FACULTY OF BIOSCIENCE ENGINEERING

TRITORDEUM AS AN ALTERNATIVE GRAIN FOR BREWING

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Preface

Throughout this bachelor thesis new insights in brewing were learned. Also, it changed our view on traditional beer and the beer industry. We would like to thank our tutor prof. dr. ir. Jessica De Clippeleer and our two promotors dr. ir. David Laureys and dipl. ing. Jonas Trummer. They helped us a lot during our writing and practical work and without them completing our bachelor thesis would have been impossible. We also would like to thank the University of Ghent for giving us our raw materials and letting us use their labs and instruments.

Abstract

Hexaploid tritordeum is a hybrid organism made by crossbreeding barley (*Hordeum Vulgare*) and wheat (*Triticum aestivum, Triticum dicoccum and Triticum monoccum*). Tritordeum has desirable flavours and aromas and a lower gluten percentage. Because of this tritordeum can be an interesting alternative grain for brewing.

The main objective of this bachelor thesis is verifying if unmalted tritordeum can be used in the brewing process, by using the Congress mash method. Using unmalted grains is economically favourable, because the malting process can be avoided.

The mixtures that were used contained 0, 10, 20, 40 and 100 % of unmalted tritordeum without or with enzymes. The rest of the mixtures is composed of barley malt. All tests were performed in triplicates to notice unexpected abnormalities and form a more accurate conclusion by using the average of these results.

After performing a Congress mash, the obtained wort is filtered and fermented with bread yeast. The fermented and unfermented mixtures are analysed for different standard characteristics (pH, extract content, free amino nitrogen, alcohol percentage, colour, saccharification time).

This resulted in the conclusion that unmalted tritordeum (up to 40%) is useful in addition to barley malt as an alternative grain. However, more research needs to be conducted to see if this percentage can be increased. Obtaining wort from 100% unmalted tritordeum was not possible.

Key words: *unmalted tritordeum, brewing, Congress mash, saccharification time, standard characteristics*

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Introduction

The hybrid breed of grains called tritordeum is already used in a variety of different applications. Tritordeum has specific qualities that other cereals do not possess. Their lower concentration of gluten is one of their best benefits. Tritordeum could be a product for people who want to reduce the intake of gluten, but not want to switch to other kinds of products (Luis Vaquero, 2017)(Martín, n.d.).

In this thesis tritordeum was tested as an alternative grain for brewing beer. Unmalted tritordeum was used. This skips the malting process increasing the economic viability of the brewing process.

To test the grain, it was used in a Congress mash with and without added barley malt. The produced wort was then subjected to different tests before and after fermentation to criticize the quality. The tested standard characteristics are pH, extract content, free amino nitrogen, alcohol percentage, colour and saccharification time.

Structure wise the thesis starts with a literature study; in this part the brewing process and the standard characteristics are discussed. It's important to understand the core basics of brewing and the impact of the different parameters. Tritordeum is examined as well, by discussing the different properties of the grain.

In materials and methods, the experimental setup is explained as well as the Congress mash process. The used tests were iodine test, fermentation, free amino nitrogen determination and the other standard characteristics were determined.

Finally, the results are discussed and compared to the literature study and interpreted. After this a conclusion is given.

1. Literature study

1.1 The brewing process

The art of making fermented alcoholic beverages, including beer, exists for over thousands of years now. Still it's changing every day and new flavours, colours and processes are developed. In order to analyse what the influences of subtle changes are, it's important to understand the whole process of beer production. Brewing is a complex process, where a lot of different steps are important to get the desired results. The first important step in the process is choosing the right raw materials. The main ingredients in beer production are hops, water, malt and yeast. There is a very wide selection of different types and varieties. This makes choosing the right materials very important. The addition of different kinds of grains as starch containing materials can add different flavours and different colours (J,D. ,1977).

1.1.1 Malting

Overall, the starchy raw materials used for brewing must be adjusted and processed in order to get the necessary sugars for fermentation. The first step in this process is malting the grains. The malting begins with raw barley, oats, rye or any other starch containing grain. The raw grain is steeped in water and germinated afterwards. During the germination, the natural enzymes become active; these enzymes will be crucial in the next steps of beer production. The grain is then dried in a kiln and sometimes roasted, if a darker malt and therefore a darker beer is desired (Yadav, 2018).

1.1.2 Saccharification process

The following definition will be used for saccharification in the entire thesis: "the process of breaking down starch into its monosaccharide components". The most important enzymes to realise this are the diastatic enzymes alpha and beta amylase. The function of amylase enzymes is to hydrolyse starch into sugar units. The presence of alpha and beta amylase is both utilised in the brewing process, more specifically for fermentation. There are significant differences between alpha and beta amylase on a physical and functional level (Georg-Kraemer, Mundstock, & Cavalli-Molina, 2001).

Alpha amylase also known as 4-α-D-glucan glucanohydrolase and glycogenase is a starch hydrolase. This means it can split α-1,4'-glycosidic linkage of long-chain carbohydrates, e.g. it can break down amylose to produce maltose and maltotriose. These sugars can be further broken down to glucose, which is the sugar that is primarily metabolised by the yeast in the fermentation process. It's commonly found in the digestive systems and saliva of animals and in fungi; therefore, it's an important enzyme in the digestion process of starch rich food. There is a need of calcium to create an active alpha amylase. It has an optimum activity at a pH between 6,7 and 7,0 and a temperature between 65°C and 67°C. During the malting process the Alpha amylases are produced in the aleurone layer of the grain ("Malt Sensory Methods," n.d.)(Canales & Pollock, 1979)(Peterson, 1998)(Goode et al., 2005).

Beta amylase, also known as 1,4-α-D-glucan maltohydrolase, glycogenase and saccharogen is produced by bacteria, fungi and plants. Contrary to alpha amylase it cannot be produced by tissues or cells of animals. Here calcium is also essential to create an active beta amylase. The optimum working environment of beta amylase is a pH between 4,0 and 5,0 and a temperature between 52 and 62°C. Its most important function is involvement in seed germination and fruit ripening. It can only act from the non-reducing end of the substrate to catalyse the hydrolysis of the second α-1,4'-glycosidic bond; this way it produces the disaccharide maltose. It acts slower than alpha amylase. It's sensitive to high temperatures and heavy metal ions, but contrary to alpha amylase it's stable at a low pH (e.g. 4). It breaks down starch into maltose ("Difference Between Alpha and Beta Amylase | Comparison of physical and functional characteristics," n.d.)("Beta-Amylase - an overview | ScienceDirect Topics," n.d.).

1.1.3 Mashing process

Mashing is the next important step in the brewing process. This process consists of combining the malt grist and water, also known as liquor, and heating it to temperatures usually between 40°C and 80°C. First the malt is sent through a grist mill. Here the husks of the kernels are cracked open and because of this the starches needed during the mashing process are exposed. During the mashing process the natural enzymes in the milled malt become active. They start breaking down the starches, converting them to sugars. These sugars will later be metabolised by the yeast and transformed into alcohol and CO_2. The temperature plays an important role in this step. By controlling the temperature, the working rate of the enzymes and which enzymes will be the most active can be regulated. Mash temperatures can be gradually increased or allowed to rest at a certain temperature, this is a part of the experience the brewer's craftsmanship brings. The addition of other unconventional grains (e.g. tritordeum) to the mashing process can add different enzymes in different concentrations. This can have a huge impact on the end results. After one to two hours of mashing, the result is a mixture of water, grains, sugar and enzymes. This mixture is not called wort yet, as it first has to be filtered (Hough, Briggs, Stevens, & Young, 1982).

Because of the specific optimum that alpha and beta amylase have, the usual range for mashing is 65-70°C. In this range alpha and beta amylase will work well together. The lower border of this range aids fermentation and the higher border contributes to the body. Because beta amylase has a higher activity in the lower range and can more optimally break down the starch into fermentable sugars. Contrary, alpha amylase has a higher activity in the higher range, and this is the enzyme that contributes to the body by liquefication. Liquefication is the concept of making starch ready for further enzymatic activity (Zannini et al., 2013).

The Congress mash method is a standardized small-scale mashing procedure employed to assess malt quality. The Congress mash is normally performed with the use of an automated mash bath ("Malt Sensory Methods," n.d.).

During another step of the mashing process, mashout, the mash is heated up until 76 to 77°C by adding hot water or by heating up the mash tun. This step isn't crucial but can be used to decrease the viscosity of the mixture and hereby increasing the flow. This helps to decrease the time for the sparging step. The decrease in viscosity is a result of heating the sugars and making them more soluble (Chris Colby, 2002).

The last step is the sparging step. In this step water is sprinkled on top of the mash to extract most of the sugars that are in the mash. This should be done at the correct water temperatures; these temperatures are between 65 to 68°C. This is to avoid extracting tannins (Palmer, 1999)("Sparging - BrewWiki," n.d.).

Now the wort is ready to be lautered, the next step in this process is called recirculation. In this step, the wort at the bottom of the mash is recirculated back to the top of the mash. Recirculation makes a big difference in quality and clarity of the beer. This also helps to achieve a mash that will function as a filter and will capture proteins and mash debris (Palmer, 1999).

1.1.4 Lautering process

Lautering is a process where the sugar rich wort is separated by filtration, this sugar rich wort is also called 'the sweet wort' from the mash. A lautering tun is the device used for the separation of the extracted wort by using filtration. The lautering tun contains a thin slit that will act as a barrier to hold back the solids (Christiansen, Barsh, & Luther, 1993).

1.1.5 Boiling and fermenting

After the lautering process the liquid wort is obtained and the solid spent grains will remain in the tun. Once the wort is clear, it's sterilized through a boiling process in a kettle, which halts enzyme activity and condenses the liquid. During this step hops are added. The addition of hops defines a big part of the taste and bitterness of the beer. Hops can be added early in the boiling step, with a longer boiling time resulting in more bitterness. This is due to more intensive isomerisation of the hop alpha acids. After the boiling step the hop solids are removed, the wort is cooled down and the fermentation can start by adding yeast. Different kinds of yeast can be used to produce different kinds of beer, with different types and aroma profiles. During the fermentation, the sugars in the wort are mainly converted into ethanol and carbon dioxide. The temperature during the fermentation is generally maintained from 14°C to 20°C. This depends on the yeast strain used in the fermentation process. For example higher temperatures employed for ale yeast result in more esters or fragrant organic compounds (Hough J.S., Briggs D.E., 1982).

1.1.6 Beer conditioning

The next step in the process is beer conditioning. This will mature and smoothen the beer and by-products of fermentation will diminish. The conditioning step is very diverse for different types of beer and can take from 1 week up to 6 weeks or more. Different methods of ageing are used and can add to the flavour of the beer. After this the beer is filtered and bottled or put in cans (Bogdan & Kordialik-Bogacka, 2017)("Beer 101: The Fundamental Steps of Brewing | Page 3 | The Beer Connoisseur," n.d.).

1.2 Effect of unmalted grains on the mashing process

The mashing is the second step of the brewing process. In this step, the enzymes that were activated during the malting step start actively breaking down the starch into sugars. For most beers, barley malt is used as the most prominent grain, because it provides the ideal wort base. An adjunct is primarily employed in brewing to provide carbohydrates that can ultimately be broken down into fermentable sugars. High starch content is clearly desired in the use of a brewing adjunct. However, the grains will have different concentrations of enzymes or even have other enzymes than barley. Therefore, the addition of different kind of grains can alter the enzyme activity drastically. It's a necessity to consider what the consequences for the final product will be, before adding different grains. The wort needs to have a standard quality: the amount of sugars, proteins and nitrogen are very important, as well as the starch solubility and the viscosity (Glatthar, Heinisch, & Senn, 2005)(Glatthar et al., 2005)(Malomo, Adebanjo, Ogunmoyela, Oluwajoba, & Adekoyeni, 2012)(Peterson, 1998).

1.2.1 Viscosity

The mash viscosity depends on the temperature used during the mashing process. The temperature is mostly used as a parameter that has an impact on the viscosity. The viscosity of the wort generally needs to be as low as possible. There are different factors that impact the viscosity. Arabinoxylan and mixed linkage β-glucan have been recognized as factors that contribute to wort viscosity, decrease wort and beer filtration rates and cause subsequent problems such as haze formation or reduced extraction efficiency (Glatthar et al., 2005).

1.2.2 Starch solubilisation

The sugar concentration in wort needs to be high enough to make sure that an effective fermentation can take place. In general, high protein content always reduces the amount of wort-fermentable sugars. Adding grains that contain large amounts of proteins can lead to a reduction of the fermentation (Sammartino, 2015).

1.2.3 Wort-soluble nitrogen

Wort-soluble nitrogen is nitrogen that is dissolved in the wort. Wort-soluble nitrogen can include amino acids, proteins, peptides and nucleic acids. A part of the wort-soluble nitrogen is essential for the nutrition of yeast and the beer foam stability. Although the biggest part of the wort-soluble nitrogen isn't needed; it can possibly have a negative influence on the production of beer. It can lead to fining problems and a lower beer stability. Adjuncts can be utilised to reduce the levels of soluble nitrogen in wort, which could help to reduce these negative effects ("Nitrogen in Wort prior to Pitching - Murphy and Son," n.d.).

1.2.4 Economic side

Aside from all the advantages for the brewing process, unmalted grains are added for an economical reason. Unmalted grains are a lot cheaper, for the obvious reason that the whole malting process is skipped. In countries where there is no barley or grain cultivation, all grains must be imported and malted which leads to higher production costs. Using a higher percentage of unmalted grains can help reduce the costs, so companies can still be competitive on the native and international market (Or, Corn, Moerman, 1989).

1.3 Enzymes

Enzyme activity mostly depends on pH and temperature. Therefore, an automated control can be a huge benefit for this process and adjustments can be made where needed. An important factor is minimizing expense (Jacob & Mitteleuropische Brautechnische Analysenkommission, 2011).

During the brewing of beer there are always interactions with enzymes which have a certain purpose. The amount of enzymes depends on the use of a certain type of grain and if the grain is malted or unmalted (Jacob & Mitteleuropische Brautechnische Analysenkommission, 2011).

1.3.1 No enzyme addition during mashing

If there is no enzyme addition during mashing, the enzymes that are already in the materials should be used to their full capacity. Therefore, enzyme denaturation should be considered, because enzymes are very temperature dependent. All the enzymes needed for conversion are present in the final barley malt. The malting process develops enzymes that hydrolyse starches and proteins during malting and mashing, which helps create better clarity, head retention and body. The most important ones are alpha and beta amylase. The active enzymes in the mashing process are described in table 1 (Djurle, 2017)("Enzymes in Beer: What's Happening In the Mash | American Homebrewers Association," n.d.).

Table 3: Enzymes that could be utilised in the mashing process.

Name	Temperature	pH range	Description
Phytase (Acid) Rest	30 – 52°C	5,0 – 5,5	Any type of phosphatase that will catalyse the hydrolysis of phytic acid. Will not reduce the pH a lot by itself. More of a historical method used with pale malt in Pilsen due to water devoid of minerals.
Beta-Glucanase Rest	35-45°C	4,5-5,0	Beta-glucans are carbohydrates found in the protein layer in grains. Found in rye, wheat, oats and under modified malts. Not needed for well modified grains. Good to use if you're using 25% or more of un-malted barley, wheat, rye and oats.
Proteinase (Protein Rest)	44-55°C	4,2-5,3	Optimal from 50°C. Breaks down proteins, polypeptides and peptides to make them smaller, improving clarity without negatively affecting head retention or body. Breaks down long-chain proteins to medium and short-chains. Typically done for 15-30 minutes.
Peptidase Rest	46-57°C	Below 5,3	Breaks down polypeptides and peptides to amino acids. In fully modified malts, it has done its work during the malting process.
Cytases	44-55°C	5,5	Solubilize protective cellulose cell walls of barley grains by hydrolysing galactan, araban, xylan and mannan giving access to the starch. Good for under

			modified malt, and unmalted barley, wheat, rye and oats if using more than 25%.
Beta-Amylase	55-65°C	4,0-5,0	Creates small sugar chains that are highly fermentable by splitting maltose from one end of the starch and leaves the lowest finished gravity and lightest body. One of the diastatic enzymes required for saccharification. (exoglucanase)
Alpha-Amylase	65-72°C	6,7-7,0	Produces glucose, maltose and unfermentable dextrins. Leaves the highest finished gravity and fuller body. Can have a slower working speed than beta-amylase. Most active at 70°C. (endoglucanase)

1.3.2 Enzyme addition

When the amount of enzymes inside the grains are low, technical enzymes or enzyme mixtures can be added. This will increase the total soluble nitrogen and free amino nitrogen values while the viscosity decreases. Ceremix is an example of a commercial enzyme mixture (Chekina, Meledina, & Khlynovskij, 2015).

Table 2 gives an overview on technical enzymes that were used when mashing with 100% raw barley substrate and what the result was.

Table 4: Mashing 100% raw barley with commercial enzymes.

Commercial enzyme	Result
Exogenous protease (*B. subtilis*) (efficiency decreased with amount added)	Increase in total soluble nitrogen levels, α-amino nitrogen levels, wort colour, and extract recovery levels.
Exogenous β-glucanase (*B. subtilis*)	Reduced high-molecular-weight wort β-glucan levels.
Exogenous α-amylase (*B. subtilis*)	The highest positive impact on mash separation.
Exogenous α-amylase	Higher wort glucose and maltotriose levels and lower maltose levels.
Exogenous high-heat thermostable α-amylase (*B. licheniformis*) with exogenous α-amylase (*B. subtilis*)	Necessary for complete starch conversion and maximum extract recovery from the raw barley substrate.

The endogenous malt enzymes have a very poor raw barley protein-hydrolysing ability. Furthermore, the endogenous malt amylases have a very poor raw barley starch hydrolysing ability (Goode et al., 2005).

1.4 Standard characteristics

1.4.1 Exract content

The measurement of the concentration of solids dissolved in wort is given by the Plato scale. This scale works with degrees Plato (°P) and quantifies the concentration of extract. This extract consists mostly out of sugars but also contains other soluble materials. Every 0,4% of alcohol is given by 1°P. A 1°P wort will contain 1 gram of extract for every 100 grams of brewery wort. When making a normal lager beer an alcohol percentage of around 4,3% to 4,5% is preferable. This means the Plato values have to be around 10,75°P to 11,25°P (Martínez-Moreno, 2017)("Plato gravity scale | Craft Beer & Brewing," n.d.).

Beer based on 40% unmalted Compana barley, 40% unmalted Glacier barley, 40% unmalted high amylose barely, 40% unmalted ND 6655 Durum wheat and 40% unmalted Yecora wheat respectively have an extract content (°P) of 9,50; 7,95; 7,60; 8,15 and 9,22. The other 60% was malted barley acquired from a local company (Koszyk & Lewis, n.d.).

1.4.2 Free amino nitrogen

Free amino nitrogen (FAN) is a measurement for the concentration of ammonium ions, individual amino acids and peptides that are one to three units long. These small peptides and amino acids are used by the yeast as nutrition to synthesize cellular proteins and cell compounds during the fermentation process. Brewing scientists suggest that using a FAN measurement is a good way to predict if the quality and the stability of the beer is desirable ("free amino nitrogen (FAN) | Craft Beer & Brewing," n.d.).

The concentration of the free amino nitrogen needs to be high enough in the wort, so the mixture is be able to ferment, but it can't be too high as well, because this can cause the formation of fusel alcohols. These are undesirable mixtures of several alcohols. They can be classified into two groups, the hazardous alcohols like methanol and aroma alcohols like isopentanol (Hill & Stewart, 2019).

The free amino nitrogen concentration is determined to which extent the proteolytic enzymes can act. These enzymes are activated during the malting process. Carboxypeptidases play a central role in producing FAN compounds, these proteolytic enzymes hydrolyse most of the proteins that are found in high concentrations in the endosperm and the aleurone layer of the grains. Not all the FAN in the wort is produced during the malting step, some of it's produced during the mashing process. Endoproteinases are not hydrolysed in the malting step. These can be used to produce FAN during the mashing step. By adding cereal adjuncts like unmalted tritordeum the amount of free amino nitrogen in the wort solution and in the fermented solution can decrease (Otter & Taylor, n.d.).

The FAN value should be higher than 160 mg/L to ferment properly, the FAN levels of beers depends on which kind of beer is produced. For example commercial all-malt lager wort contains an average FAN value of 180-190 mg/L (Mccaig et al., 2014).

Beer based on 40% unmalted Compana barley, 40% unmalted Glacier barley, 40% unmalted high amylose barely, 40% unmalted ND 6655 Durum wheat and 40% unmalted Yecora wheat respectively have a FAN value (mg glycine/100mL wort) of around 6,5; 7,0; 8,5; 5,2 and 7,2. The other 60% was malted barley acquired from a local company (Koszyk & Lewis, n.d.).

1.4.3 Saccharification time

A simple test to see if the saccharification process is completed is the iodine test. First you remove a small amount of liquid from the mash and add a couple drops of iodine. If there is still some starch left it will turn into a purple/black colour. Contrary, if all the starches are converted to sugars, the colour appears brown. It's important to note that iodine is toxic, so you should never add the sample back to the mash. For commercial breweries it's easier to automate this, but for smaller brewers low-cost temperature control systems can be a solution, because the automated systems are costly (Briggs, Boulton, Brookes, & Stevens, 2004).

For beer brewed with different species of malted barley (e.g. Tiffany, Luxor, Tolar and Jersey) a saccharification time of 10-15 minutes, verified by the iodine test was obtained (Brewing Trials with Spring and Winter Barley Varieties, n.d.).

For beer brewed with different percentages (up to 50%) of different extruded unmalted cereal adjuncts (e.g. corn starch, Debranned Sorghum, Sorghum and Sorghum Bran) no complete saccharification time was observed. Malted barley was used for the other 50% (Or et al., 1989).

1.4.4 pH

The pH is very important during practically the whole process. During the mashing process the pH regulates the working rate of the enzymes. During the fermentation the pH must have the right value to make sure the yeast can work in optimal conditions. In the end the pH has to be good so the beer doesn't taste too sour and the beer can preserve well (Guido et al., 2007).
To create the right conditions during the mashing process, the pH must be between 5,2 and 5,5. The pH will naturally be around these marks but can be adjusted by adding an acid or alkaline solution. If it's not possible or feasible to add extra solutions, pH can be regulated by slightly changing the working temperature, which will end up activating different enzymes that can alter the pH (Kaneda, Takashio, Tamaki, & Osawa, 1997)(Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003).

After fermentation the pH will be reduced to a value between 4,1 and 4,3 because yeast utilizes sugars in the wort. If the pH is between 4,6 and 4,9 this may be a sign of autolysis of the yeast. A lower pH between 3,4 and 3,7 can suggest infection of acid-producing bacteria. Temperature is a parameter that correlates to the pH value, if the fermentation isn't performed at a low enough temperature the values won't be representative (Hanna instruments, n.d.).

Beer based on 40% unmalted Compana barley, 40% unmalted Glacier barley, 40% unmalted high amylose barely, 40% unmalted ND 6655 Durum wheat and 40% unmalted Yecora wheat respectively have a pH value of 5,62; 5,79; 5,75; 5,96 and 6,00. The other 60% was malted barley acquired from a local company (Koszyk & Lewis, n.d.).

1.4.5 Alcohol percentage

During fermentation, the yeast strains produce ethanol, this gives beer its standard characteristics. The alcohol percentage is the amount of alcohol compared to the total volume of the beverage. The range of alcohol in beer can go up to 16%. For normal lager beers, the volume is around 4,5%. The alcohol percentage depends on which kind of beer needs to be produced ("Yeast and Alcoholic Beverages: Beer, Wine and Liquor," n.d.).

Beer based on 40% unmalted Compana barley, 40% unmalted Glacier barley, 40% unmalted high amylose barely, 40% unmalted ND 6655 Durum wheat and 40% unmalted Yecora wheat respectively have an alcohol percentage of 2,57%; 2,30%; 2,07%; 2,23% and 2,92. The other 60% was malted barley acquired from a local company (Koszyk & Lewis, n.d.).

1.4.6 Colour

Among the various ingredients, the colour of beer is determined mostly by the grains, and more specifically by the treatments throughout the process. barley on its own doesn't have a lot of pigmented substances, so it does not add a lot of colour. The malting phase can determine the extent of the colour formation in large proportions. The heat operations on the grains have a major impact ("Beer: A Quality Perspective - Google Boeken," n.d.).

During the heating process, the Maillard browning reactions take place. These are reactions that are heat induced and take part in the predominantly endosperm of the grains. The main components of these reactions are reducing sugars (maltose mostly), free amino acids and amino acid groups. During the reactions melanoidins are formed as end products. Melanoidins are brown, heterogeneous polymers (with a high molecular weight) and they form the main base for the beer colour. There are a lot of factors that regulate these reactions, such as temperature, pH and time. Being able to adjust these parameters, to end up with a good colour for your beer is essential. (Van Boekel, 2006).

The Maillard reactions are so complicated that not all steps are completely known. The components formed add to the flavour of the beer. Especially when brewing a darker beer with a lighter taste, these reactions can be a nuance. While the reactions give the colour, they also make the beer significantly more bitter and even a little sour (Wang, Qian, & Yao, 2011).

For American/European light lager beer a colour value between 2 and 4 °SRM or 4 and 8 EBC is expected. For American/European 100% malt Pilsner a colour value between 3 and 9 °SRM or 6 and 18 EBC is expected (Shellhammer, 2009).

Beer based on 40% unmalted Compana barley, 40% unmalted Glacier barley, 40% unmalted high amylose barely, 40% unmalted ND 6655 Durum wheat and 40% unmalted Yecora wheat respectively have a colour value (°SRM) of 2,69; 3,02; 2,67; 2,44 and 3,02. The other 60% was malted barley acquired from a local company (Koszyk & Lewis, n.d.).

1.5 Tritordeum

1.5.1 History

In 1971 professor Antonio Martin created the first tritordeum line. Hexaploid tritordeum is a hybrid organism made by breeding barley (*Hordeum Vulgare*) and wheat (*Triticum aestivum, Triticum dicoccum and Triticum monoccum*). In the first stages of breeding tritordeum, the problem was that the yield was low; much lower than the yield of wheat. By mostly backcrossing, they created the tritordeum known today (Martín, n.d.).

1.5.2 Chemical composition

Tritordeum has a very varied chemical composition. The average nutritional data from tritordeum is: 12% water, 14% protein, 65% carbohydrates, 1,9% ashes, 2,2% fat and 12% fibres (Agrasys, 2018).

Starch is a polymeric carbohydrate that harbours a large amount of glucose. This glucose is essential for the brewing process where the glucose is converted by the fermentation process into alcohol. The starch level of tritordeum is lower than some other cereals, as you can see in figure 1 it has a lower starch concentration than soft wheat and Durum wheat. In another article Daniela Mikulíková concluded that tritordeum has a lower starch concentration than wheat and rye but a higher concentration than oat and barley ("Properties Tritordeum," n.d.)(Republic, 2006).

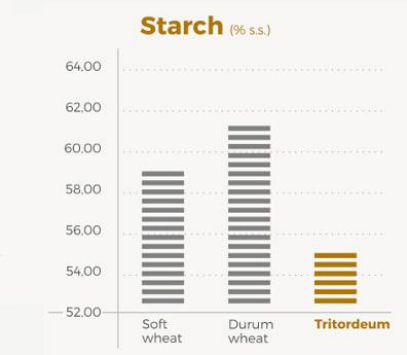

Figure 2: The amount of starch in different cereals (Soft wheat, Durum wheat and Tritordeum).

The monosaccharides and disaccharides of tritordeum are made up by four main monosaccharides, these are: arabinose, xylose, glucose and sucrose. These are metabolized in the fermentation process by yeast to produce the alcohol in the beer. Mannose and galactose are also found in tritordeum but in lower concentrations ("Malt Sensory Methods," n.d.).

Dietary fibres are found in plants and that can't be completely broken down by digestive enzymes. The main types are non-starch polysaccharides like: lignin, arabinoxylan, β-Glucan, fructan and cellulose (Englyst, Bingham, Runswick, Collinson, & Cummings, 1989).

Tritordeum and other cereals have a large amount of phenolic acids in their outer coat. These phenolic acids can have antioxidant traits (Piazzon et al., 2012).

Tritordeum harbours a big amount of minerals like calcium, copper, iron, manganese, magnesium zinc, sodium, phosphor and potassium (Alvarez, Ballesteros, A. Sillero, & Martin, 1992).

The concentration of gluten found in tritordeum is a lot lower than other cereals like wheat. ELISA tests done on proteins in tritordeum could tell us that the gluten concentration is 41 to 49% lower than in wheat. During researches on bread made with wheat and tritordeum, there was found that the bread respectively contained 93 979 and 48 667 mg kg^{-1} gluten. This was determined by competitive R5 ELISA. The difference in gluten concentration means that tritordeum has lower levels of ω-gliadins in comparison to wheat. ω-gliadins are glycoproteins that will interact with glutenin and form gluten (Luis Vaquero, 2017).

Eating cereals like tritordeum can have beneficial effects on the human health. Tritordeum contains vitamins, antioxidants, dietary fibres, unsaturated fatty acids and minerals. These are all products that are beneficial for the human health in moderation. Dietary fibres have favourable effects on the bowel movement and on bad cholesterol. This prevents high cholesterol levels and can help prevent cardiac diseases. Beside dietary fibres they also contain carotenoids that could improve visual activity (Brown, Rosner, Willett, & Sacks, 1999).

1.5.3 Applications

Tritordeum can be used as a new alternative for breakfast cereals, as it's rich in taste. It has a higher moisture content than the average cereals. Because of its traits tritordeum can for example be used to make porridge (Erlandsson, n.d.).

It also has applications in bakery; more specifically in breadmaking. The bread is more yellow than normal bread, softer and more elastic. The tritordeum bread also has a very rich taste (Erlandsson, n.d.).

Beer can be made from 100% tritordeum, but tritordeum can also be mixed with other grains to produce beer. For example, there is a commercialized beer that is made from 100% tritordeum. The beer is called "Birra di Tritordeum Ale". This is a beer made by Forn Baltà; a company located in Barcelona, Spain (Team, n.d.)(Vaquero et al., 2018).

2. Materials and method

2.1 Experimental setup

Figure 2 shows a visual image of the structure of the practical work. The samples of the different mixtures of grains were done in triplicate. After the mashing, different tests were performed as well as a small-scale fermentation.

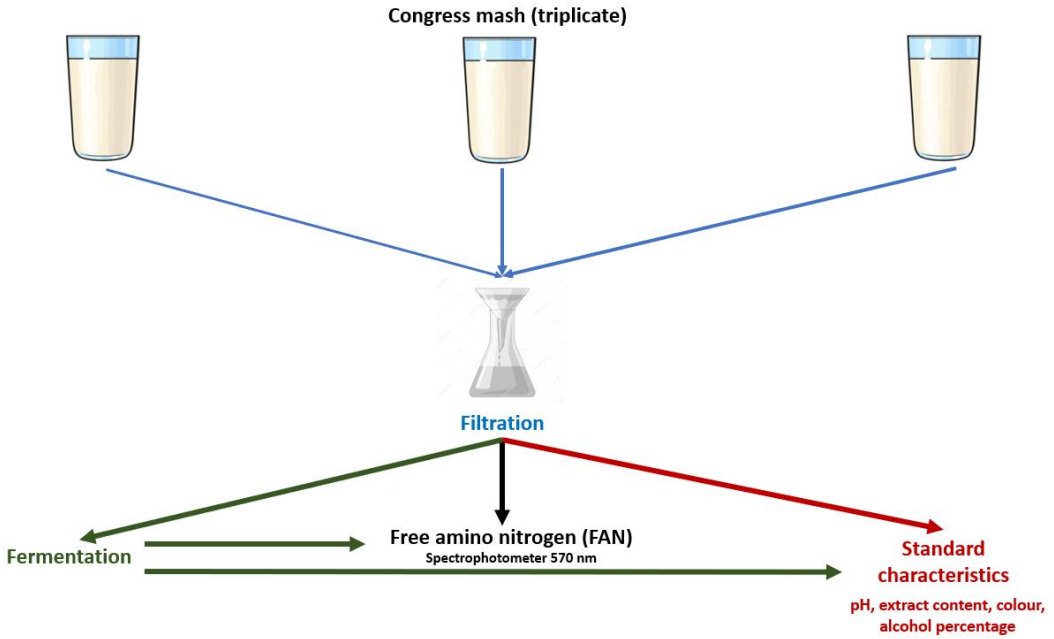

Figure 2: Visualization of the structure of the practical work.

In the practical work of our bachelor thesis a Congress mash was made from different mixtures of unmalted tritordeum and barley malt. The following mixtures were used:

- 100% barley malt
- 100% unmalted tritordeum
- 100% unmalted tritordeum + technical enzymes
- 10% unmalted tritordeum + 90% barley malt
- 20% unmalted tritordeum + 80% barley malt
- 40% unmalted tritordeum + 60% barley malt

The E-Mast (MegaZyme) enzyme mixture was used for the preparation of the technical enzymes. This mixture consists of one type of alpha amylase with a specific activity of 750U/mL and two types of beta amylase with a respective specific activity of 5700U/mL and 98U/mL. To prepare the technical enzymes, 2mL of E-Mast mixture was added to 98mL of 0.5% NaCl and mixed thoroughly.

After the Congress mash method, the obtained wort was filtered with a coffee filter. Part of the wort was immediately used to determine its standard characteristics (colour value, pH, extract content and alcohol percentage) and to start a lab-scale fermentation experiment. Another part of the wort was frozen for determination of the free amino nitrogen content (FAN). After fermentation, the standard characteristics (colour value, pH, extract content and alcohol percentage) and the FAN content of the fermented wort were determined (A. Martin & Moll, 1984).

2.2 Congress mash method

The raw materials used were malted barley and unmalted tritordeum. The malted barley was acquired from a company called Boortmalt. The unmalted tritordeum was acquired from Wiro Nillesen.

Barley malt and unmalted tritordeum grains were milled at 0,2mm and were distributed over 3 cups. In each cup, 50g of milled grains were added, as described in the protocol, as well as 200mL of water at 46 °C to obtain a water-flour mixture of 45°C. After this, the cups were kept at 45°C for 30 min in the Congress mash machine (Lochner Labor + Technik GMBH). After this, the mixture was heated up at 1°C/minute until 70°C. Then, 100mL of water at 70°C was added and the mixture was kept at 70°C for one hour. In figure 3 the temperature profile for the Congress mash method is given as a function of the time (A. Martin & Moll, 1984).

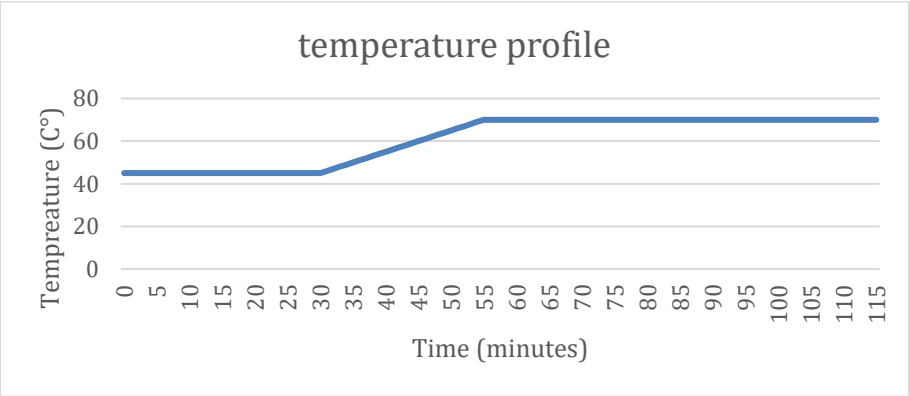

Figure 3: The temperature profile during the mashing process in function of the time.

After this, the mixture was cooled down and the stirrer was washed with a little amount of water. Then the content of the beaker was watered down to contain 450mL of mixture. After this, the mixture was filtered using a coffee filter and a large measuring cylinder. The first 100mL of filtrate was recirculated to establish the filter cake. This was to make sure the wort was clear enough to use in the Anton Paar Alcolyzer Plus. After the recirculation the filter cake was thick enough, this improved the filtration. At the same time as the 100mL was recirculated, the timer was started. When 250mL was obtained after the recirculation the filtration time was stopped. Three different samples were taken from the filtrate, one for measuring the standard characteristics, one for the FAN test and one for the fermentation. The filtration time provides a crude estimate of the filtration characteristics of the obtained wort (A. Martin & Moll, 1984).

2.3 Iodine test

The iodine test was used to confirm the presence of starch. First the iodine solution was prepared. The iodine solution was prepared by dissolving 1,27g of iodine crystals and 2,5g of potassium iodine in water and was then diluted to 500mL. Then every 5 minutes during the mashing process, more specifically during the hour the mash is at 70°C, a drop of iodine solution is mixed with a small amount of the mash. If the colour remained brownish, no starch was present. If the colour changed to blue/purple/black, there was still starch left (A. Martin & Moll, 1984).

2.4 Lab-scale fermentation

For the lab-scale fermentation, 200mL of filtered wort was transferred to a 500mL erlenmeyer and 15 g of standard bread yeast was added, acquired from the company Bruggeman. The flask was sealed with a water lock and incubated for 72hours at 25°C. The erlenmeyers weren't shaken (A. Martin & Moll, 1984).

2.5 Standard characteristics

The Anton Paar Alcolyzer Plus was used to determine pH, extract content, alcohol percentage and colour value. This machine can determine the value of all these characteristics so it's ideal to use in this thesis (GmbH, n.d.).

2.6 FAN test

First a ninhydrin colour reagent was prepared. This was done by dissolving 10g disodium hydrogen phosphate, 6g potassium dihydrogen phosphate, 0,5g ninhydrin and 0,3g fructose in water. The pH had to be between 6,6 and 6,8, in case it wasn't 85% phosphoric acid was added. The solution was diluted in 100mL distilled water. Also, a diluting solution and glycine standard solution were prepared. The diluting solution was made by dissolving 2g potassium iodate in 600mL water and by adding 400mL 96% ethanol. The glycine standard solution was made by dissolving 0,1072g glycine in 100mL distilled water. After the preparation of all the reagents the procedure could start. First 1mL of the sample was diluted to 100mL with distilled water. 2mL of this diluted sample was brought into a test tube together with 1mL ninhydrin colour reagent. The samples were then placed in a boiling water bath for 16minutes and after it cooled down for 20minutes in 20°C water bath. 5mL diluting solution was added afterwards, the sample was mixed and measured at 570nm in a spectrophotometer. This was also done for the blank and the standard sample. The standard sample didn't contain any wort but contained the glycine standard solution (A. Martin & Moll, 1984).

3. Results and discussion

3.1 Starch degradation

The extract content (original gravity) of 100% unmalted tritordeum was much lower than 100% barley malt (figure 4). When the technical enzymes were added to 100% unmalted tritordeum, the extract content increased substantially, yet did not reach the extract content of the wort obtained with barley malt. Therefore, it can be concluded that without enzyme addition unmalted tritordeum does not have enough active alpha and beta amylase to degrade the starch.

When the mixtures with malted barley are compared, there is a slight decrease of extract content in the mixtures with a higher concentration of unmalted tritordeum.

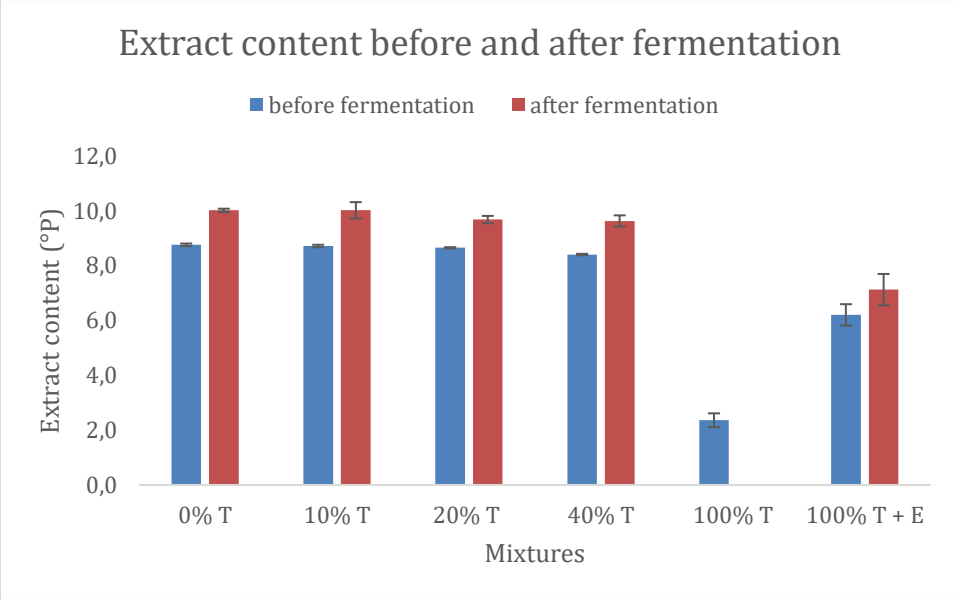

Figure 4: The extract content (°P) of the wort after the Congress mash method and after fermentation for 0, 10, 20, 40 and 100 % of unmalted tritordeum without or with added enzymes (+E). The rest of the mixtures is composed of barley malt.

The filtration time after the Congress mash method to obtain 250mL of 100% unmalted tritordeum without technical enzyme addition was higher than 2 hours. This wasn't an efficient filtration and the volume needed to ferment this mixture was not reached. So, the fermented mixture of 100% unmalted tritordeum won't be included in the entire section results and discussion. This could be because the starch wasn't broken down as much as in other mixtures. The long starch chains will complicate the filtration process and will furthermore result in a longer filtration time in comparison to the short chains formed in the other mixtures.

For 40% unmalted tritordeum before fermentation an extract content of 8,4°P was found, this is practically the same as the average extract content from the five mixtures mentioned in the literature study (8,48) (Koszyk & Lewis, n.d.).

After fermentation an extract content of 9,6°P was obtained. This is closer to the range of normal lager beer. This is favourable for the brewing process, because unmalted grains were used and a similar extract content is reached. This is economically interesting (Martínez-Moreno, 2017).

The unmalted tritordeum does not add many active enzymes, so the active enzymes mainly come from the barley malt. The extract content drops slowly, so it's still completely possible to brew with up to 40% unmalted tritordeum or maybe even higher (further research needs to be conducted). The extract content is high enough to start a successful fermentation process with up to 5 % alcohol and the saccharification process is validated by the iodine test for all mixtures composed with additional barley.

The saccharification time (validated by the iodine test) increased as more unmalted tritordeum was added (Figure 5). The samples up to 40% unmalted tritordeum had a much lower saccharification time. In less than 5 minutes the starch in the samples with 0% and 10% unmalted tritordeum were converted into smaller sugar components. The samples with 20 and 40% unmalted tritordeum had a conversion time around 10minutes, which is still very fast. In the two mixtures with 100% unmalted tritordeum, one with technical enzyme addition and one without technical enzyme addition, the starch wasn't fully broken down after one hour. There is no information how long it would take to break down the starch fully or if it's even possible. It's possible that the gelatinization temperature of raw tritordeum is higher than the temperatures used here. This could explain the long saccharification time. The conclusion is that without malted barley, it will be hard to break down all the starch from the unmalted tritordeum. Malting tritordeum might increase the enzyme activity of tritordeum and therefore decrease the saccharification time.

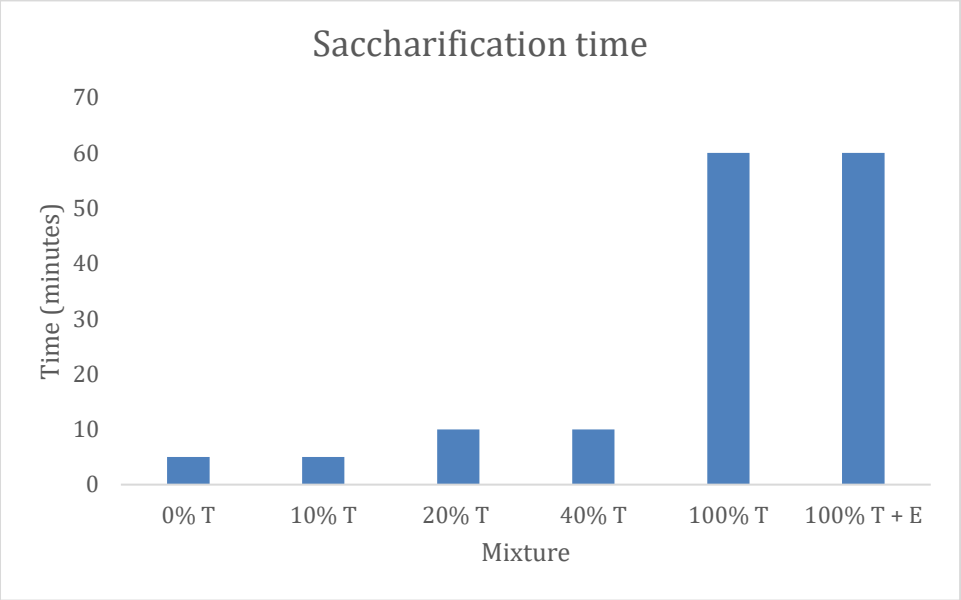

Figure 5: The time until complete saccharification during the Congress mash method for 0, 10, 20, 40 and 100 % of unmalted tritordeum without or with added enzymes (+E). The rest of the mixtures is composed of barley malt.

For malted barley the experimental saccharification time was faster than found in the literature for different species of barley (10 to 15minutes) (Brewing Trials with Spring and Winter Barley Varieties, n.d.).

With up to 40% unmalted tritordeum roughly the same time was achieved as brewing with different species of malted barley (Mikyška A., Psota V.& Hrabák M. , 2012).

For the 100% unmalted tritordeum mixtures with or without added technical enzymes the saccharification wasn't completed. This was also found for other cereal adjuncts up to 50%.

Tritordeum could be a better alternative cereal than the other ones stated in the literature research (Or et al., 1989)(Delcour, Hennebert, Vancraenenbroeck, & Moerman, n.d.).

3.2 Free Amino Nitrogen

The free amino nitrogen values of the samples after fermentation were overall moderately high (figure 6). The FAN values of the 100% tritordeum wort with and without added technical enzymes are low compared to the literature. Here is stated that at least 160 mg/L is needed for an optimal fermentation. The values of the other wort mixtures (0%, 10%, 20% and 40%) are high enough to perform an optimal fermentation. The FAN values after fermentation are a lot higher than reported in the literature (Koszyk & Lewis, n.d.).

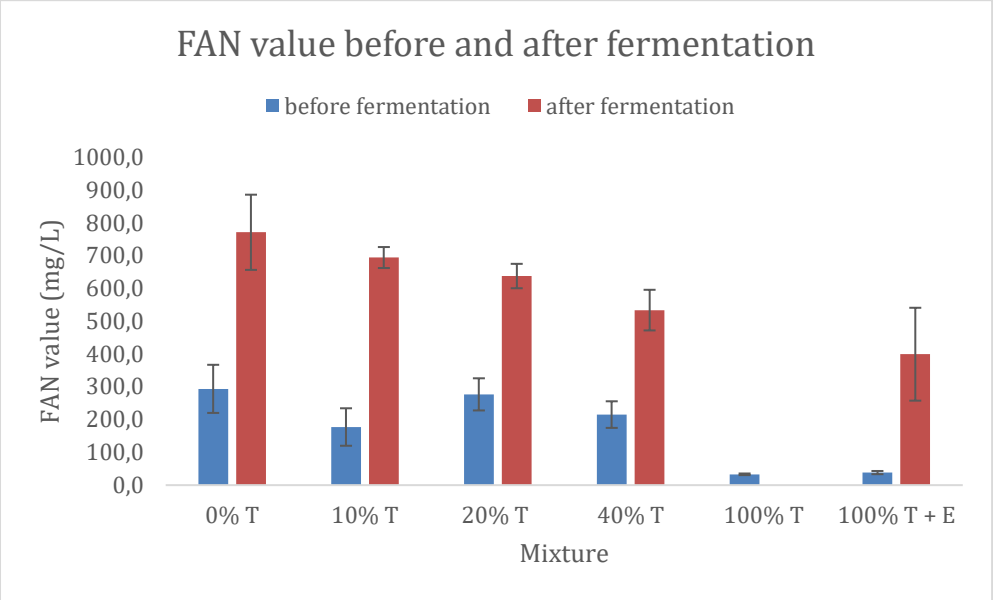

Figure 6: Free amino nitrogen (FAN) values after the Congress mash method and after fermentation for 0, 10, 20, 40 and 100 % of unmalted tritordeum without or with added enzymes (+E). The rest of the mixtures is composed of barley malt.

A possible reason for this is that a high amount of yeast was added to start the fermentation process. This might've had a large influence on the FAN value. It can be concluded that the addition of 15g dry yeast was too much. In a source for small scale brewing was found that 11,5g dry yeast would be enough to ferment 19L. Using these numbers, the addition of 0,15g of yeast per 200mL would be enough ("Dry Yeast in Home Brewed Beer | Home Brewing Beer Blog by BeerSmith™," n.d.).

Also, there aren't enough measuring points to make a clear FAN graph, only measured before and after the fermentation (after 72 hours). So, it's possible that the FAN value is this high because of the by-products formed during fermentation that are also measured, and this could give an incorrect value. These by-products could be lactic acid or ketons like diacetyl for example. A desired FAN value might have been somewhere in between the two measuring points ("Fermentation Byproducts and Yeast History | Spike Brewing," n.d.).

Another option could be that there was yeast autolysis as a result of the high yeast addition. This means that the yeast cells would "self-destruct" as a result of their enzymes. The

components inside the cell will mix with the mixtures and this could be another reason why the FAN values of our fermented mixtures are this high. (“Autolyse,” n.d.)

3.3 pH

Adding more unmalted tritordeum increased the pH of the resulting wort after the Congress mash. Adding technical enzymes did not influence the pH of the resulting wort.

The overall pH decreased from 6,2 in the wort to 4,3 after fermentation (figure 7). The pH decreased most in the wort from 100% unmalted tritordeum with technical enzymes. The higher amount of side products (e.g. acetic acid and carbon dioxide) formed during the fermentation has led to a more acidic environment.

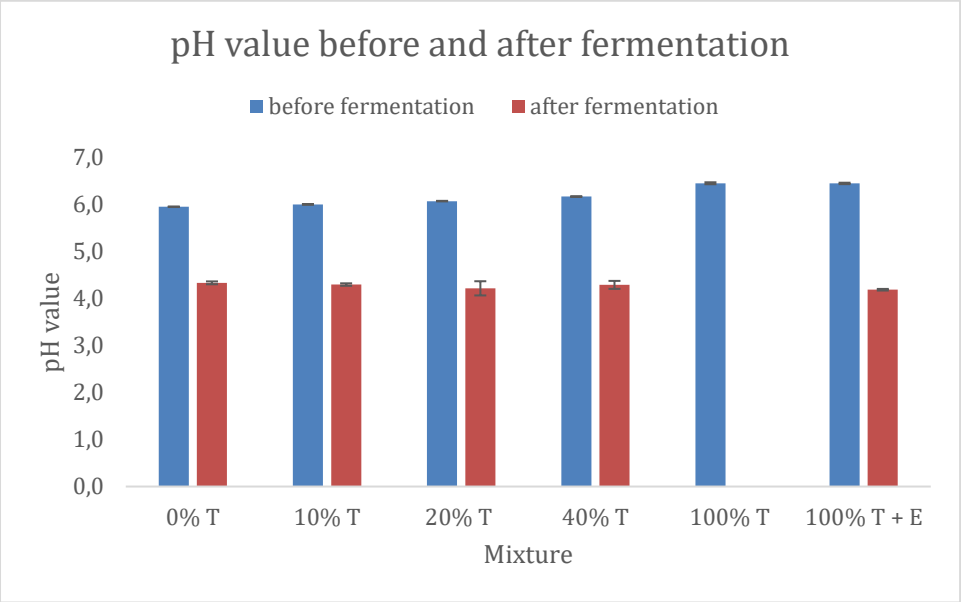

Figure 7 : pH values after the Congress mash method and after fermentation for 0, 10, 20, 40 and 100 % of unmalted tritordeum without or with added enzymes (+E). The rest of the mixtures is composed of barley malt.

In the literature, a pH value between 5,2 and 5,5 was found for mashing. This was found to be an optimal environment for yeast and for the wort mixtures. The wort mixtures had a higher pH value than expected. The fermented mixtures had a pH close to the desired pH described in the literature for normal lagers (Koszyk & Lewis, n.d.).(Raines-Casselman, n.d.)

For 40% unmalted tritordeum after fermentation a pH of 4,3 was found, this is 1,5 lower than the pH from the five mixtures mentioned in the literature study (5,82) (Koszyk & Lewis, n.d.).

Unmalted tritordeum resulted in a pH value that leans closer to the value of normal lagers than to the value of other unmalted mixtures, which is a good thing. So, it can be a good alternative for grains used in brewing (Koszyk & Lewis, n.d.).

3.4 Alcohol percentage

The fermented mixture with 100% unmalted tritordeum and technical enzymes has a lower alcohol percentage (figure 8). This can be explained because there was less extract of starch or because of the lower starch concentration of tritordeum as seen in the literature (“Properties Tritordeum,” n.d.).

The alcohol percentage slightly raises from the 0% to the 40% unmalted tritordeum mixture. The extract content however was lower. In the mixtures with higher concentrations of unmalted tritordeum there were more fermentable sugars, this enabled the yeast to get a higher alcohol production. In conclusion this means the addition of unmalted tritordeum can aid with the fermentability of the wort (“Properties Tritordeum,” n.d.).

The average alcohol percentage was around 4%. In the literature, the average alcohol percentage of lagers is around 4,5%. It can be concluded that the fermented mixtures up to 40% unmalted tritordeum are close to this value and therefore it could be a good alternative grain (Logan, Case, & Distefano, 1999).

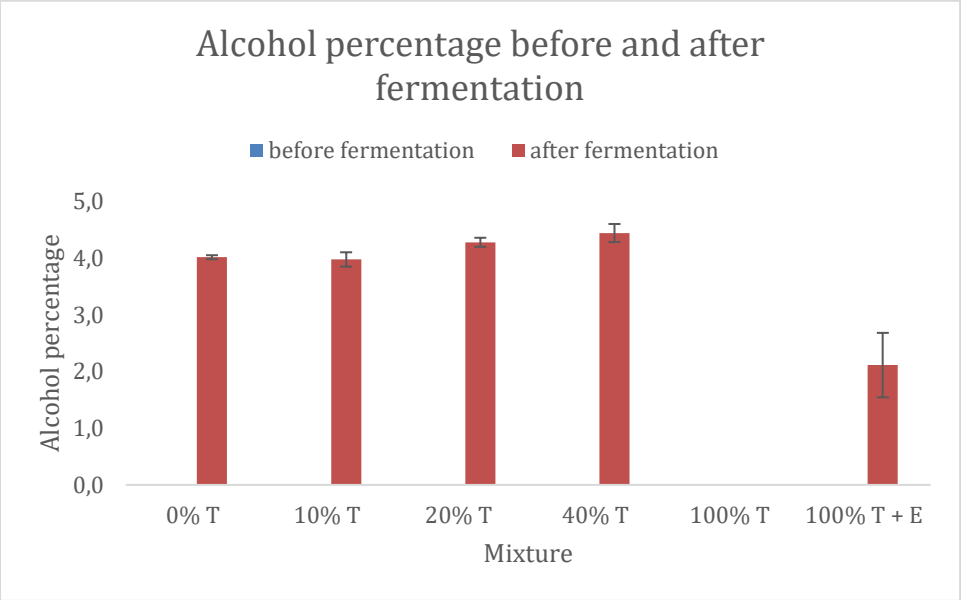

Figure 8: Alcohol percentage measured after the Congress mash method and after fermentation for 0, 10, 20, 40 and 100 % of unmalted tritordeum without or with added enzymes (+E). The rest of the mixtures is composed of barley malt.

For 40% unmalted tritordeum after fermentation a value of 4,4% was found, this is almost double the amount of the five mixtures mentioned in the literature study (2,42%). Unmalted tritordeum seems to result in an alcohol percentage that leans closer to the value of normal lagers than to the value of other unmalted mixtures, which is a good thing. So it can be a good alternative for grains used in brewing (“Yeast and Alcoholic Beverages: Beer, Wine and Liquor,” n.d.)(Koszyk & Lewis, n.d.).

3.5 Colour value

It isn’t possible to draw a conclusion form the values of the colour measurement using the ECB value of the Anton Paar Alcolyzer Plus. The colour values were very spread out even in between the samples of the same mixtures. It could be because the filters used in the filtration step were not optimal, so the wort was to cloudy for the colour sensor in the Anton Paar Alcolyzer Plus assuming nothing was wrong with the sensor.

4. Conclusion

4.1 Main findings

Tritordeum was tested as a possible alternative grain for brewing. The reason for this is that it has specific qualities that other cereals do not possess (e.g. different aromas, flavours and chemical composition). Tritordeum is concluded to be a possible alternative grain for the brewing process. Although, it isn't possible to brew with 100% unmalted tritordeum, because the starch isn't entirely degraded by the natural enzymes leading to an unsuccessful filtration. Adding technical enzymes to the 100% unmalted tritordeum mixture makes brewing possible, but a lower alcohol percentage is the result.

It's possible to brew with up to 40% unmalted tritordeum with the rest of the mixture composed of barley malt. This could be concluded because the saccharification time was positive. Also, the desired alcohol percentage was obtained because the extract content was closer to the lager beer range than the range of other unmalted grain mixtures. The same conclusion was drawn for the pH value.

Because of the positive saccharification time it could be concluded that there is potential for even higher percentages of unmalted tritordeum to be used, but further research needs to be conducted.

The FAN values were too high after fermentation, but this probably is because of an execution error.

4.2 Recommendations

For further tests malted tritordeum could be used instead of unmalted tritordeum. This would be more conventional and probably lead to different results. Malted grains are easier to use during the brewing process but are more expensive. Different mixtures with even higher percentages of unmalted tritordeum could be useful to test the potential limit.

During the FAN test too much yeast was used, so this test needs to performed with the correct amount in further research.

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