findings of Shaluk et al. (2019) the higher moisture content during steeping in the S2-regime could explain some of the higher activity in these malts compared to the S1-malts. The moisture content found in the specifications from the maltster of the commercial malt showed a higher moisture content compared to the malts produced in this project (Fig. 10). That correlates well with the higher mean of the commercial malt's β–Glucanase activity. The level of activity in commercial tritordeum malt was not different from the S2G3-malt and so it seemed that β–Glucanase was induced at a higher level during higher moisture content in steeping. Since the activity in the raw tritordeum was different from all the tritordeum malts, it indicated that malting of tritordeum increased activity of β–Glucanase as shown in Rimsten et al. (2002).

The commercial barley malt had the highest activity of β–Glucanase and was higher than all the other malts.

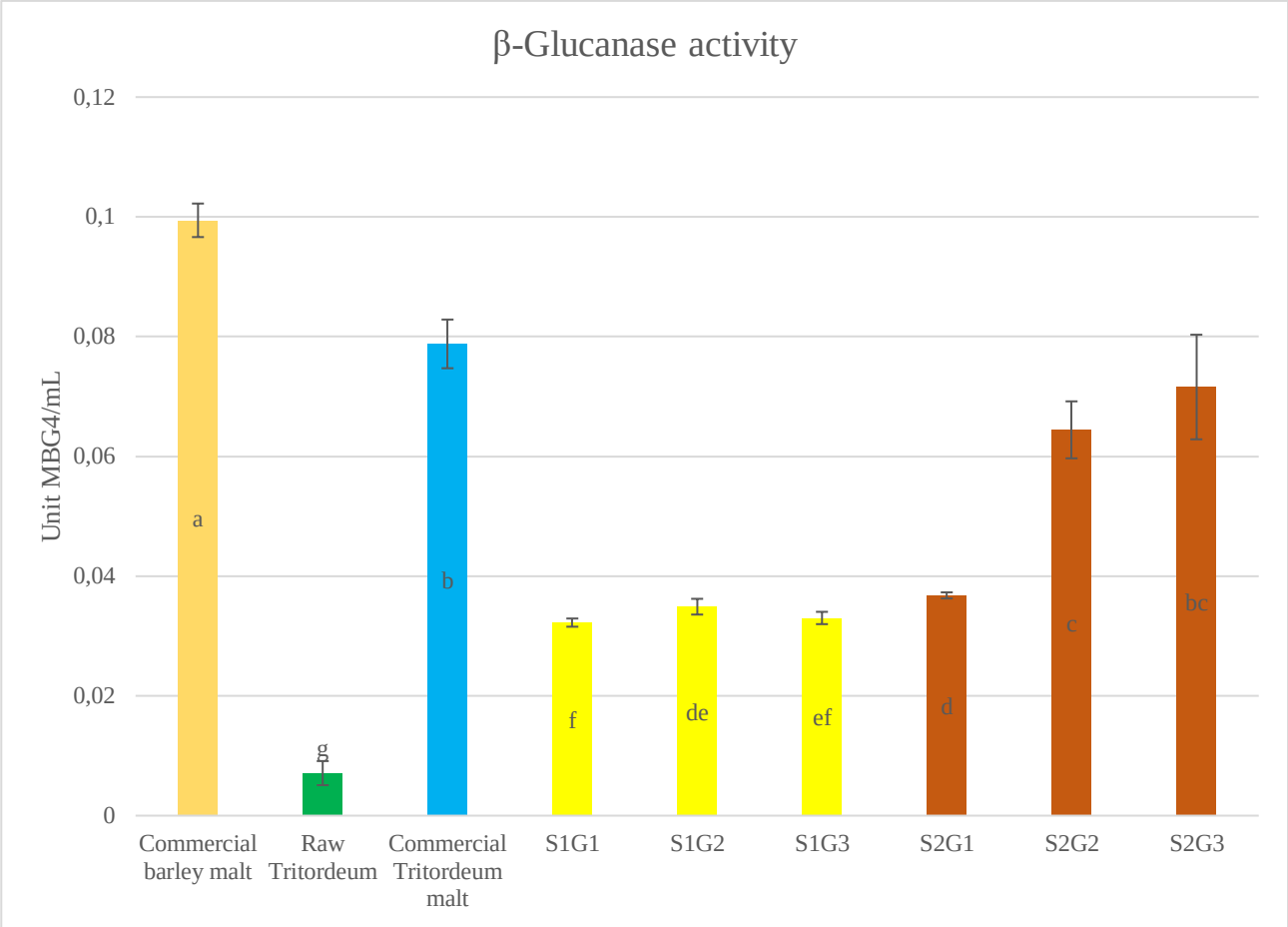

Figure 17 β–Glucanase activity in malts and raw tritordeum measured in MBG4-units per mL according to Megazyme MBG4-method. The colours indicate a unique steeping regime. Mean values labelled with the same letter are not significantly different ($p<0.05$).

The provided malt flour in the Megazyme assay that was used as a standard to indicate how precise the assay had been performed gave a mean value that was 13.27 % lower than the labelled value from Megazyme. After a correspondence with Megazyme it was recommended that the assay had to be improved if the activity-values could be comparable to other studies. However, the relative difference between the kernel flours would be useable to conclude from in terms of differences in activity between the kernels. Megazyme normally discard an assay if the standard's value is more than 2 % off the labelled value. The reason why our assay was off the labelled value could be assigned to factors such as imprecise pipetting, difference in filtration-equipment and extraction difficulties.

Amylase assays

The extracts analysed in the α -and β-Amylase assays were of the same origin. Filtration issues during the extraction process resulted in uneven pipetting of extract produced for raw tritordeum, S2G1-malt

and S2G3-malt. Because of the unknown amount of extract, the readings were not reliable compared to the other malts. Therefore, the activities measured for raw tritordeum, S2G1-malt and S2G3-malt will not be included in the discussion and conclusion of this report.

The standard readings also deviated from the labelled value from Megazyme, just as it was mentioned in the section **β–Glucanase**. The standard for α-Amylase activity was 18.2 % higher than the labelled value, and the standard for β-Amylase was 15.5 % lower than the labelled value. Thus, the comparison to other studies and analysis become difficult as explained in the section **β–Glucanase**. It was not possible to do a rerun of the assays in this project due to the time limitation. The assays recommended equipment were not all available at location and irregularities in the results could be addressed to the lack of this.

α-Amylase

An apparent difference between tritordeum malt and barley malt was observed (Fig. 18) where α-Amylase activity in commercial barley malt exceeded commercial tritordeum malt by 400 %. Figure 18 shows that the readings for the insufficient enzyme extracts from raw tritordeum, S2G1 -and S2G3-malts were lower than the other kernels, and it further confirms the non-conformity measured of enzyme extract made for these kernels. S2G2-malt was lower than the S1-steeped malts and indicated that the α-Amylase was developed to a greater extent with a S1-steeping regime. The S1G3-malt even exceeded the commercial tritordeum malt in activity, and α-Amylase seemed to increase with longer germination time. However, the S1G1 -and S1G2-malts were significantly lower than commercial tritordeum malt.

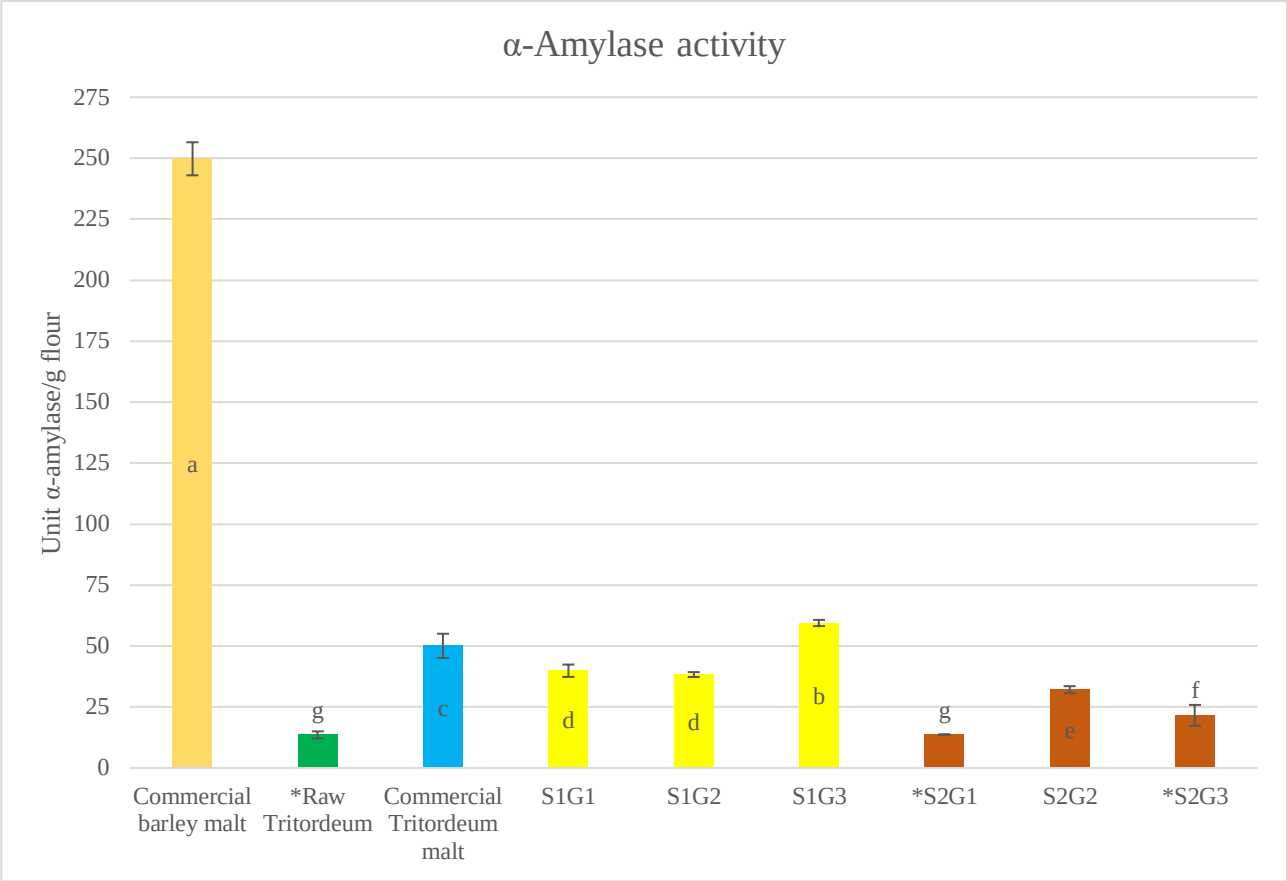

Figure 18 α-Amylase activity in malts and raw tritordeum measured in α-Amylase-units per gram flour according to Megazyme Ceraplha-method. * indicates an unknown amount of extract and therefore unreliable result. The colours indicate a unique steeping regime. Mean values labelled with the same letter are not significantly different ($p<0.05$).

β-Amylase

Barley malt and tritordeum malt exhibited a difference in β-Amylase activity. While barley malt was highest in α-Amylase activity (Fig. 18), the tritordeum malts were higher in β-Amylase activity (Fig. 19). This supports the high diastatic power, 449 WK, for commercial tritordeum in the analysis from AETCM (table 1) compared to barley malt specifications from Bestmalz, which was only 250 WK (appendix 6). β-Amylase activity did not change from S1G1-malt to S1G3-malt, but both were significantly higher than S1G2-malt. Since only the S2G2-malt was assayed properly from the malts with S2-steeping, it did not seem that germination time from four to six days had an impact on β-Amylase activity. According to Kunze (2014) β-Amylase levels increase considerably from the second to the third day of germination and it is proposed that this also was the case for tritordeum.

Of the commercial tritordeum malt and the S1-steeped malts only the S1G2-malt was slightly different from the others. Of the tritordeum malts, the S2G2-malt was only different from the S1G1-malt. As the section **Water uptake during malting regimes** showed, the germination time for the

commercial malt was five days, and there seemed to be a consistence between the tritordeum malts, that suggested that after 4 days of germination the β-Amylase level did not increase significantly.

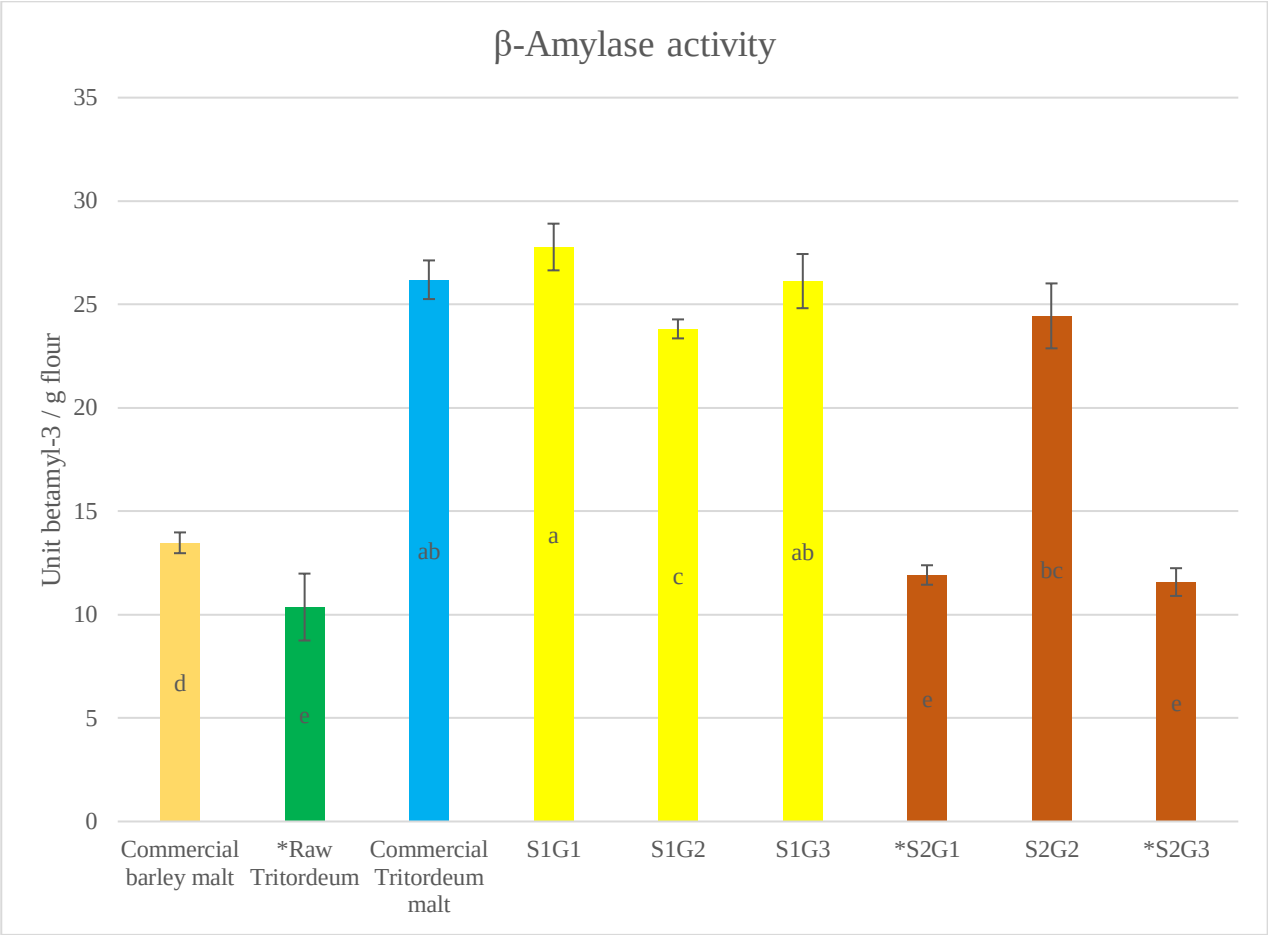

Figure 19 β-Amylase activity in malts and raw tritordeum measured in betamyl-3-units per gram flour according to Megazyme betamyl-3-method. * indicates an unknown amount of extract and therefore unreliable result. The colours indicate a unique steeping regime. Mean values labelled with the same letter are not significantly different ($p<0.05$).

S1-steeping versus S2-steeping

When comparing steeping regime S1 to steeping regime S2 differences were found in several parameters. During the germination the S1-malts did not lose moisture in the kernels which is a general trade for successful malting (Swanston and Taylor. 1990, Kunze. 2014) whereas the S2-malts' moisture content decreased with germination time after not being significantly different from S1-malts after steeping. A decrease in moisture content could be linked to a less modified kernel as the extract and FAN were lower in S2-malt by comparison to S1-malt and the commercial malts (Fig. 13, Fig. 16). According to Kunze (2014), moisture content in barley is essential during germination, and a barley malt should not have decreased more than 1 % in moisture content from the end of steeping

to the end of germination. At least 8 % of moisture was lost on average from the S2-malt, and it is proposed that this was a cause for the lower modification in S2-malts.

Using the analysis from Centro di Eccelenza per la Ricerca sulla Birra (CERB) and Asociación Española de Técnicos de Cerveza y Malta (AETCM) (Table 1) as references, it was likely that the steeping regime caused the decrease in water content during the germination. In CERB, water content of tritordeum fell from 44 % to 41.9 % during 7 days of germination (Fig. 20). The steeping regime used included three wet steeps and two air rests just like the S2-steeping. However, they used 4-hour intervals, where the S2-steeping deployed 6-hour intervals (see section **Design of sampling**). Contrary, the water content of tritordeum at AETCM increased during germination (Fig. 20). The steeping regime at AETCM resembled that of S1G2.

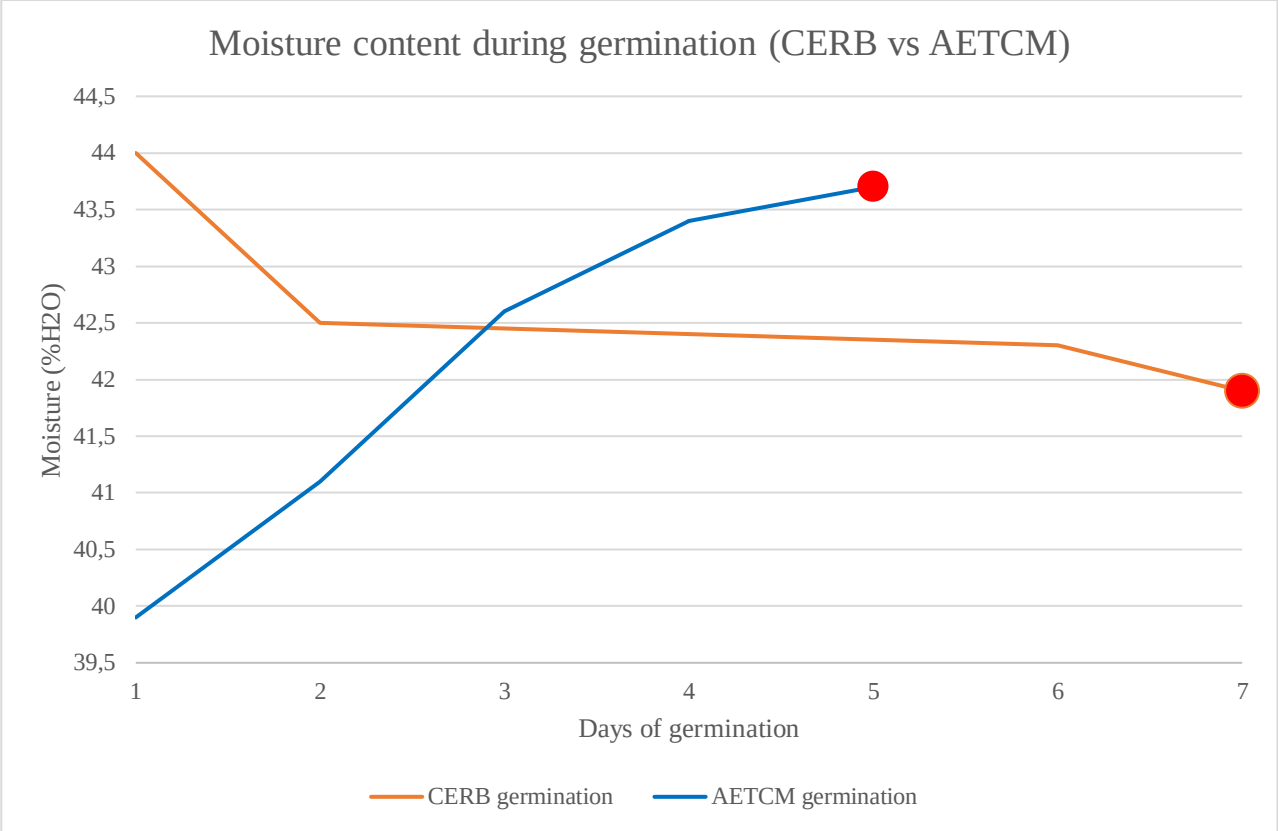

Figure 20 Moisture content in tritordeum kernels during germination regimes at Centro di Eccelenza per la Ricerca sulla Birra (CERB) and Asociación Española de Técnicos de Cerveza y Malta (AETCM). The red circle indicates end of germination in the respective regime.

The malt from CERB also scored lower in extract, Kolbach-index and higher in acidity compared to AETCM malted tritordeum. As mentioned in the chapter **Material and method**, a space limitation caused a change of refrigerator between S1-malting and S2-malting. Though the models were similar,

if the cooling effect was less in the refrigerator used for S2-malting, it could have caused higher evaporation in the kernels. This could then have led to some of the decrease in moisture content.
When extract, amylase activity, acidity and FAN were studied, it indicated less modification in the S2-regime. The extract content was significantly lower in S2-malts compared to S1-malts, though still much closer to the commercial malts and S1-malts than the unmalted, raw tritordeum (Fig. 13). The lower extract in S2-worts could be linked to lower amylase levels resulting in less amylolysis (conversion of starch into smaller branches of soluble sugar-units) during germination and mashing. This hypothesis is supported by the fact that α-Amylase activity for the S2G2-malt was the lowest of the tritordeum malts (Fig. 18). The β-Amylase activity of S2G2, however, was only significant different to the S1G1-malt and the mean value was higher than the S1G2-malt (Fig. 19). As the S2-steeping was only represented in the analysis through one wort (S2G2), it made it more difficult to compare the two steeping regimes in terms of amylolytic activity.

The acidity of the S2-worts (pH=5.90 to pH=6.06) was lower than the S1-worts (pH=5.74 to pH=5.87) (Fig. 15). This indicated that the amylases would be less active at the lower acidity. As mentioned earlier, α-Amylase's activity optimums lie within the interval pH=5.0 to pH=5.5 (Bertoft et al. 1984) which S1-wort was nearer.
Though FAN-levels in the S2G2 -and S2G3-worts were not significantly different to the S1G1-wort, the mean values of S1-worts were all higher than the S2-worts (Fig. 16). As the S1G3-wort even exceeded the commercial barley and tritordeum worts, it looked like the S1-steeping was preferable for high FAN-production, when compared to S2-steeping.
The germinative energy recorded for the S2-regime was difficult to interpret, because the counted germinated kernels decreased from three days to five days (Fig. 11) and the kernels counted on the third day of germination deviated greatly between the triplicates. However, the mean percentage of germinated S2-steeped tritordeum was significantly lower than the S1-steeped tritordeum. Considering the levels of extract, FAN and enzymatic activity, it connected well to the notion that S2-steeped tritordeum germinated slower than S1-steeped tritordeum.

All in all, S1-steeping produced a more favourable malt where FAN, extract and amylolytic power were superior. The S2-steeping was higher in β–Glucanase and β-glucan would presumably be lower in the S2-malts. As Shaluk et al. (2019) found in their steepings of barley, the moisture content during steeping lowers the level of beta-glucan. Since the hydration level seemed to be higher in S2-kernels

during most of the steeping, it suggested that it increased the activity of β–Glucanase. The signs of lower modification in S2-malt do however indicate a high level of β-glucan and high viscosity. Measurements of β-glucan are needed to answer this.

Effects of germination time

Just like other studies found (Bryce et al. 2010, Farzaneh et al. 2017), germination time had a strong effect on the level of e.g. FAN, β–Glucanase and extract. Regarding FAN, the mean values increased with germination time for both S1 -and S2-malts (Fig. 16). Because of the relatively large difference from the malts to the raw tritordeum, the FAN-level must have risen very early in the germination. This finding heavily indicates a continuous proteolytic activity during the first six days of germination. Knowing that the commercial tritordeum malt had germinated for five days, it was striking that it was 120.8 mg/L and the wort from the S1G2-malt was 120.4 mg/L. The relative increase from the fifth to the sixth day of germination for the S1-malts advocated that the protein breakdown still happened at a great rate in this stage of germination. The data for the S2-malts did not show this development clearly, because the FAN-level was not significantly different from the fifth to the sixth day of germination. Instead a relatively large increase from day four to five was observed for S2-malts, where it increased from 58.27 to 88.56 mg/L.

For the S1-regime, the β–Glucanase activity did not follow a trend within the germination times observed, albeit some small significant differences between the samples. From the fourth day of germination until the sixth day it more than doubled in activity, from 0.037 to 0.072 U-MBG4/mL. An increase in activity with germination time was also observed by de Sa and Palmer (2004) when they germinated barley at different germination times. However, the deviation in the triplicates caused the S2G2 -and S2G3-malt not to be different in β–Glucanase. As only S2G3-malt was not significantly different from the level of β–Glucanase measured in commercial tritordeum malt, it was proposed, that with a S2-steeping regime increasing germination time will produce a tritordeum malt higher in β–Glucanase. For a malt with the S1-steeping, the germination time does not seem to heavily affect β–Glucanase level after four days of germination. Since the raw tritordeum was lower than all malts, activity of β–Glucanase must start early in the germination, like that of barley (de Sa and Palmer. 2004).

The amount of extract measured in each wort increased the longer the malt had been germinating. In figure 21 the interval from 65 to 80 % on the y-axis of figure 13 has been plotted to reveal how the extract correlated with germination time for both S1 -and S2-malts. The mean extract value for S1G3 is higher than S1G2, though not significantly different.

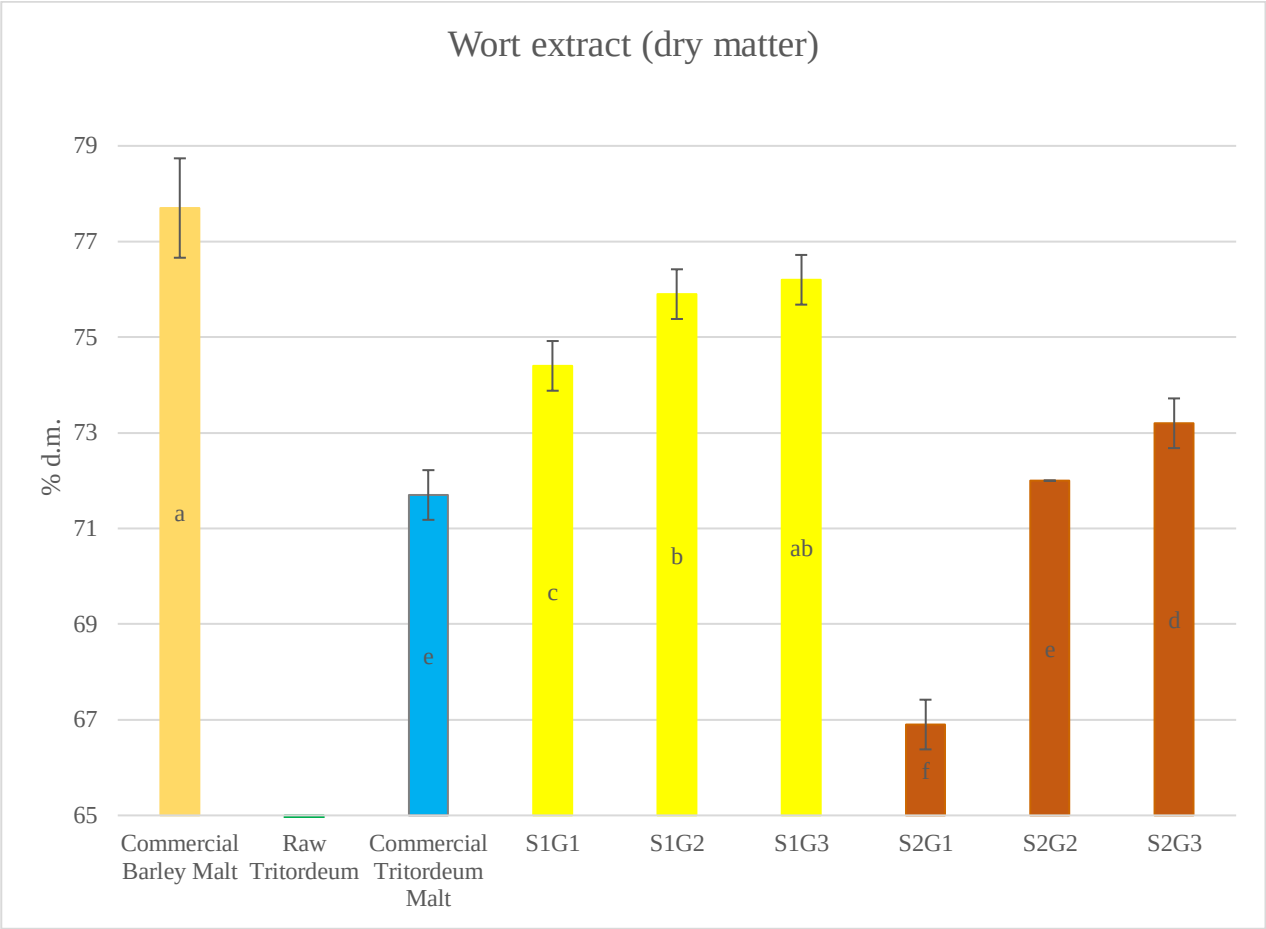

Figure 21 Zoom of figure 13. Malt extracts of commercial malts, raw tritordeum and tritordeum malts. The colours indicate unique steeping regimes. Mean values labelled with the same letter are not significantly different ($p<0.05$).

β-Amylase did not seem to increase from day four to six for both S1 -and S2-malt, but an increase from raw tritordeum to the malts indicated that the β-Amylase development happened earlier in the germination as mentioned in section **β-Amylase**.

α-Amylase rose significantly from the fifth to the sixth day of germination for the S1-malts, though not changing significantly between the fourth and fifth day. Since only one S2-malt was evaluated, it was not revealed if an increase on the sixth day also was apparent for the S2-malts. Assuming the same development for S2 as S1, it could make sense to let tritordeum germinate for six days or more to ensure a higher amylase level.

Acidity in worts decreased with germination time for both S1 -and S2-malts (Fig. 15). In combination with raw tritordeum wort being lower in acidity than all the malts, these data indicated that increasing germination time lowers pH and hence increases acidity. After the fifth day of germination, a decrease in acidity was seen for the S2-wort.

To summarize, germinating tritordeum for four days (G1) would result in abundant amounts of β-Amylase, but give a lower quality malt than the longer germination time. In comparison to five days of germination the additional sixth day did not change acidity and β-Amylase by a large margin, but it increased α-Amylase, β–Glucanase, extract and FAN. Hence, most parameters were in general at their peak after six days of germination.

Beer brewing prospects of tritordeum

When selecting a base malt for beer brewing, based on typical parameters the main factors are extract and processability (Zembold-Gula et al. 2009, Bryce et al. 2010, Farzaneh et al. 2017). The malts produced in this project have shown promising results for tritordeum in all these areas compared to the most common European base malt, barley pilsner malt. A simple steeping regime of 30 hours including two wet steeps and an air rest combined with a germination regime of six days produced a tritordeum malt, abbreviated S1G3, with an extract not different from a commercial barley pilsner malt. The amylolytic activity of tritordeum malt showed that barley malt was far higher in α-Amylase, but behind all tritordeum malts when it came to β-Amylase. This could have an impact on the amount of monosaccharide content in the tritordeum wort, since α-Amylase is responsible for cleaving starch into residues that can be converted into maltose and glucose by β-Amylase. Yeast prefers to feed on monosaccharides, and glucose concentration affects flavour compounds in fermented wort (Engan. 1972). Further research on the fermentation of tritordeum wort would be needed to assess aroma development.

In terms of protein residue, only FAN was measured in this project. Tritordeum malt (S1G3) even exceeded commercial barley malt with its significantly higher FAN-concentration. The β–Glucanase level in tritordeum could lead to higher viscosity and filterability. However, analysis from maltsters (Table 1) yielded a low β-glucan content in tritordeum malt compared to barley. Hence, the lower β–Glucanase content is expected to be enough to secure a successful filtration. Acidity in S2G2 and S2G3 malts were close to that of commercial barley malt.

CONCLUSION

Variations in steeping regime had a significant effect on malt quality of tritordeum. Using a simple, single-air rest steeping regime (S1) yielded a congress wort from tritordeum malt with levels of extract, free amino nitrogen (FAN) and β-Amylase that were higher than that of wort produced from tritordeum malt with two air rests (S2). The single-air rest steeping regime also resulted in acidities in the congress worts suitable for enzyme activity compared to the double-air rest steeping regime. Only β–Glucanase activity was higher for the double-air rest steeping regime.

Germination time had a positive effect on FAN and extract, where both types of tritordeum malt increased the FAN-content and extract with increasing germination time. Acidity increased from day four to five of the germination but did not change from day five to six. Other parameters showed different patterns depending on the steeping regime of the malts. β–Glucanase level increased with germination time in malts with double-air rest steeping but not for the malts with a single-air rest steeping. An increase in α-Amylase activity was observed between the fifth and sixth day of germination for the singe-air rest steeped malts.

The tritordeum malt with one air rest (S1) and six days of germination (G3) was not significantly different in extract to a commercial barley malt. It even exceeded the barley malt in FAN-level and in β-Amylase level, which are crucial parameters for a high-quality brewing malt. The barley malt was much higher in α-Amylase level and β–Glucanase level though. Saccharification time for all the tritordeum malts besides one were less than 10 minutes, and the α-Amylase activity measured could be enough for a successful mashing (note the high extract) in tritordeum malt. The results suggest that tritordeum malt has potential to be a new competitive base malt or adjunct in the brewing industry.

FURTHER STUDIES

Many parameters in tritordeum malt analysis are left to be studied. Like Cozzolino et al. (2013) studied fatty acid's effect in barley water uptake, it would also be relevant to make similar studies for tritordeum. Viscosity, β-glucan level and Kolbach-index can tell us about the processability of tritordeum wort. Limitations made it difficult to measure malting yield in this project, and so it would be interesting to study malting yield in tritordeum malting just like Farzaneh et al. (2017) did with barley. Sugar composition, fermentability (attenuation) and metal analysis can reveal the potential for traditional beer fermentation. Organoleptic assay after fermentation would reveal what flavour chart tritordeum beer has compared to traditional beer styles.

Experiments with different recipes of tritordeum as a base malt or an adjunct to other malts could shed light on the enzymatic power that can be applied with tritordeum's high β-Amylase content, but also as an alternative to wheat in wheat beers. Other steeping and germination regimes would also be of high relevance, and after the findings of Swanston and Taylor (1990) and Bryce et al. (2010), the effect of a steeping regime without air rests would be interesting to try. Also the laboratory standard steeping regime described in Bryce et al. (2010) and de Sa and Palmer (2004) would be useful to malt with, since it could make results more comparable to other studies. The effect of temperature, water chemistry and kilning regimes were not investigated in this report, and it would also be necessary in the search for optimal malting conditions for tritordeum. For instance, it would be interesting to steep tritordeum at higher temperatures like Montanuci et al. (2014) and Reeves (1980) did with barley. Alfeo et al. (2018) found that low kilning temperatures had a positive impact on FAN-level and enzymatic activity in durum wheat. At last the breeding of tritordeum varieties with high qualities in beer production should be an ongoing process like it is with barley.

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APPENDICES

Appendix 1

Appendix 2

Appendix 3

Equipments

Moisture analyser: Radwag MAC50 (d=1mg).

Scale: Radwag AS 220.R2 Analytical Balance

Spectrophotometer: VWR UV-1600PC

Temperature/ventilation chamber: Q-cell wersja 240

Oven: Memmert UP400

Congress masher: 1-cube Standard R12

Filter paper: Mascherey-Nagel MN 614.25 * Ø 320 mm

Glassware: Erlenmeyer flasks (2-3 L). bluecap bottles (200 ml – 2 L)

Vortex: Labnet International VX-200 Vortex Mixer

Scissor mill: Praca Przerwa Typ WZ-1

Densitymeter: Anton Paar Easydens

EBC-colour: Colour pod Lovibond

pH-meter: Elmetron CP-401

Appendix 4

Chemicals

FAN-assay

Na_2HPO_4

KH_2PO_4

Ninhydrine

Fructose

KIO_3

EtOH (96 %)

Glycine

B–Glucanase assay (MBG4-method. Megazyme. Ireland)

Glacial acetic acid 1.05 g/ml

5 M NaOH

2 M NaOH

2 M HCl

Sodium azide 0.02 % w/v

NaH2PO4.2H2O

B-TRIS500

Amylase assays (CERALPHA -and Betamyl-3-method. Megazyme. Ireland)

G-LCYST200

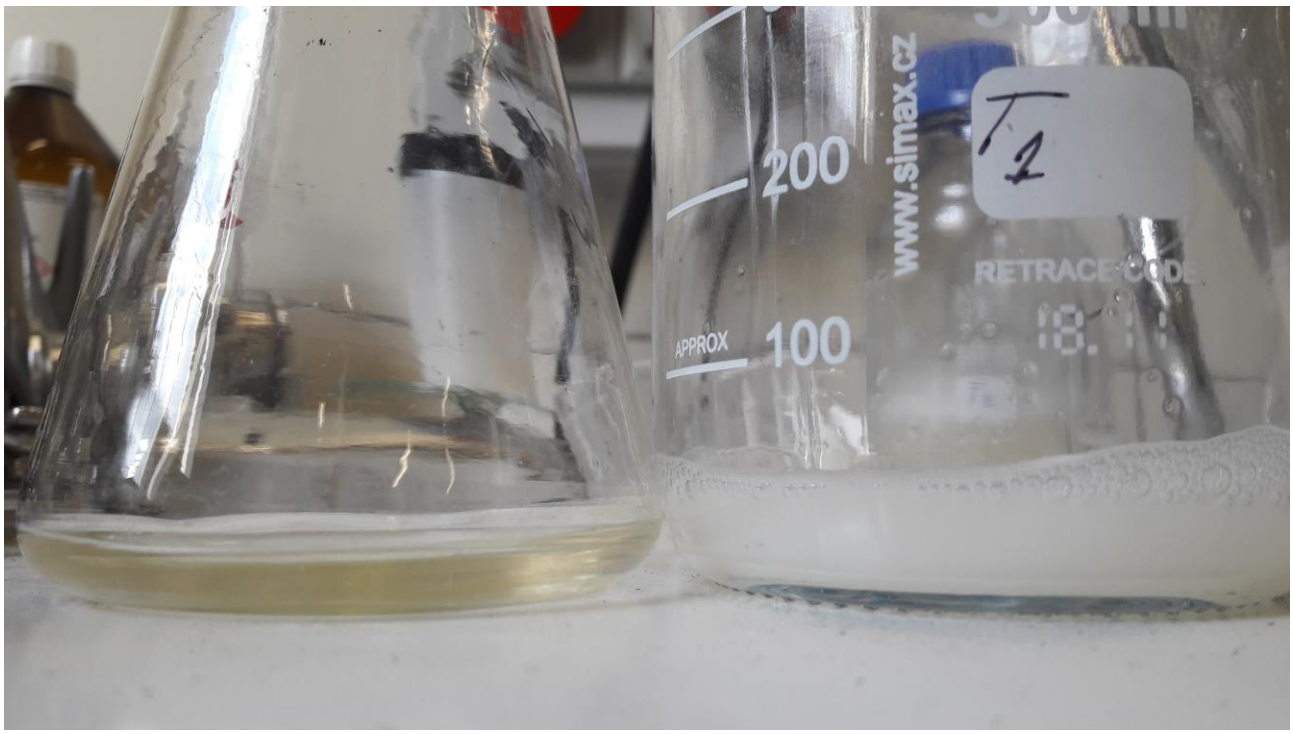
4 M NaOH

4 M HCl

B-TRIS500

Appendix 5

Unfiltered wort of raw tritordeum on the right side and the filtered one on the left.



Appendix 6

Specifications of finished pilsner malt from Bestmalz representing the malt referred to as commercial barley malt in this project.

SPECIFICATION	UNIT	MINIMUM	MAXIMUM
Moisture content	%		4.9
Extract fine grind, dry basis	%	80.5	
Fine-coarse difference EBC	%		2.0
Viscosity (8.6%)	mPa·s		1.60
Friability	%	81.0	
Glassiness	%		2.5
Protein, dry basis	%	9.0	11.5
Soluble nitrogen	mg/100g Malz-Trs.	610	780
Kolbach index	%	36.0	45.0
Wort color	EBC	3.0	4.9
Wort color	L	1.6	2.3
Wort pH		5.7	6.1
Grading > 2.5mm	%	90.0	
Diastatic power	WK	250	

Appendix 7

Commercial tritordeum moisture data.

hours	humidity
0	11.3
4	33.2
8	33.3
12	33.3
16	33.3
20	33.4
24	33.4
28	39.1
32	39.3
36	39.4
40	39.5
44	39.6
48	39.7
52	39.9
56	40.2
60	40.4
64	40.5

68	40.6
72	40.8
76	41.1
80	41.3
84	41.5
88	41.6
92	41.9
96	42.3
100	42.6
104	43
108	43.1
112	43.1
116	43.2
120	43.3
124	43.4
128	43.5
132	43.6
136	43.6

140	43.6
144	43.7
148	43.7
152	43.8
154	41.1
156	38.5
158	37.35
160	34.7
162	30.4
164	24.5
166	20.2
168	16.3
170	11.77
172	9.1
174	7.12
176	5.9