S. Nsogning Dongmo, S. Niebauer, M. Zarnkow and T. Becker

Different Influences on Lautering Performance and Wort Quality Attributes when Brewing with 100 % Unmalted Barley

The use of wort from unmalted barley in beer production offers many challenges to the brewers. The experimental data summarized in this paper aims to examine the influence of grist quality of two unmalted barley varieties on mash filterability and some wort quality attributes when brewing with 100 % unmalted barley. Milling settings (four-roller mill, six-roller mill and hammer mill), barley conditioning and two barley varieties were considered. First of all, seven series of grits of varying quality from two varieties (Beatrix and 2300/Irlbach) were produced with various milling settings, and conditioning. Mashing was carried out following a specific mashing program. Enzymes consisting of α-amylase, β-glucanase/arabinoxylanase, protease, pullulanase and lipase were provided by Novozymes. The influence of grist quality, and variety on mash lautering efficiency and the quality of the resultant wort were investigated.

Although all the experiments showed long lautering time, the longest was recorded from the mash filter experiment. Husk conditioning and coarse grist size (four-roller mill for Beatrix variety and six-roller mill for 2300/Irlbach variety) significantly improved the lautering efficiency. The coarser grist from barley Beatrix variety also delivered wort with high turbidity values. The increase of wort pH might have also contributed to the increase of the turbidity level. Furthermore, increasing the amount of protease and pullulanase amount was shown to have great influence on the turbidity decrease. The fluctuations in wort quality were partly imputable to barley variety. Mashing temperature ranges of 55-90 °C and 70-100 °C for 2300/Irlbach and Beatrix variety respectively, were suitable for considerable decrease in the viscosity. Concerning the hot break materials sedimentation in boiled wort, the 2300/Irlbach variety experiment sedimented very well after 20 minutes rest, while the Beatrix variety experiment sedimented after 40 minutes rest.

Milling setting, grain conditioning and barley variety were found to have considerable impact on the quality of the final wort produced from 100 % unmalted barley. Based on the attributes studied in this work, we can conclude that careful dosage of enzymes using conditioned six-roller mill 2300/Irlbach variety when brewing with 100 % unmalted barley can lead to wort with acceptable quality.

Descriptors: 100 % unmalted barley, barley variety, grist quality, commercial enzymes, lautering performance, wort quality

1 Introduction

In addition to malted barley, unmalted cereal starches are commonly used in brewing processes as carbohydrates sources to generally increase extracts and to impart special characteristics to the final product [32]. The use of unmalted cereal starches in brewing presents several advantages. Prominent among them is the role they play as a cheaper extract source than malt [12]. Their use in brewing has been determined by several factors among which are their availability, as well as the handling equipment and brewhouse procedures [7]. In some countries like Japan and Kenya, unmalted cereal starches cost approximately half of what malt does due to the high amount of energy required in the malting process and malt loss which occurs during the process [6].

Barley has been chosen as a favored grain for brewing since 2000 BCE [20] and it offers several advantages to brewers as compared to others cereals (e.g. rice, corn, wheat). Nowadays, barley is still used because of its high extract content, the amylolytic potency of its resulting malt, and the role it's husk plays in facilitating filtration [21]. Malting is defined as the controlled germination of cereals employed to ensure a given physical and biochemical change within the grain, which is then stabilized by drying. The main biochemical change during germination is enzyme synthesis [13].

Barley can be used in quantities of up to 40 and 50 % of the total grist as an adjunct without additional enzymes [16, 34, 30]. At 40 % barley adjunct, the malt enzymes still possess sufficient enzymatic

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activity to effectively carry out the necessary cytolytic, proteolytic and amylolytic processes of the total grist; eliminating the need for additional enzymes [34]. However, the malt quality plays a decisive role. At 50 %, the malt amylase hydrolyses all the starch [25]. Compared to the malt beers, the resulting beer achieves a comparable or higher extract yield and final attenuation owing to the combined effectiveness of malt and microbial enzymes. Higher proportions of unmalted barley have also been shown to provide a better oxidative stability of wort and to increase the antioxidant potential of the resulting beer [16]. Nonetheless, when brewing with higher amount of adjuncts and unmalted barley, a slight decrease in total polyphenols, free amino nitrogen content, and extract yield was reported. At the same time, an increase of higher molecular weight proteins and β-glucan content, harshness, lautering problems, and longer mashing periods was also reported [5, 9, 17]. When the portion of unmalted barley exceeds 50 %, the addition of enzymes from an external source is imperative. The purpose of enzyme addition is not only to achieve an acceptable extract yield from the grist, but also to guarantee good filterability and fermentability of wort [29].

Commercial enzymes have routinely been used as a supplement to malt enzymes to ensure trouble-free brewhouse performance when using undermodified malt or barley/malt blends [33]. As the amount of unmalted cereal starch increases, depending on the origin (corn/barley), the amount of commercial enzymes required increases as well [33]. Since industrial enzymes production became established in the 1960s, the portion of unmalted cereal starch was able to be raised from 25 % initially to the 100 % possible today [28].

The most important enzymes in the brewing system include α- and β-amylase, proteases, peptidases, β-(1,3)(1,4)-glucanases and lipases [4, 19]. β-Amylase and some other important enzymes in the brewing process are available in the raw barley [22]. Other enzymes supplemented during the process, are easily commercially accessible in industries from bacterial culture preparation [15, 23]. Along, pullulanase has been reported to contribute to the increase of brewhouse yield [8].

Beers produced from 70 % unmalted barley proportion combined with commercial enzymes achieved comparable or higher final attenuation limit than the ordinary beer [17]. When unmalted barley is hydrolyzed with a suitable enzyme blend it can deliver almost the same wort and beer quality as barley malt [35]. Furthermore, in combination with exogenous enzymes, barley can also deliver sufficient free amino-nitrogen, comparable aroma and taste profile even if 100 % of the malt is replaced [35]. The ability of the preserved natural enzymes content of grains to degrade their natural specific substrates is one of the main advantages of using 100 % unmalted barley [22]. Barley starch gelatinizes at a relatively low temperature similar to malt starch, allowing it not only to be easily used without major changes to the mash program but also to the grain β-amylase content to be fully utilized in combination to α-amylase [22]. Additionally, barley has same basic composition as malt.

Table 1 varieties, location, and supplier of barley

Varieties	Location	Supplier
Beatrix	38895 Böhnshausen	Nordsaat Saatzucht GmbH
2300/Irlbach	94342 Irlbach	Saaten-Union GmbH

This led to the use of only unmalted barley (as a substitute to the malt and unmalted cereal starches) and industrial enzymes (to compensate malting) during brewing process. The combination of industrial microbial enzymes with barley endogenous enzymes allowed the implementation of a fully comparable brewing process with unmalted barley [35]. It has been possible to efficiently brew beer with a satisfactory quality from 100 % unmalted barley and an appropriate amount of exogeneous OndeaPro® enzymes. Similarly, a taste evaluation done at the Technical University Berlin showed no significant difference in beer quality between 100 % malt beers and 100 % barley beers and blends [33].

In order to optimize the extraction of malt components, it is obligatory to mill malt prior to mashing during brewing process. Milling increases the physical contact between the substrate and the enzyme molecule enabling hence an efficient enzymatic hydrolysis of polymeric compounds in malt (mainly starch, proteins, and non-starch polysaccharides) to low-molecular wort components [18]. With some adjustments of milling setting as well as some changes in the control attributes of the lauter tun, a brewing process with 100 % barley can be achievable [35]. Klopper [15] reported that installation of special barley mills, longer mashing times, and heat treatment must be envisaged when brewing with barley as raw material. Moreover, careful optimization of enzyme types and dosage levels are also of great importance [11].

Another important parameter to consider is the choice of a suitable barley malt variety. Given one malt variety, the multitude of choice in the setting of various process parameters during brewhouse operations can give rise to worts with varying concentrations [11].

Since, wet milling has been reported to be highly satisfactory [8], the aim of this work is thus to examine the influence of grist quality of two barley varieties on mash filterability and some wort quality attributes when brewing with 100 % unmalted barley and exogenous enzymes.

2 Materials and methods

2.1 Materials

Barley and water

Two varieties of spring barley obtained from two suppliers in Germany were used as shown in table 1. The barley Pilsner malt came from the company Weyermann® GmbH & Co. KG Brau-, Röst- und Caramelmalzfabrik (Bamberg, Germany). The characteristics of the Pilsner malt, Beatrix and 2300/Irlbach variety are shown in table 5. Softened water with residual alkalinity of 3.2 meq/l from the chair of Brewing and Beverage Technology, Technische Universität München was used for the mashing process.

Table 2 Experiments on barley Beatrix and 2300/Irlbach varieties – pilot batches (P) (4RM= four-roller mill; 6RM= six-roller mill; HM= hammer mill; LT= lauter tun; MF= mash filter; n.a. = not applicable)

Experiments	P1	P2	P3	P4	P5	P6	P7.1	P7.2
Barley variety	Beatrix	Beatrix	Beatrix	Beatrix	Beatrix	Beatrix	2300/Irlbach	Beatrix
Type of grist	6RM	6RM	HM	4RM	4RM	6RM	6RM	6RM
Lautering method	LT	LT	MF	LT	LT	LT	LT	LT
Conditioning [%]	n.a.	2.0	n.a.	2.0	2.0	2.0	2.0	2.0
Moisture [%]	13.1 ± 0.2	13.1 ± 0.1	13.1 ± 0.1	13.1 ± 0.2	13.1 ± 0.2	13.1 ± 0.1	15.0 ± 0.2	13.1 ± 0.1
Grist, air-dried [kg]	9.0 ± 0.1	9.0 ± 0.2	9.0 ± 0.2	9.0 ± 0.2	14.0 ± 0.3	12.5 ± 0.2	14.4 ± 0.3	14.1 ± 0.1
Grist, dry matter [kg]	7.8 ± 0.1	7.6 ± 0.2	7.8 ± 0.2	7.6 ± 0.2	11.9 ± 0.3	10.6 ± 0.2	12.0 ± 0.3	12.0 ± 0.1
Mash liquor [kg]	36.0 ± 0.2	36.0 ± 0.3	36.0 ± 0.3	36.0 ± 0.2	49.0 ± 0.2	44.0 ± 0.2	49.0 ± 0.2	49.0 ± 0.3
Sparg. water/dry matter ratio	4.6 ± 0.05	4.7 ± 0.05	4.6 ± 0.1	4.7 ± 0.05	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.05	4.1 ± 0.1

Enzymes

Enzymes were supplied by the company Novozymes, based in Bagsvaerd, Denmark as enzyme mixture (Termamyl® SC, Ultraflow Max® and Neutrase®) and individual enzymes (Pullulanase and Lipase). Termamyl® SC was a liquid enzyme preparation containing a heat-stable α-amylase. Ultraflow Max was an enzyme preparation containing β-glucanase and arabinoxylanase. Neutrase® was a protease. These enzymes were added during the mashing process.

2.2 Experimental procedure

Milling

For mash filter grist, a hammer mill (HM) model Broyeur Mono 8 manufactured by Huizenscheßens N. V. in Dendermonde, Belgium was used. A modern roller-mill in the pilot plant of the company Bühler in Uzwil, Switzerland was used for the production of lauter tun grits. This roller-mill has the advantage that it was possible to change rapidly rollers to produce four-roller mill and six-roller mill grits. For the production of six-roller mill grist, the grist was separated from husks using a small Rotostar MPAR sifter after the second milling step for further milling of the coarse grits. Conditioned dry milling was carried out by grain immersion in water at 2 % by weight for 10 minutes just before milling through a tumbling mixer of the Bühler Company. Pilsner barley malt used was milled in a two-roller mill MIAG-laboratory mill (Baunschweig, Germany). Samples obtained after milling (Table 2) were packed in paper bags and stored at 1-6 °C for further use in worts production.

Table 3 Enzyme dosage used for mashing process

Enzyme	Specific activity	Activity added
α-Amylase	120.0 KNU-S/g	300.0 KNU-S/Kg
β-Glucanase	700.0 EGU/g/	210.0 EGU/Kg /
Arabinoxylanase	250.0 FXU-S/g	75.0 FXU/kg
Pullulanase (NS26062)	400.0 PUN/g	1600.0 PUN/Kg
Protease	0.8 AU-N/g	0.9 AU-N/Kg
Lipase	10000.0 PLU/g	2000.0 PLU/Kg

KNU: Kilo Novo Unit (Alpha Amylase Unit); EGU: Endoglucanase Unit; FXU: Fungal Xylanase Unit; PUN: Pullulanase Unit Novo; AU: Anson Unit; PLU: Propyl Laurate Unit

Mashing and lautering

Brewing was conducted in the 60 L pilot scale of the chair of Brewing and Beverage Technology, Technische Universität München. The mash program temperature-time profile was used (Fig. 1). Seven lauter tun experiments with Beatrix and 2300/Irlbach barley varieties and one mash filter experiment with Beatrix barley variety were carried out as shown in table 2. The process was alternated by connecting a portable Meura pilot mash filter to the system. The quantity of grist, the amount of mash liquor, the spargation water/dry matter, and the enzyme dosage is given in table 2 and 3, respectively.

During lautering, flow rate, turbidity, and the position of the lauter rakes was constantly recorded. The grain bed was sparged three times with water at different ratios (Table 2). Wort was collected in the kettle and boiled for 90 minutes after pH adjustment to 5.3 with lactic acid according to preliminary tests. Lactic acid was supplied by the company VWR International GmbH, Germany. After 20 minutes rest, wort was cooled to 0 °C for further analysis. Spent grains samples were dried at 50 °C to moisture content lower than 10 % for analysis.

Influence of enzymes on unboiled wort trub

Total wort samples from P7.1 (2300/Irlbach) and P7.2 (Beatrix) experiments were collected from the kettle immediately after lautering was completed and prior to boiling. For each variety, six brown NRW bottles were filled, each with 500 ml wort and placed in a 54 °C water bath for 20 min. Enzymes were added to the bottles, which were sealed with crown corks and shaken. The enzyme dosage is given in table 4. The turbidity was measured at 90° angle repeatedly at regular interval of time with a LabScat Dual Angle Turbidimeter manufactured by Sigrist (CH). In the sixth bottle of wort, which serves as reference sample, no enzymes were added. Components responsible for lauter tun wort trub were estimated.

Influence of temperature on mash viscosity

In order to assess the influence of temperature on mash viscosity, a rapid visco-analysis (RVA) was performed. In this method, a temperature program was followed and it consisted of increasing the mash sample temperature considerably to the gelatinization peak followed by a decrease of the temperature to the initial point.

Mash samples collected from P7.1 (2300/Irlbach) and P6 (Beatrix) experiments at the beginning of the lautering were heated from 50 to 92 °C without addition of enzymes and cooled back to 50 °C within 13 minutes. The mash viscosity influenced by the temperature was recorded.

2.3 Analytical procedures

The analyses on barley kernels, malt, grist, and wort were performed according to the standard methods of the Mitteleuropäische Brau- und Analysenkomission (MEBAK) [2, 24, 31]. Sieving tests, moisture content, protein content and extract yield were carried out according to MEBAK [24]. pH, colour, turbidity, β-glucan, FAN, viscosity, total, degradable and soluble extract were determined according to MEBAK [2]. Total and soluble extract were measured in the spent grains. DMS and DMS-P were determined according to MEBAK [14]. Hot break materials in the boiled wort were assessed qualitatively by sedimentation.

3 Results and Discussion

Characteristics of barley

Characteristics of barley malt, 2300/Irlbach and Beatrix varieties are shown in table 5. Except the friability, which was slightly lower than the typical value, the Pilsner malt characteristics complied with the typical values of the conventional malt. The friability measures the evenness of modification of the malt [4]. 2300/Irlbach variety has showed higher water content of 1.8 % and lower protein content of about 1 % than Beatrix variety. For both varieties, the water content was much higher than Pilsner malt water content and the typical value. It is also noteworthy that 2300/Irlbach variety had a higher dry extract than Beatrix variety.

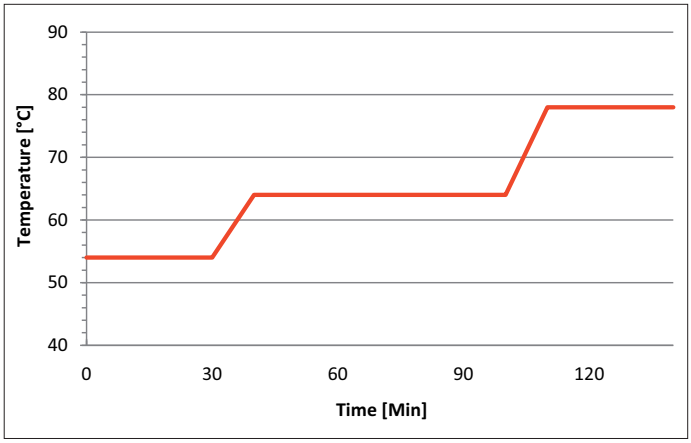

Fig. 1 Mash program

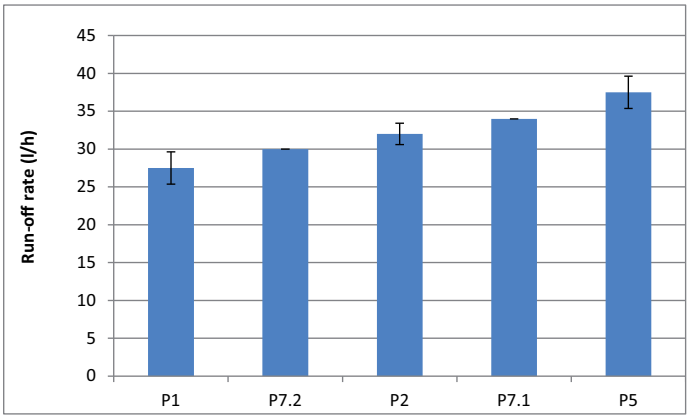

Fig. 2 Lautering efficiency of selected experiments dependent on run-off rate (l/h)

Milling

Conditioned milling method has been developed to preserve malt husks for a fast separation in the lauter tun [4]. The comparison of husk percentages in barley varieties (Table 6) shows that higher values (over 50 %) than the typical value for optimal lauter tun grist (18 %) were obtained from both conditioned and non-conditioned four-roller mill grits. Six-roller mill grits, regardless of the variety, yielded also husk percentages slightly higher than the lauter tun ideal percentage. Compared to conditioned six-roller mill Beatrix variety grits, six-roller mill 2300/Irlbach variety grist yielded 10 % higher husk and lower fine grist percentage. Conditioned grist experiments with same roller-mill setting exhibited lower husk percentage than the not conditioned ones.

The purpose of grain conditioning is to raise husk moisture content making it flexible to resist to the strength of milling process [5]. This result points out the fact that husk percentage is not only related to barley grains conditioning but also to the roller-mill setting. Higher husk volume was obtained from six-roller mill grits regardless of conditioning and variety.

The percentages of fine powder and flour were lower than the typical value in all experiments. Higher percentages were obtained from six-roller mill conditioned experiments. It should be noted that 2300/Irlbach variety grain had 2 % more moisture content than Beatrix variety. Water might seep into the endosperm during conditioning, causing an increase in the moisture content, which led to the inclusion of more fine grist in husk fraction. Thus, the fraction of fine grist, powder and flour was reduced as observed with conditioned experiments.

Lautering

The specific lautering performance according to Narziss [26], normally expressed in liters per hour based on the area of the false bottom, is used to compare lauter tuns with different designs and dimensions. In figure 2, experiments are ranked in ascending order of the lautering performance. The highest lautering efficiency was obtained from conditioned four-roller mill Beatrix variety experiment (P5) followed by conditioned six-roller mill 2300/Irlbach variety experiment (P7.1). The difference between P5 and P7.1 experiments could be due to the difference in grist composition. It should be mentioned that conditioned four-roller mill grits yielded also the highest husk fraction, while six-roller mill conditioned grits yielded low husk fraction (lower than barley malt husk fraction) but high husk volume. However, experiment P1 from non-conditioned six-roller mill Beatrix variety grist, which exhibited lowest husk fraction, showed consequently the lowest lautering efficiency.

This result supports the report that husks have an indispensable drainage function during lautering and should therefore be as intact as possible [10]. When comparing these results to those of the husk fraction, we can conclude that the higher the husk fraction, the higher the lautering efficiency. Conditioned dry milling has been reported to reduce cycle time with poorly modified malt by up to 10 minutes in a 3 hours lauter tun cycle [42]. This concurs with the lowest lautering efficiency obtained from the six-roller mill non conditioned Beatrix barley experiment P1.

A comparison within Beatrix variety (experiments P5 and P2) leads to stipulate that conditioned four-roller mill grist is more suited for an efficient lautering when brewing with 100 % unmalted Beatrix barley variety. Lautering time for all the experiments were longer than the 90 minutes (data not shown) lautering time recorded by Steiner et al. for 100% unmalted barley [37]. This result is in contradiction to the reported results that barley brews could be filtered 20-30 minutes faster than malt brews [41]. When comparing results from experiments P7.1 and P7.2 which were conducted in same conditions, it can also be concluded that variety affects the lautering efficiency of barley.

Turbidity

Despite completely opening the valve to allow rapid run-off and recirculation of the cloudy wort at the beginning of lautering, the lauter worts displayed high levels of turbidity. Table 7 shows that brew experiment from mash filter (P3) exhibited the highest value of turbidity (over 100 EBC). This result could be attributed to the high lauter pressure inside the mash filter which leads to the presence of fine particles in the wort causing turbidity. It could also be attributed to high values of first wort pH recorded. They were higher than the typical values of 5.3-5.6 and the value of 5.4 reported by Steiner et al. [37]. Four-roller mill experiments (P4 and P5) initially exhibited high turbidity values but through the dilution effect of spargation, the turbidity decreased to approximately 50-60 EBC (data not shown).

On the contrary, the lowest turbidity, less than 20 EBC, was obtained from experiment P7.1 (from 2300/Irlbach variety) followed by six-roller mill conditioned Beatrix barley experiments (P6 and P2). This result can be partly explained by the difference in the grist composition. Water and protein contents of 2300/Irlbach variety grist were respectively 2 % higher and about 1 % less than the values of Beatrix variety grist. The turbidity is primarily due to the presence of proteins and polypeptides. Denatured proteins bind to lipids in wort to form complexes which cause wort turbidity and an increase in pH [4, 10, 19]. On the other part, Bjerl [5] reported that particle size of barley grist affects considerably protein degradation. This result can be related to the high lautering efficiency exhibited by 2300/Irlbach variety.

The basic information derived from the above mentioned results is that wort turbidity is not only closely related to barley variety, respective to their protein content, but also to husk conditioning and milling setting when using 100 % unmalted barley.

Influence of enzymes on trub in unboiled wort

Lautering with experiment P6 became blocked during second spargation that is why some attributes were measured. For this reason, different amounts of enzymes were added to separate unboiled kettle worts in order to follow up the breakdown of the particulate matter causing the turbidity. Figure 3 shows that in barley variety 2300/Irlbach wort, protease was able to reduce the turbidity from 36 to 15 EBC within 45 minutes while pullulanase reduced it slightly from 36 to 28 EBC within 2 hours. However, lipase, α-amylase, β-glucanase and arabinoxylanase had no effect on the wort turbidity in both varieties. On other side, the increase of turbidity with these enzymes at the end in both varieties and in control experiments might be due to the formation of a cold trub.

Unlike 2300/Irlbach variety, pullulanase did not reduce the turbidity in Beatrix variety wort. Protease, contributed to the slightly decrease of turbidity from 70 to 49 EBC within 1 hour. As it can be seen, it was possible to hydrolyze barley organic polymers, responsible for wort turbidity, into small size compounds. Nonetheless, a part of wort colloids involved in haze formation was evidently not accessible to these enzymes. Haze formation includes covalent building of insoluble complexes which are not dissolved by heating during brewing process [36].

Those complexes are proanthocyanidins from the testa tissue of the barley grain, dextrins and polyphenols [38]. Lysophospholipids, which also tend to produce complexes with starch helices, have been conjectured to be the root of lautering problems and turbid run-off [14]. Pentosans can create gels in a dissolved state or as attached side chains bound to starch kernels and proteins in the grain bed, thereby slowing wort run-off [27]. Additionally, non-hydrolyzed starch can undergoes retrogradation and compounds-producing gums can develop into reversible networks.

Referring to protease and pullulanase activity, it can be then concluded that compounds responsible for turbidity include mainly proteins and some cell-wall polysaccharides, since pullulanase is an endo-type enzyme that cleaves α-1,6-glucosidic linkages in polysaccharides. The use of lipase in this work did not reduce the turbidity. However, it does not mean that lipids are not involved in haze formation.

An increase of protease dosage can help to reduce considerably wort turbidity. We can also conclude that there is a difference in

Table 4 Enzyme dosage for the enzymatic analysis of turbidity in both varieties

Bottle	1	2	3	4	5	6
Enzyme	α-Amylase	β-Glucanase/ Arabinoxylanase	Pullulanase	Protease	Lipase	Reference sample
Activity added	25.20 KNU-S	70.00 EGU/ 25.00 FXU-S	132.00 PUN	0.08 AU-N	1000.00 PLU	–

Table 5 Characteristics of malt, Beatrix and 2300/Irlbach barley varieties used in the experiments (ND = not detected)

Analysis	Units	Typical values [26]	Pilsner malt	Barley variety Beatrix	Barley variety 2300/ Irlbach
Water content	%	4.5	4.3 ± 0.1	13.1 ± 0.2	14.9 ± 0.1
Extract air – dried	%	77.8	78.2 ± 0.3	66.6 ± 0.2	68.4 ± 0.3
Dry extract [%]	-	81.5	81.7 ± 0.3	76.6 ± 0.3	80.3 ± 0.4
Protein content	%	10.2	10.5 ± 0.2	10.9 ± 0.1	10.1 ± 0.1
Colour (Hellige)	EBC	2.8	3.3 ± 0.1	ND	ND
Boiled colour	EBC	4.8	5.5 ± 0.3	ND	ND
pH value	-	5.93	5.84 ± 0.05	ND	ND
Saccharification	min	10-15	10-15	ND	ND
Soluble Nitrogen	mg/100g MTs	653	706 ± 15	ND	ND
Friability	%	> 85	83 ± 0.7	ND	ND
Viscosity 8.6 % calculated	mPa·s	1.540	1.510 ± 0.05	ND	ND
Viscosity 12.0 % calculated	mPa·s	-	1.800 ± 0.05	ND	ND
ELG	%	-	41.9 ± 0.3	ND	ND
Transparency	%	-	1.8 ± 0.05	ND	ND

polysaccharides content and compounds causing turbidity in wort among 2300/Irlbach and Beatrix variety.

Viscosity and hot break material

During mashing, β-glucan is enzymatically hydrolyzed into smaller oligosaccharides; an increase in β-glucan content leads to an increase of wort viscosity [37, 40]. In our experiments, a comparison of β-glucan content from conditioned and non-conditioned six-roller mill experiments (Table 8) showed that conditioning has an influence on the degradation of β-glucan in wort. One reason could be the fact that the contact between intact kernel enzymes and substrates might have been impaired. β-Glucan concentrations of experiments P1 and P2 were well above the typical value which is 200 mg/L. This result is correlated with the result of the influence of enzymes on the trub, recommending an increase of enzyme dosage when brewing with 100 % unmalted barley. However, Walker et al. [43] reported that the amount of β-glucan in barley does not predict extract potential.

Viscosity of all the experiments were lower than the typical value (< 1.6), and the values reported by Steiner et al. (1.59 and 2.09) for unmalted barley [37]. The lowest viscosity value recorded for experiment P5 (Table 8), which exhibited highest lautering efficiency, corroborates with Darcy's law stating that filtration rate is inversely proportional to the viscosity. The low viscosity values recorded in this work may be partly due to the wide temperature range and the 60 minutes maltose rest employed in the mash program. In addition, bacterial amylases and proteases have shown β-glucanase activity [9], since the mash pH values of our experiments were similar to the 5.6 optimum pH of bacterial β-glucanase. Bjerl [5] also reported that wort viscosity does not represent a problem when brewing with unmalted barley and enzymes. P7.1 and P7.2 experiments exhibited also identical and low viscosity values. Variety may not affect viscosity of wort when brewing with unmalted barley.

Different hot break material formation was observed in each barley variety (Fig. 4). Wort produced using barley variety 2300/ Irlbach (P7.2) sedimented very well after boiling and subsequently formed a very stable cone of hot break materials after 20 minutes rest. On the other side, barley variety Beatrix experiment (P7.1) sedimented very slowly, and it was necessary to lengthen the sedimentation rest to 40 minutes. The color of all the experiments were within the typical range (7-11 EBC) [37] except experiment

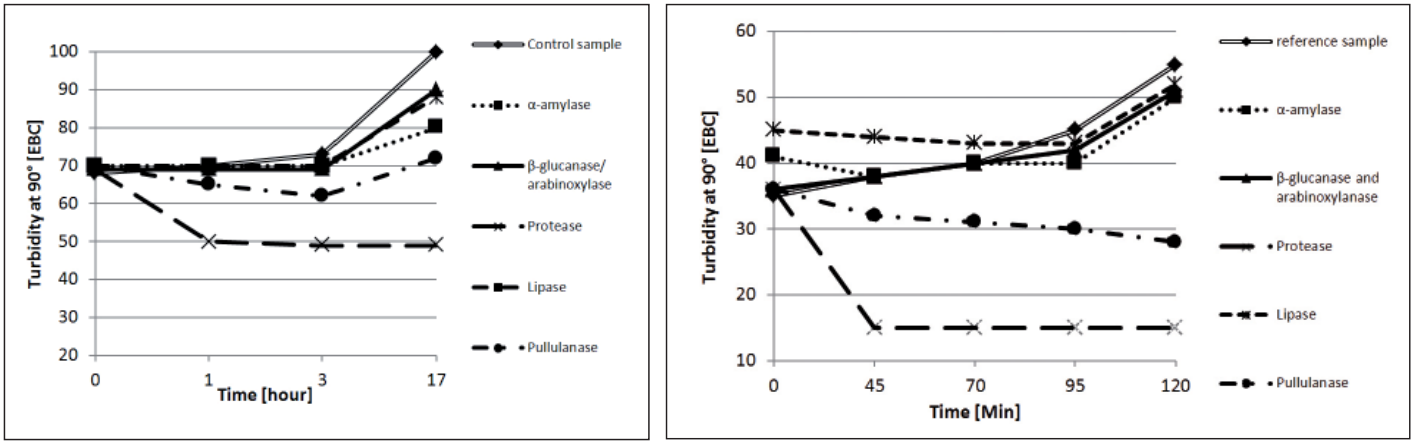

Fig. 3 Enzymatic hydrolysis of trub in the unboiled wort over time by exogenous enzymes; left, 2300/Irlbach variety; right, variety Beatrix

Table 6 Analysis of grist fractions from Beatrix and 2300/Irlbach barley varieties: conditioned (cond.) and not conditioned (not cond.), four-roller mill (4RM) and six-roller mill (6RM) grits

Fraction	LT Typical values	2RM malt grist	4RM grist (not cond.)	4RM grist (cond.)	6RM grist (not cond.)	6RM grist (cond.)	6RM grist (cond.) 2300/ Irlbach
Husk volume [ml/100g]	-	-	320 ± 43.0	330 ± 27.0	580 ± 38.0	540 ± 31.0	410 ± 27.0
Husks [%]	18 ± 1.0	39 ± 3.0	51 ± 3.0	57 ± 5.0	26 ± 3.0	20 ± 2.0	31 ± 2.0
Coarse grits [%]	8 ± 0.5	15 ± 1.5	24 ± 2.0	18 ± 2.0	8 ± 1.5	5 ± 0.5	7 ± 0.5
Fine grists I [%]	35 ± 3.0	19 ± 2.0	16 ± 1.0	14 ± 1.0	41 ± 3.0	38 ± 3.0	34 ± 2.0
Fine grists II [%]	21 ± 2.0	11 ± 0.5	5 ± 0.5	5 ± 1.0	15 ± 0.5	27 ± 2.5	18 ± 2.0
Flour [%]	7 ± 0.4	5 ± 0.5	2 ± 0.1	2 ± 0.1	3 ± 0.2	5 ± 0.1	4 ± 0.3
Fine powder [%]	11 ± 0.7	11 ± 1.2	4 ± 0.5	4 ± 0.3	7 ± 1.1	6 ± 1.0	8 ± 0.8

P7.1 which was slightly lower than the typical range. During wort boiling, proteins coagulate and can be removed in the whirlpool. No more than one-third of barley total protein content passes into the finishing beer through wort [38]. Bearing in mind the fact that 2300/Irlbach variety had 1 % less protein than Beatrix variety, the clear sedimentation of the wort from 2300/Irlbach variety can find here an explanation.

Influence of temperature on mash viscosity

Figure 5 shows that mash viscosity decreases with increase of temperature until the gelatinization temperature peak. It increases afterwards with temperature decrease in both varieties. This demonstrates the strong temperature-dependence of the mash viscosity. At gelatinization temperature peak, the mash viscosity peak of 2300/Irlbach barley variety was lower than that of barley malt and Beatrix variety. Lowest viscosity was obtained from 55-90 °C and 70-100 °C temperature ranges in 2300/Irlbach and Beatrix variety, respectively.

This result has revealed that the mash program used, in reference to mash viscosity, was suitable for 2300/Irlbach variety. Nevertheless, the 55 °C temperature considered in the mash program, exhibited high mash viscosity for variety Beatrix. This result could also be related to the low lautering efficiency exhibited by Beatrix variety comparing to 2300/Irlbach variety. As conclusion, different mash program for 2300/Irlbach and Beatrix variety should be used when brewing with 100 % unmalted barley.

Extract and FAN

Extract yield is the weight percent of raw material that will appear as extract when the material is mixed or mashed with water while the extract is the amount of soluble particles in a solution. As it can be seen in table 8, conditioning of barley grains and milling setting do not have significant influence on the extract yield and the amount of different material.

Unlike the statement that the hammer mill give more than 100 % extract [3], in this work the hammer mill yielded the lowest extract. However, the highest soluble extract content in the spent grains was likewise recorded for the hammer mill experiment. Contrary to the results of Steiner et al. [37] showing that even when exogenous enzymes were used the extract yield was still a little too low, experiments P7.1 and P5 as well as P2 yielded more than 100 % extract as well as lowest soluble extract content in the spent grains. The high extract yield obtained may be due to the fact that during 60 minutes saccharification rest employed in the mashing program, cell wall degrading enzymes were still acting to provide more extract.

Soluble and degradable extract values were all under the typical values of 0.8 %. Furthermore, with regard to the high activity and heat stable exogenous enzymes used, low values of degradable and soluble extract were expected. Goode et al. [11] similarly reported that an increase in the enzymes dosage rate resulted in a corresponding exponential increase in extract recovery levels when mashing with 100 % unmalted barley. Additionally, the same authors showed that after using optimal level of commercial enzymes, the spent grains were still starch positive.

Moreover, the soluble extract values varied widely between the experiments while degradable extract values were quite low and did not vary widely between the experiments. With reference to soluble extract which is dependent on the spent grains structure, the previous assertion that there is a difference in structure between barley varieties is argued.

Wort color is a consequence of products formed by Maillard reaction from free amino nitrogen (FAN) and reducing sugars. A low FAN content could be limitating for yeast nutrition and should therefore

Fig. 4 Sedimentation of hot break materials in P7.2 and P7.1 experiments after 20 minutes rest; left, Beatrix variety; right, Irlbach variety

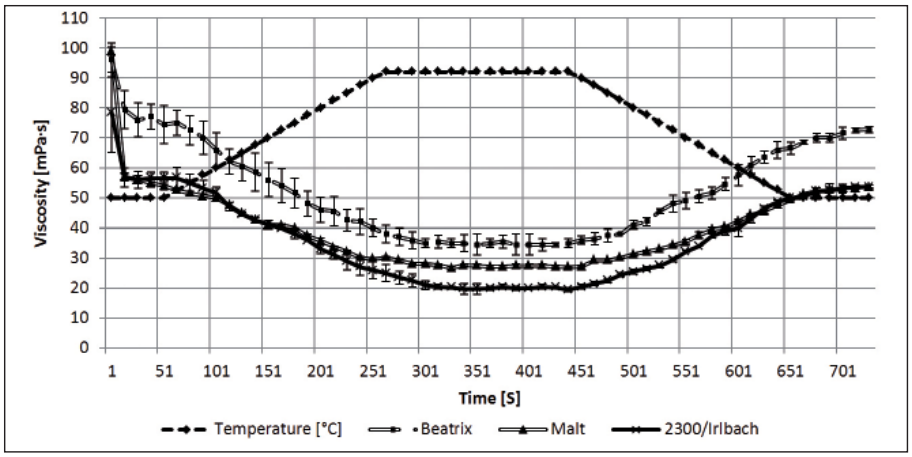

Fig. 5 Temperature-dependence of mash viscosity of 2300/Irlbach and Beatrix variety and malt

Table 7 Turbidity and pH values of the strong wort of different experiments

Analysis	P1	P2	P3	P4	P5	P6	P7.1	P7.2
Turbidity at 90°/25° [EBC]	54 ± 27/ 64 ± 28	78 ± 34/ 53 ± 40	187 ± 18/ 200 ± 0	115/139	83 ± 1.4/ 89 ± 2	38/43	11/15	85/89
Wort pH	6.2 ± 0.05	6.2 ± 0.05	6.2 ± 0.1	6.2 ± 0.05	6.2 ± 0.05	6.2 ± 0.05	5.9 ± 0.05	6.0 ± 0.1

be in the normal range. Except 2300/Irlbach variety FAN value, all the FAN values were lower than the typical values of malt wort (120-150 mg/L) recommended for optimum yeast nutrition by Narziss and Back [26] and the values of 200-250 mg/L recommended by Steiner et al. [37]. On the other side, some experiments of Beatrix variety met the FAN typical range (108-170ml/L) reported by Schoenenberg and Kreisz [35] for 100 % unmalted barley.

DMS-P and DMS

Dimethylsulphide (DMS) is an off-flavor compound in beer. It is formed during wort boiling where it could also be removed by evaporation. Prolonged cooking thereby contributes to reduce the amount of DMS [44]. Its precursor DMS-P is broken down in a thermal reaction to form free DMS. Barley variety and wort pH are some factors that determine the DMS-P level. The DMS-P value of lager malt and wort should be less than 7 ppm and 100 µg/l, respectively [39]. The value of DMS was under the above-mentioned threshold in both varieties. Additionally, a comparison between Beatrix and 2300/Irlbach variety as shown in table 9 indicates that the DMS-P value was higher in 2300/Irlbach than in Beatrix variety while the DMS values were relatively similar in both varieties. Longer cooking time or other processes such as rapid wort cooling for the purpose of DMS evaporation would therefore be unnecessary.

4 Conclusion

The aim of this work was to investigate on the influence of grist quality of two unmalted barley varieties on mash filterability and wort quality attributes when brewing with 100 % unmalted barley.

This research work shows that milling setting and barley variety considerably affect wort quality and lautering performance when 100 % unmalted barley is used. Lautering efficiency was significantly improved by husk conditioning and coarse grist size (four-roller mill for Beatrix variety and six-roller mill for 2300/Irlbach variety). High turbidity exhibited by coarse grist was found to be attributable only to barley Beatrix variety. Furthermore, according to our enzymatic turbidity test, an increase of protease and pullulanase amount was shown to have great influence on the turbidity reduction. Mashing temperature ranges of 55-90 °C and 70-100 °C for 2300/Irlbach and Beatrix barley variety, respectively, were found to be suitable for considerable decrease of mash viscosity. There was a rapid sedimentation of hot break materials in boiled wort produced from 2300/Irlbach barley experiment as compared to Beatrix barley variety experiment.

Whether these differences in wort quality are attributable to specific barley provenance or perhaps possible poor storage conditions are beyond the scope of this investigation. For the use of 100 % unmalted barley in brewing process, grains conditioning and the

Table 8 Analysis of wort obtained from the experiments

Analysis	Units	P1	P2	P3	P4	P5	P6	P7.1	P7.2
Extract yield	%	98.0 ± 1.4	100.5 ± 0.7	92.5 ± 4.9	104.0 ± 3.2	101.0 ± 0.0	–	101.0 ± 4.9	–
Total extract of total wort	%w/w	9.0 ± 0.1	8.6 ± 0.1	9.0 ± 0.0	9.2 ± 0.5	10.8 ± 0.0	–	11.0 ± 0.2	10.9 ± 0.2
Viscosity at wort cooling (p=8 %w/w)	mPa·s	1.445 ± 0.007	1.451 ± 0.001	1.465 ± 0.007	–	1.420 ± 0.000	–	1.430 ± 0.050	1.430 ± 0.050
β-glucan (p=12 % w/w)	mg/L	373.0 ± 27	403.0 ± 31	–	–	–	–	–	–
Color of wort	EBC	9.4 ± 0.1	9.7 ± 0.5	8.4 ± 0.3	–	8.0 ± 0.4	–	6.8 ± 0.5	7.8 ± 0.4
FAN (p=12 % w/w)	mg/L	94.0 ± 6.4	107.0 ± 19.1	98.0 ± 0.7	–	114.0 ± 4.9	–	125.0 ± 6.5	119.0 ± 8.9
Brewhouse yield (dry matter)	%	75.0 ± 1.4	77.0 ± 0.0	68.0 ± 0.0	79.0 ± 1.2	77.0 ± 0.0	–	78.0 ± 0.4	77.0 ± 0.3
Degradable extract	%	0.60 ± 0.04	0.60 ± 0.01	0.48 ± 0.01	0.61 ± 0.02	0.60 ± 0.02	–	0.57 ± 0.01	0.53 ± 0.01
Soluble extract	%	0.60 ± 0.01	0.47 ± 0.14	1.76 ± 0.77	0.56 ± 0.01	0.51 ± 0.06	–	0.42 ± 0.04	0.70 ± 0.01

Table 9 DMS and DMS-P values of Beatrix and 2300/Irlbach barley variety

Experiment	Barley variety	DMS [µg/l]	DMS-P [µg/l]	Sum [µg/l]
P7.1	2300/Irlbach	13.0 ± 1.1	54.0 ± 5.4	67.0 ± 6.5
P7.2	Beatrix	14.0 ± 0.8	34.0 ± 4.2	48.0 ± 5.0

use of 2300/Irlbach variety at six-roller milling setting was found to be preferable. However, further investigations on the use of 2300/Irlbach variety with different milling settings, would be beneficial. When brewing with Beatrix variety, a four-roller milling setting should be considered and further investigations on the mash program could be useful. Additionally, an increase of protease and pullulanase dosage is substantial to the quality of the final wort.

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