Brewing with fractionated barley

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Brewing with fractionated barley

Laura H.G. van Donkelaar

Thesis

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Contents

Introduction and thesis outline

Beer is a globally consumed beverage, which is produced from malted barley, water, hops and yeast. While beer brewing probably started in the ancient Egyptian culture, the current traditional recipe dates back to the 14th century (Kunze, 2010). The main ingredients and processing principles have not changed much since then. Although it is possible to substitute malted barley with other starch or sugar sources such as sorghum, unmalted barley or sugar syrup, this does not happen in many premium brands due to the German 'Reinheitsgebot'. This is a German purity law dating from the 15th century that states that the only ingredients permitted for beer are water, hops and barley (Stahleder, 1987). Many people and breweries still value this law. In recent years, however, the use of unmalted barley and exogenous enzymes has become more popular due to advantages such as simplified processing and reduced environmental impact (Steiner, Auer, Becker, & Gastl, 2012).

1.1 Barley as a raw material

Barley is the main raw material in the brewing process. A cross section of the barley kernel is depicted in **figure 1**. The barley grain consists of three main structures, which are the bran, germ and endosperm. The bran protects the barley kernel from microorganisms and environmental conditions. It has several layers with different composition and functions. The outer layers are the husks, which consist mainly of cellulose in which components like polyphenols and bitter substances are localised (Kunze, 2010). Most arabinoxylans that are present in the barley are located in the bran. The germ is the part of the kernel that initiates the growth of the acrospires, and is rich in lipids. The endosperm is the main energy storage of the kernel. It consists for 77% of starch (van Donkelaar, Noordman, Boom, & van der Goot, 2015), which is surrounded by a matrix of storage proteins (Kunze, 2010). This matrix with starch network is surrounded by cell walls, which consist for 75% of β-glucans and 20 % of arabinoxylans (Jadhav, Lutz, Ghorpade, & Salunkhe, 1998). In the brewing process, starch is broken down into fermentable sugars, which are needed by the yeast to produce alcohol. Around the endosperm, the aleurone layer is located. This layer is about 3 cells thick. The cell walls consist for 26% of beta glucans and 71% of arabinoxylans. These cells are rich in protein, and many enzymes are produced and activated in these cells during germination. These enzymes are essential in the brewing process to hydrolyse, amongst others, cell wall material, proteins and starch.

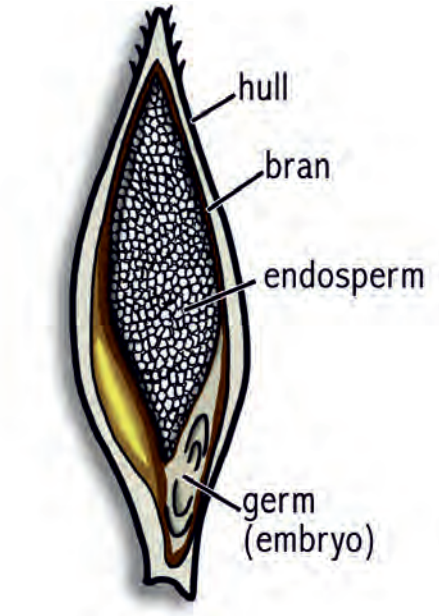

Figure 1. Schematic representation of the barley kernel and its structure (by MR Illustrations).

1.2 Beer production

Barley is produced in ± 140 million tons a year (International-Grains-Council, 2014), and the main food application is brewing (M. Edney, Wrigley, & Batey, 2010). Barley is malted before it is brewed into beer. During malting the barley is wetted and germinated to induce enzyme synthesis, after which it is dried by kilning. The malted barley is milled and mashed with water. After mashing, the mash is filtered, boiled with hop and fermented into beer. **Figure 2** outlines a schematic representation of the malting and brewing process.

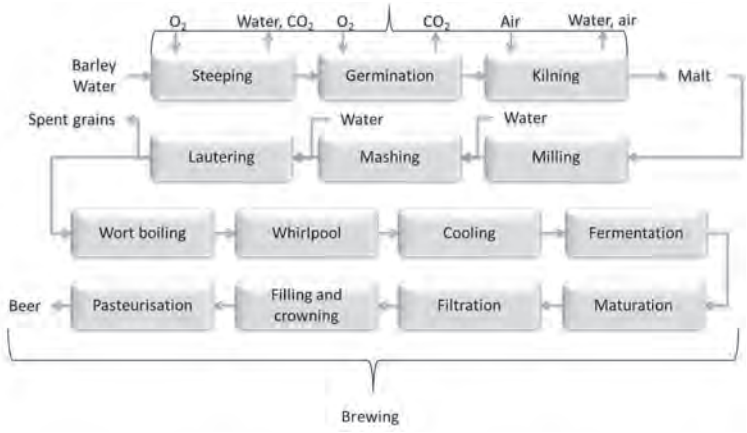

Figure 2. Schematic representation of the brewing process

1.2.1 Malting

Native barley hardly contains active enzymes except *β*-amylase. Therefore, barley is malted to produce and activate enzymes that are needed in the brewing process. The malting process has three steps; steeping, germinating and kilning. During steeping, the barley is hydrated for about 40 hours, leading to a water content increase from 11% to about 46% (F. G. Priest & Stewart, 2006). (T. O'Rourke, 1999a). The water in the barley activates the barley kernel, after which the embryo starts to produce the hormone gibberellic acid. This hormone stimulates the enzyme synthesis, e.g. of ***α***-amylase in the scutellum and aleurone layer (Higgins, Jacobsen, & Zwar, 1982; R. L. a. A. Jones, J.E., 1971). Besides gibberellic acid, also auxins (Indole-3-acetic acid) stimulate enzyme production, e.g. *β*-glucanase to break down cell walls (Stuart, Loi, & Fincher, 1986).

The enzymes synthesized during germination are necessary for the barley kernel to break down the endosperm structure, for the hydrolysis of starch into sugars that are metabolised during fermentation. Cytolytic enzymes cause cell-wall disruption through hydrolysis of fibre components like *β*-glucan and hemicellulose. As a result, the starch and proteins inside the endosperm cells become more accessible to the other enzymes, such as the *α*- and *β*-amylases and proteases. The purpose of proteolytic enzymes in brewing is the production of free amino nitrogen for the yeast to grow during fermentation. The main starch degrading enzymes are ***α***-amylase and ***β***-amylase (Steiner et al., 2012).

Kilning is a drying step performed after germination to stop the germination, to induce Maillard reactions to give flavour and colour, to preserve the enzyme activity and to preserve the barley itself. Drying is performed by hot air and starts at a lower temperature (50°C) to minimize the thermal inactivation of enzymes. Once the water activity is lowered and the thermostability of the enzymes has increased, the temperature of the air is increased (T. O'Rourke, 1999a). It is important to start kilning at the right moment to have an optimal balance between enzyme synthesis and low material losses due to respiration and plant growth. After kilning, the rootlets are removed and the malt is stored prior to further processing.

1.2.2 Beer production

The brewing process consists of several unit operations as depicted in **figure 2**. After malting, the malt is milled before brewing to increase the surface area of the substrates, allowing for a more efficient hydrolysis. Generally, a hammer mill or roller mill is used, depending on the filter that is used later on in the process. The milled malt is mixed with water in the mashing vessel to form the mash. Enzymes dissolve from the malted barley kernels into the water and start hydrolysing their substrates. The mashing starts at a temperature of about 54 °C (± 30 minutes) to allow glucanases and proteases to work. Glucanases break down the cell walls and proteases degrade the protein matrix, so that the starch becomes more accessible. The mash is subsequently heated to 64°C (± 60 minutes) to gelatinise starch and induce its hydrolysis by β-amylase and α-amylase. Beta-amylase is an exoenzyme with an optimum temperature of 60-65 °C. It catalyses the hydrolysis of the 1-4 linkages to the non-reducing chain ends of amylopectin, producing maltose and dextrins. Alpha-amylase is an endoenzyme with an optimum temperature of 72-75 °C which hydrolyses 1-4-α-glucosidic linkages in amylose and amylopectin, producing glucose, maltose, maltotriose and oligosaccharides (Briggs, Brookes, Stevens, & Boulton, 2004; Stewart, 2013). The final step of the mashing is at 78°C (± 10 minutes) to inactivate the enzymes and microorganisms.

The mash is transferred to a filtration unit after mashing. Traditionally, a lautertun is used in which the mash is filtered over the husk particles originating from the barley kernels. Therefore it is important that the husk is kept intact as much as possible during the milling process. A roller mill is most suitable for this purpose (T. O'Rourke, 1999b). However, polyphenols, bitter components and arabinoxylans will solubilize into the wort when the husk is included in the mash, leading to a

risk of off-flavour- and/or physical instability and process inefficiency. The other option for filtration is a mash filter. Here the mash is put onto a mesh over which a pressure difference is generated. This filtering process allows a much finer milling, which can be achieved by using a hammer mill (T. O'Rourke, 1999b). The filtration separates the suspension into the filtered mash called wort and the solid residue, called the (brewer's) spent grains, which is mostly used as animal feed.

From the filtration unit, the filtered mash is transferred into the wort boiler. Here, the wort is boiled with hops that give the beer its characteristic bitter flavour. Furthermore, boiling leads to sterilisation of the wort, strips off undesirable volatiles and causes excess proteins and tannis to form a precipitate that can be removed by use of a whirlpool (Eaton, 2006; Stewart, 2013). The wort is then cooled and fermented by yeast (*Saccharomyces cerevisiae*). The yeast needs sufficient levels of fermentable sugars, amino acids and lipids to grow. During fermentation, fermentable sugars are converted into alcohol and carbon dioxide. Also other flavour and volatile components are produced that contribute to the beer flavour. Subsequently, the beer is filtered to remove some of the polyphenols and proteins that interact to form complexes. These complexes cause a haze, which is considered a quality defect in beer. After filtration, the beer is ready to be bottled, pasteurized and distributed.

1.3 Waste and Trade-offs in the brewing process

Half of the waste in cereal agriculture is generated before the product is transported to the consumer (Gustavsson, Cederberg, Sonesson, Van Otterdijk, & Meybeck, 2011). This is due to inefficient harvesting, storage or processing. Material losses in processing can be reduced by minimizing the amount of waste streams and creating valuable by-products from those waste-streams. Besides raw material, energy and water can be saved by more efficient processing. This is also the case for the brewing process.

During malting, for example, submersion of the grain in water is alternated with air-resting periods. Each submersion can require up to 900 litres of water per ton of barley, resulting in large quantities of water with a high biological oxygen demand that needs to be discarded (F. G. Priest & Stewart, 2006; Willaert, 2006). In **figure 3**

the mass streams of the malting process are visualized. It shows the large amounts of water that are used, and that the water which is taken up by the barley has to be evaporated again. The latent heat for this process is about 1638 kJ/kg barley. Furthermore there are significant raw material losses due to respiration and plant growth. Circumventing the malting process would save raw material, water and energy. The use of unmalted barley, however, is challenging since it only contains very low levels of endogenous enzymes and since its chemical composition is different from that of malt.

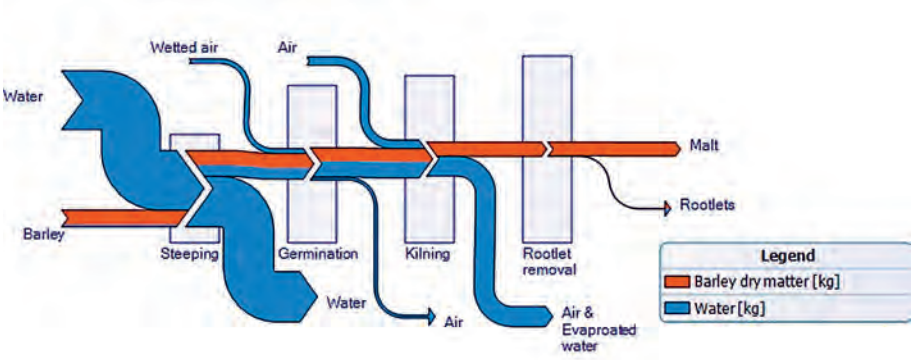

Figure 3. Mass streams during the malting of barley. The diagram excludes air for germination and kilning.

In malting as well as brewing, trade-offs are made. The main trade-off in malting is between sufficient enzyme development (longer germination time) and minimized raw material losses (shorter germination time). In case of brewing, especially mashing, limited protein hydrolysis, but maximal hydrolysis of starch and β-glucan is required. Because the hydrolysis of these components occurs at the same temperature, keeping the temperature of the mash for a longer time at 54 °C will increase the breakdown of β-glucan but also that of protein (T. O'Rourke, 1999b). Other issues, like fat oxidation, protein-polyphenol complex formation and increased viscosity may occur due to the components naturally present in malt. The breakdown of these components and the amount of breakdown products can be influenced by adapting the mashing program.

Besides these trade-offs, also the generated waste streams are related to process efficiency and product quality. The largest waste stream in the process is the spent grains. **Figure 4A** shows how the mass of this stream is proportional to the main product stream (e.g. the wort). The spent grains contain many valuable

components like dietary fibres, proteins and polyphenols (Aliyu & Bala, 2013). A main problem, though, is that the spent grains consist for about 80% of water that contains dissolved extract, which will be lost. Furthermore the stream requires drying to avoid spoilage and to allow further processing. One could think of recovering components from the spent grains, or to reduce the amount of spent grains. One way to do the latter would be to take away part of the husk before brewing. This removal can be done by pearling, an abrasive milling technique. The removed husk remains dry this way, which makes it easier to transport and handle. Also, there will be less spent grains that take up less water leading to decreased losses. However, taking away too much husk would impair the filtration, especially when a lauter tun is used. Also some components from the husk dissolve into the mash during brewing. Taking away the husk affects the beer composition, which could influence the taste and appearance of the final product.

1.4 Fractionation as a pre-treatment for brewing

Fractionation of the raw material can help solving inefficiencies in the brewing process. In the current brewing process, about 25% of the malt dry matter ends up in the spent grains, which means that a significant part of the original barley kernels is not used in the final product. In addition, separation of the kernels into fractions to subject each to its most optimal treatment may enhance the overall yield of the brewing process. Separation of the barley in a starch-rich faction and a protein-rich fraction, allows adding the two fractions to the mash at different temperatures or at different times, which would allow for more complete starch hydrolysis, while limiting protein hydrolysis. Furthermore, fractionation can be used to reduce the amount of undesirable components. Arabinoxylans and anthocyanogens are examples of components that can have undesired effects in beer. These components are mainly located in the husk of the barley, and removal of this part before the brewing process will lead to lower levels of these components in the mash. An additional advantage could be that the unprocessed husk fraction might more easily be used for other applications, such as functional foods. **Figure 4** shows the first steps of the brewing process for brewing with unmalted barley **(figure 4a)** and pearled unmalted barley **(figure 4b)**. The addition of exogenous enzymes is necessary in this process because the malting step was omitted, and native barley does not contain sufficient enzyme activity to hydrolyse its components. In this

diagram the wet spent grains steam is reduced by 58% and the total water use would be reduced by 8% compared to the traditional process.

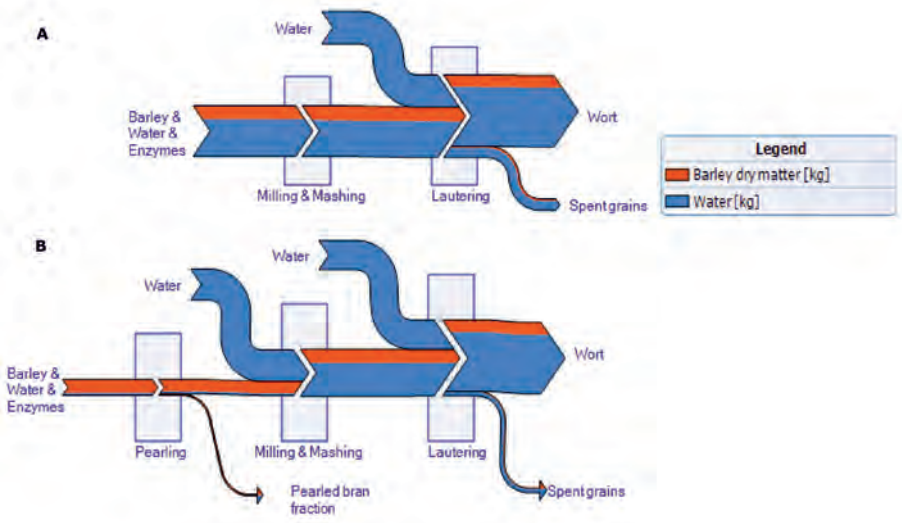

Figure 4. Mass streams during the first two steps of brewing in the conventional process (A) and a process with an added pearling step (B).

1.5 Traditional processing and sustainability

The beer production process has been largely optimized; malting is optimised to have high enzyme activity with minimal material losses (Cook, 2013) and mashing is designed for optimal conversion of components given the enzymes present in the malt. Yeast strains are cultivated to have optimal conversion of carbohydrates to ethanol, to give the right flavour pattern or to be temperature resistant (F. Priest & Campbell, 2011). Only minor improvements can be achieved by optimizing these processing steps further without adapting the types of operations. Nevertheless, inherent in the traditional process are the production of large waste streams, such as the spent grains and waste water during malting. Kilning, furthermore, is an inherently energy intensive operation. Therefore, the environmental sustainability (resource efficiency) of this process can only be improved by fundamentally changing these steps.

There are many possible options to improve this process, and before implementing any of them, one should assess if these options would contribute to a better overall efficiency. The efficiency of the use of resources and emission of wastes, two aspects of the sustainability of an operation, can be measured in various ways. Life cycle analysis is widely applied: it assesses the environmental impact of a product over its life or production cycle, in this case from raw material to end product. It may consider amongst others raw material input, waste production, energy requirements and emissions of greenhouse gases (Roy et al., 2009). It is, however, difficult to compare energy and mass streams in this analysis, and therefore difficult to compare processing options that have different balances of energy and mass streams. Exergy analysis, having an objective thermodynamic basis, is an analysis which can be used to objectively compare mass and energy streams with each other. This analysis is becoming more common to assess the environmental sustainability of processes (Apaiah, Linnemann, & van der Kooi, 2006; Dincer & Ratlamwala, 2013), even though it does not directly take into account for example toxicity of waste streams or their effect on global warming. The outcome of both analyses depend on the system boundaries and allocation of environmental load.

1.6 Aim and outline of this thesis

We hypothesize that fractionation of the unprocessed barley may result in significant improvements in the brewing process. **Figure 4b** shows that removal of the bran from the barley will reduce the amount of water needed in the process. It will reduce the volume of spent grains, hence further reducing wastes and energy required for drying the spent grains. A further step would be to omit the malting process, thereby even further reducing the use of water en energy (since no kilning would be required). Of course, a major question here is what the impact is of these changes to the process, and whether one can still brew high-quality beer. The overall aim of this thesis was therefore to investigate how barley can be fractionated to optimize the beer brewing process in terms of its use of resources, while maintaining the quality of the resulting beer. **Figure 5** shows a schematic outline of the content of this thesis.

The thermo-mechanical properties of the barley constituents starch and protein were investigated, to see if we could create optimal conditions for separating them

from each other by milling. **Chapter 2** describes the glass-to-rubber transition of protein and starch isolated from barley. The hypothesis of this chapter is that dry fractionation by milling is facilitated by milling under conditions in which the protein is in a rubbery state and the starch in a glassy state. Two measurement methods were used to measure the glass transition temperature (Stuart et al.); differential scanning calorimetry (DSC) and thermo-mechanical compression tests (TMCT). The methods gave different results due to the differences in moisture content range, heating rates and possibly conformational changes of the protein. The importance of correcting the T_g lines for the moisture distribution inside the endosperm over the various components was made clear. After this correction, the glass transition lines of starch and protein were closer together. Because the two glass transitions are found to be very close in the native material, the expectation is that achieving good separation between the components based on having one glassy component and one rubbery component is challenging.

For this reason, another dry fractionation technique, pearling, is considered. Pearling is an abrasive method that removes the outer layer of a particulate material. In case of barley, removal of the husk before brewing by pearling seems advantageous, because the husk contains undesirable components when it comes to beer. Besides, pearling would facilitate the use of those husks in other applications, and will reduce the energy required for drying them. The effect of using pearled barley in the brewing process is investigated.

Chapter 3 describes the chemical composition of the barley and of fractions that were pearled off. Pearling was shown to selectively remove insoluble fibre, ash, protein and polyphenols, while the β-amylase activity and starch content of the remaining kernel was hardly affected. The water holding capacity of the barley fractions was related to the fibre content. This indicates that when the fibre content is reduced in the spent grains, the spent grains will take up less wort, leading to less wort and sugar losses in this waste stream.

Chapter 4 describes a comparison between a traditional brewing process and an enzyme-assisted brewing process with respect to their use of resources. The use of exogenous enzymes is found to be more efficient than producing enzymes through the malting process. In the analysis, we proposed to use the cumulative exergetic content of the enzyme production rather than just the chemical exergy

of the enzymes. This cumulative exergetic content of the enzymes was ± 30 times higher than their standard chemical exergy. This conclusion shows that the cumulative exergetic costs of minor components should be taken into account if a process uses significant quantities of these components. This can be achieved by extending the system boundaries to include the production process of the purified components.

Chapter 5 describes brewing tests using malted, unmalted and pearled unmalted barley kernels. Brewing with unmalted barley saves material, energy and water in the malting stage but may result in complications during processing. Pearling mitigates these problems. Exogenous enzymes were used to compensate for the low enzyme activity in unmalted barley. Lautertun filtration and mash filtration were considered as filtration methods. Principle component analysis was performed on the chemical composition of the wort and the various spent grains, to investigate the effect of the malt-to-barley ratio, the degree of pearling and the filter method. A window of operation for working with pearled barley was determined for brewing with a mash filter as a filtration method.

Chapter 6 concludes with a general discussion of all findings presented in this thesis. It furthermore discusses the possibility to use pearling as a pre-treatment before malting. Finally, an outlook on future work in this area is given.

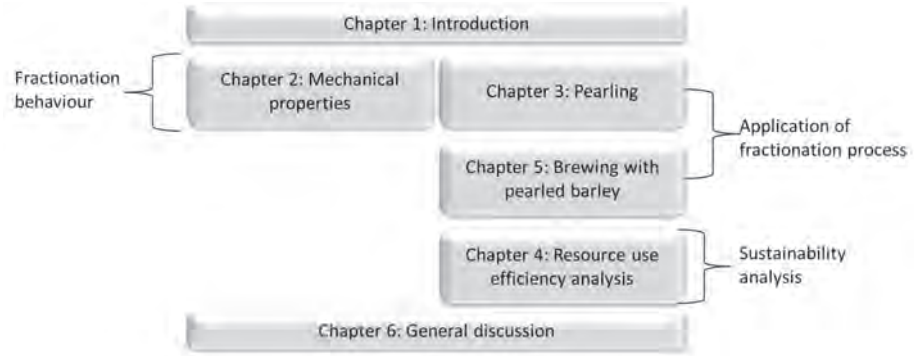

Figure 5. Schematic outline of the content of this thesis.

Glass transitions of barley starch and protein in the endosperm and isolated from

Highlights

- State diagrams should be corrected for moisture distribution in a material
- The combination of DCS and TMTC gives a better view on glass transition in biopolymers
- Glass transition lines of protein and starch in barley are close together
- Fraction behaviour of barley is influenced by the state of its constituents

This chapter has been published as: Laura H.G. van Donkelaar, José Torres Martinez, Hans Frijters, Tom R. Noordman, Remko M. Boom, Atze-Jan van der Goot (2015), Glass transitions of barley starch and protein in the endosperm and isolated from, Food Research International 71, 241-246

2.1 Abstract

When studying the glass-to-rubber transition inside natural materials, it is important to take into account the moisture content but also the moisture distribution over the components in the material. We measured the T_g of protein and starch isolated from barley at different moisture contents using differential scanning calorimetry (DSC) (heating rate 10°C/min) and by thermo mechanical compression tests (TMCT) (Heating rate 2°C/min). The measurement of the T_g of partially crystalline materials, such as barley starch, is more difficult using TMCT because the mechanical effect of expansion of these materials is smaller. For both measurement sets the glass transition lines were modelled using the Gordon-Taylor equation. The lines were adapted for the differences in moisture content over the endosperm by using the sorption isotherms of isolated barley starch and protein and whole barley endosperm. The glass transition lines measured by TMCT were closer together than the ones measured by DSC.

Keywords: Dry fractionation; Glass transition temperature; Thermomechanical compression test; Differential scanning calorimetry; Gordon-Taylor equation; Barley

2.2 Introduction

In many food processes, the raw materials contain components that influence the production process and product quality both positively and negatively. The negative effects can be reduced by removing or lowering the content of the components that cause them. The latter can be achieved by processing the raw materials into fractions with just the right composition to convey the right functional properties for their use in the final product.

Dry fractionation is a relatively simple approach to obtain various fractions from a raw material. It offers the opportunity to enrich materials while retaining the components' native functionality (Schutyser & van der Goot, 2011). Milling is the first step in dry fractionation to separate materials into the constituent components. Separation of the components is crucial to obtain high yields. Milling is followed by separation techniques such as sieving or air classification. (Andersson, Andersson, & Aman, 2000; Knuckles & Chiu, 1995; Knuckles, Chiu, & Betschart, 1992; Liu, 2009; Sundberg, Tilly, & Aman, P., 1995;). The efficiency of sieving and air classification depends on many factors but detachment of the components is still a prerequisite. Barley is one of the cereals for which dry fractionation has been investigated, with the aim to enrich in starch and β-glucans (Knuckles & Chiu, 1995; Knuckles et al, 1992; Létang, Samson, Lasserre, Chaurand, & Abécassis, 2002; Sundberg et al., 1995; Vasanthan, 1995, Yu, Stringfellow, & Inglett, 1994).

The physical state of the components is one of the factors that influences detachment. Components can be in a glassy state, in which they are brittle, or in a rubbery state, in which they are ductile (Sperling, 2005). The glassy state is a nonequilibrium solid state, in which the molecules have no ordered structure, leading to a larger volume to be occupied than when in crystalline state (Roos, 2007; Slade et al., 1995). The glass transition is a second-order reaction and the glass transition temperature (Stuart et al.) is influenced by the water content and the temperature (Abiad, Carvajal, & Campanella, 2009). It can be reasoned that when one material, for example the protein matrix of barley, is in the rubbery state and one material, for example barley starch, is in the glassy state, milling could be more effective in separating these two components. The rationale is that, in this case, breakage would occur between the protein matrix and the starch, resulting in detached components. This hypothesis was successfully applied to

peas; increased detachment was found when the protein was in the rubbery state (Pelgrom, Schutyser, & Boom, 2012).

Different methods for measuring glass transitions are described in the literature but differential scanning calorimetry (DSC) is the most widely used method (Abiad et al., 2009). DSC measures the heat flow and heat capacity in heating and cooling to identify the temperature of the transitions. The change in heat capacity measured by DSC for starch dispersions is small, which makes the detection of the Tg more difficult than that of protein (Biliaderis, 1991). Tg's measured by DSC of starch with a moisture content of 7% and higher have been reported in literature (Chung, Woo & Lim, 2004; Sun et al., 2002). . A relatively new method to measure glass transitions of food and pharmaceutical powders is the thermomechanical compression test (TMCT), established by Boonyai et al. in 2007 (Boonyai, Howes, & Bhandari, 2007). This test uses a probe that applies a constant force on the sample while the sample is being heated. The glass transition is then characterized by displacement of the probe, caused by the increased mobility of materials entering the rubbery state (Boonyai, Howes, & Bhandari, 2007; Pelgrom et al., 2012).

The T_g of an isolated component at a certain moisture content, however, cannot be taken directly as the T_g of that component in a barley kernel at that same overall moisture content. This is because the components have the same water activity but therefore a different moisture content, which makes that the moisture distribution inside the barley kernel is not homogeneous between its components. Therefore, isotherms of the material and the components should be used to estimate local moisture content in the barley, which will influence the glass transition lines of the components. Microscopic imaging or compression tests can be used to test the effect of the temperature and the moisture content on the breaking behaviour of the material.

The aim of this study is thus to understand whether differences in the state of components can be used to detach components in barley more effectively during milling, focusing on the two main components of the barley endosperm of the variety Sebastien: starch (77 w/w% dm) and protein (8.2 w/w% dm) (van Donkelaar, Noordman, Boom, & Van der Goot, 2015). The glass transition curves of isolated starch and protein are presented and discussed. The results obtained by DSC are compared with the results obtained by the TMCT method. The curves were

adapted using the isotherms of barley endosperm, isolated starch and protein. Compression tests were done and visualized using scanning electron microscopy (Jane, Kasemsuwan, Leas, Zobel, & Robyt).

2.3 Materials and methods

2.3.1 Materials

Barley (*Hordeum vulgare*) of the variety Sebastian was used (harvested in France in August 2012, stored at 4°C).

2.3.2 Isolation of starch and protein

Starch was obtained by extraction from the barley. A dough was prepared using barley flour (water/flour ratio 1:2.5). The dough was washed (flour/water ratio 1:20) and the wash water was sieved through a 53-µm sieve. The suspension was then centrifuged (1400×*g*, 20 min). The supernatant and the grey top layer of the residue were removed. The residue was freeze dried and its starch content, measured using a total starch assay (Megazyme, Total Starch [AA/AMG], Ireland), was 94±2.7%. The moisture content of the samples was adjusted by mixing water and dry starch, and equilibrated for at least 72 h to ensure a homogeneous moisture distribution. Per measurement, 5 gram of sample was prepared.

Protein was recovered using the method described by Wang et al. (2010) with some modifications. Pearled barley flour was defatted using hexane in a ratio of 1:10 w/w%. The defatted flour was mixed with alkaline solution (pH 11.5, ratio 1:10 w/w%) and stirred at 20°C for 30 min. After centrifugation (8500×*g*, 15 min at 23°C), the pH of the supernatant was adjusted to pH 5 using 0.5 M HCl to precipitate proteins. The supernatant was centrifuged (8500×*g*, 15 min, 23°C) and the residue was freeze dried, ground and stored at 4°C. The Dumas method (Nitrogen analyser, FlashEA 1112 series, Thermo Scientific, Interscience), conversion factor 5.83, was used to determine the purity of the protein (96.6±1.2%). The yield was ~50%. The moisture content of the samples was adjusted at least 72 h before analysis by placing them in a climate chamber at a fixed temperature and moisture content.

2.3.3 Glass transition measurements

DSC analysis (Diamond DSC, PerkinElmer, Waltham, Massachusetts, USA) was used to measure the glass transition temperatures. About 15 mg of sample was placed in a stainless steel pressure cup. The DSC analyser was calibrated using gallium and indium; an empty stainless steel pan served as a reference and nitrogen was used as a carrier gas. The samples were cooled to –60°C at a rate of 10°C/min and heated to 160°C at a rate of 10°C/min. This was repeated once and measurements were analysed for the glass transition midpoint using Start Pyris Software (version 11.0) using the second heating curve.

TMCT was performed using the setup as described by Pelgrom et al. (2012), with some modifications. Two grams of sample was placed on the bottom of the concentric cylinder (diameter 20 mm, height 40 mm). A water chamber in the sidewalls and a solid bottom was used to control the temperature. A ramp of 5–80°C was created by connecting the water chamber to a water bath (Julabo FP50-HE, Julabo, Seelbach, Germany). A heating rate of 2°C/min was used. The temperature in the bottom of the cylinder was recorded continuously (Testo 175 T3, Testo GmbH & Co., Lenzkirch, Germany). A 15-mm cylindrical probe attached to a texture analyser (Instron-5564Series-Table-Model- Systems-Twin-column-design, Canton USA) equipped with a 2000 N load cell, exerted a constant force of 30 N on 2 g of sample. The force–displacement curve was measured with the Bluehill 2 Texture Profile Analysis software. For each sample, the force–displacement curve was corrected for thermal expansion of the equipment by subtracting the force–displacement curve of maltodextrin (Aldrich Chemical Co.; dextrose equivalent, 13–17). Maltodextrin was chosen as reference because it has a high T_g value (T_g >180°C), and it is physically and chemically stable.

Modelling the glass transition lines for both the DSC and TMCT methods was done using the Gordon-Taylor equation:

$$T_g = \frac{w_1 T_{g1} + k\, w_2 T_{g2}}{w_1 + k\, w_2}$$

where w_1 and w_2 are the weight fractions of water and barley isolate, respectively; T_{g1} and T_{g2} are the glass transition temperatures of water and barley isolate (Gordon & Taylor, 1952). T_{g1} is taken to be 136 K although this value has been disputed (Yue & Angell, 2003).

The *k* parameter relates to the strength of interactions in the sample components and is either fitted or defined by

$$k = \frac{\rho_1 T_{g1}}{\rho_2 T_{g2}}$$

where ρ_1 and ρ_2 are the density of water and barley isolate, respectively, and T_{g1} and T_{g2} are the glass transition temperatures of water and barley isolate (Ford & Timmins, 1989). The density of the components was defined by Singh & Heldman (2008):

$$\rho_{water} = 916.89 - 0.13071 T_{g1}$$

$$\rho_{starch} = 1599.1 - 0.11046 T_{g\,starch}$$

$$\rho_{protein} = 132.99 - 0.51840 T_{g\,protein}$$

The models with and without *k* as a fitting parameter were compared by comparing the Akaike information criterion (AIC) corrected for a finite data set (Akaike, 1973):

$$\mathrm{AIC} = n \ln\left(s^2\right) + 2p + \frac{2p(p+1)}{n \quad p \quad 1}$$

$$s^2 = \frac{\mathrm{RSS}}{n}$$

where *n* is the number of experiments, *p* is the number of parameters, *s* is the standard deviation and RSS is the residual sum of squares. A model with a lower AIC value describes the data with less data loss compared to a model with a higher AIC value.

2.3.4 Sorption isotherms

Dynamic vapour sorption (DVS) was used to measure the sorption and desorption isotherms of isolated barley starch, isolated barley protein, and barley endosperm. The samples were put in a stainless steel mesh basket which was placed in a DVS Advantage apparatus (Surface Measurement Systems NA, Allentown, PA). The temperature of the DVS was set at 25°C. The material was subjected to a range of relative humidities (RH); the RH was increased stepwise to approximately 90%, decreased in steps of 10% to 0% RH, and increased again to the starting RH. When

the mass of the isolates did not change by more than 0.002 mg/min for 10 min or when the material was subjected to a certain RH for a maximum of 360 min, it was assumed that the sample was at equilibrium. The sample was on an RH of 0 for 600 min. All the components in the endosperm were assumed to have the same water activity. The isotherms were used to read the moisture content of the components at a certain endosperm moisture content. The Tg lines were adapted by plotting the glass transition temperature of the components at this determined moisture content was plotted versus the moisture content of the endosperm.

The relationship between the moisture content (M) on dry weight and the water activity (a_w) was described with the Guggenheim, Anderson and De Boer (Bottega et al.) equation (Anderson, 1946; de Boer, 1953; Guggenheim, 1966):

$$M = \frac{CkM_0 a_w}{(1 - ka_w)(1 - ka_w + Ca_w)}$$

where M_0, k, and C are the parameters. The parameters were obtained by least square minimization of the difference between the measured moisture content and the predicted moisture content. The non-linear regression routine was implemented in Matlab.

2.3.5 Compression tests

Compression tests were done by compressing one whole barley grain (n=20) at 20 mm/min using a texture analyser (Instron, described earlier)). The moisture content of the samples was adapted at least 120 h before the analysis by placing them in a climate chamber at a fixed temperature and moisture content. The compressive load (N) during compression was plotted versus the compressive extension (mm). The experiment was performed at moisture contents of 9.2% and 20.3% and at 20°C and 50°C.

Microscopic images of the compressed barley were obtained using table-top SEM equipment (PHENOM Pure; Phenom-World BV, Eindhoven, The Netherlands). Samples were placed on a holder for non-conductive materials and analysed at a magnification of 900×.

2.4 Results and discussion

The Tg values of protein and starch isolated from barley were measured by DSC and TMTC. Interpretation of the data is an important step in both DSC and TMTC analysis. With DSC data, one can look at the onset, peak and the end temperature of the transition. The glass transition temperature range may be small or very broad depending on the composition of the material. For starches, especially at low moisture contents, the Tg is hard to interpret (Zeleznak & Hoseney, 1987; Biliaderis, 1991), because changes in heat capacity when passing the glass transition are small for starch in comparison with other components, such as proteins **(Figure 1)** (Liu et al., 2010). The horizontal lines indicate the glass transition range. Also TMCT data can be difficult to interpret.

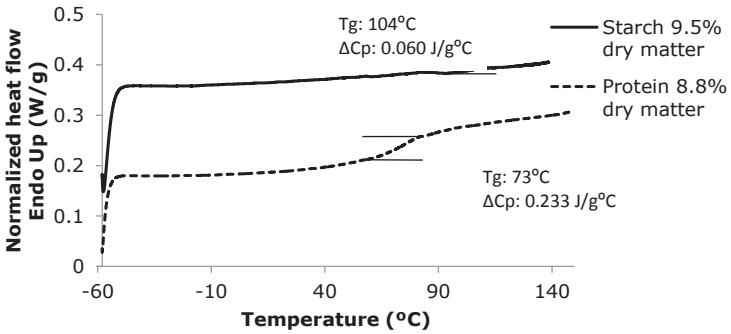

Figure 1. Differential scanning calorimetry curves of starch (9.5% dry matter) and protein (8.8% dry matter).

Figure 2 shows extension versus the temperature of barley starch isolate, barley protein isolate, and barley endosperm. The temperature range between the displacement curve being horizontal and the temperature where the curve goes up determined the range of the glass transition (Boonyai et al., 2007). The figure shows that the barley protein isolate expands more and has a smaller glass transition temperature range compared with the starch isolate. This means that the glass transition temperature is easier to identify. The line for the barley endosperm has the least steep slope and no meaningful glass transition temperature could be determined from this figure. This is probably because barley endosperm contains too many components with different glass transition temperatures, leading to a very broad glass transition range.

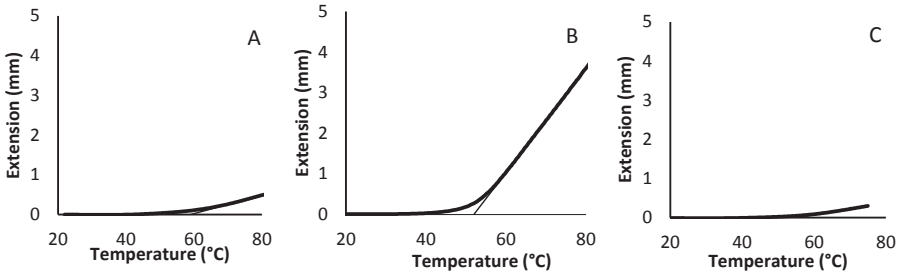

Figure 2. Extension versus temperature when compressing isolated barley starch (A), isolated barley protein (B) and milled barley endosperm (C) measured at moisture contents of 13.4% 13.3% and 12.4% weight/dry weight respectively.

Besides having a broader range for the T_g, starch expands less than protein. The reason for this might be related to the crystallinity of the starch (relative crystallinity 20-36%) (BeMiller & Whistler, 2009). The glassy part of the granule material will expand above Tg, but the total starch expansion is reduced by the presence of the crystalline parts that will hardly expand upon temperature increase (Roos, 2007). Besides, the rigid structure of the crystalline part reduces the mechanical effect of the glass transition.

The data measured by DSC and TMTC were interpreted using the Gordon-Taylor equation **(Figure 3)**. The use of T_{g2} and *k* as fit parameters resulted in lower AIC values than the use of only one fit parameter **(table 1)**. This indicates that the model using 2 fit parameters describes the data more accurately. The DSC data are more accurately described by the Gordon-Taylor equation than the TMCT data. The *k* values that were obtained for starch by DSC measurements and TMCT measurements were similar with the model using one fit parameter, but were higher when two parameters were fitted. The k values for protein were higher than that of starch. For all models, the glass transition temperature of proteins is lower than that of starch, which was expected given the data in the literature (Cuq, Abecassis, & Guilbert, 2003; Pelgrom et al., 2012).The T_g lines of barley starch and barley protein as measured by the DSC were similar to the T_g lines for wheat starch and wheat protein as published by Cuq et al. (2003) (Cuq, Abecassis, & Guilbert, 2003).

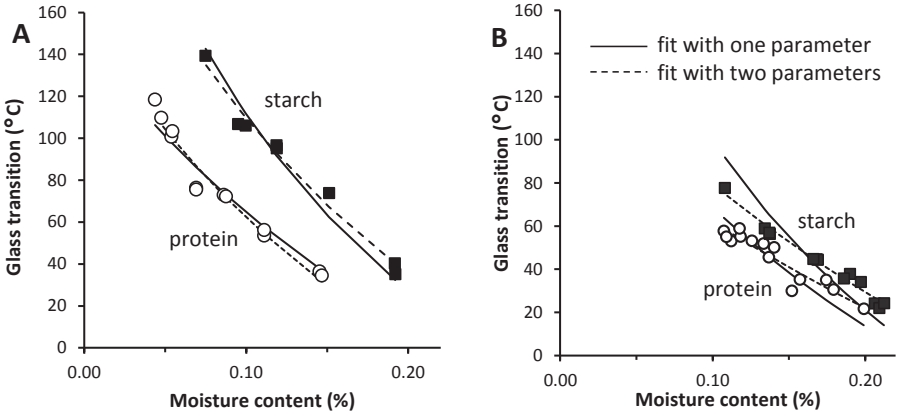

Figure 3. Glass transition lines of starch and protein isolated from barley as measured by differential scanning calorimetry (A) and thermo mechanical compression tests (B).

Table 1. The k (parameter), Tg0 (glass transition temperature of dry starch/protein) and Akaike information criterion values ± standard deviation for the glass transition temperature lines as modelled from differential scanning calorimetry and thermo mechanical compression tests data according to the Gordon-Taylor equation.

Model	*k*	T_{g0} (°C)	AIC
DSC			
Starch *p*=1	0.16±0.00	284±7.03	65.1
Starch *p*=2	0.21±0.00	240±3.01	56.5
Protein *p*=1	0.27±0.00	147±2.67	90.6
Protein *p*=2	0.22±0.01	161±2.26	86.2
TMCT			
Starch *p*=1	0.17±0.01	258±23.81	113.2
Starch *p*=2	0.35±0.01	148±3.45	49.2
Protein *p*=1	0.27±0.00	154±3.20	100.7
Protein *p*=2	0.44±0.01	112±1.51	82.5

p=1 means that only the glass transition temperature of the starch or protein was a fit parameter; *p*=2 means that *k* was also a fit parameter.

The glass transition line of isolated barley starch as measured by DSC is higher than that measured by TMCT. The reason for this might be the difference in the heating rate; it is known that the glass transition depends on the rate of heating or cooling. A lower heating rate would result in a lower measured glass transition temperature (Abiad, Carvajal, & Campanella, 2009).

Another factor that causes differences between the T_g obtained by DSC and TMCT is the range of moisture content at which they were measured. The samples that gave the data used to construct the T_g curve for barley starch isolate had a moisture content between 7% and 19% for DSC, and between 11% and 21% for TMCT. With TMCT, it was not possible to measure the protein glass transition temperature at moisture contents lower than 11%. This results in a flatter glass transition line for samples measured by TMCT.

Another possible explanation for the difference between results for DSC and TMCT could be related to conformational changes in the molecules due to heating during DSC (Bengoechea, Arrachid, Guerrero, Hill, & Mitchell, 2007; Furukawa, 1995; Mizuno, Mitsuiki, Motoki, Ebisawa, & Suzuki, 2000). In general, conformational changes increase with temperature increase. The first heating step in the DSC measurement could thus result in changes, which is usually associated with reduced mobility and therefore a higher T_g value. However, Bengoechea et al. (2007) found a higher T_g for soy, casein and gluten proteinwhen using a phase transition analyser (Gupta, Abu-Ghannam, & Gallaghar) compared with DSC. Similar to TMCT, the PTA method makes use of a piston to apply a constant force to a sample while increasing the temperature. They hypothesized that conformational changes related to cross-linking (which can occur during the first heating cycle of the DSC measurement) can result in a lower measured T_g due to increased molecule mobility. Also earlier studies showed that cross-linking does not necessarily increase the T_g and that the T_g of biopolymers might be determined by the state of the non-covalent bonds (Furukawa, 1995; Mizuno et al., 2000).

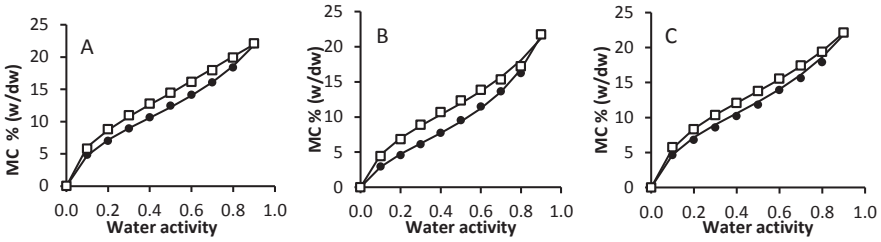

Figure 4. Measured (de)sorption data (symbols) and Modelled GAB (de)sorption isotherms (lines) of barley starch isolate (A), barley protein isolate (B) and barley endosperm (C). Closed circles: sorption. Open squares: desorption.

Protein and starch within the barley grain will have the same water activity but not necessarily the same moisture content. Therefore we measured the isotherms of isolated barley starch, isolated barley protein and barley endosperm, and modelled them using the GAB equation as shown in **figure 4**. The values for the parameters from the GAB equation are shown in **Table 2**. We used the isotherms to adapt the state diagram; this extends the methodology (Abiad et al., 2009). The isotherms showed that proteins take up less water than the starch granules, which causes the protein to be dryer than expected from the moisture content of the endosperm. For example, when the moisture content of the endosperm is 11.7%, the moisture content of the starch is 12.2%, while that of the protein is 9.3%. This means the glass transition of the protein inside the endosperm is higher than expected when considering the overall moisture content only.

Table 2 The values for the parameters from the Guggenheim, Anderson and De Boer equation for the isotherms of isolated barley starch, isolated barley protein and barley endosperm at 25°C. C and k are dimensionless constants, M_0 stands for water content in weight/dry weigh.

	M_0	C	k
Sorption isotherm			
Starch	9.76±0.60	12.02±2.04	0.64±0.03
Protein	6.96±0.77	7.35±2.46	0.77±0.03
Endosperm	9.00±0.78	12.48±3.38	0.68±0.04
Desorption isotherm			
Starch	13.24±1.15	13.34±2.08	0.50±0.04
Protein	9.57±1.77	12.07±6.04	0.64±0.08
Endosperm	11.67±0.43	14.02±1.20	0.56±0.02

Figure 5 shows the state diagram that is adapted by calculating the moisture content of the separate components at different endosperm moisture content, and plotting the glass transition temperature of the calculated moisture content versus the moisture content of the endosperm. The glass transition lines of protein and starch are now closer together. For example, the T_g of starch shifts from 90°C to 87°C, and the T_g of protein shifts from 48°C to 63°C in the state diagram constructed by DSC and at a moisture content of 0.12. The window of operation for milling in which the protein is already rubbery, but the starch is still glassy, is smaller. It might be that the small differences between the glass transition temperature of barley protein and starch, at the water content of which they are present in the barley endosperm, makes it difficult to use this phenomenon in dry fractionation.

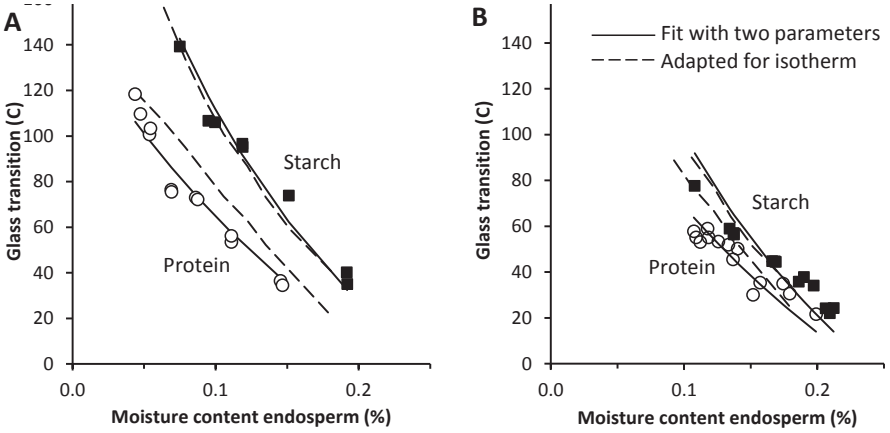

Figure 5. Adapted state diagram of starch and protein isolated from barley measured by differential scanning calorimetry (A) and thermos mechanical compression tests (B), adapted using the isotherms.

We have now sufficient information to assess the hypothesis stated in the introduction, that the fracture behaviour is significantly changed by the glass transition and that hence the separation of components can be influenced by choosing the overall state in between the glass transitions of the two main components (starch and protein). To assess these phenomena in the native material, we compressed single barley kernels and looked at the surface of the fraction with SEM **(figure 6)**. We compressed the kernels in three areas of the state diagram: when both starch and protein were rubbery, when both were glassy, and when the protein was rubbery and the starch was glassy. Structures of the husk, aleurone layer, cell walls, starch and protein were indicated in the figure.

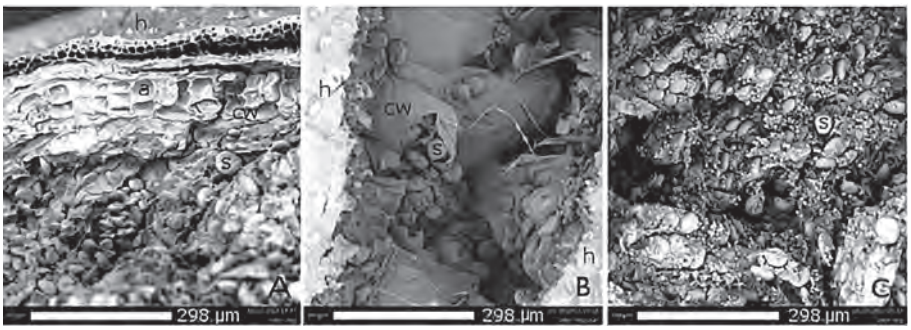

Figure 6. Scanning electron microscopy pictures of the fracture surface of compressed barley (900x). A: MC 9.2% at 20C, B: MC 20.3% at 20C. C: 20.3% at 50C. Husk (h), aleurone layer (a), cell walls (M. Edney et al.), starch (s) and protein matrix (p) can be distinguished.

Differences in fracture behaviour were observed when the barley kernel was fractured at different temperatures and moisture contents. When the protein and starch were both in the glassy phase **(figure 6A)**, the surface of the fracture was straight and clearly brittle. This is especially well visible in the husk and aleurone layer. Also it is visible that the fracture went through the cells in the endosperm. When both components were in the rubbery state **(figure 6B)**, the surface was ruptured and no clean fracture was visible. The starch and protein matrix are indicated in the figure, but no clear cell wall structure can be distinguished. In **figure 6C**, the fracturing conditions were chosen such that the protein was in the rubbery state and the starch was in the glassy state according to the state diagram constructed with data obtained by DSC. For the diagram constructed with the data obtained with TMCT, the conditions were such that both components were in their glass transition. The breaking surface is now in between a brittle fracture and a ruptured, deformed surface. The picture shows that a cell was ruptured and its content is leaking out.

2.5 Conclusions

DSC and TMTC gave different glass transition lines for isolated barley starch and isolated barley proteins, as modelled by the Gordon-Taylor equation. These differences may be attributed to the different heating rate, the different range of moisture content, or in the case of protein, conformational changes. The sorption isotherms of both components were measured and used to correct for the unequal sorption behaviour. After this correction, the glass transition lines were closer together. Fracturing of single, whole barley kernels showed that below the glass transitions, the fracture surface showed a brittle fracture, and above it, a deformed fracture surface indicating ductile behaviour. Since the two glass transitions are found to be very close in the native material, we expect that achieving good separation between the components based on having one glassy component and one rubbery component will be difficult.

2.6 Acknowledgements

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Pearling barley to alter raw material composition before brewing

Highlights

- Pearling is an effective method to remove undesired components, like arabinoxylans and polyphenols, before brewing
- The use of pearled barley has potential to decrease water and energy consumption in the brewing process
- The amount of insoluble fibres in a barley fraction is linearly related to the water binding capacity of that fraction
- The potential of using pearled barley in the brewing process is described

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3.1 Abstract

Partly replacing malt with unmalted barley is a trend in. The use of unmalted barley, however, leads to issues due to its higher content of undesired components. Pearling, an abrasive method to remove the outer layers of the barley kernels was shown to reduce insoluble fibre, ash, protein and polyphenols content, while the β-amylase activity and starch content of the remaining kernel were hardly affected. Removing de outer 5% of the kernel, for example, results in a reduction of 15% of the insoluble arabinoxylans, 23% of the insoluble fibre content and a reduction of 25% of the water binding capacity of the non-starch components. It also reduces the ash content by 19% and the polyphenol content by 11%, while only 0.20% of the starch is pearled off. Reducing the arabinoxylan content lowers the mash viscosity, which will facilitate the filtration step after mashing. Lower polyphenol content reduces the haze formation potential. Lower fibre content reduces the volume and water holding capacity of the spent grains, which implies that less wort and sugar are lost during filtration. That the bran fraction remains dry, moreover, implies a reduction in energy required to dry the spent grains.

3.2 Introduction

Traditionally, beer is brewed with malted barley. In recent years, the partial replacement of the malted barley by unmalted barley was investigated (Doode, Wijngaard, & Arendt, 2005; Lowe, Ulmer, Van Sinderen, & Arendt, 2004; Steiner et al., 2012). The use of barley instead of malt in the brewing process has potential advantages, such as energy and water savings in the malting stage and a reduced raw material use due to the fact that no starch is used for germination. (D.L. Goode, Wiltschko, Ulmer, & Arendt, 2005; Steiner et al., 2012). The main disadvantage of using barley instead of malt is the lower enzymatic activity in barley. Consequently, fewer components will be hydrolysed during malting, leading to a reduced mashing or hydrolysis rate. Besides, less hydrolysis at the stages of the mashing process will lead to more residual cell wall materials like β-glucans and arabinoxylans (Choct, 1997). This might lead to more water absorption in the filter bed when filtering the mash, due to the high water holding capacity (WHC) of β-glucans and arabinoxylans. In addition, these components increase the mash viscosity, which also leads to longer filtration times. Finally, more water uptake by the spent grains leads to higher extract loss (D.L. Goode et al., 2005; Lowe et al., 2004). A solution for the disadvantages described above could be the (partly) removal of those undesired components before the wet processing takes place.

The native barley kernel contains several tissues that are highly structured. The kernel consists of an embryo and endosperm surrounded by several tissue layers, being aleurone, testa, pericarp and husk. The embryo contains most of the lipids. Starch granules are located in the endosperm and are embedded in a protein matrix. The cell wall that surrounds the protein matrix is rich in β-glucans (75%) and also contains arabinoxylans (20%) (Jadhav et al., 1998). The endosperm contains the enzyme β-amylase, which facilitates the breakdown of starch during mashing (Buttimer & Briggs, 2000). The bran consists of the pericarp layer and the aleurone layer. The aleurone layer, which is rich in protein, surrounds the endosperm and has thick cell walls, which are rich in arabinoxylan (71%) and also contain some β-glucan (26%). The husk layer, the outer layer of the barley, has a different composition as it is rich in polyphenols and cellulose. Polyphenols are important flavour components in beer, but they can also form complexes with proteins causing haze in the final product (Langstaff & Lewis, 1993; Siebert, Carrasco, & Lynn, 1996). Most arabinoxylans present in the kernel are located in

the husk and bran. Ash, most of which is also present in the husk of the barley, has been reported to be mostly silicate, which incidentally contributes to beer haze. However, it should be taken into account that some of the minerals, like zinc salts, provide trace elements in beer (Kunze, 2010).

When looking at the composition and the structure of the barley kernel, it seems that removal of the outer part of the barley kernel before mashing could improve its mashing and filtering performances. This can be achieved through pearling, which is an abrasive milling method. Pearling first removes the husk, followed by the pericarp, testa, aleurone layer and the embryo. The remaining material is then enriched in endosperm components like starch and β-amylase. A reduction of the outer layers implies a reduction in spent grain, because the husk makes up a large part of the spent grain dry matter. Spent grain contains about 80% of water and is therefore less compact and heavier than the original husk and bran. Besides this, less water and therefore less water soluble components are lost in the waste stream. Another advantage of pearling can be that it reduces the range and total amount of micro-organisms on the barley kernel (Flannigan & Dickie, 1972; Ríos, Pinson-Gadais, Abecassis, Zakhia-Rozis, & Lullien-Pellerin, 2009). When removing (some of) the husk before mashing, the mash cannot be filtered over the husk as is done in the traditional process. If insufficient material is left to use as a filter bed an alternative filtering process, like using more modern mash filters, would be necessary (T O'Rourke, 1999).

Pearling is mostly used to characterize barley (A Iwami, Kajiwara, & Omori, 2003; A. Iwami, Kajiwara, Takashita, & Omori, 2005; Klamczynski, Baik, & Czuchajowska, 1998; Lampi, Moreau, Piironen, & Hicks, 2004; K Liu, Barrows, & Obert, 2009; KS Liu & Moreau, 2008; Madhujith, Izydorczyk, & Shahidi, 2006; Marconi, Graziano, & Cubadda, 2000; Quinde, Ullrich, & Baik, 2004; Sumner, Grebre-Egziabher, Tyler, & Rossnagel, 1985; Wang, Sosulski, Sosulski, & Ingledew, 1997; Yeung & Vasanthan, 2001). Two of these studies (Marconi et al., 2000; Yeung & Vasanthan, 2001) describe the pearling of different barley varieties, and analysed the composition of the pearled kernels and the material pearled off. Liu et al found that pearling had a significant effect on the enrichment of functional lipids in the kernels (KS Liu & Moreau, 2008). Madhujith et al found that most antioxidant activity was present the outer 25 % w/w of the kernel related to the presence of polyphenols in this outer layer (Madhujith et al., 2006). Wang et al reported that ethanol production

from cereal grains increased in efficiency upon pearling, calculated as the ratio between theoretical ethanol yield and actual ethanol yield, when the outer part of the kernel was removed by pearling. In relation to brewing, pearling was used to correlate the hardness of the barley kernel to the kernel yield after pearling on the one hand and to conversion (as a measure for fermentation performance) on the other hand (A Iwami et al., 2003). Furthermore they found that the degree of pearling could be used to influence the taste of the Japanese distilled beverage *shochu* (A. Iwami et al., 2005). A patented process includes a device for dehusking cereal grains with the claim that pearling before malting and/or brewing could be beneficial for the beer quality. Besides, it was claimed that dehusking resulted in lower milling energy and decreased pollutants on the grain surface (Gehrig, Menger, & Keller, 2012).

Based on the information given above, it can be hypothesized that pearling might be beneficial in brewing, though it is not clear which degree of pearling (i.e. the w/w percentage of material removed by pearling) is optimal. Furthermore, it has not yet been investigated how pearling affects the amount of spent grain waste and losses in the process. In this paper, pearling is considered as a method for altering the composition of barley used as a raw material for brewing. Barley of the variety Sebastian was pearled to remove 5%, 10%, 15% and 25% of the weight of the kernels, leaving an endosperm fraction of about 75% of the original kernel weight eventually. In addition to the chemical composition, the WHC off all fractions was determined. The appearance of the fractions was visualized using scanning electron microscopy (Jane et al.). Furthermore, the potential advantages and implications of using pearling to modify the raw material for the brewing process are discussed.

3.3 Materials and Methods

3.3.1 Materials

Barley (*Hordeum vulgare*) of the variety Sebastian was used (France, harvested May 2013) in all experiments.

3.3.2 Sample preparation

Barley kernels with a moisture content of 13.1% were pearled in a Satake TM05 pearling machine. The samples were made according to the following procedure; First, about 5 % w/w was removed from the barley kernel. This was repeated 10 times (n=10; identical pearling runs) to obtain sufficient material for further processing. The pearled bran was called fraction 1. The 10 batches of pearled kernels were combined and thoroughly mixed after which the kernels were pearled to remove another 5% (n=8, fraction 2), 5% (n=7, fraction 3) and 10% (n=7, fraction 4) of the original weight respectively. The endosperm fraction obtained after the 4th pearling step consisted of kernels with approximately 75% of the weight of the original kernels. This fraction was called fraction 5. For further analysis (except for dry matter, water activity and some water holding capacity analysis) fractions 1 and 2 were milled in a laboratory mill (Fritsch, type pulverisette 14 equipped with a 500 µm screen). Fraction 5 and whole barley were milled in the same equipment for all analyses except for the dry matter determination. Fraction 3 and 4 were already sufficiently fine. The yield of the fractions was defined as the weight percentage (as is) of the kernel that was pearled off as per pearling session.

3.3.3 Methods

The dry matter content was determined by oven drying at 105°C overnight, and expressed in % of the total weight. The concentration of all other components was expressed in % w/w per gram dry matter, unless stated otherwise. The ash content was measured by burning the samples at 525ºC overnight. The water activity was measured in a dew point water activity meter (AquaLab Dew Point water activity meter 4TE).

The starch and mixed β-glucan content and β-amylase activity were measured using assay kits purchased from Megazyme Intl. Ireland Ltd. (Wicklow, Ireland). For the starch measurement, method B (AOAC official method 996.11) was used, for β-glucan EBC method 3.11.1 was used and for β-amylase the betamyl-3 method

was used. The absorbance was measured using a spectrophotometer (Beckman Coulter DU®720). β-amylase activity was expressed in U/g dm.

The protein content was measured by DUMAS. Conversion factors as reported by Merrill and Watt, 6.31 for the bran fractions (1-4), 5.70 for the endosperm fraction (5) and 5.83 for whole barley were used to calculate the protein content (Merrill & Watt, 1973). Different conversion factors were used to account for the difference in protein composition in the husk and endosperm.

The fat content was measured by means of a soxhlet extraction (Buchi-extractor) with petroleum ether as a solvent. The fat content was calculated by expressing the mass of dry extract as a percentage of the mass of defatted barley flour.

Polyphenols were extracted by combining the supernatants of two subsequent extractions of 0.4 g of sample in 4 ml of water at 80°C for 1 hour. The polyphenol content was approximated by using the Folin-Ciocalteu method; 100µl of aqueous extract was mixed with 2900µl of water, 200µl of Folin–Ciocalteu-reagents and 800µl of 20% sodium carbonate solution. After incubation in the dark at 40°C for 30 minutes, the absorbance was measured in a spectrophotometer at 765 nm (Varian, Cary 50 Bio UV/visible spectrophotometer). Gallic acid was used for the calibration curve and polyphenol content was expressed in mg/g gallic acid equivalent (Ragaee, Abdel-Aal, & Noaman).

The arabinoxylan content was determined by measuring the neutral sugar content in triplicate according to Englyst and Cummings (Englyst & Cummings, 1984). The samples were treated for 30 min with aqueous 72% H_2SO_4 at 30ºC as a pre-hydrolysis step followed by hydrolysis with H_2SO_4 (1.0M, 3 hr, 100°C). The monosaccharaides were derivatized to their alditol acetates and analysed by gas chromatography (Focus-GC, Thermo Scientific, Waltham, MA, USA). Inositol served as an internal standard. Arabinoxylan content was calculated as the sum of the amount of arabinose and xylose, and expressed in mg/g dm.

The insoluble fibre content was assumed to be the rest fraction calculated by subtracting the amount (% w/w per dm) of starch, β-glucan, protein, fat and ash content from the total amount of dry matter (100%). It was assumed that this fraction consisted only of fibres.

The water holding capacity (WHC) of the non-starch material was determined by weighing ±1 gram of sample into 14 ml test tubes. To focus the non-starch materials, starch was removed through heating the samples in 10 ml of an alpha amylase solution, SIGMA A-3403 from ***Bacillus licheniformis***, at 80ºC for 30 minutes. Then the samples were centrifuged at 2000g at 20°C and the supernatant was decanted. WHC was expressed in gram of water per gram of dry starting material.

Microscopic images were obtained by using table top SEM equipment (PHENOM Pure). The material of fraction 1-4 was used as such. Fractions 5 and whole barley were milled before analysis.

Mass flow (Sankey) diagrams were constructed using the program e!Sankey.

3.4 Results

3.4.1 Pearled fractions composition

Barley kernels were pearled to remove 5.3 % w/w, 4.4 % w/w, 5.1 % w/w and 10.1 % w/w of the original weight (fractions 1 to 4 respectively). The 5th fraction consisted of the remaining kernels having about 75% of the original kernel weight. **Figure 1** shows SEM images obtained from whole barley and fractions 1 to 5. Barley starch has a binominal distribution and can be identified by SEM as large disc-shaped granules of 15-32 µm and small granules of 2-3 µm (Jane et al., 1994). The images suggests that the outer fractions contained less starch granules and more fibrous material, while most starch granules are present in fraction 5, which represented the middle of the kernel. Besides, some fibrous fragments are still present in this fraction.

Table 1 shows the yield and composition of the barley and the pearled fractions. The dry matter content was higher in the husk fractions than in the endosperm fraction. Some water may have been evaporated during pearling. The starch concentration increased from the outer fractions to the inner fraction. The low starch content in fraction 1 indicates that hardly any endosperm is removed at this degree of pearling. The protein content was high in fractions 3 and 4 suggesting that the aleurone layer, which has high protein content, is mostly present in these fractions. It is in accordance with the β-glucan values, which are higher in these

fractions. Aleurone cells are known to have a thick cell wall consisting of β-glucan and arabinoxylans. The endosperm contained 4.47 % w/w β-glucan. Since cell wall material is one of the three main structures in barley endosperm besides protein matrix and starch granules, the relatively high β-glucan level in this fraction was expected.

Fat levels are especially high in fraction 3, which indicates that the most germs are pearled off when removing this fraction. The deviation of the mass balance of fat is high. This might be caused by an increased extractability of fat in pearled fractions 3 and 4 compared to the whole barley, which was milled. Pearling damages the cells and less lumps of material remain in the pearled fractions, which probably facilitates the extraction of fat. Ash content was highest in the husk fractions, as was the content of insoluble fibres. The insoluble fibres are mainly cellulose, lignin and pentosan (including arabinoxylan) compounds that are predominantly present in the husk. The values obtained for the insoluble fibre content of barley bran (as a sum of the former mentioned three components) are in line with values previously reported e.g. (A. Andersson, Andersson, & Aman, 2000; Olkku, Salmenkallio-Marttila, Sweins, & Home, 2005).

Table 1. Yield and component content of the mayor components in barley fractions and whole barley. Fraction 1 stands for the outer layer of barley (5 % w/w), fraction 2 (5 % w/w), 3 (5% w/w), and 4 (10% w/w) are the fractions that were subsequently pearled off and fraction 5 the inner 75% w/w of the barley.

Fraction	Yield (%)	DM	Starch	Protein	β-glucan	Fat	Ash	Ins. Fibre
Fraction 1	5.3 ± 0.1	91.0 ± 0.1	2.5 ± 0.1	5.7 ± 0.6	0.3 ± 0.3	1.2 ± 0.2	7.0 ± 0.1	82.9 ± 0.7
Fraction 2	4.4 ± 0.2	91.1 ± 0.2	7.8 ± 0.5	10.8 ± 0.5	1.3 ± 0.5	3.7 ± 0.1	6.59 ± 0.2	68.8 ± 0.9
Fraction 3	5.1 ± 0.2	90.1 ± 0.2	24.3 ± 2.1	18.3 ± 0.4	3.1 ± 0.3	7.2 ± 0.3	5.78 ± 0.2	39.5 ± 2.2
Fraction 4	10.1 ± 0.2	90.0 ± 0.3	44.6 ± 2.1	17.0 ± 0.3	4.9 ± 0.3	4.2 ± 0.0	3.79 ± 0.0	23.9 ± 2.2
Fraction 5	75.2 ± 0.2	87.2 ± 0.1	77.2 ± 7.3	8.2 ± 0.2	4.5 ± 0.4	0.9 ± 0.1	0.85 ± 0.1	6.3 ± 7.3
Whole barley	100	86.9 ± 0.0	62.7 ± 2.8	9.8 ± 0.4	3.6 ± 0.4	1.3 ± 0.1	2.2 ± 0.2	18.2 ± 2.9
Dev. from mass balance		-1.1	-1.6	0.2	-0.5	-0.4	0.2	1.7

Table 2 shows the measured contents of polyphenols, β-amylase activity, arabinoxylan content and the values for water activity of the barley and the barley fractions. Polyphenols are mostly present in the inner part of the husk layer. This

fraction might contain the testa, which contains a lot of proanthocyanidins. Also the arabinoxylans are mostly present in the outer fractions, which is in line with previous studies (Dervilly et al., 2002; Oscarsson, Andersson, Salomonsson, & Åman, 1996). The β-amylase activity was mostly present in the endosperm, and its activity was very low in the outer 10% of the husk. This complies with literature in which it is stated that β-amylase is present in the endosperm already before germination (in contrast to for example α-amylase, which is synthesized in the aleurone layer during germination) (Chrispeels & Varner, 1967). The water activity follows the dry matter content of the fractions. Fractions with a higher dry matter content showed a lower water activity.

Table 2. Component composition minor components in barley and barley fractions ± standard deviation. Fraction 1 stands for the outer layer of barley (5 % w/w), fraction 2 (5 % w/w), 3 (5% w/w), and 4 (10% w/w) are the fractions that were subsequently pearled off and fraction 5 the inner 75% w/w of the barley.

Fraction	Polyphenols GAE (mg/g)	β-amylase activity (U/g)	Insoluble Arabinoxylans (mg/g dm)			Water Activity (%)
			Arabinose	Xylose	Total	
Fraction 1	6165 ± 28	-1.0 ± 1.7	51 ± 4	98 ± 17	148 ± 16	0.46 ± 6E-3
Fraction 2	9319 ± 371	1.6 ± 0.2	76 ± 2	138 ± 23	213 ± 25	0.42 ± 2E-3
Fraction 3	7045 ± 213	9.4 ± 0.2	76 ± 4	94 ± 6	170 ± 9	0.51 ± 6E-3
Fraction 4	4034 ± 494	16.2 ± 0.2	38 ± 3	53 ± 4	91 ± 7	0.52 ± 2E-3
Fraction 5	1906 ± 203	25.7 ± 4.0	10 ± 1	13 ± 2	23 ± 3	0.55 ± 6E-4
Whole barley	2461 ± 186	23.9± 0.2	21 ± 1	43 ± 5	64 ± 5	0.62 ± 8E-4
Deviation from mass balance	-469	-0.96	0	12	12	0.08

3.4.2 Pearled fractions water holding capacity

Table 3 shows the WHC of the barley and barley fractions. When looking at the WHC of the fractions per gram of dry starting material, the WHC in the outer fractions was higher than in the endosperm. This can be explained by the lower amount of non-starch material in the endosperm; when the starch was hydrolysed, less material was left to hold the water. The WHC of the unmilled fractions 1 and 2 were higher than when milled. This indicates that the destruction of the structure by milling reduces the WHC of the husk. In traditional brewing, the husk is kept mostly intact, so the WHC of the unmilled fractions 1 and 2 is probably more representative for the WHC of the husk of grist/spent grains.

Table 3. WHC of pearled barley fractions and whole barley**.** Fraction 1 stands for the outer layer of barley (5 % w/w), fraction 2 (5 % w/w), 3 (5% w/w), and 4 (10% w/w) are the fractions that were subsequently pearled off and fraction 5 the inner 75% w/w of the barley.

Fraction	WHC (g/g dm flour)	
	milled	Unmilled
Fraction 1	3.5 ± 0.1	5.4 ± 0.4
Fraction 2	2.7 ± 0.1	3.1 ± 0.1
Fraction 3	1.8 ± 0.0	
Fraction 4	0.8 ± 0.0	
Fraction 5	0.7 ± 0.0	
Whole barley	0.9 ± 0.2	

3.5 Discussion

Currently, brewers tend to replace part of the malt by barley. This replacement, however, might give rise to difficulties due the fact that some components present in barley might cause processing problems. Therefore, it is beneficial to alter the starting composition of the barley through removing the undesired components while keeping the desired components, such as starch and enzymes. Starch is the most important component in barley for beer brewing, and losses of starch should be minimized. Also the β-amylase activity should not be compromised. The diagram in **figure 2** summarizes the compositions of the pearled fractions, pearled kernels and whole barley.

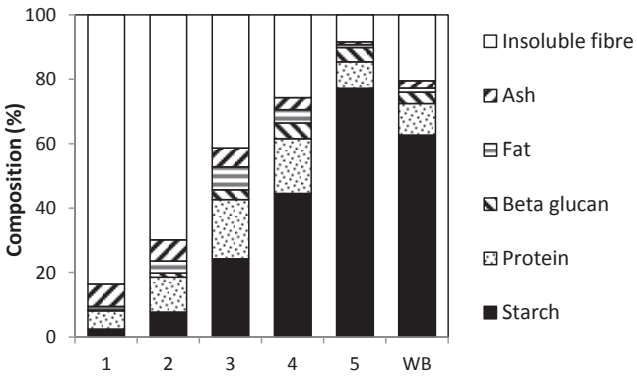


Figure 2. Composition of barley and barley fractions

Figure 3 depicts the percentage of components removed from the raw material versus the percentage of starch removed. The figure shows that over 40% of fibre, about 30% of ash and arabinoxylans and about 25% of polyphenols will be removed, when abasing about 10% of the kernel. Remarkably, less than 1% of the starch will get lost under those conditions. In addition, almost no β-amylase activity was measured in the outer two layers, while the activity of the whole barley was almost equal to that of the endosperm.

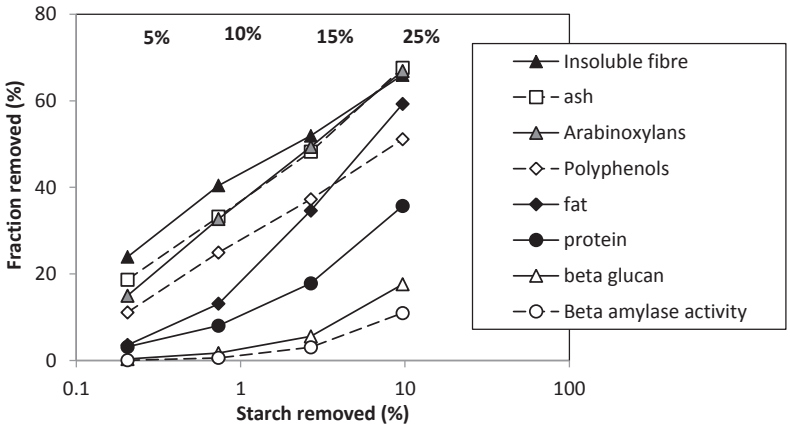


Figure 3. The removal of several components versus starch removal when pearling barley. Percentages in the top stand for the degree of pearling.

3.5.1 Water holding capacity (WHC)

Removing fibres, like arabinoxylans, can reduce the waste via the spent grains by reducing the water holding capacity of these spent grains. In **figure 4A** the WHC of the barley fractions (g/g dm) is plotted versus the amount of insoluble fibres present it becomes clear that fractions with higher fibre content had a higher WHC. The graph showed a linear correlation (R^2 of 0.977) confirming that the fibres are a major water holding component in the spent grains. Extrapolating this graph to 100% insoluble fibre suggests that the water binding capacity of the pure insoluble fibre would be around 5.6 g/g dm. This corresponds to a water content of water saturated fibres of 85%. Brewers spent grains indeed usually have a water content of around 80% (Mussatto & Roberto, 2006), likely caused by its high fibre content. Reducing the amount of insoluble fibres in the system will directly reduce the WHC of the spent grains, which could result in a reduced extract lost. Though arabinoxylans are part of the insoluble fibres, they did not show a linear relation

with the WHC. This might be because the differences in arabinose/xylose ratio between the fractions yields a difference in the WHC of the arabinoxylans in the fractions (Sternemalm, Höije, & Gatenholm, 2008).

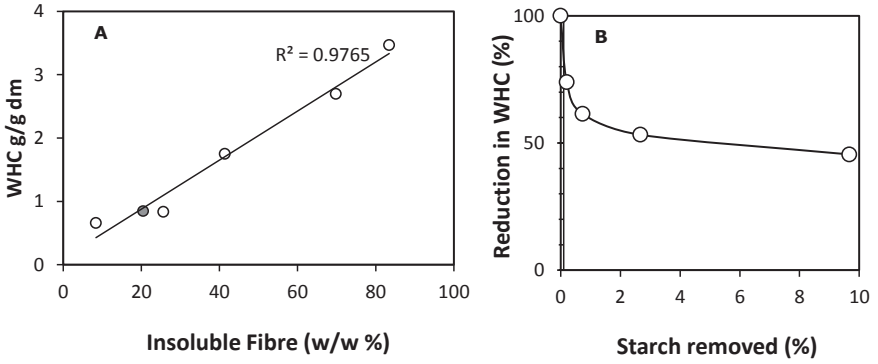


Figure 4. WHC versus fibre content (A) and reduction in WCH versus the amount of starch removed by pearling (B). Open circles: pearled fractions. Closed circle: whole barley.

3.5.2 Implications of pearling for brewing

The previous section indicates that removing the outer part of the kernel may have beneficial effects for the beer brewing process. Pearling can be used to partly remove polyphenols, fibres, arabinoxylans and ash, while hardly influencing the starch content and β-amylase activity. A lower amount of fibres will reduce the WHC of the spent grains and as a result less wort will remain in the spent grains. This will reduce the spent grain waste and water requirements, and less wort will get lost. **Figure 4B** illustrates the reduction in WHC versus the amount of starch removed.

When removing about 0.20% of the starch, which corresponds to a degree of pearling of 5%, the WHC of the flour as determined in this study was reduced by about 25%. It is assumed that this leads to a similar reduction in spent grains and wort losses. The reduction of polyphenol content after pearling would potentially facilitate the filtration of polyphenols at the end of the process and can reduce haze formation because of less complex formation with proteins. When pearling to a degree of 10%, the WHC of the flour will be reduced by about 40%. In this case 0.73% of all starch will be lost in the bran fraction, so in this case it might be worthwhile to try to recuperate the starch from this fraction.

Pearling might affect some of the characteristics of the final product. The characteristic which is expected to be most affected is colour, because of the reduction in polyphenols. Polyphenols also influence flavour stability, because of their antioxidative properties. At a degree of pearling of 5%, however, the reduction in polyphenols is only 11%. Therefore the changes in colour are not expected to be large at this degree of pearling. Another important characteristic is the body of the beer, and β-glucan is an important component that provides this body. At a degree of pearling of 5%, only 0.32% of beta glucan is removed from the raw material, so the body of the beer is not expected to change.

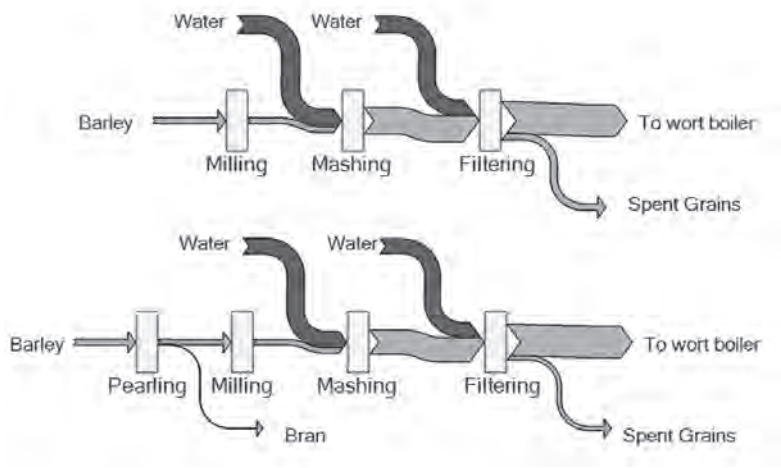


Figure 5. Mass flow diagram of the conventional brewing process and a process that includes pearling away 10% of the barley kernels.

Figure 5 illustrated that an additional side stream of bran is generated with pearling. However, this side stream is still dry, which might give it a better applicability in for example bread to improve its structural or nutritional properties (Izydorczyk, Chornick, Paulley, Edwards, & Dexter, 2008). Barley contains 33 to 66 µg/100 g folate, most of which is found in the bran (Schoenlechner, Wendner, Siebenhandl-Ehn, & Berghofer, 2010). Other possibilities would be to use the dietary fibre, polyphenols or arabinoxylans from the bran as functional ingredients in healthy foods or even animal feed.

The dry mass losses of the spent grains and the bran are approximately equal to the amount spent grains losses without pearling, but the bran remains dry. This will lead to energy savings due to reduced or even no drying or reduced transportation costs. The removal (some of) the husk before mashing might negatively influence the filtering of the mash over the husk, as is done in the traditional lautering process. If insufficient material is left to use as a filter bed, an alternative filtering process, like using more modern mash filters, would be necessary (T O'Rourke, 1999).

3.6 Conclusions

Pearling was shown to selectively remove insoluble fibre, ash, protein and polyphenols, while the β-amylase activity and starch content of the remaining kernel was hardly affected. Removing the outer 5 % w/w of barley takes away 15% of the arabinoxylans and reduces the water holding capacity of the non-starch components by 25%. Reducing the arabinoxylan content reduces the mash viscosity, which might facilitate the filtration step after mashing. Pearling the outer part of the barley kernel also reduces the polyphenol content of the starting material. This might reduce haze formation in the end product.

The water holding capacity of the barley fractions is related to the fibre content. This indicates that when the fibre content is reduced in the spent grains, the spent grains will take up approximately 25% less wort, leading to less wort and sugar losses in this waste stream. In addition, the fact that the bran fraction remains dry would mean a significant reduction in energy required to dry the spent grains.

Going up to a degree of pearling of 10% would enhance all these advantages but would also increase the starch lost in the bran fraction from 0.20% to 0.73%.

3.7 Acknowledgements

This research took place within the ISPT framework (Institute for Sustainable Process Technology). We want to thank ISPT and Heineken for their financial support and fruitful discussions. We want to thank Arjen Punt and Henk Schols (WUR) for their assistance with the measurements on the arabinoxylans.

The use of enzymes for beer brewing: thermodynamic comparison on resource use

Highlights

- The exergetic production costs of enzymes are ±30 times their standard chemical exergy
- These costs of enzymes should be taken into account in exergy analysis
- Enzyme-assisted brewing is more exergy efficient than brewing with malted barley
- Enzyme-assisted brewing saves raw material, water and energy

This chapter has been submitted as: Laura H.G. van Donkelaar, Joost Mostert, Filippos K. Zisopoulos, Remko M. Boom, Atze-Jan van der Goot, The use of enzymes for beer brewing: thermodynamic comparison on resource use.

4.1 Abstract

The exergetic performance of beer produced by the conventional malting and brewing process is compared with that of beer produced using an enzyme-assisted process. The aim is to estimate whether the use of an exogenous enzyme formulation reduces the environmental impact of the overall brewing process. The exergy efficiency of malting was 77%. The main exergy losses stem from the use of natural gas for kilning and from starch loss during germination. The exergy efficiency of the enzyme production process ranges between 20% and 42% depending on if the by-product was considered useful. The main exergy loss was due to the high power requirement for fermentation. The total exergy input in the enzyme production process was 30 times the standard chemical exergy of the enzyme, which makes it exergetically expensive. Nevertheless, the total exergy input for the production of 100 kg beer was larger for the conventional process (441 MJ) than for the enzyme-assisted process (354 MJ). Moreover, beer produced using enzymes reduced the use of water, raw materials and natural gas by 7%, 14% and 78% respectively. Consequently, the exergy loss in the enzyme production process is compensated by the prevention of exergy loss in the total beer brewing process.

Keywords: Exergy, Enzymes, Brewing, Unmalted barley, Biotechnology

4.2 Nomenclature / list of symbols

m	mass [kg]
x	mass fraction of component [-]
h	Enthalpy [kJ/mol]
Q	heat [kJ]
W	work performed by the system
Ex	exergy [kJ]
c_p	specific heat capacity [kJ/kg K]
T_0	reference temperature [K]
T	temperature [K]
R	ideal gas constant [kJ/mol K]
m_x	average molar mass of the stream [kg/mol]
P_0	reference pressure [Pa]
P	pressure [Pa]
b_0	standard chemical exergy [kJ/kg] for which the values can be found in **appendix I**
x_i	mass fraction of component *i* [-]

4.3 Introduction

Brewing is a traditional process, which can still be further optimized with respect to environmental impact (Olajire). Several sustainability analyses have been performed on the process (Cimini & Moresi; Cordella, Tugnoli, Spadoni, Santarelli, & Zangrando, 2008; Hospido, Moreira, & Feijoo, 2005) and studies have been aimed at the re-use or prevention of by-product streams to minimize water and raw material losses and energy use (Aliyu & Bala, 2013; Köroğlu, Özkaya, Denktaş, & Çakmakci, 2014; Pérez-Bibbins, Torrado-Agrasar, Salgado, Oliveira, & Domínguez, 2015; Simate & Hill, 2015; van Donkelaar et al., 2015). Even though it does not take into account every aspect of sustainability, exergy analysis is based on the second law of thermodynamics and, therefore, is considered as an objective method to compare material and energy losses occurring in a system both quantitatively and qualitatively (Dincer & Ratlamwala, 2013). As formulated by Szargut, exergy is the amount of work obtainable when some matter is brought to a state of thermodynamic equilibrium with the common components of its surrounding nature by means of reversible processes, involving interaction only with the components of nature (Szargut, 1980). Exergy analysis has been used to analyse, optimize, and compare various food processes and food production chains in terms of their resource use efficiency (Apaiah et al., 2006). An improvement of the exergetic or thermodynamic efficiency of a process reflects a reduction on its overall use of resources and hence its environmental impact (Rosen, Dincer, & Kanoglu, 2008). Exergy analysis can be applied to many different food production chains to identify improvements, and to compare the thermodynamic performance of existing processes to potential alternatives. This was done for example in vegetable oil (/and protein) production (Berghout, Pelgrom, Schutyser, Boom, & van der Goot, 2015; Özilgen & Sorgüven, 2011), in a fish-oil microencapsulation process (Aghbashlo, Mobli, Rafiee, & Madadlou, 2012), dairy processing (Quijera & Labidi, 2013), an isoflavone extraction process (Jankowiak, Jonkman, Rossier-Miranda, Goot, & Boom, 2014), and the use of plant based ingredients for fish feed (Draganovic et al., 2013) amongst others. The analysis shows if the use of an alternative process is in fact more efficient.

The outcome of an exergy analysis can be influenced by the system boundaries, which are chosen by the analyst, i.e. wider system boundaries imply a more complex but also a more complete analysis (Zisopoulos, Rossier-Miranda, Van Der

Goot, & Boom, 2015). Besides, the allocation of the exergetic content of the streams will also influence the outcome of the analysis. In this paper, these aspects will be demonstrated when describing the exergetic production costs, or cumulative exergy consumption (CExC), of enzymes.

The conventional brewing process has 3 main process stages. The first stage is malting, during which enzymes are synthesized in the barley kernel. In this stage the endosperm is modified: cell walls are broken down to render the protein and starch inside the cells more accessible. The second stage is mashing, during this stage the enzymes hydrolyse starch into fermentable sugars and proteins into amino acids. The third stage is fermentation, during which yeast ferment the sugars into alcohol. Brewing with unmalted barley grains more attention because of the economic advantages and its potential for water and energy savings. Additionally, material losses due to respiration are prevented (Steiner et al., 2012). In this paper, we analyse the both beer brewing processes with exergy analysis.

A disadvantage of brewing with unmalted barley is the low amount of available endogenous enzymes present in the native kernel. Therefore the addition of enzyme formulations is necessary. These formulations usually contain a combination of α-amylase, pullulanase, proteases, lipase, β-glucanase, and xylanase. The effectiveness of these formulations has been investigated and documented in various reports. No negative effect on beer quality was found when 50% or up to 100% of the malt was replaced by unmalted barley (Evans et al., 2014; D.L. Goode, Wijngaard, & Arendt, 2005; Kunz, Müller, Mato-Gonzales, & Methner, 2012; Steiner et al., 2012).

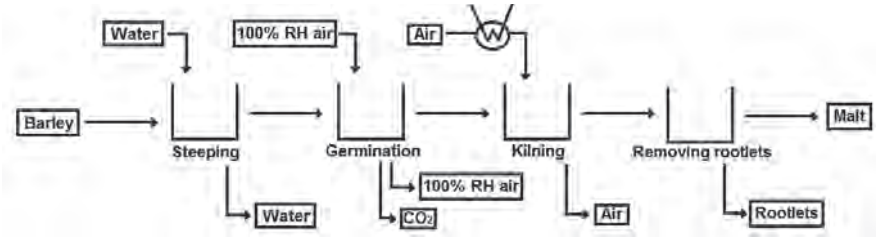
One should take into account that the production of an enzyme formulation also requires resources and produces waste. This raises the question if the use of enzymes requires less resources compared to the malting process. In many studies the standard chemical exergy of purified ingredients like enzymes, protein isolates or other isolated or purified ingredients is used in exergetic assessments, neglecting the CExC of these components. The aim of this paper therefore is two-fold. It assesses the exergetic performance of traditional beer brewing by the conventional malting and brewing process, and compares it to an enzyme-assisted brewing process. It also estimates the CExC of the enzyme formulation used in the enzyme-assisted brewing process.

4.4 General description of the brewing production chain

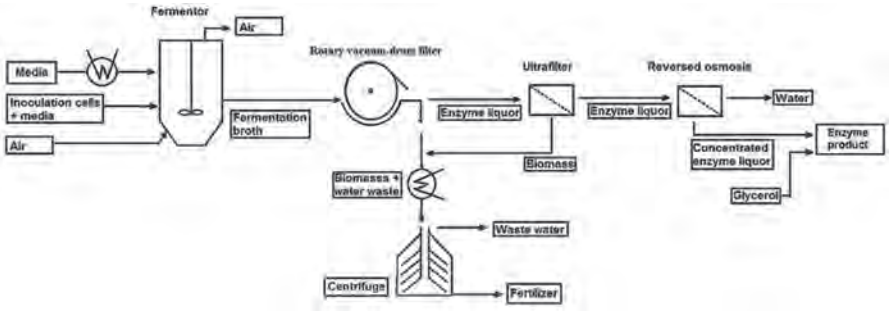
4.4.1 System boundaries

In the brewing process, the malting process was taken into account when malt was used, while enzyme production was considered in the enzyme-assisted brewing process. The compositions of the various streams in both processes are listed in **appendix II.** The process configurations of the analysed processes are shown in **Figure 1.** The production of the growth medium used in the enzyme production process is not considered in the analysis, which means that only the chemical exergy for the ingredients present in the medium was taken into account. The same counts for glycerol, as this product is currently produced as a by-product of biodiesel. All exergy input for this process was attributed to the biodiesel and not to the glycerol used in the enzyme formulation.

A. Process flowchart of the conventional malting process



B. Process flowchart of the enzyme production process



C. Process flowchart of industrial brewing

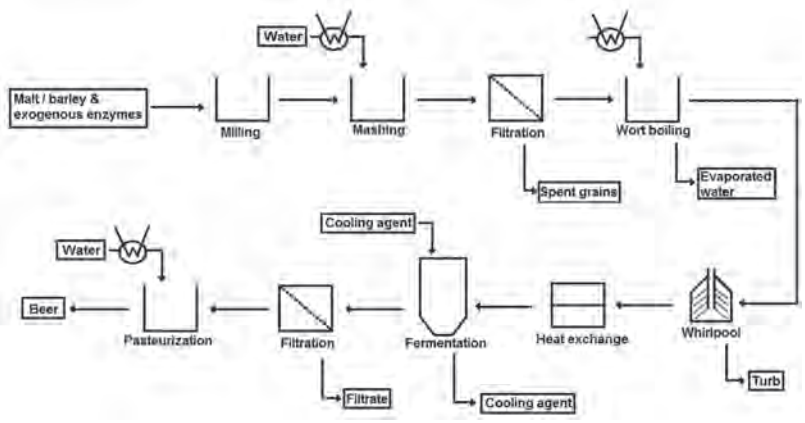


Figure 1. Process flowchart of: A) the conventional malting process, B) the enzyme production process, C) the overall industrial brewing process

The data and assumptions made for the enzyme production process, malting process and brewing process and the associated references are listed in **Appendix III**.

4.4.2 Exergy analysis

Mass and energy balances were calculated with Eq. (1) and Eq. (2),

$$\sum m_{in} - \sum m_{out} = 0 \sum m_{in} - \sum m_{out} = 0 \quad (1)$$

$$\sum (mh)_{out} - \sum (mh)_{in} = Q - W \sum (mh)_{out} - \sum (mh)_{in} = Q - W \quad (2)$$

The exergy was categorised into the chemical exergy (Eq. 6), and the physical exergy (Eq. 3) composed of the thermal and pressure exergy (Eq. 4 and Eq. 5). The exergy loss was defined as the difference between the total exergy input and the total exergy output (Eq. 7), and consisted of both the wasted exergy (i.e. theoretically usable but lost to the environment) and destroyed exergy (irreversibly lost) (Eq. 8). The chemical exergy efficiency of a process chain was defined as the total output chemical exergy over the total input exergy (Eq. 9). The rational exergy efficiency was defined as the useful chemical exergy output over the total exergy input (Eq. 10). Dry enzyme, malt and beer were considered useful exergy output. It

was debatable whether the fertilizer and enzyme formulation are to be considered as useful; we will discuss this in the results section.

$$Ex_{physical} = Ex_{thermal} + Ex_{pressure} Ex_{physical} = Ex_{thermal} + Ex_{pressure} \quad (3)$$

$$Ex_{thermal} = m \cdot c_p \cdot [(T\text{-}T_0)\text{-}T_0 \cdot \ln\left(\frac{T}{T_0}\right)] Ex_{thermal} = m \cdot c_p \cdot [(T\text{-}T_0)\text{-}T_0 \cdot \ln\left(\frac{T}{T_0}\right)] \quad (4)$$

$$Ex_{pressure} = \frac{R \cdot T_0}{m_x} \cdot [\ln\left(\frac{P}{P_0}\right)] Ex_{pressure} = \frac{R \cdot T_0}{m_x} \cdot [\ln\left(\frac{P}{P_0}\right)] \quad (5)$$

$$Ex_{stchem} = m \cdot \sum_{i=1}^{n} (b_0 \cdot x_i) Ex_{stchem} = m \cdot \sum_{i=1}^{n} (b_0 \cdot x_i) \quad (6)$$

$$Ex_{loss} = Ex_{in}\text{-}Ex_{out} Ex_{loss} = Ex_{in}\text{-}Ex_{out} \quad (7)$$

$$Ex_{loss} = Ex_{waste} + Ex_{destruction} Ex_{loss} = Ex_{waste} + Ex_{destruction} \quad (8)$$

$$\text{Total chemical exergy efficiency} = \frac{Total\ Ex_{chem}out Total\ Ex_{chem}out}{Total\ Ex_{chem}in\ \ Total\ Ex_{chem}in} \quad (9)$$

$$\text{Useful chemical exergy efficiency} = \frac{Useful\ Ex_{chem}out Useful\ Ex_{chem}out}{Total\ Ex_{chem}in\ \ \ Total\ Ex_{chem}in} \quad (10)$$

Mass and energy flows were visualized by Sankey diagrams and exergy flows were visualized by Grassmann diagrams, using e!Sankey 3.1 (ifu Hamburg GmbH, Hamburg, Germany).

4.4 Results and Discussion

Figure 2 shows the mass flows in the conventional malting process. The malting process consists of a steeping step in which water is added. This water is partially taken up by the grains. After germination the malt is dried with hot air to evaporate this water again. At the end of the process rootlets are removed.

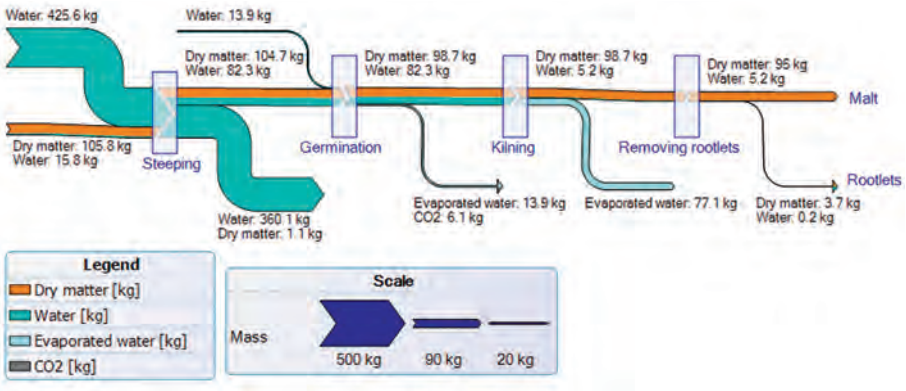


Figure 2. Sankey diagram showing the mass of the streams of the conventional malting process for the production of 100 kg malt. The diagram excludes air (germination uses 3111 kg dry air, kilning uses 9535 kg dry air and cooling the kilned barley uses 288 kg dry air).

During germination, a small part of the starch is lost due to respiration. Nevertheless, this raw material loss is one of the main disadvantages of the conventional malting process. Less starch left in the malt means less starch is hydrolysed during brewing and therefore less beer is produced from the same amount of raw material. Another disadvantage is the required addition of water during steeping. About 456.5 kg of water is required during steeping and germination of 100 kg of malt. The water that is taken up has to be evaporated during kilning to ensure shelf life and facilitate transportation, requiring 537MJ for kilning 100 kg malt. This value is in line with a study by Kribs et al. which reported an energy consumption of 500 MJ/100 kg malt for a conventional kilning process (Kribs & Spolek, 1997).

The Grassmann diagram in **Figure 3** shows the exergy flows of the conventional malting process. The process can be considered as exergy efficient (77%) since the destroyed exergy is relatively small compared to the (chemical) exergy of the main product stream. The total exergy loss for processing is 518 MJ/100 kg malt, of which 380 MJ is destroyed and 138 MJ is wasted. The main losses are due to the high quality energy (natural gas) used for removing water in the kilning process.

In addition, about 7% dry matter is lost during malting due to respiration and the removal of rootlets.

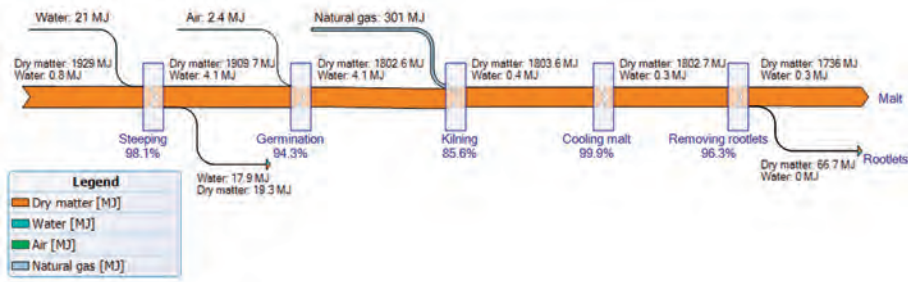


Figure 3. Grassmann (exergy flow) diagram of the conventional malting process for the production of 100 kg malt

A potential alternative to malting is the use of unmalted barley in combination with exogenous enzymes (Steiner et al., 2012). The losses in the malting processes would be prevented, but materials and energy are needed to produce the enzyme mixture. Enzymes are produced in an industrial fermentation process in which yeast convert part of the protein present in a fermentation broth into enzymes. After fermentation, the enzymes are separated from the other biomass by a rotary vacuum drum filter. The biomass is sterilized, dried, and sold as a fertilizer. The enzyme liquor coming out of the drum filter is subsequently purified by ultrafiltration and concentrated by reverse osmosis. The enzyme liquor (7% protein, 93% water) is then mixed with glycerol to stabilize the enzyme solution that is the final product with a glycerol concentration of 30%.

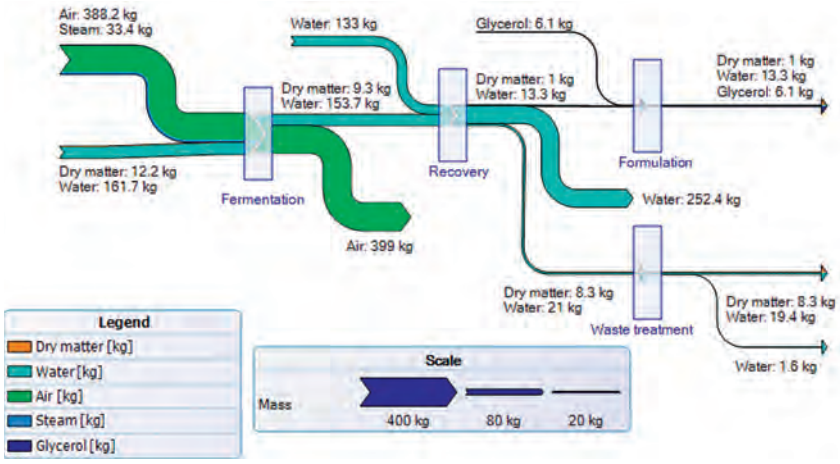


Figure 4. Sankey diagram showing the mass of the streams of the enzyme production process for the production of 1 kg of enzyme. Diagram is excluding cooling water (3974 kg and 133kg of cooling water in the fermentation and in the waste treatment, respectively).

Figure 4 shows the main steps in the enzyme production process, which are: fermentation (including sterilisation of the medium and fermenter), recovery (including the concentration in the drum filter and the purification by ultrafilter and reverse osmosis), formulation (mixing the purified enzyme solution with glycerol), and waste treatment (including sterilisation and concentration). It was shown that aeration and cooling require most natural resources (air and water). The side stream can be considered either as a waste or as a useful by-product (e.g. fertilizer) (Nielsen, Oxenbøll, & Wenzel, 2007). **Figure 5** illustrates the exergy flows of the enzyme production process. The total exergy used in the production process of the enzyme is about 30 times the chemical exergy of the enzyme itself (676 MJ per kg dry enzyme). Clearly, the exergy input of enzymes used in a process is considerably higher than their standard chemical exergy only. The CExC of these ingredients should be taken into account when assessing the thermodynamic performance of the overall system. The system boundaries affect the outcome of the exergy analysis and have to be extended to include the production of at least the purified ingredients (if not all raw materials).

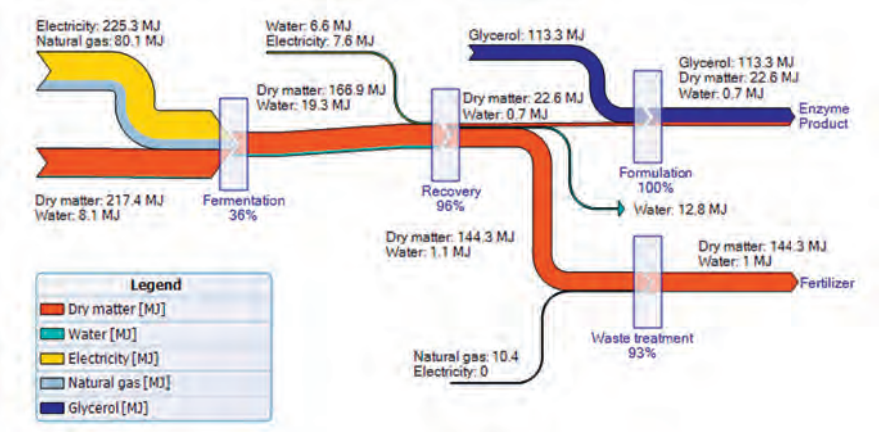


Figure 5. Grassmann diagram of the enzyme production process for the production of 1 kg of enzyme. The standard chemical exergy of all heating and cooling agents are not illustrated.

The largest exergy destruction in the enzyme production process occurs during fermentation, due to the high power consumption of 2500 W m^3. When calculating the exergy efficiency of the process one has to decide how to attribute the loss of exergy to the produced products. The exergy efficiency of the total enzyme production process when the fertilizer stream is considered as a useful stream is 42%. However, when all exergy loss is allocated to the enzyme product, the

efficiency of the process becomes 20% and even 3.4% when only the dry matter of the enzyme is considered. Here, we consider the enzymes as the main product of the process, making the fertilizer a side stream of this process. The selection of this side stream as a by-product or waste generated during the enzyme production process is arbitrary and, thus, debatable. Fertilizers are usually meant to enrich the soil in certain elements, for example nitrogen. However, in this particular side stream the amount nitrogen is reduced compared to the medium, and, though the amount is still sufficient to be used as a fertilizer, one could argue that this process is an inefficient way to produce fertilizer. In fact, the starting material would be a more efficient fertilizer. Second, fertilizer in general can be produced in much more efficient ways than in this process. Therefore, we decided to attribute all exergy losses to the production of the enzyme formulation itself and not to the fertilizer side stream.

Figure 6 shows the amount of wasted and destroyed exergy per process step for both the conventional malting and the enzyme-assisted process. The exergy losses in the enzyme-assisted process are smaller than those in the malting process when the amount of enzymes or malt necessary for the production of 100 kg of beer are compared. The main reason is related to the small required dosing of only 33 gram enzyme mix, which contains only 1.6 gram of dry enzyme, per 100 kg of beer. Even if we assign all resources that used to the enzymes, which accumulates to 676 MJ per kg enzyme, the small dosage of enzyme mix leads to a lower cumulative exergy consumption. The exergy losses for mashing, brewing and fermentation are similar in both processes.

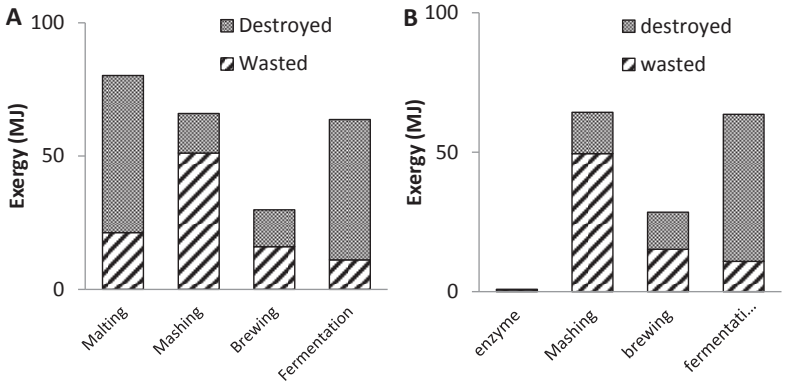


Figure 6. Wasted and destroyed exergy in the different process stages of the industrial brewing process for producing 100 kg beer when: (A) conventional malting process is used, and (B) when enzymes are used.

Figure 7 depicts the percentage of wasted, destroyed and used exergy per process. Circumventing the malting step does not only reduce the total exergy input of the process but also prevents about 60 MJ/100 kg beer of exergy destruction. The reduced exergy input is partly due to the reduced water and energy use, and partly due to the lower amount of raw material needed. The latter is related to the fact that some starch is used during malting, and, therefore, more barley is needed to produce the same amount of beer.

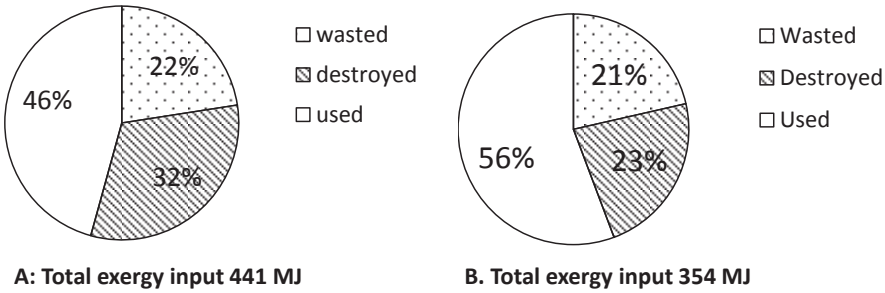


Figure 7. Total exergy used, destroyed and wasted for the production of 100 kg of beer by using the: (A) conventional malting process, or the (B) enzyme-assisted process.

The exergy efficiencies of the complete processes are 45.7% for the conventional brewing process and 55.6% for the enzyme assisted process. Besides this, the total exergy input of the enzyme assisted process is also lower, implying that the use of enzymes instead of malting means a considerable improvement in exergetic sustainability of the process. If the fertilizer would be taken into account as useful output of the process, the exergetic efficiency would increase from 55.6% to 55.7%, which is a negligible increase, and this decision therefore does not affect the outcome of the analysis when the whole process is taken into account.

Figure 8 shows the raw material use, water use, natural gas and electricity consumption, and exergy input for the production of 100 kg beer. The raw material use, water use and natural gas consumption were reduced by 14%, 7% and 78%, respectively. The air use was reduced by almost 2000 kg. The electricity input is the only parameter that increased, but only by 2.6%. These factors together resulted in a total decrease of 24**%** in total exergy input. Consequently, the use of raw barley brewed with the addition of exogenous enzymes is exergetically more efficient compared to the conventional brewing process.

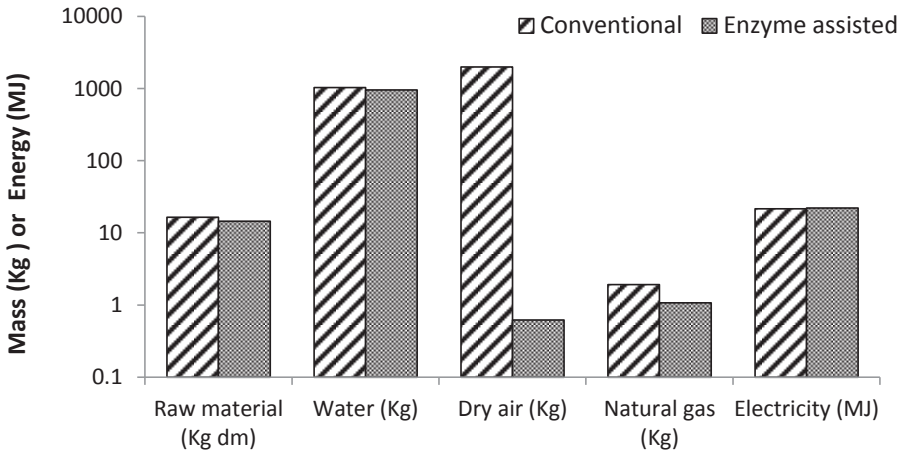


Figure 8. Amount of raw materials, water, dry air, natural gas and electricity used in the production of 100 Kg beer when using the: (A) conventional malting process, or the (B) enzyme-assisted process.

In the enzyme assisted process, only 1 MJ of the total 354 MJ of exergy necessary to produce 100 kg of beer is due to the enzyme production process. This is only 0.31% of the total exergy input of the process, and therefore the CExC of enzymes does not significantly contribute to the total CExC of beer. The amount of enzyme needed to make the enzyme assisted process equally efficient as the malting process would be more than 80 times as much as what is used at the moment. This would be a very unrealistic value. As these amounts of enzymes will never be used in enzyme assisted processes, it can be concluded that enzymes are useful to make processes more resource efficient.

4.6 Conclusions

We compared two processes for making beer at industrial scale. One process is the conventional process, while the other process is an enzyme-assisted brewing in which the malting step is omitted. The analysis showed that the enzyme-assistant process has a reduced impact on the environment. Circumventing the malting step reduces the use of water by 7%, of raw materials by 14%, and of natural gas by 78%.

The CExC of specific additives (for example enzymes), can be considerably higher than just their standard chemical exergy. In case of enzymes, we found that the

CExC of enzymes is about 676 MJ/kg dry enzyme, which is 30 times the standard chemical exergy value. Whether the CExC considerably affects the outcome of the analysis depends amongst others on the amount of the ingredient required. In the case of brewing though, only a little amount enzyme is needed, which makes that their use in the brewing process is still about 1.24 times more effective in terms of exergy input than the conventional malting process.

4.7 Acknowledgements

This research took place within the ISPT framework (Institute for Sustainable Process Technology). We would like to thank ISPT and Heineken for their financial support and fruitful discussions.

Appendix

Appendix I. Standard Molecular mass, chemical exergy and heat capacity of the used components

Material	Molecular Mass [kg/mol]	Standard Chemical Exergy [J/Kg][a]	Heat Capacities [J/kgK]
Water	0.01802	4.994E+04	4190
Steam	0.01802	5.272E+05	1840
Air	0.02896	-1.290E+03	1010
Carbohydrates (other)	227000 (of starch)	1.764E+07	1420
Carbohydrates (glucose)	0.1802	1.626E+07	1420
Proteins	3000 (of gluten)	2.261E+07	1550
Fat	0.2564 (of palmic acid)	4.309E+07	1680
Ashes	0.06005 (of K_2CO_3)	3.164E+04	837
Ethanol	0.04607	2.952E+07	2390
CO2	0.04401	4.516E+05	780
Glycerol	0.09202	1.850E+07	1629
O2	0.03200	1.241E+05	919
N2	0.02801	2.463E+04	1040
ammonia	0.01703	1.980E+07	4520

[a] Calculated from (Szargut, 1989)

Appendix II. Composition of process streams

Component	Composition (%)							
	growth medium[a]	*enzyme formulation*[b]	*Barley*[c]	*Malt*[d]	*Fertilizer*[e]	*Spent grains*[f]	*Beer*	
							Conventional process	*Enzyme assisted*
Water	93	65.1	13	5	70	80	92.33	92.47
Protein	2.8	4.9	9.57	11.09	13.97	5.6	0.86	0.79
Carbohydrates	3.5		73.95	79.87	11.63	11.7	1.71	1.59
of which starch			75.7	74.7				
of which fibres			24.3	25.3		100	100	100
Fats			1.52	1.76		1.64	0	0.00
Ash	0.7		1.96	2.27	4.4	1.06	0.22	0.20
Glycerol		30						
Ethanol							5	5

[a] (A. Jones et al., 2008)
[b] Protein content of commercial enzyme formulation was measured by DUMAS (conversion factor 6.25)
[c] We assumed these values based on our own measured values in combination with (Kunze, 2010)
[d] Calculated from barley compositions and assumptions on malting (Appendix III).
[e] All water and dry matter that does not end up in the enzyme formulation stream will end up in the fertilizer stream
[f] (Kunze, 2010)

Appendix III. Assumptions per process and process unit

General assumptions
- Reference environment: T_o=283.25 K, P_o=101.325 kPa, RH=82% (0.0064 kg moisture/ kg dry air) - All processes are adiabatic (no heat losses to the environment) - Steam of 403.15 K and 2.7 bar was used for heating duties and produced from environmental water heated by natural gas (the embedded exergy in this water is 0) - Steam leaving the system was at 383.15 K and 1.4 bar - Environmental water was used for cooling - Cooling below 283.25 K was done by ammonia of 253.15 K (ammonia was reused so its standard chemical exergy was not taken into account)

Assumptions malting process		
Process unit	Assumptions	References
Steeping	- Dry matter loss during steeping is 1% (no compositional change)	(Kunze, 2010)
	- The water used is 3.5 times the amount of barley	(Kunze, 2010)
Germination	- 5.8% of the dry matter is lost due to respiration	(Kunze, 2010)
Kilning	- Kilning is done with hot air in 3 stages; drying to 23% moisture using air of 328.15 K (air out = 303.15 K), then to 12% moisture using air of 343.15 K (air out is increasing from 303.15 to 333.15 K) and finally to 5% moisture using air of 363.15 K (air out increasing to 353.15 K).	(Lewis & Young, 1995)
	- Germination happens at 290.15 K and 100% RH	
	- The final moisture content of the malt is 5% w/w	(Kunze, 2010)
Cooling	- Cooling is done by outside air (RH =18.2%) that heats up till 308.15 K	(Kunze, 2010)
		(Kunze, 2010)

Assumptions enzyme production process		
Process unit	Assumptions	References
General	- All enzymes in the exogenous enzyme mixture for brewing are produced in a similar way	
Fermentation	- Sterilisation of the medium is at 394.15K	
	- Fermentation takes 6 days in a fed-batch stirred tank reactor at 303.15K	
	- The extracellular enzymes are produced by ***Bacillus subtilis*** (54kg dm/m^3)	(Alber et al., 2002; Kløverpris, Elvig, Nielsen, & Nielsen, 2009; Nielsen et al., 2007) (Gill, Appleton, Baganz, & Lye, 2008; Nielsen et al., 2007)
	- Agitation takes 2500 W/m^3	
	- Enzyme yield is 0.1 kg enzyme/ kg substrate	
	- Cooling water of the sterilized medium leaves at 368.15 K	
Recovery	- Downstream processing losses are 16.5%	
	- Electricity use of the rotary vacuum filter is 0.03 MJ, for the ultrafilter is 1.6 MJ, and for the reversed osmosis is 6 MJ	(Albaek, Gernaey, Hansen, & Stocks, 2011)
	- All pump efficiencies are 80%	
Formulation	- 30% (w/w) is needed to stabilize the enzymes	
Biomass treatment	- Biomass and waste water receive a heat treatment at 394.15 K. Afterwards they are cooled, cooling water leaves at 368.15 K	
	- Waste biomass and waste water are separated by a centrifuge till a 30% dry matter substance is obtained. The centrifuge uses 0.5 MJ/m^3	(Bradbury & Jakoby, 1972)

Assumptions brewing process		
Process unit	Assumptions	References
Milling	- Milling malt and barley consumes 6.5kWh/ton and 10.45 kWh/ton respectively	(Kløverpris et al., 2009) (Steiner et al., 2012)
Mashing	- Enzymes from malt and the exogenous enzymes are able to break down all starch in the brew (2 g/kg barley)	(Kløverpris et al., 2009)
	- Conventional brewing uses 2.5 m3 water/ton grist and barley brewing uses 2.2 m3 water/ton grist.	(Kløverpris et al., 2009)
	- All starch is hydrolysed into fermentable sugars	
Lautering	- 0.64 m3 sparging water/ton mash is used (345.15K)	(Kløverpris et al., 2009)
	- 14% of the wet weight ends up in the spent grains	
Wort boiling	- 4% water is evaporated during wort boiling	
Coarse break & whirlpool	- 7g/L is removed (80% water, 74% (dry matter) carbohydrates, 12% (dry matter) proteins and 13% (dry matter) fats	
	- Cooling water heats up to 366.15 K. Additional cooling to 280.15 K by ammonia.	
Fermentation & maturation	- Temperature during fermentation is 280.15 K, cooled by ammonia	
	- Only ethanol is formed, no higher alcohols	
	- 2% of the fermentable sugars are used for yeast anabolism.	(Kunze, 2010)
Filtration Pasteurisation	- 2.25% w/w (wet weight) is removed as yeast after fermentation	
	- All yeast is removed	
	- Water is added to bring the beer to a 5%w/w alcohol	
	- No evaporation of water or alcohol occurs	

Combining unmalted barley and pearling gives good quality brewing

Highlights

- Brewing with pearled unmalted barley can result in beer of good quality
- The malt-to-barley ratio is more important for beer quality than the degree of pearling
- The mash filter is more suitable for brewing with pearled barley than the lauter tun

This article is in press as: Laura H.G. van Donkelaar, Jos A. Hageman, Serhat Oguz, Tom R. Noordman, Remko M. Boom, Atze-Jan van der Goot (2016), Combining unmalted barley and pearling gives good quality brewing, Journal of the Institute of Brewing, 122:2

5.1 Abstract

Brewing with unmalted barley can reduce the use of raw materials, thereby increasing the efficiency of the brewing process. However, unmalted barley contains several undesired components for brewing and has a low enzymatic activity. Pearling, an abrasive milling method, has been proposed as a pre-treatment for barley to remove some of its undesired components, while maintaining its β-amylase activity. The potential of combining pearling with using barley/malt mixtures for brewing was studied. Filtration was done either in a mash filter or a lauter tun. The effects of the different barley/malt ratios, degree of pearling and two different filter types on compositional and quality parameters were assessed. It was concluded that a mash filter is optimal for this type of process, and a window of operation could be identified in which optimal use is made of the raw materials while maintaining the end product quality, judged on basis of 4 quality parameters.

5.2 Introduction

Brewing with unmalted barley is receiving increased attention because it has economic advantages and has potential for water and energy savings *(Steiner et al., 2012)*. The main point of attention for brewing with unmalted barley is the lower enzymatic activity when preparing the wort. A lower enzyme activity means slower or less starch conversion. This results in a lower amount of fermentable sugars and thus a lower process yield. Furthermore, using barley instead of malt, increases the presence of β-glucans and arabinoxylans in the mash. This can lead to higher mash viscosities and thus longer filtration times *(D.L. Goode et al., 2005)*. The different raw material composition might also affect the quality of the end product. Polyphenols can, for example, form complexes with certain proteins leading to haze *(Asano, Shinagawa, & Hashimoto, 1982)*.

The potential of raw barley for brewing has been investigated since the 1960's, and recent advances in brewing enzyme development have again raised interest for the topic *(Evans et al., 2014; D.L. Goode et al., 2005; Steiner et al., 2012)*. Goode et al (2005) reported that increasing the malt-to-barley ratio increases the extract recovery levels, the wort α-amino nitrogen levels and the fermentability, while it decreases the viscosity and β-glucan levels. The endogenous malt enzymes were reported to have very poor raw barley protein and starch hydrolysing ability. Steiner et al ***(1)*** brewed with 100% barley using the commercial enzyme mixture Ondea Pro and obtained beer of a satisfactory quality in an efficient way regarding lautering and filtration, though the free amino nitrogen (FAN) content (Zhao et al., 2006) and total nitrogen content were lower compared to beers brewed from malt. Evans (2014) confirmed this, and concluded that even if the FAN content of barley brewed beer is lower, the quality of the FAN is higher due to its amino acid composition. This results in an increased amino acid utilization, and the yeast growth becomes nitrogen dependent at lower FAN content. Wort prepared from barley had a higher fatty acid content than wort brewed with 100% malt, which might affect the flavour and foam stability of the beer. These lipids were probably extracted from the embryo and aleurone layer *(Evans et al., 2014)*. Kunz et al ***(5)*** used an enzyme cocktail containing the same enzymes as the commercial enzyme mixture Ondea Pro. Though they found some minor differences in the wort composition they concluded that the use of barley up to 50% had no negative effect on the beer quality and flavour. Also the original extract and final

attenuation in worts produced with between 0%-90% barley and an enzyme cocktail were comparable *(Kunz et al., 2012)*. In other words, partial replacement of malt by barley is possible, provided sufficient enzymes are present in the mash.

Pearling, an abrasive milling method, has been proposed as a treatment for barley to remove some of its undesired components *(Palmer, Barrett, & Kirsop, 1970; van Donkelaar et al., 2015)*. Donkelaar et al. ***(7)*** showed that pearling to a degree of 5% reduced the insoluble arabinoxylan content by 15%, the insoluble fibre content by 23% and the water holding capacity of the non-starch components by 25%. It also reduced the ash content by 19% and the polyphenol content by 11%, while only 0.20% of the starch was removed. The reduced arabinoxylan content resulted in a lower mash viscosity, which facilitates the filtration step after mashing, yielding a reduced loss of wort and sugars after filtration. The fraction removed during pearling, being the bran fraction, remained dry, which makes it better applicable for other purposes (e.g. for extraction of functional ingredients).

Brewing with pearled barley has been suggested (Palmer et al., 1970). Palmer et al. malted pearled barley in the presence of gibberellic acid, and obtained a greater action of hydrolytic enzymes during malting compared to malt form unpearled barley. They speculated that such malts will allow the use of larger quantities of unmalted adjunct in brewing. Alternatively, suitable adjustment of the malting or mashing procedure would allow malts from abraded barley to yield worts of lower fermentability and nitrogen content. Brewing with pearled barley is already applied in the production of Shochu, a beverage produced from pearled barley, fermented by mould and yeast. Iwami et al showed that the degree of pearling affects the quality of the product, and that the colour and flavour of Shochu can be changed by the degree of pearling of the barley *(A. Iwami et al., 2005)*.

The effect of pearling barley would have on beer brewing with malt/barley combinations has not yet been demonstrated. In this paper worts produced using different ratios of malted and unmalted barley and different degrees of pearling of this unmalted barley are compared. A lauter tun and a mash filter are compared for suitability as filtration methods. Using Principal Component Analysis (PCA) and linear models, influences of these three variables on composition, extract, quality and processing parameters were compared. Also the spent grain fraction of these brews was compared. The consequences for the processing were discussed.

5.3 Materials and methods

5.3.1 Material

Barley (*Hordeum vulgare*) (86.7% m/m dry matter) and malted barley (95.6% m/m dry matter) of the variety Sebastian was used (France, harvested in the summer of 2013, stored at 4ºC).

Brewing water was prepared by dissolving 2.68 mM CaCl2 in demineralized water. The enzyme mixture Ondea Pro (Novozymes) was used in allexperiments. This product is a mixture of α-amylase, pullulanase , β-glucanase, xylanase, endo-protease and lipase *(Steiner et al., 2012)*.

5.3.2 Methods

Pearling: Barley kernels were pearled in a Satake TM05 pearling machine until the desired degree of pearling (i.e. the w/w percentage of material removed by pearling) was reached.

Brewing: the brewing tests were performed in a laboratory scale equipment which consisted of a 5 L mashing vessel equipped with a heating mantle and a mixer, a filter (lauter tun or mash filter) and a wort boiling vessel which was equipped with a heating spiral of stainless steel and placed on a balance. The mash was transferred from the mashing vessel to the filers using a transfer vessel. The lauter tun was equipped with a heating mantle and raking knives of which the rotation and height were continuously recorded. After transfer of the mash into the lauter tun the mixture was allowed to rest (10 min) and was then circulated (10 min). and the wort was filtered and after the first wort the sparging water was added. The raking knives were placed to loosen the top layer of the filter bed. The filtered mash was pumped to the wort boiler. After boiling the wort was cooled to < 10°C and samples were taken. The temperature during mashing, the pressure difference of the filter, the density (brix) of the filtered mash, the temperature of the wort and the weight of the wort were continuously recorded.

Table 1. Barley to malt ratios and degree of pearling of the barley used in the lauter tun and mash filter brewing experiments

Barley/Malt ratio	degree of pearling barley (%)	Filter type
100/0	0	Lauter tun
100/0	5	Lauter tun
80/20	0	Lauter tun
80/20	5	Lauter tun
80/20	10	Lauter tun
65/35	10	Lauter tun
50/50	0	Lauter tun
50/50	5	Lauter tun
50/50	10	Lauter tun
100/0	0	Mash filter
80/20	0	Mash filter
80/20	5	Mash filter
80/20	10	Mash filter
50/50	0	Mash filter
50/50	5	Mash filter
50/50	10	Mash filter

Table 1 shows the raw material composition for mashes filtered by lauter tun or mash filter. The barley and malt were milled before mashing using a roller mill (distance between rolls 2 mm) in case of lauter tun filtration. If a mash filter was used, a hammer mill with a sieve of 1.5 mm was used. A total of 950 g of raw material (barley plus malt) was used and the mashing-in ratio was 1:3.2. When malt was substituted with pearled barley, the weight of the barley was reduced with the pearling percentage. The enzyme mixture Ondea Pro (Novozymes) was used in a concentration of 2g/kg barley. Ondea Pro was added to the mashing water before addition of the barley and malt. The mashing process followed the scheme shown in **figure 1**. The mashing starts with a 30 minute period at 54 ºC to allow proteases and β-glucanase to work. Then the temperature is increased to 64 ºC to allow α-amylase and β-amylase to work. After 60 minutes the temperature is increased to 80 ºC to inactivate the enzymes.

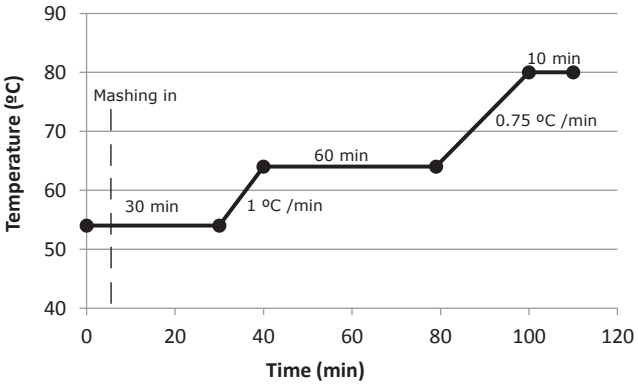


Figure 1. Mashing scheme used in all brewing experiments

Filtration: a lauter tun (diameter = 140 mm) or a mash filter (Meura 2001, Meura technologies, 0.0254 m^2 filtration surface) was used in a filtration step. When using a lauter tun a total amount of water for brewing of about 3225 ml was used and for the mash filter about 5440 ml was used.

Wort boiling: after mashing, the mash was transferred to the wort boiler and boiled with 6 grams of hop extract (Joh. Barth & Sohn GmbH & Co. KG) and 7.2 ml HCl (1M) to a final weight of 4213g ± 42g in case of the lauter tun. For the worts filtered by the mash filter, the end volume of the wort was 5070g ±78g. The wort was cooled to <10ºC and samples were taken. The spent grains were collected, weighed, and mixed for homogenisation before analysis.

Analysis methods wort: Free arabinose (%), free xylose (%), total arabinose (%), total xylose (%), arabinoxylan (%) were measured by Eurofins (Eurofins laboratories). Total polyphenols (AU/10ml) and anthocyanogens (AU/10ml) were measured using the molybdate method and McFarlane method respectively *(Baier et al., 1992; McFarlane, 1961; McFarlane, Sword, & Blinoff, 1963)*. S-methylmethionine (µg/L) and free dimethyl sulphate (µg/L) were analysed according MEBAK. All other wort parameters (bitterness (BU), colour (visual method), colour after boiling (visual method), high molecular protein (HMP) (mg/L), free amino nitrogen (Zhao et al., 2006) (mg/L), total nitrogen (total UV digestion, mg/L), glucose (%m/V), fructose (%m/V), maltose (%m/V), maltotriose (%m/V), total fermentable sugars (%m/V), pH of the wort, viscosity (mPa.s), extract (%m/m), apparent final attenuation limit (AFAL) (%), apparent extract after final attenuation (AEAFA) (%(m/m), high

molecular weight β-glucan (fluorimetric method, mg/L), (4-vinylguaiacol (mg/L), chloride (mg/L), nitrate (mg/L), sulphate (mg/L), phosphate (mg/L), calcium (mg/L), magnesium (mg/L), potassium (mg/L), sodium (mg/L) and zinc (mg/L)) were analysed according Analytica EBC, ed.1998, sec.4, malt.

All data were standardized to a wort extract of 11.5 ºP.

Analysis methods spent grains: Total weight (g), total moisture (%m/m), washable extract on dry matter (%m/m) and total extract on dry matter (%m/m) were measured according to Analytica EBC, ed. 1998. From this data the dry weights of the spent grains was calculated.

5.3.3 Statistical analysis

A low-dimensional summary of the data was obtained by applying Principal Component Analysis (PCA). PCA converts a set of correlating variables into a smaller number of orthogonal variables called the principal components. Usually a small number of principal components suffices to capture a large part of the variance present in a data set. In a two-dimensional plot, this small set of principal components is plotted with the original variables related to these components. This provides a visual summary of the important sources of variation in the data set. Data were standardised (mean of zero and standard deviation of 1) prior to PCA.

To find significant differences that can be attributed to the experimental design, linear models and significance testing were used. All responding (dependent) variables, such as the compositional and quality variables, were fit to a multiple linear regression model using Matlab (R2012A) using barley/malt ratio, degree of pearling and filter type as explanatory (independent) variables. This model also takes interaction between these three variables into account:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_1 x_2 + \beta_5 x_1 x_3 + \beta_6 x_2 x_3 + \beta_7 x_1 x_2 x_3 + \varepsilon \qquad (1)$$

Here, β_0 is the general intercept, x_1 is a dummy (integer) variable indicating the filter type, x_2 is the barley/malt ratio, x_3 is the degree of pearling, while all other terms accommodate the different interactions between the 3 explanatory variables.

Significance tests were used to determine if any of the 3-way, 2-way or main effects were significant ($p<0.05$). If significant effects were found, a graphical representation of Fischer's LSD method for pairwise comparisons was used to gain insight into the nature of the differences. For this graphical overview, all treatments (see table 1) are plotted stacked on to each other together with their confidence intervals. For any pair of treatments having non overlapping confidence intervals, indicate a significant difference ($p<0.05$).

5.4 Results and discussion

The worts and spent grains produced with different ratios of malt and barley, different degree of pearling and filtered with a mash filter or a lauter tun, were analysed on their chemical composition and filtration time. A graphical overview of the pair-wise comparisons is presented in appendix 1. All differences induced by filter type are related to the filter as well as the milling process used before mashing and the amount of water used. Roller milling before the lauter tun gives a coarser particle size distribution compared to milling with a hammer mill before the mash filter. Differences could also be related to the increased agitation by the knives in the lauter tun, which could facilitate extraction.

5.4.1 Wort analysis

Starch hydrolysis products and attenuation

The detailed results for this section are summarized in the graphs in **appendix I-a**.

The extract was not affected by the malt-to-barley ratio when a mash filter was used, but increased with increasing malt-to-barley ratio when using a lauter tun. At the same time, the amount of extract was larger with a lauter tun than with a mash filter. This was because of the lower water usage when a lauter tun (end volume 4200g) is used compared to when a mash filter is used(end volume 5070g), which made these brews more concentrated.

All values of the other chemical component analyses of the wort values were standardized to extract values of 11.5 ºP. The fraction of total fermentable sugars were not influenced by filter type but decreased with an increasing amount of

barley and with the degree of pearling. This was not expected, as only a minimal amount of starch was pearled off *(van Donkelaar et al., 2015)*. The lower fermentable sugars might however be caused by the milling procedure, as milling pearled material gives different particle sizes and particle composition. This in turn could have impaired the breakdown of starch resulting in less breakdown component. That the extract increased while the total fermentable sugars did not increase when using a lauter tun instead of a mash filter, may imply that a larger amount of non-sugar components or unfermentable sugars was extracted into the mash when a lauter tun was used.

Both the glucose and fructose levels decreased with an increasing amount of barley, just like the total fermentable sugars. The glucose levels were increased with a mash filter relative to a lauter tun. Maltose and maltotriose levels were not influenced by the amount of barley added, but decreased with an increasing degree of pearling.

Apparent final attenuation limit (AFAL) was higher in wort filtered with a mash filter, which may be related to the higher glucose levels. AFAL increased with the barley concentration for the lauter tun, while the wort produced with a mash filter was not affected by the amount of barley. Pearling increased the AFAL, which seems contradictory to the amount of total fermentable sugars.

Apparent extract after final attenuation (AEAFA) was only influenced by filter type and higher for worts filtered with a mash filter.

Proteins

The results for this section are depicted in the graphs in **appendix I-b**.

FAN concentrations decreased with increasing barley concentrations, which is in line with literature *(Evans et al., 2014; Steiner et al., 2012)*. No effect of the degree of pearling was observed, which means that the protein present in the outer 10% of barley, which is about 8% of the total protein present in barley (van Donkelaar et al., 2015), did not significantly contribute to the FAN content in wort. Fan is necessary for the yeast for fermentation, wort of 12 ºP should contain 140-150 mg/l FAN (O'Connor-Cox, Paik, & Ingledew, 1991). Beers produced in this experiment had a

lower FAN content, so the increase of FAN due to a higher barley content was not expected to have a negative effect on the beer quality.

High molecular Protein (HMP) increased with increasing barley concentrations, because more protein breakdown occurred in malted barley during germination. No effect of the degree of pearling was observed in either HMP or the total nitrogen content. HMP are important for the foam stability of the beer, and therefore adding barley is expected to increase the foam stability of the beer. Steiner et al. found a higher foam stability in beers brewed with unmalted barley. They attributed it to a higher β-glucan content (Steiner et al., 2012). The total nitrogen content was lower in the brews with a lower malt-to-barley ratio. This suggests that more protein was lost in the spent grains if more barley was used. In malted barley, proteins are already partly broken down during germination, increasing their solubility and extraction.

Polyphenols

The results for this section are depicted in the graphs in **appendix I-c**.

The total polyphenol content was only influenced by the amount of barley added to the brew. It decreased with more barley, which is logical because malt generally has a higher polyphenol content than barley as polyphenols are synthesized/ released during the malting process *(Maillard, Soum, Boivin, & Berset, 1996)*. Pearling removes the polyphenols in the outer layer of the husk and therefore a decrease was expected with an increased degree of pearling, but this effect was not observed for the total polyphenols.

The anthocyanogens also decreased with increasing barley content, although their content in barley is believed not to be affected by malting unless it gets in contact with oxygen *(Pollock, Pool, & Reynolds, 1960)*. The anthocyanogen content did decrease with the degree of pearling. Anthocyanogens are mostly present in the aleurone layer, and after pearling off more than 10% this layer is damaged and partially removed *(van Donkelaar et al., 2015)*. The anthocyanogens might also have been oxidized because of the damaged outer layer of the barley. Anthocyanogens are one of the groups of polyphenols which are involved in the haze formation in beer, so a decrease of these components indicates a decrease in risk of haze formation.

When regarding complex formation between HMP and anthocyanogens, they seem to show an opposite concentration effect for pearling and malt to barley ratio. Depending on the variety and batch of barley a brewer might want to reduce the amount of either one. If the brewer wants to decrease anthocyanogens in wort, pearling to a degree of 5% to 10% is a way to remove about 20% w/w to 33% w/w of them respectively in the case of the variety Sebastian.

Arabinoxylans, β-glucan and viscosity

The results discussed below are depicted in the graphs in **appendix I-d**.

The free arabinose levels increased with increasing malt addition and decreasing degree of pearling. The free xylose was only influenced by the degree of pearling, and increased with an increasing degree of pearling. This was expected because arabinoxylans are located mostly in the outer layers of the barley kernel. That arabinose and xylose behave differently with respect to pearling can be explained by the lower arabinose-to-xylose ratio in the husk compared to the endosperm ***(Ullrich, 2010)***. However, the total arabinose, total xylose and arabinoxylan contents of the mashes were not influenced by any of the process variations, even though when the outer 5% of barley are removed, about 15% of the arabinoxylans are removed. Pearling away the outer 10% takes away about 33% of total arabinoxylans *(van Donkelaar et al., 2015)*. This decrease is not observed in the wort because only a small part of the arabinoxylans is water-soluble *(A. A. Andersson et al., 2008)*.

The concentration of ß-glucans increased with more barley, in worts produced with a mash filter, because the β-glucan concentration of barley is higher than that of malt. However, the concentration of β-glucan was not affected when using the lauter tun. Possibly the different milling and subsequent coarser particle size for the mash filtered with the lauter tun allowed for less extraction of β-glucan.

The viscosity was higher in the mash filter compared with the lauter tun, and increased with the barley concentration. The changes in viscosity are not directly related to the arabinoxylan levels. They do, however, show a similar trend as do the glucans when using a mash filter. Pearling did not affect the viscosity though there was a 3-way interaction between filter type, barley percentage and degree of pearling.

Flavour components

Results for this section are depicted in the graphs in **appendix I-e**.

The bitterness was not affected by the filtration method, the amount of barley added or the degree of pearling. Hop components are the main cause for bitterness, which are added after filtration, and therefore not influenced by the variations in the process.. S-Methylmethionine (SMM) is a precursor of dimethyl sulphide (DMS), which can cause an off flavour in beer. Both components were present in low concentrations. The SMM concentration was lower with more barley and more pearling. This was expected because the SMM content of barley increases during germination *(João Pimenta, Kaneta, Larondelle, Dohmae, & Kamiya, 1998)*. Insufficient data were available for DMS for any conclusions on this component.

4-vinylguaiacol is a component in beer which can cause an off flavour if its taste is not desired in a certain beer type. The primary source of 4-vinylguaiacol in beer is ferulic acid, which is converted by yeast *(McMurrough et al., 1996)*. The content of free ferulic acid is low in barley but highest in the aleurone layer, and this content generally increases with malting *(Szwajgier, Pielecki, & Targonski, 2005)*. Our results are contradictory to this, because the amount of 4-vinylguaiacol increased with more barley. More pearling increases this effect with a mash filter but not with a lauter tun.

Summarizing, the amount of barley and pearling seem to have an opposite effect on SMM compared to 4-vinylguaiacol. The filtration method does not affect the measured flavour parameters.

Minerals and pH

The results for this section are depicted in the graphs in **appendix I-g and I-h**.

The chloride and nitrite concentrations increased while the sulphate, potassium, and magnesium concentrations decreased with the barley concentration. In general a decrease in minerals was expected because rootlets, which are removed at the end of malt production, have a relatively high mineral content compared to the grain *(D. Liu, Pomeranz, & Robbins, 1975)*. The phosphate concentration was

higher in wort filtered by a mash filter while the zinc concentration was higher in those filtered by lauter tun.

The calcium and magnesium concentrations increased with pearling. This is in line with earlier work that showed that magnesium is more abundant in the endosperm than in the outer layers *(KeShun Liu, Peterson, & Raboy, 2007)*. The sodium concentration was not affected by the process variations.

The pH of the mash was not affected by any of the variables. The pH of the wort often decreases with the calcium concentration, but in this case there was no connection.

Appearance

Results for this section are depicted in the graphs in **appendix I-f**.

The colour of the wort before boiling was not influenced by the filtration method but decreased in intensity with more barley and more pearling, because malting (and kilning) leads to Maillard reaction products that give darker colour, and pearling reduces the polyphenol content. The colour after boiling was only affected by the addition of barley. Pearling was expected to have an effect, but this was not observed.

5.4.2 Processing parameters

Results for this section are depicted in the graphs in **appendix I-i**.

The filtration time increased with more pearling and with the addition of more barley. This was attributed to a denser filter bed due to less husk material because of the pearling, and less breakdown of the barley components compared to malt. The different particle size distribution of barley compared to the malt also affects the density of the bed. Pearling leads to coarser particles, and therefore a shorter filter time would be expected with an increased degree of pearling. This was not the case, probably because of a lower amount of spent grains leading to a filter bed that was less deep. The viscosity increased with an increasing barley concentration, which may also explain the longer filtration times.

5.4.3 Spent grains analysis

Results for this section are depicted in the graphs **in appendix I-j**.

The dry weights of the spent grains are only affected by pearling and decreases with more pearling, because the fibres that normally make up for most of the dry matter of the spent grains were pearled off.

The total moisture content of the spent grain was only affected by the filter type and not by pearling, indicating a similar water holding capacity (WHC) for filter beds resulting from pearled and unpearled barley. The moisture content of the spent grains was higher in the lauter tun, so the WHC of the spent grains produced with a lauter tun was higher. This is also be seen in the total and washable extract values, which were higher for the lauter tun.

In the lauter tun, the extract values were increased by more pearling and more barley, while in the mash filter these increases were not observed. In general there were more water and extract losses when a lauter tun was used, which is in line with the literature *(Kunze, 2010)*. The lower losses with a mash filter are related to the larger amount of water used for sparging and a lower extract in the last runnings of brews filtered in a mash filter. Furthermore it was visually observed that the sticky gray layer on top of the lauter tun filter bed was thicker when more barley was used. Possibly, some of the extracted components in the lauter tun accumulated in this layer, forming a gel-like substance with a high water binding capacity. The different milling methods used for the lauter tun and mash filter also had an influence on the density and thus on the performance of the filterbed. However, a lower extraction in the mash filter would have been expected based on the milling method, because hammer milling leads to finer particles and therefore a more dense filterbed. Apparently the use of more water to wash the filter bed of the mash filter had more effect than the density of the bed.

5.4.4 PCA Analysis

A PCA analysis was done on the data, leading to 4 important principle components (PC). Those 4 PC's explained 65.9% of the variance in the data. **Figure 2** shows a biplot of the first and second PC of the principle component analysis. In this figure, three clusters can be distinguished, grouped by the malt-to-barley ratio that was used. The lower the PC1 value and the higher the PC2 values, the higher is the malt-to barley-ratio.

PC1 explains 27.9% of the variation. A high PC1 corresponds with high amount of minerals, SMM and losses in the spent grains. PC2 explains 17.2% of the variation. A high PC2 value indicates degradation of high molecular weight components (e.g. high amounts of FAN, glucose, fructose, total fermentable sugars, free arabinose). The higher the PC2 value, the higher is the component conversion and extraction efficiency of components into the wort. For a good quality wort, it would be desirable to have a low PC1 value and a high PC2 value. PC1 increased and PC2 decreased with decreasing malt-to-barley ratios, which means that more losses occur and less fermentable sugars end up in the wort when less malt is used. This is because malt components are already partially converted and are thus better accessible for enzymatic hydrolysis. The PC1-PC2 biplot shows no differences, with respect to the filtration method or the degree of pearling.

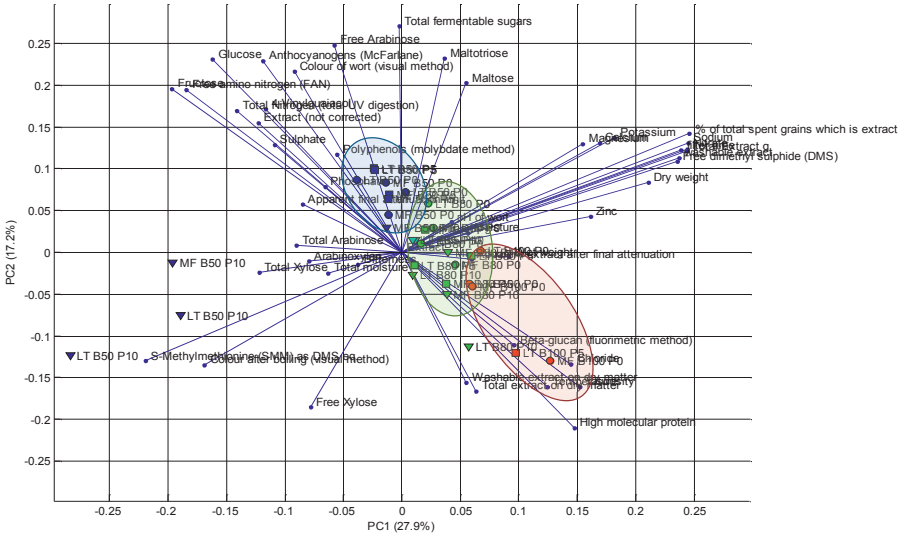


Figure 2. Biplot of principle component 1 and 2. Dark blue: 50% barley. Light blue: 65% barley. Green: 80% barley. Red: 100% barley. ●: 0% pearling. ■: 5% pearling. ▼: 10% pearling.

Figure 3 shows the biplot of PC3 and PC4. PC3 explains 11.7% of the total variance between the variables. Brews with a high PC3 value have higher values for the extract and colour, and have more mass lost in the spent grains. Low PC3 values are related to high levels of most minerals and a higher viscosity, while high PC3 values relate to high extract and colour. PC4 explains 9.1% of the variance between

the variables. High PC4 values are related to high arabinoxylan and FAN levels, while low PC4 levels correspond with higher levels of total fermentable sugars. High PC3 values and low PC4 values seem to be favourable for a good quality wort, although it should be kept in mind that together these parameters only explain 20.8% of the variance in the data.

This biplot shows a clear distinction between the brews prepared with a lauter tun (positive PC3 values) and brews prepared with a mash filter (negative PC3 values). Values for PC4 were on average higher for mash filter brews compared to lauter tun brews.

In general, brews prepared with a lauter tun have a higher extract and colour while brews with a mash filter have higher arabinoxylan content and viscosity. The higher extract of worts prepared with a lauter tun is attributed to a lower water usage in this system, which makes the wort more concentrated. The agitation in the lauter tun during lautering may also have effect, by enhancing the extraction of components into the mash.

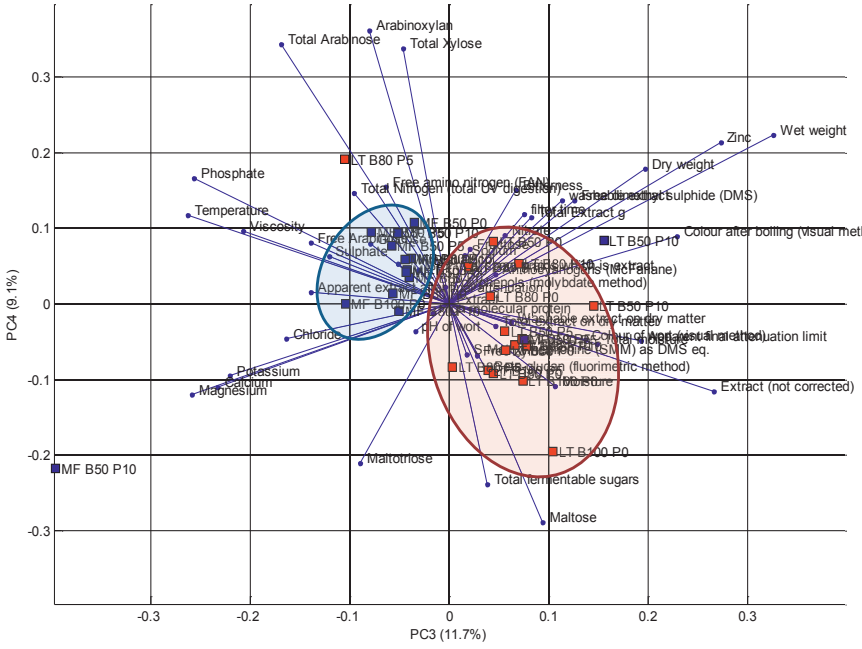


Figure 3. Biplot of principle component 3 and 4. Red: Lauter tun. Blue: Mash filter.

In general, by using these biplots, it is possible to estimate the characteristics of a brew when using a certain filter or malt-to-barley ratio. No different clusters for the degree of pearling were visible in the biplots. This complies with the preceding general analysis which showed that the malt-to barley-ratio was the variable with the highest impact. Several parameters were affected by the filter type, or were only affected by pearling or the malt to barley ratio in one of the filters. Pearling affected the least parameters, which may indicate that pearling indeed may be practically applicable.

5.4.4 Process design

To determine which process parameters and which filtration system should be used, the focus was on 4 variables. (1) The total amount of dry matter which is extracted into the wort was calculated as a measure of the extraction efficiency. (2) The conversion potential of the wort for the yeast was calculated, by calculating the percentage of dry matter which ends up in the wort in the form of fermentable sugars. Furthermore two values were considered to be minimized, which are (3) viscosity and (4) anthocyanogens. Minimizing viscosity increases the filter efficiency. The effect of pearling and the malt-to-barley ratio on these 4 variables is shown in the contour plots in **figure 4**. The uniform white in the upper right corners indicate that no data were available for the 100% pearled barley samples and no estimations were made for these areas of the graph.

The colour patterns are different for the lauter tun and for the mash filter. This indicates that pearling and the malt-to-barley ratio affect the variables in a different way for the two filtering methods. The reasons are the differences in particles size distribution of the barley and malt before mashing, the agitation in the filter bed and the different densities of the filter beds.

With the mash filter more material is extracted into the wort and the percentage of the dry starting material that ends up in the wort as sugars is higher. This seems contradictory to the statistical data and PCA analysis which show that the extract values for the lauter tun are higher, but this can be explained by the smaller amount of water that is used in the lauter tun, resulting in less but also in a more concentrated wort.

This also explains the higher losses in the spent grains for mashes produced in the lauter tun. The higher anthocyanogen content in mash filter worts is most probably explained by the finer milling that enhances the extraction of this component. Because of the differences between the filter methods different values for the processing variables should be considered, or if possible the filter method has to be adapted to the processing variables.

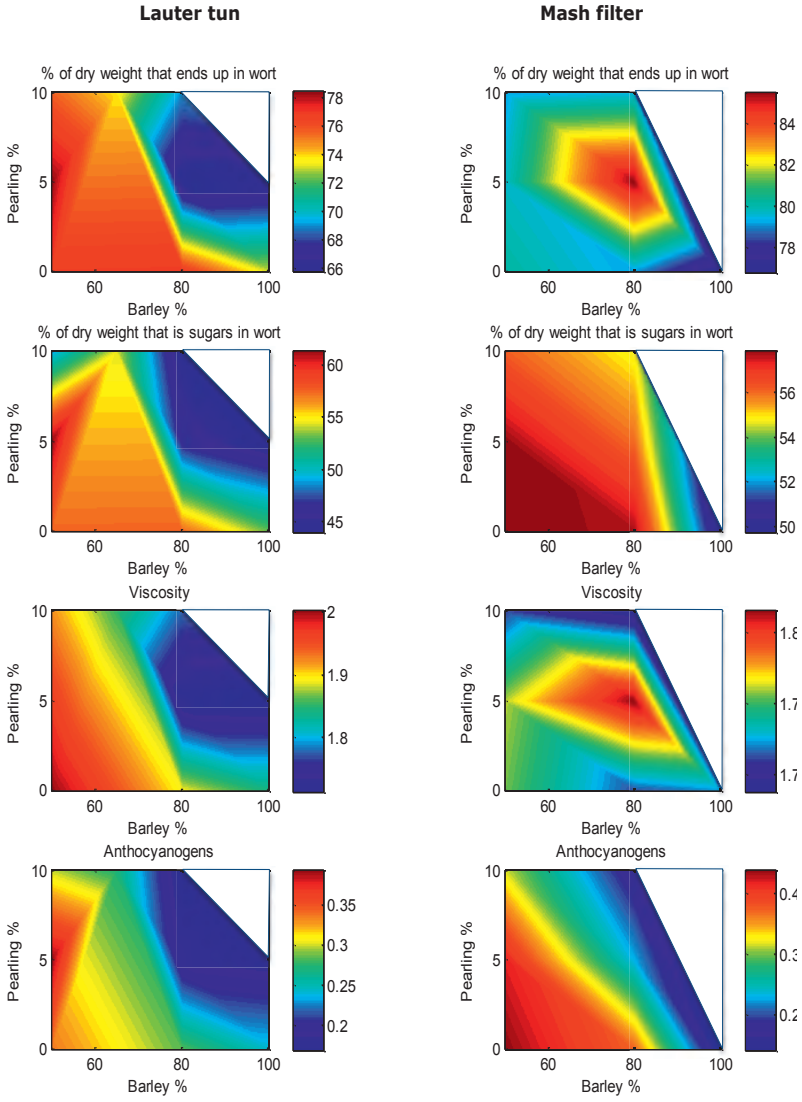


Figure 4. Contour plots showing the effect of pearling degree and barley percentage on the 4 selected parameters

To determine the optimal brewing parameters minimal or maximal values were set for a good quality wort for the 4 variables (total dry matter extraction, conversion potential, viscosity and anthocyanins). For the total amount of dry matter which was extracted into the wort the minimum value was 75%(m/m) and for the percentage of dry matter which ends up in the wort as fermentable sugars the minimum value was 50%%(m/). For the viscosity the maximum value was 1.75 mPa.s while the maximum value for the anthocyanogens was 0.2 AU/10mL. **Figure 5** shows the areas where the values were above or below the minimal or maximal values for the 4 variables respectively. The common overlap of all 4 areas overlap yields the window of operation for brewing.

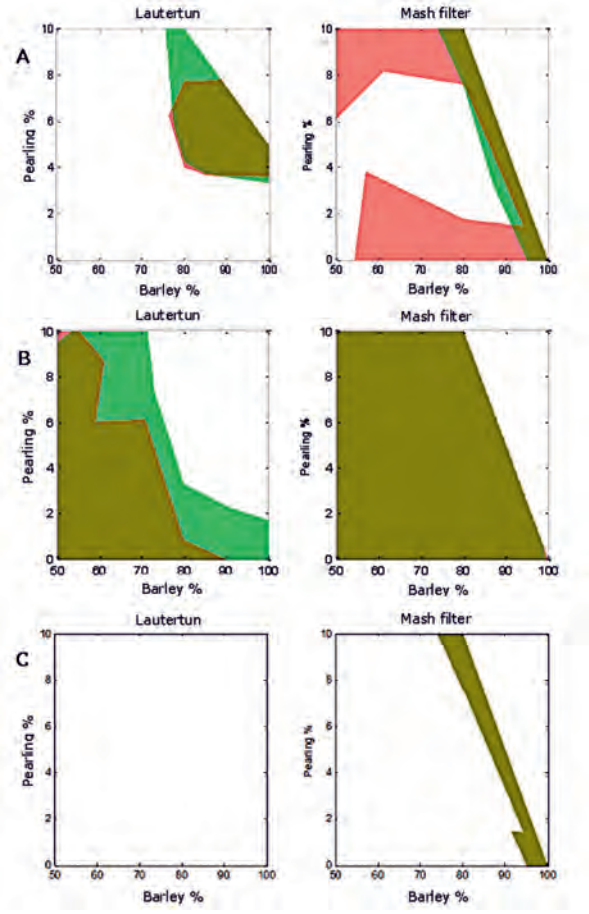


Figure 5. Contour plots showing the optimal combinations of degree of pearling and malt-to-barley ratio for the lauter tun and the mash filter. Fig 5a. Light green: dry matter which is extracted into the wort >75% (m/m). Pink: dry matter which ends up as sugars in the wort >50% (m/m). Fig 5b. Light green: anthocyanogen content <0.2 AU/10ml. Pink: Viscosity < 1.75mPa.s Dark green means overlap of the pink and light green areas. Fig 5c. When both dark green areas of fig 5a and fig 5b overlap, this area represents the allowed window of operation for the filter.

In **figure 5a** as well as **figure 5b** there is overlap for the parameters using both filter methods. For the lauter tun, however, there is no area in which both the overlap areas of **figure 5a** and **figure 5b** match. This means that the window of operation in which the extraction is optimized is not the same one as the one in which the viscosity and anthocyanogen content are minimized, see **figure 5c**. Thus there is no window of operation that would allow the use of a lauter tun.

In the case of the mash filter there is an area in which these overlap, see **figure 5c**: from 74%-80% barley with a degree of pearling of 10% up to using 95-99% of barley with a degree of pearling of 0%. This means that for the mash filter there is a window of operation for brewing in which all four selected parameters are within an acceptable range. Thus, for using unmalted barley in combination with pearling, it may be concluded that a mash filter is more suited than a lauter tun.

It should be noted that, at the moment, the milling, mashing and filtering systems are optimized for brewing with unpearled malt. This means that they are not optimal for these new processing conditions. Therefore, optimized processes are not optimal for implementing improvements. The systems should be optimized for the new situation, and this may well lead to a wider window of operation.

5.5 Conclusions

A brewing study was done on the use of mixtures of barley and malt in combination with pearling of the barley to reduce the amount of spent grains and optimise the use of the raw materials, while maintaining the quality of the wort.

The malt-to-barley ratio is the most important process parameter. Increasing the amount of barley decreases the yield and the efficiency of the process; thus its benefits should be weighed against these losses. Pearling was the least important parameter. However, pearling decreases the free arabinose, S-methylmetionine and anthocyanogen content, and increases the apparent final attenuation limit (AFAL) of the wort. As a negative effect, it decreases the total fermentable sugars and increases the free xylose and filter time.

Brews filtered with a lauter tun had similar levels of fermentable sugars but a lower AFAL compared to brews filtered with a mash filter. When using a lauter tun, the losses in the spent grains are larger with more barley. These losses are not observed in the mash filter. The use of a mash filter yields a window of operation in which acceptable extraction values are reached while anthocyanogens and viscosity stay below the upper level that was set for them. The current process, however, is optimized for brewing with unpearled malt, and the process is not optimal for brewing under the new processing conditions. It is expected that optimisation of the complete process will enlarge the window of operation, and may even open up new ones.

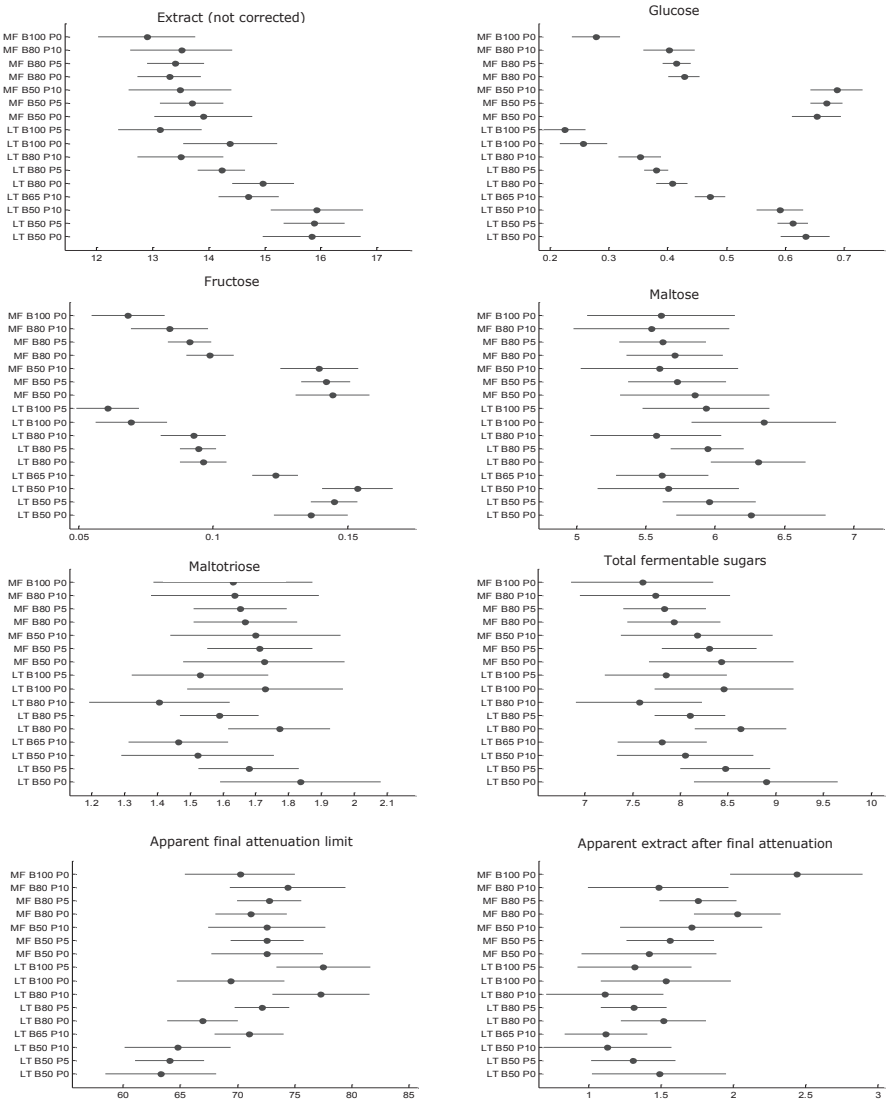
5.6 Acknowledgements

This research took place within the framework of the Institute for Sustainable Process Technology (ISPT). We would like to thank ISPT and Heineken for their financial support and fruitful discussions. We would like to thank Heineken for facilitating the brewing experiments.

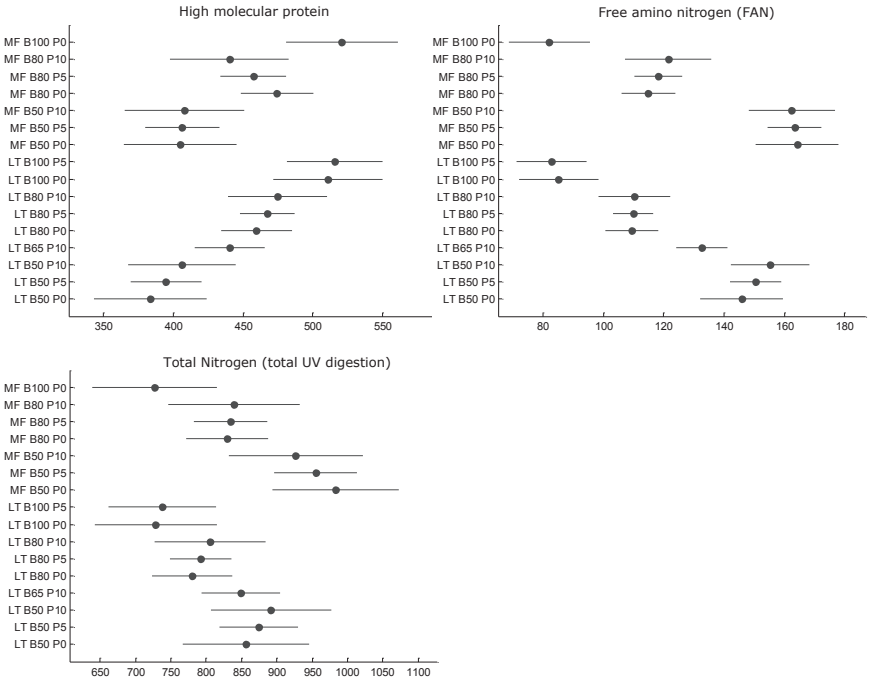
5.7 Appendix

Each plot shows the estimated treatment mean together with a 95% confidence interval. In these plots, treatments are coded as follows: MF indicates brews were filtered over a mash filter, LT indicates filtration by a lauter tun. In the second part of the coding scheme, the symbol B is followed by a number. This number indicates the percentage of barley. So in a brew with B80, 80% of the raw material was barley and 20% was malt. The number following the P in the code stands for the degree of pearling.

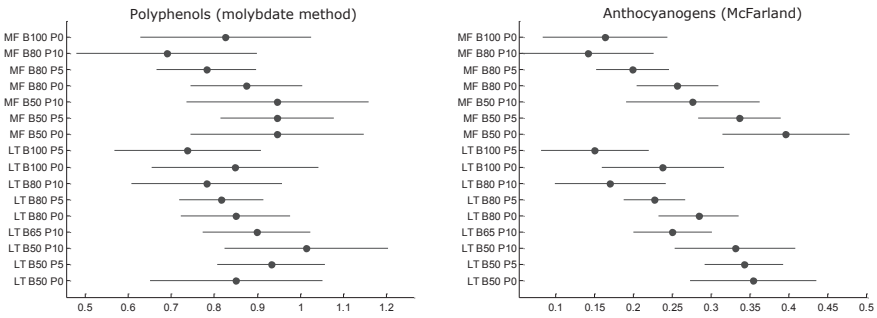
Appendix I-a: Extract (%m/m), glucose (%m/V), fructose (%m/V), maltose (%m/V), maltotriose (%m/V), total fermentable sugars (%m/V), apparent final attenuation limit (%), apparent extract after final attenuation (%m/m)



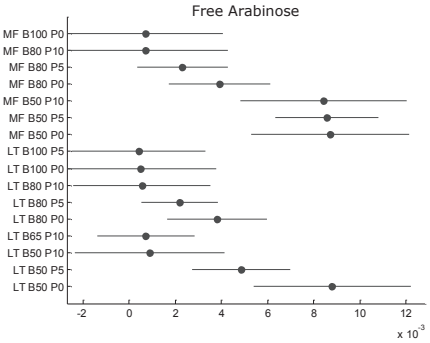
Appendix I-b: High molecular protein (HMP) (mg/L), free amino nitrogen (mg/L), total nitrogen (total UV digestion, mg/L)

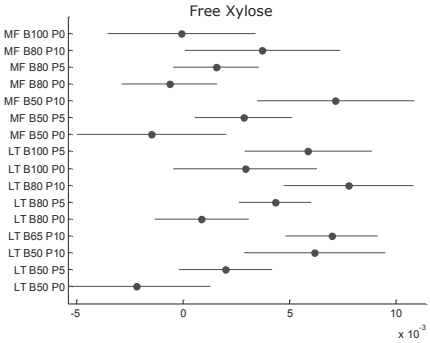


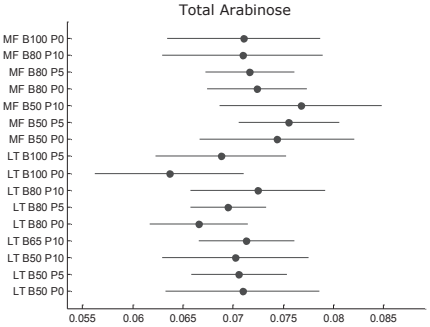
Appendix I-c: Total polyphenols (AU/10ml), anthocyanogens (AU/10ml)

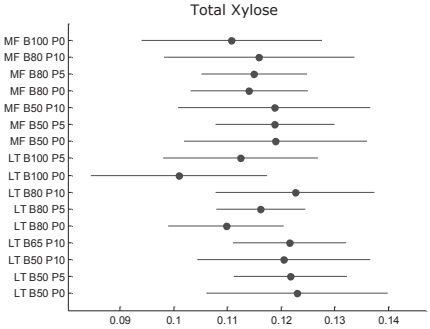


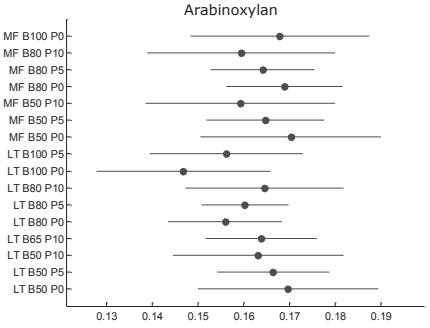
Appendix I-d: Free arabinose (%), free xylose (%), total arabinose (%), total xylose (%), arabinoxylan (%), high molecular weight β-glucan (mg/L), viscosity (mPa.s)

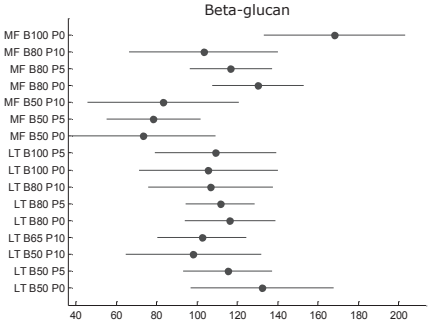


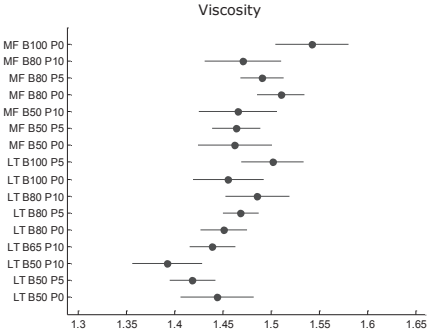




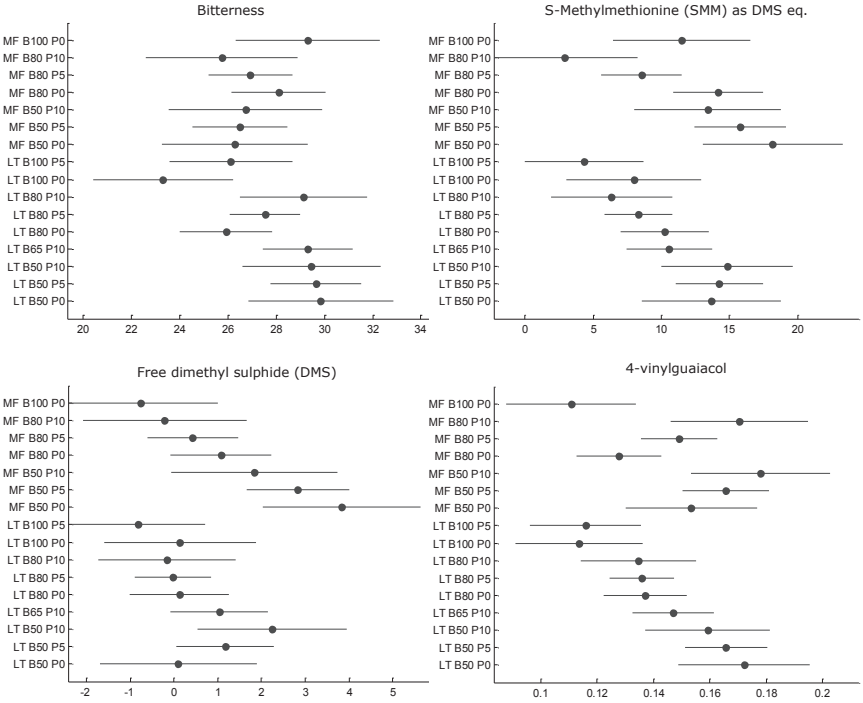




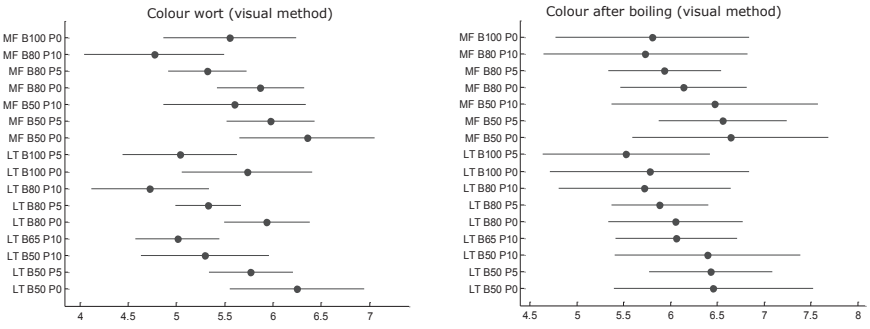




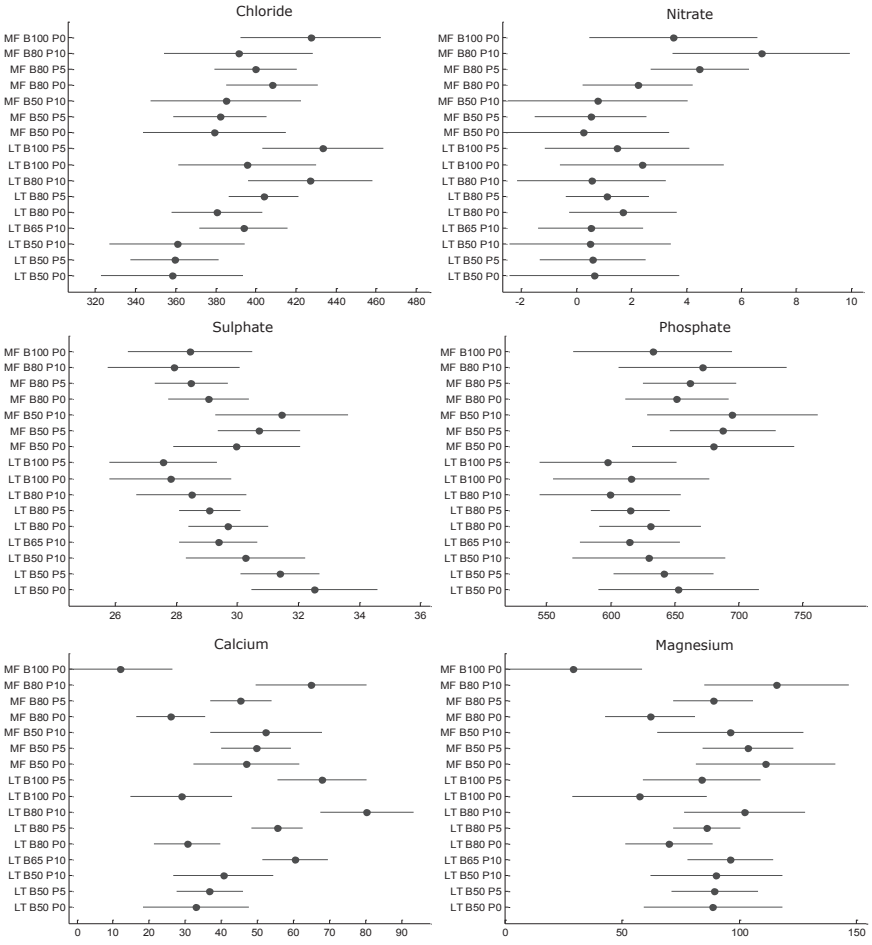
Appendix I-e: Bitterness (BU), s-methylmethionine (µg/L) free dimethyl sulphide (µg/L), 4-vinylguaiacol (mg/L)

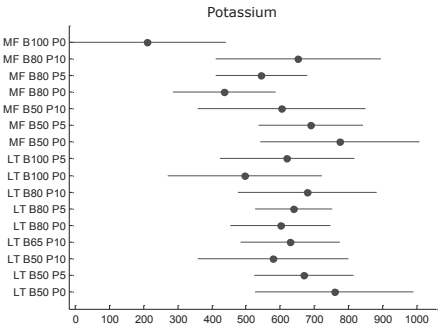


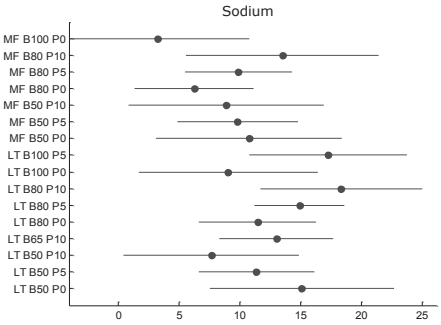
Appendix I-f: Colour of wort, colour of wort after boiling

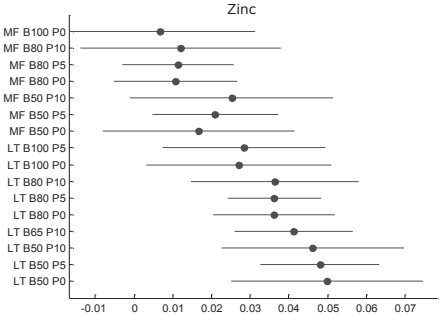


Appendix I-g: Chloride (mg/L), nitrate (mg/L), sulphate (mg/L), phosphate (mg/L), calcium (mg/L), magnesium (mg/L), potassium (mg/L), sodium (mg/L) and zinc (mg/L)

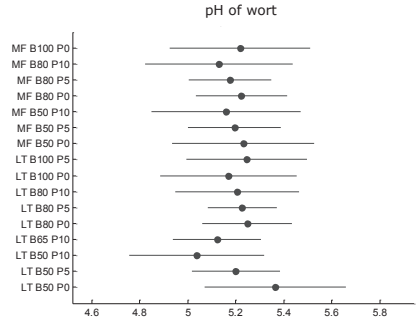




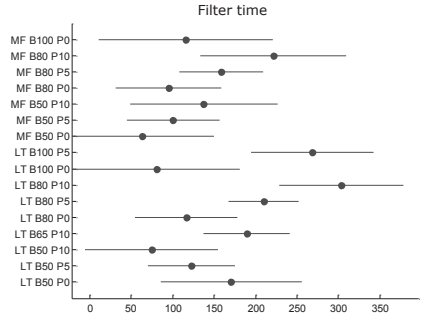




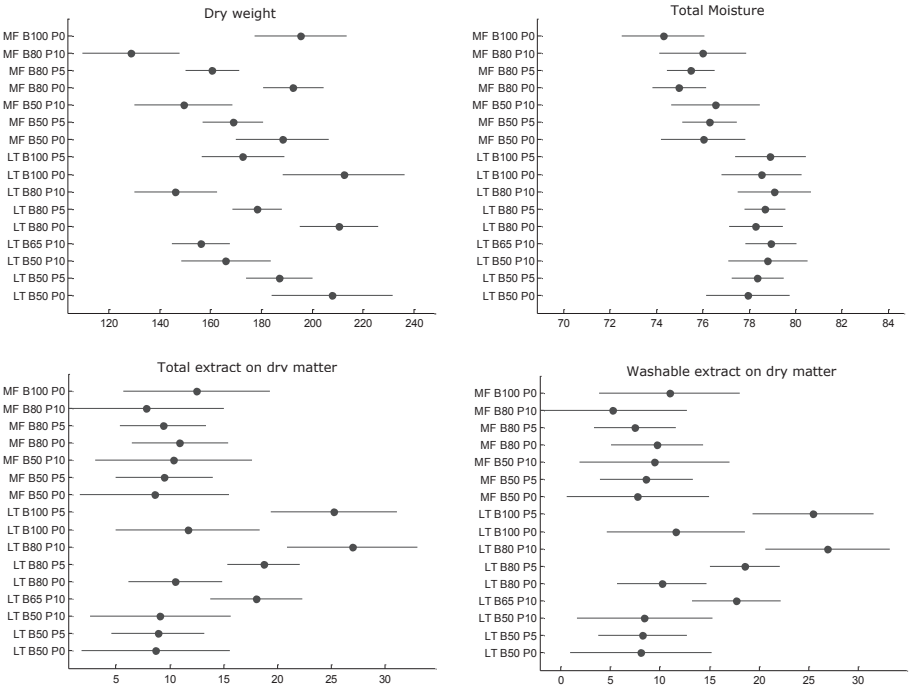
Appendix I-h: pH



Appendix I-i: Filter time (min)



Appendix I-j: dry weight (g), total moisture (%m/m), total extract (g), total extract on dry matter (%m/m), washable extract on dry matter (%m/m)



General discussion

Many traditional food production processes aim to convert one or more raw materials to one final ingredient or product. This focus often results in degraded side streams which might be utilised as by products or discarded as waste. Barley for food use, as an example, is mainly brewed for making beer. The part of the barley which is not used in the end product is called spent grains, and is discarded as waste or used as animal feed. These spent grains still contain components like polyphenols, dietary fibres and proteins. Barley thus contains potentially valuable components which do not end up in the beer. It also contains components which complicate the production process or degrade the end product quality.

The overall aim of this thesis was to investigate how barley can be fractionated to optimize the beer brewing process in terms of its use of resources, while maintaining the quality of the brewed beer. The work was based on the hypothesis that by pre-fractionation of the raw materials before use, it is possible to make better use of the raw materials, while at the same time allowing the brewing of beer of the same quality. This chapter gives an overview of the main findings of this thesis, after which it reflects on the selectivity of the pre-fractionation. The chapter concludes with an overview of how the outcome of this thesis can be utilised. In addition, it identifies other methods that can potentially further improve the fractionation or the brewing process.

6.1 Main findings and conclusions

The underlying hypothesis of this thesis is that the brewing process and the use of the raw material can be optimized by fractionating the barley components from each other. To this aim, one can make use of the properties of the barley components themselves, or of the natural distribution of components inside the barley kernel. The first option can be to make use of the thermomechanical properties of these components. Differences in the glass transition temperature (Stuart, Loi, & Fincher) have an impact on the breaking behaviour of the kernel. Knowledge of the T_g of the components in the barley kernels might thus allow for optimization of the milling process by pre-treating the barley to the right moisture content and then milling at the right temperature. **Chapter 2** describes the differences in T_g between starch and protein isolated from the barley endosperm, and indicates the importance of taking the distribution of the moisture content

inside the kernel into account. It shows how the fracture behaviour of the barley is different when compressing the kernel in different regions of the state diagram. The use of the T_g in practice was found to be challenging because of the high transition temperatures and because the T_g lines of barley starch and protein are very close in the state diagram.

In **chapter 3** we made use of the internal structure of the barley kernel to separate the components. Abrasive milling, or pearling, was used to fractionate the kernel in 5 different fractions. The first fraction is the outer layer of the barley, while the fifth fraction is the endosperm. Subsequently, 3 fractions constituting 5% w/w, 1 fraction constituting 10% w/w and 1 fraction constituting and 75% w/w of the total kernel respectively were obtained. The components were clearly distributed heterogeneously over the fractions, with the outer layer rich in fibres and polyphenols and the endosperm richer in starch. The fraction in which the aleurone layer was pearled off was especially rich in proteins. A linear relation was observed between the insoluble fibre content and the water holding capacity of the fractions.

Chapter 4 gives a resource efficiency analysis of the brewing process, and compares conventional brewing to a process in which exogenous enzymes and barley are used instead of malt. The total exergy input (cumulative exergy consumption) of the enzyme production process is 30 times as high as the standard chemical exergy of the enzyme. Therefore the cumulative exergy costs of enzymes and other purified ingredients should be taken into account when assessing the thermodynamic performance of the overall system. The use of exogenous enzymes, however, reduced the total exergy input for the production of 100 kg of beer from 441 MJ to 354 MJ. Moreover, beer produced with exogenous enzymes reduced the use of water by 7%, of raw materials by 14%, and of natural gas by 78%. The overall process efficiency increased from 45.7% to 55.7%.

In **Chapter 5** the effect of using pearled and unpearled barley during brewing is investigated using a microbrewery system. The malt-to-barley ratio, degree of pearling of the barley and the filter type were varied. The malt-to-barley ratio has the biggest impact, followed by the filter type. The influence of the filter type was partly because of the chosen filtering system, and partly because of the use of the different milling methods used to grind the barley. Pearling causes a decrease

in the free arabinose, S-methylmetionine and anthocyanogen content, and an increase in the apparent final attenuation limit of the wort. As a negative effect, it decreases the total fermentable sugars and increases the free xylose and filter time. When a mash filter was used, a window of operation (e.g. a range of malt/barley ratios and degrees of pearling) could be identified within which a good quality beer could be produced as evaluated on four quality parameters.

6.2 Comparing dry fractionation processes

Pearling and milling followed by a separation technique like sieving or air classification have been considered in in chapter 2 and 3 of this thesis. Pearling makes use of the natural distribution of components inside the barley kernel, while separating the components by milling the whole kernels makes use of the mechanical properties of the individual components, disregarding their spatial distribution in the kernels. The efficiency of the methods can be compared using several criteria. One such criterion is the *purity* or the concentration of a target component that is attained. The *separation efficiency* can also be used to compare fractionation methods and is defined as the mass percentage of the total component contained in the original flour that was recovered in a fraction (Schutyser & van der Goot, 2011). The separation efficiency **ε** can be determined with equation 1 (Tyler, Youngs, & Sosulski, 1981):

$$\epsilon = \frac{\phi_f \, C_f}{C_0} \tag{1}$$

Where ϕ_f is the yield fraction relative to the total barley flour, C_0 the concentration of the target component in the original flour and C_f is concentration of the target component in the product fraction. The component shift 6 is another parameter which can be used to compare separation processes and is known as the percentage of the total component content in the original flour which shifted into or out of a particular fraction (Rezsoe, 1960; Wu, Stringfellow, & Inglett, 1994). A positive shift indicates an enrichment and a negative shift indicates a depletion of the component in the fraction. The shift is calculated according to equation 2 (Schutyser & van der Goot, 2011):

$$\delta = \left(\frac{C_f - C_0}{C_0}\right)\phi_f \quad (2)$$

In table 1 pearling is compared with milling and sieving or air classification. The data as found in our lab from milling and separation experiments, are combined with some values in literature. Details on the fraction yield and concentrations and on milling and separation methods are shown in **appendix I**.

Table 1 shows that the effectiveness of pearling compared to other milling and separation techniques is dependent on the component that is to be separated. The numbers in the table correspond to those fractions having the highest concentration / separation efficiency / shift and lowest shift. The fraction with the highest concentration is not necessarily the fraction with the highest separation efficiency or shift. Milling and sieving or air classification yields fractions with higher concentrations of starch than pearling, however, the yield of these fractions is lower, leading to lower separation efficiencies and shifts.

The protein concentration obtained by pearling was higher than that reported in literature. The yield of this fraction was low, and therefore the shift was not very high. It was however possible to obtain a fraction that was more depleted in protein than the most depleted fraction obtained by milling in our laboratories.

Milling and separation yields higher component concentrations of β-glucan and higher shifts, than pearling. This is because the localization of β-glucan in the barley kernel is mainly in the endosperm cell walls. Pearling did not detach the endosperm components from each other, therefore not yielding high β-glucan levels. Pearling did yield high levels of fibre in the outer fractions, the highest being in the outer 5% of the kernel. Also another fraction with high depletion of β-glucan was obtained; this was the endosperm which naturally does not contain many fibres. A high separation efficiency was obtained because of the high yield of the fraction.

Milling experiments in our own laboratories focussed on separating starch and protein, and for these 2 components the concentrations and separation were similar to that of the highest values found in literature. These experiments were not optimized for separating beta glucan, which generally requires more sieving or classifications. This is reflected by the lower yields and separation in our processes compared to those found in literature.

Table 1. Concentration, separation efficiency and shift of starch, protein, β-glucan and fibres in fractions separated by pearling or milling and sieving/air classification.

Component	Pearling[a]				Milling & Sieving our lab				Milling & sieving/air classification data from literature			
	Conc (w/w %)	ε	Shift High	Shift Low	Conc (w/w %)	ε	Shift High	Shift Low	Conc (w/w %)	ε	Shift High	Shift Low
Starch	77.2	92.6	17.4	-5.1	76.2[b]	67.8[b]	16.4[b]	-6.8[b]	80.0[e]	80.2[e]	13.2[e]	n.a
Protein	18.3	62.9	7.4	-12.3	14.0[c]	45.3[c]	2.4[c]	-2.2[c]	14.0[f]	50.0[f]	18. 0[f]	n.a
β-glucan	4.9	94.0	18.8	-4.9	12.9[d]	37.1[d]	25.3[d]	-50.5[d]	17.6[g]	68.7[g]	48.0[g]	n.a
Fibre	82.9	26.0	18.8	-49.2	n.a.				n.a.			

a: Satake pearling machine (van Donkelaar, Noordman, Boom, & van der Goot, 2015)
b: Hosokawa Alpine fine impact mill equipped with a pin disc (22000 RPM) and sieving
c: Hosokawa Alpine fine impact mill equipped with a fine cutter (18000 RPM) and sieving
d: Hosokawa Alpine fine impact mill equipped with a pin disc (22000 RPM) and sieving. Debranned barley was used.
e: Impact mill and air classification (Sundberg & Åman, 1994).
f: OHG mill (Brabender Duisburg) equipped with a 0.7-mm screen and air classification (Andersson, Andersson, & Aman, 2000)
g: Udy Corporation, Fort Collins, CO abrasive mill and sieving of abraded barley (B. E. Knuckles, Chiu, M.C.M. and Betschart, A.A., 1992)

Summarizing, milling is more effective than pearling for creating a concentrated β-glucan stream, and separating starch and protein from the endosperm from each other. It can yield fractions which are purer than fractions obtained with pearling. Pearling is effective to remove fibres into a stream that contains very little starch, leaving the majority of the kernel with almost all of the starch. Because of the low starch losses and high starch separation efficiency, pearling is the more appropriate method for mild separation of barley components for the purpose of efficient brewing.

6.3 Pearling

6.3.1 Effect of pearling on milling behaviour

In Chapter 5 we reported on milling barley and pearled barley before they were used for brewing. The negative effects of the filter, such as higher losses in the spent grains and decreased fermentable sugar content, were in fact due to a combination of the filter type and type of the milling equipment. A roller mill leaves a large fraction of the husk intact, and generally gives coarser particles than a hammer mill: in this mill, the flour has to pass a sieve before leaving the mill, and the particle sizes will therefore be smaller.

The particle size distribution is also influenced by the material properties of the milled material. In pearling, the husks are removed, and since these have different properties from the endosperm (Mabille, 2001), the resulting particle size distribution is different. **Figure 1** shows the particle size distribution for flour of pearled and unpearled barley milled with a roller mill and a mash filter.

In a roller mill, the pearling does not affect the particle size distribution of barley flour. With unpearled barley, however, the fraction of larger particles consists mainly of husks, which remain largely intact. These are utilised later to form a filter bed in the lauter tun (O'Rourke, 1999). For the pearled barley flour almost all husks are removed, and here, the coarse particles consist largely of endosperm. This means that more starch is located in larger particles, which reduces the accessibility of the starch for enzymatic hydrolysis during mashing. This may well be the reason for the reduced efficiency (reduced amount starch conversion into fermentable sugars) of the processes which used a lauter tun, that was found in chapter 5.

When a hammer mill was used, a larger difference in particle size distribution was observed. The reference (unpearled) sample had a relatively wide particle size distribution. Pearled barley flour gave a smaller average particle size with a peak in the size range of 0.25-0.50 mm (retrieved in the 0.250mm sieve). This may be one of the reasons why pearling has less influence on the starch conversion when a hammer mill and mash filter combination is used, compared to the roller mill and lauter tun. The particle size distribution also helps to explain why the hammer mill is more suitable to milling pearled barley than the roller mill: the particles that contain the starch are on average coarser in the roller mill, and therefore the starch is less accessible for enzymatic hydrolysis during mashing.

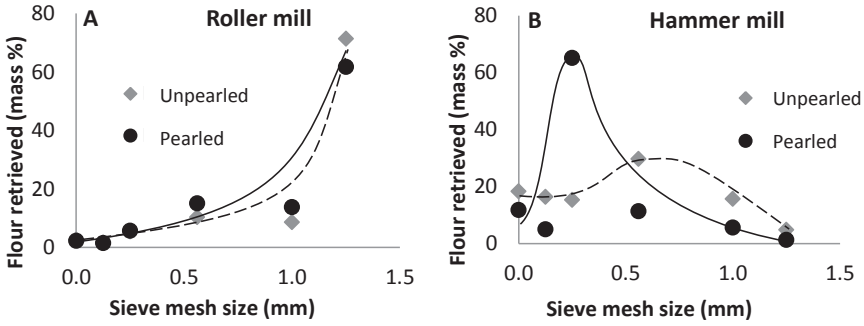


Figure 1. Particle size distribution of pearled (10%) and unpearled (=reference) barley milled by hammer mill (A) and roller mill (B). Hammer mill was equipped with a 1.5 mm sieve, the roller mill settings were 1.3 mm for the first roller and 0.5 mm for the second. Flour was sieved with a Retsch AS 400 shaker at 300 rpm (10 minutes). Lines are to guide the eye.

6.3.2 Sustainability assessment of brewing and pearling

In chapter 4 it was shown that the loss in chemical exergy accounts for the largest reduction in exergetic process efficiency. The exergetic efficiency of the process can thus only be increased by pearling if the gain in bran fraction weighs up to the loss in starch (and consequently the amount of beer that can be produced from this starch). Pearling to a degree of 5% will produce a 5 kg bran fraction per 100 kg barley, which corresponds to a standard chemical exergy content of 88.2 MJ. The loss in starch would be 0.205 kg, which in itself represents about 3.62 MJ of chemical exergy. This amount of starch mixed with water, however, would produce 2.29 kg of beer (useful end product), corresponding to 4.17 MJ exergy. Assuming that both bran and beer streams would be used for human consumption, equal amounts of exergy input could be allocated to both bran and beer streams per kg of these streams. Pearling would thus increase the total useful exergy output by about 84 MJ.

In this calculation it was assumed that the milling energy for pearling is negligible in comparison to the standard chemical exergy of the streams. It was also assumed that the efficiency of the brewing process is qualitatively unaffected by pearling, while the amount of beer that is produced is linearly related with the amount of starch going into the process. When using a degree of pearling of 10%, a theoretical increase of useful exergy of about 161 MJ could be achieved. A brewing process that uses unmalted barley as a raw material and includes pearling would therefore be an exergetically more efficient process than to a process using unpearled barley.

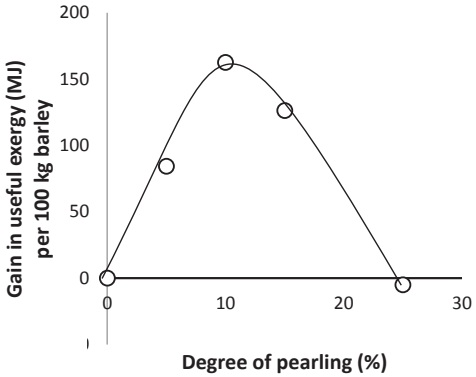


Figure 2. The gain in useful exergy output of the brewing/bran production process as a function of the degree of pearling of the barley.

Figure 2 shows the overall gain in useful exergy output as a function of the degree of pearling. Pearling up to a degree of 10% gives the most environmental gain, and pearling up to 24% would still be more beneficial than not pearling at all. Pearling up to 5% would only result in a 0.2% starch loss in the raw material. If milling and filtration processes would be optimized for processing unmalted pearled barley, the use of 5% pearled barley would probably not result in high losses for brewers. The 0.2% of starch that is lost in pearling would result in 2.1 kg of beer per 100 kg barley. The loss of this beer is the cost of producing 5 kg of bran fraction. Therefore if the bran fraction could be sold for half the price of beer, pearling would also have economic benefits. Pearling to a degree of 10% would result in a loss of 7.6 kg beer per 100 kg barley. The bran fraction would therefore have to sell for about 75% of the price of beer. The composition of the bran fraction changes with the degree of pearling: the starch content is increased with an increased degree of pearling. This change in composition will affect the usability of this fraction in other products.

6.3.3 Making use of the pearled bran fraction

For an efficient use of resources, it is important that the bran fraction that is pearled-off is used in other products. This fraction has potential to be used as functional ingredient because it contains several components like arabinoxylans, β-glucans, and polyphenols. These components can be used in products that claim beneficial health effects. It has been reported that barley bran enhanced diets lower cholesterol values in hypercholerestic rats as well as in people (Behall, Scholfield, & Hallfrisch, 2004; El Rabey, Al-Seeni, & Amer, 2013; Lupton, Robinson, & Morin, 1994). The insoluble fibres reduce the risk of colon cancer and reduce the absorption of glucose in the digestive system (Potty, 1996). Some studies have successfully tried to incorporate barley fractions in commercial foods like biscuits, pasta or bread (B. Knuckles, Hudson, Chiu, & Sayre, 1997; Marconi, Graziano, & Cubadda, 2000; Sudha, Vetrimani, & Leelavathi, 2007). It is therefore reasonable to assume that the fraction can be used for human consumption and therefore that the exergetic input for the process can indeed be partially allocated to the bran fraction. It should be noted that for the bran fraction to have potential on larger scales, breweries should make agreements with bakers and other food producers about its use. Since the brans fraction remains dry and unprocessed on microscale, it can however be easily stored and transported over longer times and distances.

6.3.4 Pearling as a pre-treatment for malting

In chapter 5, pearling was considered as a pre-treatment for brewing. In the experiments exogenous enzymes were used. If a brewery does not want to add these enzymes to their product, malting is necessary. Pearling can be combined with malting: it combines the advantages of creating a dry and valuable side stream with creating the endogenous enzymatic activity by malting. Pearling can however affect the malting process in several ways. For example, water enters the kernel mostly via the embryo region of the barley and less through the rest of the husk, especially in the beginning of the malting process. Water initiates the germination process (e.g. enzyme synthesis) (Kunze, 2010). Because pearling damages the husk, it increases the water uptake. The effect of pearling on the uptake of water is illustrated in **figure 3.** The average uptake was ±17% higher when the barley was pearled before starting the malting process. Both pearled and unpearled samples reached the moisture content of 37% - 40% which is preferred before germination (Kunze, 2010).

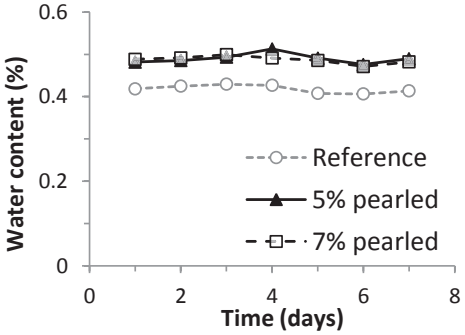


Figure 3. water content in germinating barley for pearled and unpearled (reference) samples from day 1 to 7 of germination. The sample of day 1 is taken right after steeping. Average water contents of triplicate measurements were 41.8% (reference), 49.0% (5% pearled barley) and 48.7% (7% pearled barley).

Another effect of malting relates to the bacteria and mycotoxins that are located on the bran of the barley grain, and which are largely removed by pearling. However, pearling removes the protective layer of the barley kernel, making the kernels more susceptible to any remaining microorganisms. This makes the kernels more susceptible to spoilage. Because of the damaged husk, some of the endosperm is not protected anymore which can also make the kernels stickier. The most important disadvantage of pearling might however be an impaired germination process. Both the embryo and the aleurone layer are essential in the enzyme synthesis process, and if these are damaged less enzymes will be synthesized. It is necessary that sufficient enzyme activity remains after malting when pearling is used as a pre-treatment.

Figure 4 shows the α-amylase and β-glucanase activity of germinating barley in time. The graph shows that the enzyme synthesis of these enzymes is indeed reduced by pearling. After 6 days of germination the α-amylase activity of 5% and 7% barley was only 54% and 13% of that of the reference barley, respectively. The β-glucanase activity of 5% and 7% pearled barley was 64% and 20% of the reference barley, respectively. The enzyme activity of 13% pearled barley was almost zero for both enzymes. The enzyme activation in the reference barley started after day 2, and after day 3 for 5% and 7% pearled barley. It can be concluded that pearling slows but does not inhibit enzyme synthesis in germinating barley, and that a degree of pearling of 5% already damages the kernel enough to significantly decrease the enzyme activity of the kernel.

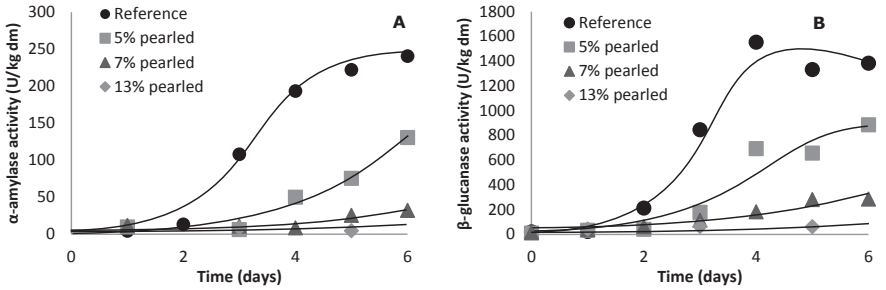


Figure 4. Enzyme activity of A. α-amylase and B. β-glucanase of germinating unpearled and pearled barley from day 0-6. The graphs give the average of two independent germination experiments. Lines are to guide the eye.

After germination, barley is kilned to add to the taste of the malt and to ensure shelf life. Kilning, however, lowers the enzyme activity of the malt due to thermal inactivation of the enzymes. The enzyme activity of β-amylase is typically reduced by 40% and β-glucanase is reduced by about 60% during kilning (Georg-Kraemer, Caierao, Minella, Barbosa-Neto, & Cavalli, 2004; Kunze, 2010). The activity of α-amylase, on the other hand, increases by about 15% (Kunze, 2010). Breweries could consider brewing with germinated but unkilned malt, also called green malt. This would eliminate an energy intensive step and prevent enzyme inactivation during kilning. This would be especially beneficial when pearled barley is considered, because of its lower enzyme activity. The use of green malt, however, would introduce challenges from a logistics, process flexibility and safety point of view: the green malt has a high water activity, and is thus more susceptible to spoilage.

Table 2. The fermentable sugar content (mg/ml), amount of dry matter which ends up in the wort as sugars (%), β-glucan content (%) of the wort produced with reference (not pearled) barley, 5% pearled barley and 7% pearled barley by mashing with a 1.5h sacharfication (64 ºC) time. No exogenous enzymes are added.

Sample	Fermentable sugar content (mg/ml)	Amount of dry matter which ends up in the wort as fermentable sugars (%)	β-glucan content (mg/L)
Reference	72.1	27.9	0.8
5% pearled	71.8	25.1	2.5
7% pearled	71.3	23.8	15.1

Table 2 shows that less breakdown occurs during brewing with pearled barley, because less sugars are produced. Even though the concentration of sugars in the wort is similar, the filtration of pearled barley was slower and yielded more and wetter spent grains compared to the reference. More β-glucan remains in the wort when the barley was pearled, indicating less breakdown of cell wall material. Therefore we can conclude that the amount of active enzymes in pearled germinated barley is reduced leading to less hydrolysis of the barley components. The reduction in fermentable sugars in the wort was, however, only 10% for a degree of 5% and 15% for a degree of pearling of 10%. This decrease in wort quality could be improved by adapting the milling process of the germinated grains or by increasing the germination time of the pearled barley.

6.4 Outline for future research

From the graph in section 6.3.2 of this discussion we can conclude that pearling to a degree of 10% is optimal in terms of exergy. From chapter 4 we know that a good quality beer can be brewed when 75% to 80% of 10% pearled barley is used together with 20%-25% of malt by using a mash filter. Therefore, a malt-to-10%-pearled-barley ratio of 20/80 is suggested in order to produce a good quality beer with a reduced environmental impact. This way only one fifth of the barley will have to be malted, while at the same time a valuable side stream is generated. The process efficiency could be improved by optimizing the milling of the pearled barley, to prevent the formation of a dense filter bed.

Because beer is a wet product, one could also consider wet fractionation as a pre-fractionation. As long as water is used as a medium for separation and the amount of water is less than the amount needed for brewing, one could separate components from each other directly before brewing. This technique would be especially suitable to separate a starch-rich slurry from the rest of the mixture, since starch is loosely embedded in the endosperm structure, which will allow the starch granules to be suspended in the water. Furthermore the density of the starch is higher than that of most other barley components, which allows gravitational or centrifugal separation of a starch slurry from the suspension. This fractionation would not reduce the amount of spent grains, but would give the opportunity to mash different fractions at different times and/or temperatures, that are optimal

for the breakdown of the components present in that fraction. A disadvantage of this separation method is that separation would have to take place directly before brewing, as the wet material has a limited shelf life and a larger volume compared to the dry material. This would reduce the flexibility of the process.

Instead of pearling hulled barley, one could consider using hull-less barley, a relatively new barley variety for the use of brewing. Using hull-less barley would allow the omission of the pearling step. In contrast to hulled barley, which has traditionally been used and adapted to optimize it for malting, the use of hull-less barley has not been optimized yet. Efforts have been made only recently to malt hull-less barley, and malts with good (low) protein and β-glucan levels and high extract have already been produced, though the focus was not yet on the enzyme activity in these malts (Edney, Rossnagel, & legge, 2011), which was found to be somewhat lower than that of hulled barley (Bhatty, 1996). Due to the absence of the husk, hull-less barley has a higher amount of starch per weight of malt, giving higher extract yields. Furthermore it contains less polyphenols and therefore gives less haze in the beer. Filtration times for mashes produced with hull-less barley, however, were longer than those of the mashes produced with hulled barley (Bhatty, 1999). We also found this to be the case for pearled hulled barley in chapter 5, where it was attributed to a denser filter bed. A disadvantage of using hull-less barley is its lower yield per area. Therefore the use of this new raw material has to be compared to using hulled barley, combined with pearling, and future studies have to elucidate which alternative may have the highest overall yield.

In this research we used pearling as a process to pre-fractionate barley before brewing, to increase resource efficiency use. Many food production processes use a raw material and leave a spent fraction after the extraction of the required components. Examples are the production of soy milk, after which the spent soybeans are left as okara. Okara has a moisture content of 80%-85%, and its dry matter mainly contains fibres (50%-60%) (Li et al., 2012). Okara is highly susceptible to spoilage due to its high water activity. Therefore it has to be dried or immediately used into other products, or it has to be discarded. Reduction of the volume of okara would therefore reduce the amount of resources needed overall, and allow better use of the fibrous fraction for other purposes, food or non-food. Just like in brewing, removing the fibres would reduce the volume of the okara, and reduce the losses in this stream. Also outside the direct realm of food production, the

concept of pre-processing may have potential. In the production of bioethanol, removal of most of the fibre and protein from corn would still allow good fermentation of the starchy part, would reduce the energy needed for drying the spent grains, and would open other applications for the fibre and protein fractions. In these cases, pearling may or may not be the most effective process, and other dry pre-processing methods may be more suitable.

It is clear from this thesis that for dry raw materials such as barley, dry pre-processing could lead to less waste, a reduction in the use of energy and water, and to better use of the other fractions. It therefore fits in the philosophy of bio-refining, in which raw materials should be completely utilised for high-value products. This can be done best by retaining the raw materials in their original state as long as possible. Pearling is an example of a process in which the internal anatomy of the raw material is used as starting point for the design of a separation process. We feel that using this principle in addition to using the different physicochemical properties of the individual components, has potential for better overall separation while leaving the quality of the components intact as long as possible. This concept thus fits naturally in any bio-refining concept, for food or non-food production, or for combined purposes.

6.5 Appendix

Data used for **table 1** of the discussion are shown in **table A1** (pearling data), **table A2** (milling experiments) and **table A3** (literature data) of this appendix. Unless mentioned otherwise, barley of the variety Sebastien with a moisture content of 13% was used for the experiments. Data used in the discussion are in bold underlined.

Table 1. Composition, separation efficiency and shift of fractions obtained by pearling. Compositional data and yield from van Donkelaar et al. (van Donkelaar et al., 2015).

Fraction composition (w/w dm %)					
Fraction	**Yield**	**Starch**	**Protein**	**Glucan**	**Ins fibre**
1	5.3	2.5	5.7	0.3	**82.9**
2	4.4	7.8	10.8	1.3	68.8
3	5.1	24.3	**18.3**	3.1	39.5
4	10.1	44.6	17	**4.9**	23.9
5	75.2	**77.2**	8.2	4.5	6.3
Whole barley	100	62.7	9.8	3.6	18.2
Component separation efficiency					
Fraction	**Yield**	**Starch**	**Protein**	**Glucan**	**Ins fibre**
1	5.3	0.2	3.1	0.4	24.1
2	4.4	0.5	4.8	1.6	16.6
3	5.1	2.0	9.5	4.4	11.1
4	10.1	7.2	17.5	13.7	13.3
5	75.2	**92.6**	**62.9**	**94.0**	**26.0**
Whole barley		100.0	100.0	100.0	100.0
Component shift (δ)					
Fraction	**Yield**	**Starch**	**Protein**	**Glucan**	**Ins fibre**
1	5.3	**-5.1**	-2.2	**-4.9**	**18.8**
2	4.4	-3.9	0.4	-2.8	12.2
3	5.1	-3.1	4.4	-0.7	6.0
4	10.1	-2.9	**7.4**	3.6	3.2
5	75.2	**17.4**	**-12.3**	**18.8**	**-49.2**
Whole barley		0	0	0	0

Table 2. Fraction yield and component content, component separation efficiency and shift for starch, protein and β-glucan. Fractions were obtained by milling (pearled) barley barley with different mills.

Starch separation of whole barley, pin mill[a]				
Fraction (µm)	**Yield (%)**	**Starch (w/w dm %)**	**ε**	**Shift**
>710	3.7	63.4	4.0	0.4
710-500	3.4	42.0	2.5	-0.9
500-250	11.8	27.6	5.6	-6.1
250-100	16.6	33.9	9.8	**-6.8**
100-50	10.9	44.9	8.4	-2.4
50-20	51.4	**76.2**	**67.8**	**16.4**
<20	2.3	69.9	2.8	0.5
Whole Barley	100	57.7	100	0
Protein separation of whole barley, fine cutter mill[b]				
Fraction (µm)	**Yield (%)**	**Protein (w/w dm %)**	**ε**	**Shift**
>250	8.0	13.3	10.4	**2.4**
250-100	29.7	10.4	30.2	0.5
100-50	13.6	11.1	14.8	1.2
50-20	47.5	9.7	**45.3**	**-2.2**
<20	1.2	**14.0**	1.6	0.4
Whole Barley	100	10.2	100	0
β-glucan separation of pearled barley, pin mill[c]				
Fraction (µm)	**Yield (%)**	**β-glucan (w/w dm %)**	**ε**	**Shift**
>250	2.9	**12.9**	9.6	6.6
250-100	11.4	12.6	**37.1**	**25.3**
100-50	8.9	12.4	28.1	19.4
50-20	59.7	0.6	9.9	**-50.5**
<20	17.1	0.4	1.6	-15.4
Whole Barley	100	3.9	100	0
β-glucan separation of whole barley, pin mill[d]				
Fraction (µm)	**Yield (%)**	**β-glucan[e] (w/w dm %)**	**ε**	**Shift**
>250	6.3	8. 3	13.9	7.6
250-100	20.0	8.8	**46.5**	**26.5**
100-50	12.1	**8.9**	28.5	16.5
50-20	60.4	1.1	17.1	**-43.3**
<20	1.2	1.0	0.3	-0.87
Whole barley	100	3.8	100	0

[a] Hosokawa Alpine mill equipped with a pin mill, 22000 RPM

[b] Hosokawa Alpine mill equipped with a fine cutter mill, 18000 RPM

[c] Hosokawa Alpine mill equipped with a pin mill, 22000 RPM . Pearled barley (±25% of its weight removed) was used in this experiment.

[d] Hosokawa Alpine mill equipped with a pin mill, 22000 RPM. Moisture content of the barley was conditioned to 7.7% before milling.

[e] Additional information on milling with unpearled barley. Not used in discussion.

Table 3. Literature data used in discussion table 1.

Component	Fraction yield (%)	Start concentration (w/w%)	Fraction concentration (w/w%)	ε	shift
Starch[a]	67.0	66.8	**80.0**	**80.2**	**13.2**
Protein[b]	32.2	9.0	**14.3**	**51.1**	**19.0**
β-glucan[c]	20.7	5.1	**17.6**	**71.4**	**50.7**

[a] Barley of the variety Hora was milled using a MLU 202 laboratory mill (Laboratoriums Mahlautomat Modell MLU 202, Gebruder Buhler Maschinenfabrik, Uzwill, Schweiz) equipped with side screens (Sundberg & Åman, 1994).

[b] Barley of the variety Golf was milled in an OHG mill (Brabender Duisburg) equipped with a 0.7mm screen at 16000 RPM, and subsequently air classified with a pilot system from Alpine AG (Ausberg, Germany) including a Circoplex classifier mill 50 (ZP) with counter rotating beaters and a Turboplex utra-fine classier 50 (ATP) with horizontally rinning classifying wheel axis (4000 RPM) (Andersson et al., 2000).

[c] Barley of the variety Klages was pearled (11 w/w% was removed), milled with an abrasive udy mill (B. E. Knuckles, Chiu, M.C.M. and Betschart, A.A., 1992).

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Summary

Beer is a globally consumed beverage, which is produced from malted barley, water, hops and yeast. In recent years, the use of unmalted barley and exogenous enzymes have become more popular because they enable simpler processing and reduced environmental impact. Raw barley, however, contains less endogenous enzymes and more undesired components for the use of beer brewing, compared to malted barley.

The overall aim of this thesis is to investigate how barley can be fractionated to optimize the use of resources for the beer brewing process, while maintaining the quality of the brewed beer. A resource use efficiency analysis was performed to verify the presumed benefits on the environmental sustainability of the proposed process change. The work was based on the hypothesis that fractionation of the unprocessed barley will reduce the amount of undesired components, which leads to improvements in the brewing process based on partial or no malting. Fractionation can be performed by milling and separation, which requires physical disentanglement of the components. This fractionation can be influenced by properties of the components of the material, such as the glass transition temperature (Stuart et al.). Fractionation by abrasive milling, also known as pearling, is another possibility: here one makes use of the spatial distribution of components in the kernels. In case of barley for brewing this technique is especially promising as most of the undesired components are in the outer layer of the kernel. In addition, the removal of bran from the barley reduces the amount of water needed in the process. It will also reduce the volume of spent grains, hence reducing wastes and energy required for drying the spent grains. A disadvantage of pearling is however that it lowers the ability of the barley kernel to produce enzymes. This leads to the need of the addition of exogenous enzymes, as is the case when the malting step is omitted.

Chapter 2 describes the glass-to-rubber transition of protein and starch isolated from the barley endosperm, for different moisture levels. The hypothesis for this chapter is that dry fractionation by milling is facilitated by milling conditions in which the protein is in a rubbery state and the starch in a glassy state. Two methods were used to measure the T_g; differential scanning calorimetry (DSC) and thermo-mechanical compression tests (TMCT). The methods gave different

results due to the differences in moisture content range, and heating rates, which may lead to conformational changes of the protein. The value of the T_g of partially crystalline materials, such as starch in barley, was not unambiguous when using TMCT because the mechanical effect of expansion of these materials was smaller. For both results, the T_g lines were modelled using the Gordon-Taylor equation. Based on sorption isotherms, it was concluded that moisture does not distribute evenly over the protein and starch in the kernel. Starch absorbs more moisture than protein at given water activities. This required a correction of the T_g lines. After this correction, the glass transition lines of starch and protein were closer together. The expectation is therefore that achieving good separation between the components based on having one glassy component and one rubbery component is challenging.

For this reason, another dry fractionation technique, pearling, was considered. **Chapter 3** describes the chemical composition of the barley and of fractions removed by pearling. Pearling was shown to selectively remove insoluble fibre, ash, protein and polyphenols, while the β-amylase activity and starch content of the remaining kernel was hardly affected. For example, removing the outer 5% of the kernel reduced insoluble arabinoxylans (15%), insoluble fibres (23%), ash (19%), polyphenols (11%) and water holding capacity of the non-starch components (25%), while only lowering starch content by 0.20%. The water holding capacity of the barley fractions was strongly related to the fibre content. This indicates that when the fibre content in the mash was reduced by pearling, the spent grains will take up less water, leading to less wort and sugar losses in this waste stream, and hence better use of the raw materials and less wastes.

Chapter 4 compares a traditional brewing process to an enzyme-assisted brewing process with respect to their resource use efficiency, which is one aspect of the sustainability of the processes. The use of exogenous enzymes is found to be more efficient than producing enzymes through the malting process. The exergetic efficiency of the conventional malting process was 77%. The main losses stem from the use of natural gas for removal of moisture from the barley in the kilning process, and from the loss of starch in the germination process. In case of the use of exogenous enzymes, it was concluded that the chemical exergy content of the enzymes was not a good measure for the exergy content of the enzymes. Instead, we proposed to use the cumulative exergetic consumption in the enzyme

production rather than just the chemical exergy content of the enzymes. This cumulative exergetic consumption in the production of the enzymes was ± 30 times higher than their standard chemical exergy. This shows that the cumulative exergetic costs of minor components should be taken into account if a process uses them in significant quantities. This can be done by extending the system boundaries to include the production process of the purified components. The exergy efficiency of the enzyme formulation production process ranges between 20% and 42% depending on whether the by-product of the fermentation broth was considered as useful as the enzyme product. Even though the cumulative exergy consumption of the process was 30 times the standard chemical exergy of the dry enzyme, the total exergy input (i.e. both wasted and destroyed) for the production of 100 kg of beer was still larger for the conventional malting process (441 MJ) than for the enzyme-assisted process (354 MJ). In addition, beer produced using exogenous enzymes reduces the use of water by 7%, of raw materials by 14%, and of natural gas by 78%. Thus, the exergy loss of the enzyme production process is more than compensated by the prevention of exergy loss in the total beer brewing process.

Chapter 5 describes brewing tests using malted, unmalted and pearled, unmalted barley kernels. Brewing with unmalted barley saves material, energy and water in the malting stage but may result in complications during processing. Pearling mitigates these problems. Exogenous enzymes were used to compensate for the low enzyme activity in unmalted barley. Lautertun filtration and mash filtration were considered as filtration methods. Principle component analysis was performed on the chemical composition of the wort and the various spent grains, to investigate the effect of the malt-to-barley ratio, the degree of pearling and the filter method. A mash filter is optimal for this type of process, and we identified a window of operation in which optimal use is made of the raw materials while maintaining the end product quality, judged on basis of 4 quality parameters.

The concluding **chapter 6** presents a general discussion of all results described in this thesis. In addition, the benefits of pearling over that of milling and fractionation, and the effect of pearling on milling properties were discussed. Furthermore, it explores the advantages in environmental sustainability that can be achieved by pearling. Pearling as a pre-treatment for malting reduces the enzyme activity of germinating barley, and therefore the mash quality.

This thesis provides insights in how pre-treatment of barley can make beer brewing more efficient in the use of resources. It stresses the need to optimally use all material streams in a process, to be able to design an environmentally sustainable process, and it shows that efficient resource use is key for achieving this. Additionally the value of enzymes as processing aids was discussed. A clear result is that one needs to include the resource use in the production of enzymes or other processing aids, when analysing the environmental sustainability of a process, since this can be significant in the overall process.

Samenvatting

Bier is een wereldwijd geconsumeerde drank, die gemaakt wordt van gerst, water, hop, en gist. Recent is er meer aandacht gekomen voor het gebruik van ongemout gerst en exogene enzymen, omdat dit voordelen oplevert zoals een versimpeld proces en een verminderde milieubelasting. Vergeleken met gemout gerst geeft ongemout gerst minder endogene enzymactiviteit, en meer componenten die ongewenst zijn tijdens het bier brouw proces.

Het algemene doel van dit proefschrift was om te onderzoeken hoe gerst gefractioneerd kan worden om het gebruik van grondstoffen in het bierbrouwproces te optimaliseren, terwijl de kwaliteit van het gebrouwen bier gewaarborgd blijft. Om de vermeende vermindering van milieubelasting van het nieuw ontworpen proces te verifiëren is een analyse van het grondstofgebruik uitgevoerd. De vermindering in milieubelasting is gebaseerd op de hypothese dat de fractionering van de onbewerkte gerst de hoeveelheid ongewenste componenten kan verminderen, wat zal leiden tot verbeteringen in een brouwproces waarin gebruik gemaakt wordt van niet of gedeeltelijk gemout gerst. De mogelijke toepassing van zo'n initieel fractioneringsproces wordt beïnvloed door de eigenschappen van de te fractioneren componenten. Eén van de bepalende eigenschappen is de glasovergangstemperatuur (Tg).

Hoofdstuk 2 beschrijft de glas-overgang van de eiwitten en het zetmeel geïsoleerd uit de endosperm van de gerst. De hypothese hier is dat droog fractioneren malen als eerste stap wordt vergemakkelijkt door maalomstandigheden te kiezen waarin de eiwitten zich in een rubbertoestand bevinden en het zetmeel zich al in de glasfase bevindt. Twee methoden zijn gebruikt om de Tg te meten; differentiële scanning calorimetrie (DSC) en thermisch-mechanische compressietesten (TMCT). De twee methoden gaven verschillende resultaten, door de verschillen in de vochtgehalten van de monsters en verschillen in verhittingssnelheid, die tot conformatieveranderingen van de eiwitten zouden kunnen leiden. De waarde van de Tg van gedeeltelijk kristallijne materialen, zoals zetmeel in gerst, was moeilijk te bepalen via de TMCT methode, omdat de uitzetting van deze materialen klein is. Voor beide meetmethoden zijn de Tg lijnen gemodelleerd met behulp van de Gordon-Taylor vergelijking. Gebaseerd op de sorptie-isothermen van de materialen is de conclusie getrokken dat de vochtverdeling in de gerst korrel niet

homogeen is. Zetmeel neemt meer water op dan eiwit, waardoor een correctie in de Tg lijnen nodig was. Na deze correctie lagen de glasovergangen dichter bij elkaar. De verwachting is daarom dat het bereiken van een goede scheiding tussen zetmeel en eiwit in gerst gebaseerd op verschillen in Tg lastig zal zijn.

Een andere optie is het fractioneren door middel van het afschuren van de buitenste lagen, ook wel pearlen genoemd. In dit geval maakt men gebruik van de structuur van de gerstekorrel. Voor brouwen met gerst is dit een veelbelovende techniek, omdat de ongewenste componenten zich veelal in de buitenkant van de gerstekorrel bevinden. Daarbij vermindert de hoeveelheid water die nodig is in het proces wanneer de buitenkant van de gerst verwijderd wordt. Ook wordt het volume van de bierbostel verminderd, waardoor er minder afval geproduceerd wordt en er minder energie nodig is om dit afval te drogen. Een nadeel van pearlen is dat het vermogen van de gerstekorrel om enzymen te synthetiseren afneemt. Dit leidt tot de noodzaak van het toevoegen van exogene enzymen, wat ook nodig is wanneer de mout stap overgeslagen zou worden. In **hoofdstuk 3** worden de resultaten van pearlen beschreven. In dit hoofdstuk wordt de chemische compositie van verschillende fracties, verkregen door pearlen, beschreven. Pearlen bleek onoplosbare vezels, eiwitten en polyfenolen van de gerst selectief te kunnen verwijderen, terwijl de β-amylase-activiteit en het zetmeelgehalte nauwelijks beïnvloed werden. Het verwijderen van 5% van de buitenkant van de gerst gaf een vermindering van het onoplosbare arabinoxylangehalte (15%), het onoplosbare vezel gehalte (23%), de polyfenolen (11%) en het waterbindend vermogen van alle niet-zetmeel componenten van de gerst (25%). Hierbij ging slechts 0.20% van het zetmeel verloren. Het waterbindend vermogen van de fracties was recht evenredig met de hoeveelheid onoplosbare vezels. Dit geeft aan dat de bostel minder water op zal nemen wanneer het vezelgehalte in de bostel verminderd wordt. Dit zal leiden tot minder verliezen van wort en de hierin opgeloste suikers, en daardoor tot een efficiënter gebruik van de grondstoffen.

Hoofdstuk 4 vergelijkt een traditioneel brouwproces met een brouwproces waarin exogene enzymen gebruikt worden. De vergelijking heeft betrekking op de efficiëntie van het gebruik van de grondstoffen, één van de duurzaamheidsaspecten van een proces. Het gebruik van exogene enzymen (dat wil zeggen: geproduceerd via fermentatie in een ander proces) bleek efficiënter dan het gebruik van enzymen die doormiddel van het mout proces geproduceerd worden. De exergetische

efficiëntie van het conventionele moutproces was 77%. De belangrijkste verliezen in dit proces kwamen door het gebruik van aardgas in de stap waarin mout gedroogd wordt, en door het verlies van zetmeel in het kiemproces. Verder bleek dat de chemische exergie van een enzym geen goede maat is voor de exergy gebruikt in de productie van een exogeen enzym. Daarom stellen we voor om hiervoor het cumulatieve exergiegebruik in de enzymproductie als maat te nemen. Dit cumulatieve exergiegebruik was ongeveer 30 maal hoger dan de standaard chemische exergie die aanwezig is in de enzymen. Dit laat zien dat dit cumulatieve exergiegebruik de juiste maat is wanneer men kijkt naar additieven die in grotere hoeveelheden gebruikt worden. Dit kan men doen door de systeemgrenzen te verbreden door het productieproces van deze additieven mee te nemen in de analyse. De exegetische efficiëntie van het enzymproductieproces varieert tussen de 20% en 42%, afhankelijk van de waarde die toegekend wordt aan nevenproduct van het fermentatie proces. Ondanks het feit dat het cumulatieve exergiegebruik van het enzym ongeveer 30 maal hoger was dan de standard chemische exergie bleek de totale exergie input van het conventionele moutproces nog steeds hoger (441 MJ) dan die van het enzym-geassisteerde proces (354 MJ). Daarnaast wordt het watergebruik met 7% verminderd in het proces met toegevoegde enzymen, wordt het gebruik van grondstoffen 14% lager en het aardgasgebruik daalt met 78%. Daarom kan geconcludeerd worden dat het exergieverlies in het enzym productie proces gecompenseerd wordt door het verminderde verlies van exergie in het totale bier brouwproces.

Hoofdstuk 5 beschrijft brouwtesten met gemout, ongemout en gepearld ongemout gerst. Brouwen met ongemout gerst bespaart water, energie en grondstoffen, maar kan tot veranderingen leiden tijdens het brouwproces. Pearlen vermindert deze problemen. Om het gebrek aan enzymactiviteit in ongemout gerst te compenseren zijn exogene enzymen toegevoegd. Als filtratie-methode zijn zowel de filtreerkuip als een modernere filter met gebruik van drukverschil over het filter overwogen. Om de effecten van de mout-gerst ratio, de mate van afschuren en de filtreermethode te onderzoeken, is een hoofdcomponenten-analyse (principal component analysis) gebruikt op de gegevens van de chemische samenstelling van de verkregen wort en bostel. Het moderne filter is geschikter voor het nieuwe proces, en laat een werkgebied toe waarin de grondstoffen optimaal gebruikt werden terwijl de kwaliteit van het bier gewaarborgd blijft op basis van vier kwaliteitsparameters.

Het afsluitende **hoofdstuk 6** presenteert een algemene discussie van alle resultaten die in dit proefschrift beschreven zijn. Verder worden de voordelen van pearlen vergeleken met die van malen en scheiden, en wordt het effect van pearlen op de maaleigenschappen van gerst bediscussieerd. Ook wordt er besproken hoe de milieubelasting van het brouwproces beperkt kan worden door toepassing van pearlen Daarnaast wordt aangegeven hoe pearlen als een voorbehandeling voor mouten de enzym activiteit in kiemende gerst vermindert, waardoor de wort kwaliteit vermindert.

Dit proefschrift geeft inzicht in hoe het voorbehandelen van gerst het bierbrouwproces efficiënter kan maken met betrekking tot het gebruik van grondstoffen. Het benadrukt de noodzaak om optimaal gebruik te maken van alle materialen in onze processtromen om een duurzaam proces te ontwerpen, en dat het optimaal gebruiken van onze grondstoffen een hoofdrol speelt in het ontwerpen van een dergelijk proces. Daarnaast is de waarde van het gebruik van exogene enzymen als hulpstof onderzocht. Een duidelijk resultaat is dat men de kosten van de productie van dergelijke hulpstoffen mee moet nemen in een duurzaamheidsanalyse.

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About the author

Laura Henrietta Gerardina was born in Bladel, The Netherlands on June 24 1988. She went to the Pius X college where she obtained her VWO diploma in 2006 with a major in science and technology.

In 2006 she started her study Food Technology at Wageningen University. In her minor thesis project at the laboratory Food Quality and Design she worked on the measurement of antioxidant activity in marula fruit and marula fruit products, and the heat resistance of these antioxidants. Laura completed her major thesis in the group Food Process Engineering. Here she analysed the environmental sustainability of the conventional starch hydrolysis process and compared it to an enzymatic starch hydrolysis process at a low water content. During her internship at the research and development department at Gloria S.A., Lima, Peru, she investigated the effect of the incorporation of starch in yoghurt products, she improved the production of lactose free milk, and worked on the development of a new milkshake product.

After completing her MSc studies, she continued working as a PhD candidate at the group Food Process Engineering of Wageningen University on the project 'Selective opening and extraction of natural raw materials'.

Publications

Laura H.G. van Donkelaar, Tom R. Noordman, Remko M. Boom, Atze-Jan van der Goot (2015), Pearling barley to alter raw material composition before brewing, Journal of Food Engineering, 150, 44-49

Laura H.G. van Donkelaar, José Torres Martinez, Hans Frijters, Tom R. Noordman, Remko M. Boom, Atze-Jan van der Goot (2015), Glass transitions of barley starch and protein in the endosperm and isolated from, Food Research International 71, 241-246

Laura H.G. van Donkelaar, Jos A. Hageman, Serhat Oguz, Tom R. Noordman, Remko M. Boom, Atze-Jan van der Goot (2016), Combining unmalted barley and pearling gives good quality brewing, Journal of the Institute of Brewing, 122:2

Laura H.G. van Donkelaar, Joost Mostert, Filippos K. Zisopoulos, Remko M. Boom, Atze-Jan van der Goot, The use of enzymes for beer brewing: thermodynamic comparison on resource use. (Submitted to Journal)

Overview of completed training activities

Discipline specific courses	
Courses	
Summer Course Glycosciences (by VLAG/GBB)	2012
Food Structure and Rheology (by VLAG)	2013
Biorefinery for food and biofuels (by VLAG)	2013
Hosokawa symposium, Ausburg (by Hosokawa)	2012
Biocatalysts in the food & drink industry (by GBB)	2013
multivariate analysis (by VLAG/Biometris)	2014
Conferences	
ISFRS, Zurich (poster)	2012
FoodBalt, Kaunas (poster)	2012
EFFOST, Uppsala (oral presentation)	2014
Food Technology, London (oral presentation)	2015
ECCE, Nice (oral presentation)	2015
EFFOST, Athens (oral presentation)	2015
General courses	
VLAG PhD week (by VLAG)	2012
Project and time management (by WSG)	2012
PhD competence assessment (by WSG)	2012
Effective behaviour (by WSG)	2012
Teaching and supervising students (by VLAG)	2012
scientific writing (WSG)	2013
Career orientation (by WSG)	2015
Career assessment (by WSG)	2015
writing and presenting a scientific paper (by WSG)	2014
Mobilizing your scientific network (by WSG)	2015
Optional activities	
Preparation of research proposal (in the Food Process Engineering group)	2012
ISPT team meetings at ISPT, Heineken, Unilever, Cosun, Wageningen University and Technical University Eindhoven (by ISPT)	2011-2015
PhD study tour to Finland and the Baltic states (poster and oral presentation)	2012
Weekly group meetings (by Food Process Engineering)	2011-2016
Organising PhD study tour to Chile and Brazil for the Food Process Engineering group	2013-2014
PhD study tour 2014 to Chile and Brazil (oral presentations)	2014

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