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Evaluation of Mashing Attributes and Protein Profile Using Different Grist Composition of Barley and Wheat Malt

This study is focused on protein profile and processability of mashes as well as wort quality by varying grist composition by increasing wheat malt amount. In the first part of this study – the screening, on mash relevant characteristics – an increase of final attenuation, viscosity, soluble nitrogen and β-glucan, whereas a decrease of arabinoxylan (AX) and protease activity during the mashing process was detected. Between 55 ° and 62 °C the amylolytic mash values extract and final attenuation rose most considerably. Proteolytic parameter declined strongly between these two temperatures. A wheat malt ratio higher than 50 % shows no benefit for brewing purpose in terms of processability and wort quality (extract and apparent attenuation limit. Due to the similar distribution of the extract graph it can be assumed that amylolytic enzyme activities and their changes during mashing of wheat malt are comparable to those of barley malt. Regarding mash temperature between 55 ° and 62 °C the cytolytic mash attributes viscosity and β-glucan denoted the biggest increase, whereas AX showed the greatest decline. A relationship between viscosity and mash attribute AX and β-glucan couldn't be detected at a sufficiently extent. Proteolytic parameter declined strongly during the first mashing stages (35 ° to 55 °C) remained almost constant from 55 ° to 78 °C. In consideration of processability and wort quality for wheat beer production insufficient soluble nitrogen (SN) values of barley malt can be compensated by good wheat malts as well as wheat malt ratio. In the second part of this study a protein profiling was performed to evaluate changes during the mashing process. Moreover, the differences in protein constitution of grist composition with increasing wheat malt amount were investigated. In mashes with increasing wheat malt no 17 kDa protein fraction was detected. Furthermore, no lipid transfer protein (LTP) 2 and 21 kDA fraction was discovered with raising barley malt amount. Wheat malt contained more turbidity active proteins with molecular weights of 21 and 25 kDa. These protein fractions comprise turbidity active and foam positive Protein Z as well as α-Amylase/Trypsin inhibitors. With increasing barley malt content the portion of foam positive LTP 1 raised up to finally more than 50 %. Low-molecular foam promoting proteins increased during mashing, whereas middle-molecular, mainly turbidity active proteins, were mostly reduced. High-molecular enzyme active proteins between 55 and 63 kDa were completely degraded in all samples.

Descriptors: wheat, mashing, protein profile, grist composition

1 Introduction

Wheat (*Triticum aestivum* L.) has been studied as a malting/brewing material to far lesser extent than barley, which remains the leading raw material in conventional malted cereal beverage production. Significant studies were published on wheat and the influence of environmental and agronomical influence on resulting malt and beer quality [38] as well as the determination of quality related aroma compounds for wheat beer production [21]. *Sacher, B.* [38] defined in his research viscosity, raw protein content, pH value as well as free amino nitrogen (FAN) as crucial quality related attributes. He also investigated that the growing region and climatic conditions are important and specific for wheat varieties. Moreover, malting regimes with falling temperatures seems to be more suitable for wheat, although the malting parameters have to be adjusted to the present wheat variety and quality. Varieties, with high viscosity values and show strong proteolytic potential at the same time, cannot be modified during malting so that they result in good malt and beer quality attributes. Compared to the commonly used malting cereal, barley, relatively less is known about the proteolytic, amylolytic and cytolytic impacts of wheat malt during mashing; although wheat proteins and enzymes are most extensively investigated, among cereal grains. Hence, the influence of wheat malt on processability, wort quality and its interaction with barley malt during mashing was barely investigated. Moreover, all fundamental and technological knowledge concerning mashing is based on barley malt. Mashing is the first biochemical process step of brewing and completes the enzyma-

List of abbreviations

AAL	apparent attenuation limit
FAN	free amino nitrogen
SN	soluble nitrogen
TN	total nitrogen

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tic degradation of high and low molecular substances of cereal grains, started during the malting process [22]. During mashing, insoluble malt fractions are transferred into soluble ones. Specific enzymes are required, similar to the germination process, to solubilize these malt compounds. These enzymes are responsible for the degradation of high molecular organic compounds to low molecular weight and soluble substances. Three modification processes amylolysis, proteolysis and cytolysis characterise mashing. Malt quality, temperature, time and pH are crucial factors for the enzymes activity and for the resulting mashing process.

Starch degradation is the most important process during mashing [14]. Starch is solubilized in three steps:

- mechanical: swelling of starch granules;
- chemical: gelatinisation of starch granules;
- enzymatical: degradation of starch granules [6, 33].

With continued heating, the starch granules become distorted and soluble starch is released into solution. Starch gelatinisation is important in mashing as it disrupts the crystalline structure of the starch, leaving a mixture of amylopectin fragments and dispersed molecules of amylose and amylopectin. These polymers are hydrolysed during mashing into fermentable sugars and dextrins. The main starch degrading enzymes during mashing are β- and α-amylases as well as limited dextrinase, maltase and saccharase [14, 33].

Starch and protein degradation has to be distinguished fundamentally during mashing. While malt starch appears as a homogeneous and relatively simple substance, malt protein substances depend of a complex mixture of all possible nitrogenous compounds (high molecular classes of insoluble native proteins, as well as amino acids) in the mashing process. At the beginning of mashing, soluble nitrogenous substances pass directly into the mash. During the mashing process, they are degraded further by proteolytic enzymes. In addition, insoluble proteins are degraded and transformed into soluble ones. Endopeptidases split proteins to polypeptides and into lower molecular compounds, whereas exopeptidases transfer degradation products into amino acids. Optimal protein degradation depends on solubility, malt enzyme activity and the mashing conditions (i.e., temperature, time and pH level of the mash) [32].

High molecular non-starch polysaccharides, such as β-glucan and arabinoxylan, are known as viscosity increasing substances in beer and it is claimed that wheat arabinoxylans have foam-enhancing properties [27]. Wheat contains much less β-glucan than barley (0.5–2%, 3–7% respectively). On the other hand, the pentosan content of wheat (2–3%) is higher and also the wheat pentosan solubility (1–1.5%) is higher than that of barley (0.7%) [4]. Hemicelluloses of wheat are therefore mainly responsible for the high wort viscosity and the pentosans have the biggest impact. The β-glucan content in wort is a key indicator for mashing. The malt quality has substantial influence on cytolysis and therefore the on β-glucan content in the resulting wort [22]. Incomplete degradation of the endosperm cell walls reduces the amount of extract yield during mashing.

Beer contains ~500 mg/L proteinaceous material, including a variety of polypeptides with molecular weights (MW) ranging from 5–100 kDa, the majority of which lie within a 10 –40 kDa size range [8, 20, 33]. During beer production, the protein type, quantity, and size distributions are all of importance in terms of filtration, foam and haze stability, as well as fermentability. [29, 35]. Barley malt protein characterisation has been the focus of many research papers, and in particular their influence beer qualities. In contrast to barley, wheat generally has higher protein content. Wheat also has an increased proportion of high molecular weight (HMW) proteins, a characteristic that has been correlated to superior foam characteristics and enhanced haze formation, when compared to barley malt beer [3, 9, 10, 40–42]. Malt proteins have a high impact on the brewing process and the resulting beer quality. It is known, that wheat varieties with low protein contents are favoured for brewing [16, 33]. However, proteolytic attributes such as raw protein content, Kolbach index, soluble and total nitrogen, as well as free amino nitrogen (FAN), characterizes malt quality and its processability during the brewing process.

The aim of the present study is primarily gain a fundamental knowledge of wheat malt during the mashing process. Moreover, the influence of wheat malt on mash relevant characteristics, processability and wort quality when using varying amount of wheat malt adjunctions to barley malt was evaluated. In spite of the comprehensive scientific publications on wheat malt, in particular during mashing, wheat malt protein research has been limited, with the focus lying on barley malt instead. The second aim of this study is to narrow that knowledge gap by evaluating the fundamental protein changes during mashing, with comparison to the well-established barley malt protein changes.

2 Experimental

2.1 Materials

The barley variety Marthe and the wheat variety Elixer were used in this study. All varieties were grown and harvested in 2012 in Germany. The raw materials were malted in a pilot scale malting plant (Chair of Brewing and Beverage Technology, Weihenstephan, Germany) according to the standard malting method MEBAK (Mitteleuropäische Brau- und Analysenkommision) 2.5.3.1 [1]. All analyses are based on approved methods for malting and brewing as described in the EBC [13] or MEBAK handbooks [1].

2.2 Mashing

The influence on mash attributes during mashing using different grist compositions of barley and wheat malt was investigated. The grist compositions where labelled according to the wheat/barley ratio [%]: A (100/0), B (75/25), C (60/40), D (50/50), E (25/75), F (0/100). A modified congress mashing regime was used to produce the different mashes according to figure 1 (see next page). For proteolytic and cytolytic degradation temperature stages with 35, 40, 45, 49 and 55 °C were used, for amylolysis preferential 62, 72 and 78 °C were chosen, with sampling in 15 min interval. Fifty grams of grist were milled in a Buhler Miag discmill, using a fine grind (0.2 mm) and coarse grind (0.7 mm). Mashing trails were carried out in stirred metal beakers (n = 3). Two hundred ml of water were added to the ground grist. The mash was continually stirred. Each mash (per beaker) was then cooled to 20 °C, stirred and rinsed. The mash volume was adjusted to 450 ml by the addition of water at 20 °C. Samples were filtrated and immediately analysed. For protein profile analysis, samples were freeze dried.

2.3 Malt and mash analysis

Analytical procedures were carried out at least in triplicate ($n \geq 3$). The means of all results were calculated. All concentrations are based on dry matter (d. m.) unless otherwise stated.

Analyses of barley and wheat malt and wort were performed according to the approved methods of the EBC, MEBAK and ASBC [43] using congress mash programs (MEBAK method 3.1.4.4.1). Extract was calculated using an Anton Paar Alcolyzer (Anton Paar, Graz, Austria), following MEBAK method 4.1.4.2.2 [1]. The apparent attenuation (amount of fermentable sugars) was detected by MEBAK method 4.1.4.10 [1]. Total protein (TP) content was analysed according to MEBAK method 1.5.2.1 and calculated as total nitrogen. The soluble nitrogen (SN) content was determined by using an automatic Kjeltec system, according to MEBAK method 3.1.4.5.2.1 [1]. The protein content was measured according to Kjeldahl method. The determined nitrogen content was multiplied with a 6.25 converting factor according to MEBAK 1.5.2.1 for barley or wheat malt. The official conversion factor for wheat (raw material) and wheat derived products (flour) is 5.7 (AACC method 46.19, and ICC 105/2 standard). However, as it is described in literature [31, 38, 44] and approved methods according AACC (method 46.18) and ICC, the protein content of wheat varieties which are used for feed purpose is obtained by multiply nitrogen content by 6.25. Moreover, in the case of comparing different cereals and wheat varieties among each other it is also valuable to use one converting factor. Due to the fact that this nitrogen converting factor 6.25 is custom for barley and malt, it has been also applied for wheat disposed as a malting and brewing cereal. The Kolbach Index (KI) was calculated from the formula according to MEBAK method 4.1.4.5.3 [1]. α-Amino nitrogen, also referred to as free amino nitrogen (FAN), was determined using a Skalar working station (Skalar, Breda, The Netherlands), following MEBAK method 3.1.4.5.5 [1]. Wort viscosity was measured using a falling ball viscometer (AMVn-Automated Micro Viscometer Anton Paar, Graz, Austria).

The proteolytic enzyme activity (U) was measured according to the method of Brijs [7], where haemoglobin was used as a substrate. After incubation, the reaction was stopped by the addition of 10 % (w/v) trichloroacetic acid. The FAN levels of the supernatants were assayed with trinitrobenzene-sulfonic acid reagent (0.3 %, v/v, in 0.2 M sodium phosphate buffer, pH 8.0) using L-leucine as a standard. For this purpose, the supernatant and trinitrobenzene-sulfonic acid reagent were incubated and the reaction was stopped with 0.2 M HCl. The absorbance was measured at 340 nm. One unit of proteolytic enzyme activity (U) corresponds to the enzyme hydrolysis that liberated 1 mg of L-leucine/h under the assay conditions.

The arabinoxylan content was measured according the modified phloroglucinol method of *Douglas* [12] and *Kiszonas* et al. [24]. To compare the samples and to avoid the influences of fermentable sugars and hexoses, filtrated AAL samples were used for analysing (diluted with water 1 : 3). For calibration 2.0 mL aliquots each of a dilution series of xylose (Sigma-Aldrich, St. Louis, MO) were prepared to 0.0, 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30 mg/mL and were added to 12 mL stoppered reaction tubes. Water was added to each tube to bring the total volume to 2.0 mL, and then 10.0 mL of the reaction reagent (110 mL of glacial acetic acid, 2.3 mL of hydrochloric acid, 5 mL of 20 % w/v phloroglucinol in ethanol, and 1 mL of 1.75 % w/v glucose in water) was added to each tube. The tubes were then placed in a boiling water bath for 25 min, after which time they were removed, cooled in an ice bath, and moved to a room-temperature (≈ 22 °C) bath. The tubes were removed, laid horizontally, and covered with aluminum foil. Absorbance of the samples was read at 558 and 505 nm with an autosampler (1.0 mL) attached to a BioSpec-1601 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). The absorbance reading at 505 nm was subtracted from that at 558 nm to remove the influence of hexose sugars.

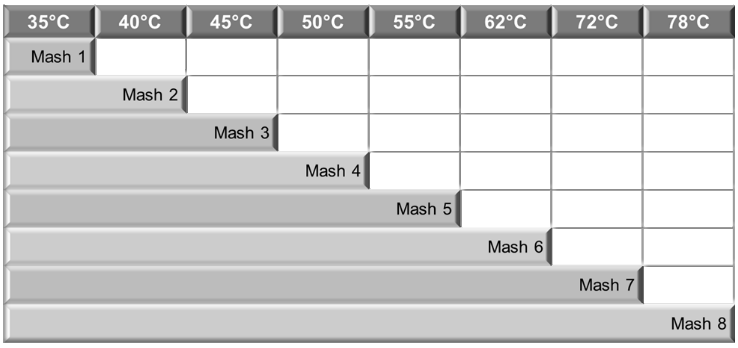

Fig. 1 **Isothermal mashing – sampling plan**

2.4 Lab-on-a-Chip analyses of the total protein extracted during individual mashing stages

To evaluate protein changes during the mashing process as well as the influence of the grist composition of barley and wheat malt, a Lab-on-a-Chip capillary gel electrophoresis system was used (Agilent 2100 Bioanalyzer, 80+-Chip, Agilent Technologies, Palo Alto, CA). Each run included a ladder comprising reference proteins of 3.5, 6.5, 15, 28, 46, 63 kDa plus an upper marker of 95 kDa and a lower marker of 1.5 kDa. Each sample contained an internal standard comprising the upper and lower marker as well. Any peak detected below 5 kDa is termed a system peak and is not included in the analysis. These electrophoretic assays are based on traditional gel electrophoresis principles that have been transferred to a chip format. The chip accommodates sample wells, gel wells and an external molecular weight ladder, and is prepared according to the manufacturer's recommendations (Agilent Technologies, Palo Alto, CA). After sample application, an integrated electrical circuit separates proteins by size intercalating dye into protein micelles as it flows through the matrix with detection by laser-induced fluorescence. Data is translated into gel-like images (bands) and electropherograms (peaks).

For analysis of the total protein content and extracted protein fractions, 40 mg of milled wheat samples were extracted, during the malting process, and prepared as described by the manufacturer before application to the Protein 80^{+}-chip (Agilent Technologies).

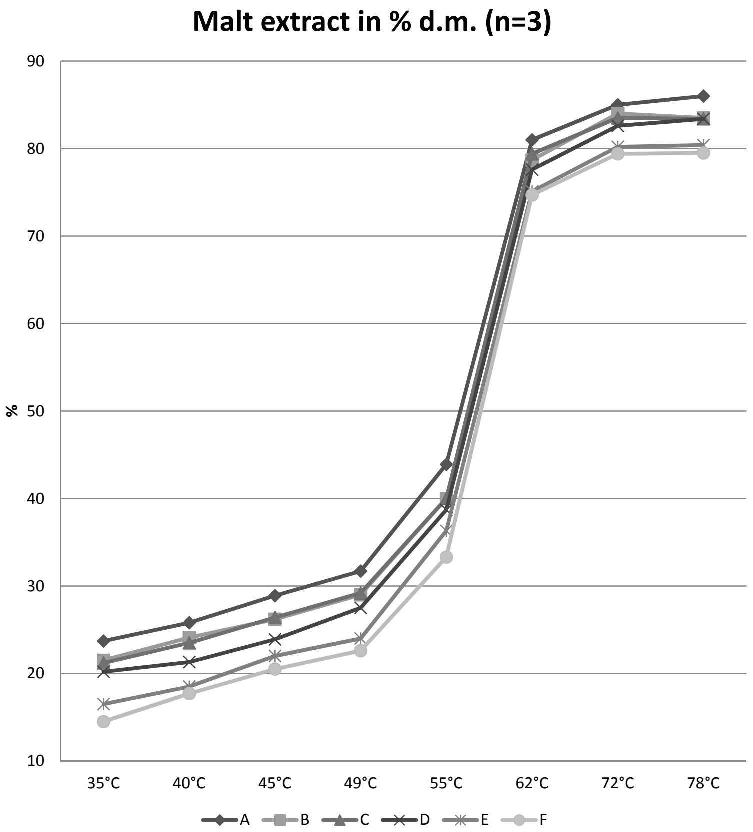

Fig. 2 Modification of extract yield during mashing process

3 Results and discussion

Samples with varying grist compositions of barley and wheat malt were taken throughout the mashing process and then analysed according EBC and MEBAK standard methods.

3.1 Amylolysis

Figure 2 and table 1 shows the devolution of extract yield during mashing and represents one of the most important amylolytic malt quality attributes [33], which are very important for the brew house yield. The fermentable extract generated during mashing is essential for a successful fermentation. The extract has a direct correlation to the soluble sugars. With normal amylolytic enzymatic activity, the potential extract (maximum extract level obtained during mashing) indicates the sugar content and therefore the later alcohol percentage [45]. The extract yield of wheat malt is higher than for barley malt due to the missing husks. With increasing wheat malt ratio an increase in extract content goes along [24, 33, 38]. This can already be seen at the mashing in temperature of 35 °C (A: 23.7 %, B: 21.5 %, C: 21.2 %, D: 20.2 %, E: 16.5 %, F: 14.5 %). During malting, amylolytic enzymes are formed which break down starch into small and soluble sugar compounds. Increased proteolytic activity increases starch availability and can also produce, given the circumstances, higher extract values [6, 34]. In this study a generally increase of the extract values of each grist composition throughout the mashing process can be detected (Fig. 2). An overall increase from about 10 % extract value during the first mashing rests from 35 °C to 49 °C is analysed for each grist composition (shown in Table 1). Between 49 ° and 55 °C and overall extract increase of 40 % can be detected for all samples. Protease and glucanases are even at 35 °C active, but the temperature range 49 ° and 55 °C enhance their activities and the starch degrading process starts. The highest increase can be detected between 55 °C and 62 °C in all samples. The extract value is increased by ca. 84 % (A) and 124 % (F). Contrary to the findings above, the higher the barley malt content of the grist composition is, the higher increase of extract can be found during 55 °C and 62 °C. It seems that amylolytic barley malt enzymes have higher activity than those of wheat malt. This is due to barley β-amylase and β-glucan-solubilase has their enzymatically optimum at 62 °C and transfer starch and high moleculare β-glucan in soluble forms. Moreover, amylolytic barley malt enzymes may contribute to interaction with wheat enzymes. However, enzyme activities seem not to be affected and influenced by the high pH values (5.9 and 6.3, A and F respectively). During mashing, as

Table 1 Amylolytic results

Malt extract in % d.m. (n = 3)

	35 °C		40 °C		45 °C		49 °C		55 °C		62 °C		72 °C		78 °C	
Sample	AV	SD±	AV	SD±	AV	SD±	AV	SD±	AV	SD±	AV	SD±	AV	SD±	AV	SD±
A	23.7	1.65	25.8	1.97	28.9	1.67	31.7	1.42	43.9	0.4	81	1.15	85	3.49	86	0.58
B	21.5	0.76	24.1	0.82	26.2	1.59	29	1.47	40	1.77	78.7	1.14	84	1.74	83.5	2.39
C	21.2	1.51	23.5	0.82	26.4	0.83	29.2	1.28	40	0.26	79.4	0.79	83.5	2.02	83.4	1.88
D	20.2	0.63	21.3	0.75	23.9	1.39	27.5	1.27	38.7	2.35	77.6	0.21	82.6	2.09	83.4	1.08
E	16.5	0.21	18.5	0.03	22	0.36	24	0.42	36.3	0.08	75.1	0.62	80.2	0.25	80.4	0.72
F	14.5	1.08	17.7	1.12	20.5	0.51	22.6	0.12	33.3	0.09	74.7	0.04	79.4	0.04	79.5	0.5

AAL % (n = 3)

	35 °C		40 °C		45 °C		49 °C		55 °C		62 °C		72 °C		78 °C	
Sample	AV	SD±	AV	SD±	AV	SD±	AV	SD±	AV	SD±	AV	SD±	AV	SD±	AV	SD±
A	63.15	0.57	65.15	0.41	66.53	0.86	68.56	0.37	73.22	0.49	78.86	0.42	79.52	0.07	79.25	0.11
B	62.23	0.54	63.39	0.08	63.6	0.64	64.53	0.11	69.8	0.11	77.8	0.11	78.77	0.18	78.45	0.14
C	62.59	0.53	63.62	0.98	64.5	0.04	65.76	0.3	70.77	0.53	78.35	0.43	79.28	0.08	78.74	0.29
D	66.74	0.13	67.43	0.03	68.84	0.23	69.59	0.09	74.81	0.14	80.54	0.18	81.19	0.2	80.45	0.02
E	62.33	0.56	65.17	0.06	66.06	0.32	66.47	0.34	73.62	0.43	81.07	0.04	81.09	0.27	80.58	0.25
F	69.08	0.06	70.15	0.06	71.25	0.06	71.57	0.35	77.04	0.3	83.25	0.07	81.91	0.09	82.29	0.13

Table 2 Cytolytic results

Viscosity mPas (n = 3)

	35 °C		40 °C		45 °C		49 °C		55 °C		62 °C		72 °C		78 °C	
Sample	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**
A	1.268	0.0042	1.2757	0.0021	1.2725	0.0033	1.2983	0.0153	1.39	0.0082	1.6619	0.007	1.7146	0.0087	1.7156	0.0341
B	1.2657	0.0215	1.2463	0.0598	1.2908	0.0027	1.2973	0.0013	1.344	0.0477	1.601	0.0283	1.6732	0.0034	1.6815	0.0001
C	1.2168	0.0625	1.2531	0.0289	1.236	0.0062	1.2777	0.0155	1.3424	0.0185	1.5792	0.0039	1.6274	0.0139	1.5815	0.0015
D	1.2411	0.0083	1.2365	0.0042	1.3001	0.0855	1.2621	0.0221	1.3404	0.0157	1.5373	0.0438	1.6102	0.0024	1.5922	0.0129
E	1.1277	0.0141	1.1397	0.011	1.16	0.0022	1.1722	0.0042	1.2505	0.0006	1.4809	0.0201	1.5313	0.0116	1.56	0.0014
F	1.1371	0.0074	1.1467	0.0037	1.151	0.0018	1.1635	0.0007	1.2216	0.012	1.4755	0.0031	1.5348	0.0121	1.542	0.0019

Arabinoxylan content mg/L (N = §)

	35 °C		40 °C		45 °C		49 °C		55 °C		62 °C		72 °C		78 °C	
Sample	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**
A	1198	8	1195	10	1260	7	1236	4	1173	6	955	8	942	14	1058	12
B	1075	19	840	19	1087	29	1086	8	1016	8	755	46	725	35	873	6
C	918	21	806	8	709	8	793	25	1122	29	865	26	917	29	813	8
D	933	104	709	40	909	19	997	12	872	8	842	20	854	17	804	4
E	892	18	913	18	915	10	923	1	886	4	728	10	690	12	658	14
F	751	27	758	0	745	9	718	3	727	18	554	4	463	29	495	7

in the germinated grain, the same enzymes or enzyme groups are active. They degrade the malt and the soluble compounds are transferred into a soluble form in the wort. The optimal values of the αamylase enzymes during mashing are at a pH level of 5.6–5.8 and a temperature of 65–75 °C [33]. αamylase attacks the starch granule from the inside and possess substrates for βamylase, which shows little activity. Between 62 ° and 78 °C only a slight extract increase in all samples can be detected. A potential extract of more than 80.0 % is expected for barley malt used for brewing purposes. The extract values for wheat are higher and are around the range of 83.0 % [33]. The final mashes showed acceptable extract values (A: 86.0%, B: 83.5 %, C: 83.4 %, D: 83.4 %, E: 80:4 %, F: 79.5 %). No or marginal differences between samples B, C and D can be seen during the single mashing stage and the final wort.

AAL represent the percentage that measures the conversion of sugars into alcohol and carbon dioxide by the fermentation process [33]. The AAL depends upon the availability of the fermentable sugars and on the yeast remaining in contact with wort [5]. This concentration is measured in terms of grams of solids per 100 grams of wort. Barley wort, obtained by congress mashing, normally has an AAL of 80.0 %. Wheat malt recorded AAL values vary between 75.7 and 82.2 %. [28, 33]. The evaluated values (Table 1) show that with an increase of barley malt a higher AAL can be achieved (A: 79.25 %, B: 78.45 %, C: 78.74 %, D: 80.45 %, E: 80.58 %, F: 82.29%) which can be referred to the more effective α-amylase activity in barley than in wheat malt [24, 33]. Barley malt starch is more degraded, and therefore more fermentable sugars are available for the yeast. However wheat malt shows higher extract values but lower AAL.

Concerning the processability and wort quality it can be stated that only marginal differences between sample B, C and D as well as E and F for amylolytic attributes could be detected. In sense of extract and AAL, a wheat malt ratio higher than 50 % shows no benefit for brewing purpose. Due to the similar deviation of the extract graph it can be assumed that amylolytic enzymes and their changes during mashing of wheat malt are comparable to those of barley malt. Moreover, it can be stated that insufficient barley malt extract values may be compensated by increasing wheat malt amount and their amylolytic enzymes. This has to be proven in further studies.

3.2 Cytolysis

Viscosity is the primary malt quality parameter for wheat as it gives an indication of its processability during beer production, especially lautering and filtration. High molecular non-starch polysaccharides, such as β-glucan and arabinoxylan, are known as viscosity increasing substances in beer and it is claimed that wheat arabinoxylans have foam-enhancing properties [27]. Wheat contains much less b-glucan than barley (0.5–2 %, 3–7 % respectively). On the other hand, the pentosan content of wheat (2–3 %) is higher and also the wheat pentosan solubility (1–1.5 %) is higher than that of barley (0.7 %). Hemicelluloses of wheat are therefore mainly responsible for the high wort viscosity and the pentosans have the biggest impact. Table 2 shows the viscosity distribution during mashing. Throughout the mashing process an increase of viscosity was detected for each sample (Sample A: 1.268 to 1.7256 mPas; Sample E: 1.137 to 1.540 mPas). Moreover, with increasing wheat malt content higher viscosity values could be determined (Table 2). At the beginning of the mashing process marginal viscosity increases could be detected, which are caused by soluble proteolytic substances and noncarbohydrate polysaccharides. The biggest increase was measured again between the 55 ° and 62 °C mashing temperatures. Amylolytic degradations starts to proceed (compare extract values), and cytolytic processes are ongoing. The solubilized β-glucan is degraded by β-1,3-glucanase. Gelatinisation starts at 62 °C and starch transferred to limited dextrins by α-amylases [33, 37]. Fermentable sugars are built which affect the viscosity increase, too. Additionally, high molecular β-glucan is solubilised by β-glucan-solubilase. The further increase of the viscosity above 72 °C is caused by α-amylases which hydrolyse limited dextrins [33].

Moreover, in literature is stated that increased water extractable arabinoxylans (WEAX) cause problems during the brewing process. Complications in the filtration and lautering processes [19, 39], as well as contributions to premature yeast flocculation [26] can occur. *Lu* et al. [30] studied the effect of arabinoxylan (AX) solubilisation on wort viscosity and filtration and compared barley malt mashes that contained wheat and wheat malt. With an increased proportion of wheat or wheat malt, a higher AX content in the final wort was observed. This can be attributed to the fact that wheat endosperm contains more arabinoxylans than barley endosperm. Furthermore, worts containing wheat malt showed the highest arabinoxylan content as some water-insoluble arabinoxylans were solubilised during malting and released into the final wort [11]. These findings can be compared with the results in this study. Sample A (wheat/barley malt ratio [%]: 100/0) contains nearly double amount of AX than sample F (wheat/barley malt ratio [%]: 0/100), 1198 mg/L and 751 mg/L, respectively at 35 °C mashing temperature. During the first mashing steps, no significant changes in AX content could be detected for all samples. AX degrading enzymes, e.g. endoxylansase and α-ʟ-arabinosidase have a temperature optimum at around 49 °C. These enzymes degrade and solubilise high molecular AX which is degraded by β-D-xylosidase at higher temperatures (Table 2). The final wort of all measured samples show the same behaviour as at the beginning of mashing. However, a difference of decreasing percentage of AX between the grist compositions could be detected. A higher barley malt ratio is followed by a higher percentage of total decrease of AX in the final mash, with an exception of sample B (A: 13 %, B: 23 %, C: 13 %, D: 16 %, E: 35 %, F: 52 %). This leads to the assumption, that AX degrading enzymes of barley malt are more effective and promoted as wheat malt ones during mashing. Arabinoxylans in wheat were not found to be extensively degraded and most of the AX were insoluble, resulting in a relatively low AX content during mashing. *Finscher* and *Stone* [18] reported that, arabinoxylans can form highly viscous solutions, especially in the presence of β-glucan. Our findings can be compared to further conclusion of Lu`s et. al [30] study. When more arabinoxylans are solubilised, it leads to an increase in wort viscosity and therefore retards wort filtration.

Additionally to viscosity and AX values, β-glucan content of the different mashes was evaluated, shown in figure 3. With an increase in barley malt content an increase of β-glucan in the final wort samples were identified (Sample [mg/L]: A: 29, B: 106.9, C: 148.2, D: 203.8, E: 290.0, F: 470). Moreover, sample E which contains 25 % of wheat malt contains at the 62 °C step ca. 20-fold and in the final wort 31 % less β-glucan than sample F. At lower mashing temperatures, β-glucan is transferred by endo-β-1,4 and β-1,3-glucanase in small substances, which cannot be detected by the method. The biggest increase only for sample F can be seen at mash temperatures between 55 ° and 62 °C. However, for sample A to E the biggest increase was measured between 62 ° and 72 °C. endo-β-1,4-glucanase is heat sensitive and is inactivated at 55 °C. However, β-glucan-solubilase activity is increasing (temp. optimum at 62 °C), and insoluble β-glucan is degraded to high molecular, viscosity increasing form [33]. This form undergoes further degradation by β-1,3-glucanase. It seems that interactions of wheat malt and barley malt enzymes occur, whereby barley malt enzyme activity seem to be lowered by wheat malt enzymes. β-glucan-solubilase of wheat malt is more active and promotes insoluble β-glucan degradation. In this study, the activity of these enzymes was favoured by the high pH values (sample pH at 35 °C: A: 6.31, B: and C: 6.18, D: 6.12, E: 6.07, F: 5.99), due to their pH optima between 6.6 and 7.7. With increasing wheat malt ratio an increase in pH could be detected.

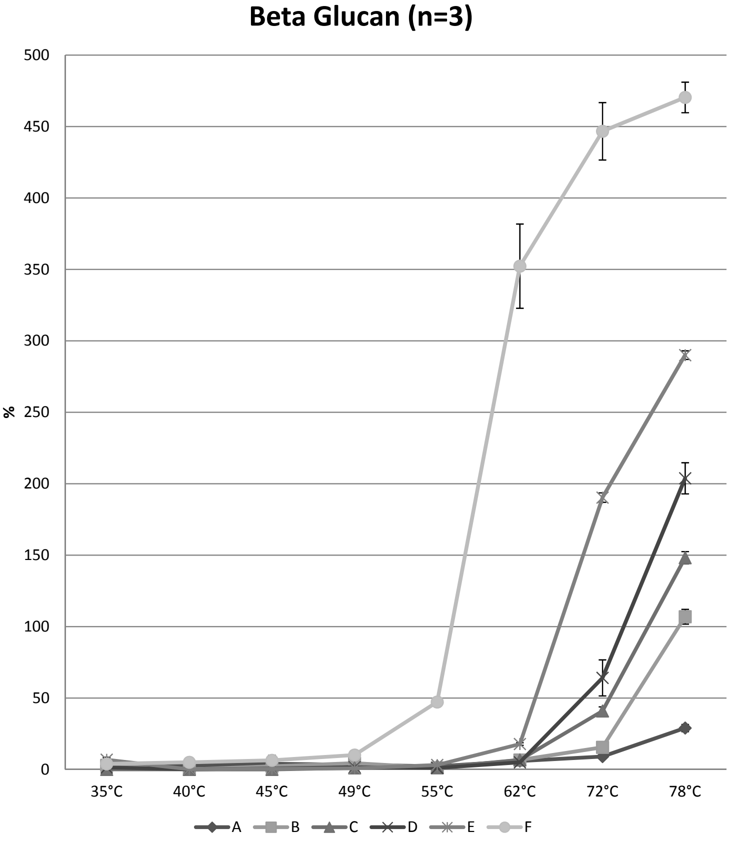

Fig. 3 **Modification of B-glucan during mashing process**

Summarising these results for processability and quality aspects, it could be proven that with increasing wheat malt ratio an increase of viscosity goes along. All analysed worts lay below the viscosity limit of 1.800 mPas and 1.560 mPas (wheat and barley, respectively). AX and β-glucan content in sum (sample [mg/L]: A: 1087, B: 979.9. C: 981.2, D: 981.2, E 948.0, F: 965.0) can be attributed to their specific viscosity. Moreover, no big differences between sample B, C and D were evaluated again.

3.3 Proteolysis

The brewing attribute for the amount of nitrogen degradation products, which are solubilised during congress mashing, is referred to as soluble nitrogen (SN) level [6] and should lie in the range of 600–800 mg/100 g for wheat malt and 650–750 mg/100 g for barley malt [38]. At the beginning of mashing, soluble nitrogenous substances pass directly into the mash. This can also be clearly seen in table 3, where continuous increases of soluble nitrogen (SN) as well as an overall increase of 130 mg/100 mg malt in all samples were evaluated. Moreover, with increasing wheat malt content, significant differences in SN values were detected between the samples (Sample from 35 °C to 78 °C respectively in [mg/100 g malt]: A: 577 to 708, B: 532 to 660, C: 512 to 648, D: 497 to 585, E: 438 to 569, and Sample F: 398 to 508). Sample A (wheat/barley malt ratio [%]: 100/0) showed constantly 200 mg/

Table 3 **Proteolytic results**

	SN in [mg/100 g malt d.m.] (n = 6)															
	35 °C		**40 °C**		**45 °C**		**49 °C**		**55 °C**		**62 °C**		**72 °C**		**78 °C**	
	AV	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**
A	577	12.2	624	0.6	653	3.4	677	5.4	721	2	726	5.6	714	3.9	708	4.6
B	532	4.3	567	3	608	1	631	1	664	0.2	670	4.5	664	2.8	660	7.7
C	512	13.7	544	4.7	587	1	625	3	649	3	668	2.1	651	0.7	648	8.4
D	438	5.7	448	6.2	507	6.6	536	6.6	560	7.1	571	8.2	613	13.8	569	8.1
E	497	8.9	523	4.2	545	3.2	571	1.4	592	0.4	585	4.6	578	1.4	585	1.1
F	396	3.9	423	0.4	458	1.6	487	5	505	0.4	510	0.1	511	8.6	508	1.9
	Protease activity in [U/g] (n = 3)															
	35 °C		**40 °C**		**45 °C**		**49 °C**		**55 °C**		**62 °C**		**72 °C**		**78 °C**	
	AV	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**	**AV**	**SD±**
A	23.003	0.323	22.893	0.369	20.594	1.877	18.44	0.081	15.121	1.106	11.211	0.871	9.912	0.363	9.078	0.469
B	23.58	0.134	23.231	4.921	21.423	2.178	19.149	0.436	15.263	1.173	10.879	0.134	9.647	0.067	9.173	0.268
C	23.84	0.369	23.39	0.737	21.834	2.622	20.025	0.402	15.873	0.242	10.34	0.081	9.398	0.766	8.415	0.134
D	25.228	0.645	23.589	0.068	22.427	1.027	20.756	0.377	16.386	0.726	11.652	1.129	9.284	0.04	9.427	0.484
E	25.672	0.068	24.196	1.407	22.718	0.081	21.292	0.887	17.299	0.484	10.56	0.068	9.518	0.103	9.349	0.068
F	25.798	1.129	25.142	1.089	23.745	1.775	22.975	0.363	17.583	0	10.197	0.686	9.826	0.484	10.397	0.403

100 g malt higher values than sample F (wheat/barley malt ratio [%]: 0/100). This is due to the higher protein content of wheat and greater proportions of high molecular protein fractions in wheat malt wort compared to barley and barley malt [2, 38]. Optimal protein degradation depends on solubility, malt enzyme activity and the mashing conditions (i.e., temperature, time and pH level of the mash) [14, 28, 32]. During the mashing process, proteins are degraded further by proteolytic enzymes and insoluble proteins are degraded and transformed into soluble ones. Endopeptidases split proteins to polypeptides and into lower molecular compounds, whereas exopeptidases transfer degradation products into amino acids. Lower molecular proteins are necessary for adequate yeast nutrition, to provide solid fermentation without forming undesirable fermentation products. Higher molecular proteins influence beer filterability and stability. It is known that proteolysis occur mainly between 35 °C and 55 °C. At around 50 °C the breakdown of proteins, gums, and phosphates is increased [14]. This can also be seen in table 3, where SN values are constantly increasing during these temperature stages, and show their maximum values at 55 °C. Moreover, between 55 °C and 78 °C SN values only marginal increase. Soluble nitrogen influences beer quality as well as the brewing process. In consideration of processability and wort quality for wheat beer production insufficient SN values of barley malt can be compensated by good wheat malts as well as wheat malt ratio. Regarding the wheat/barley malt ration it can be stated, that narrow differences for sample B and C as well as D and E could be detected. At the end of the mashing process sample A to D show adequate values, whereas sample E and F resulted in a too low SN values.

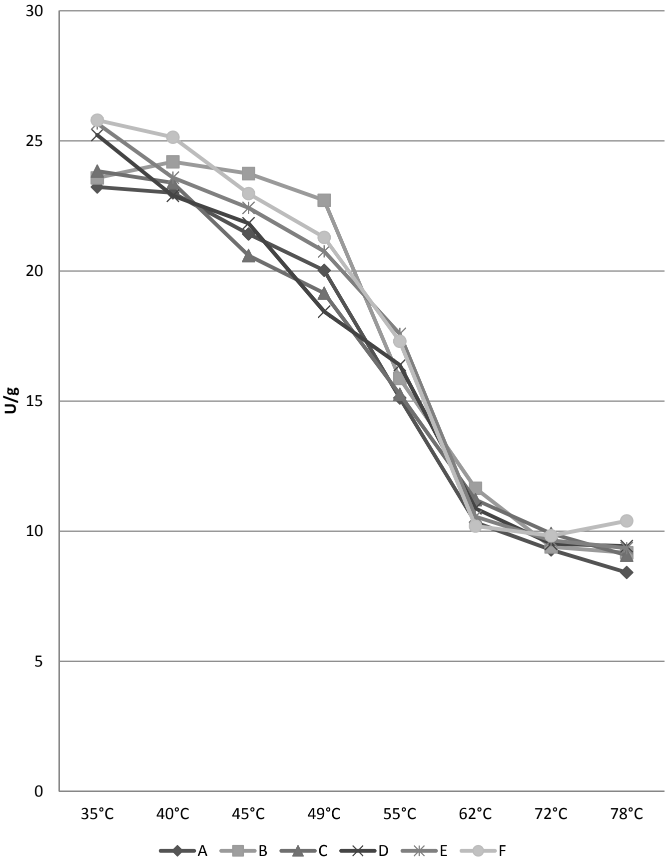

Fig. 4 **Protease activity**

Furthermore, total proteolytic enzyme activity was analysed using haemoglobin as substrate and absorption was ensured against ʟ-leucine as standard. An overall decrease during the mashing process could be detected in all samples (Sample A: 23.840 to 8.415 U/g and Sample F: 25.79 to 10.397 U/g from 35 °C to 78 °C respectively; Table 3 and Fig. 4). This is in contrast to the malting process, where an increase of proteolytic activity could be detected and was shown for other cereal grains in many researches [12, 17, 25]. Between sample A and F significant differences in protease activity could be observed, during all mashing stages. Sample F (wheat/barley malt ratio [%]: 0/100) show a 19 % higher protease activity than sample A (wheat/barley malt ratio [%]: 100/0).

Moreover, with an increase of wheat malt ratio, a decrease in protease activity was evaluated. Hence, barley malt seems to have higher proteolytic activity than wheat malt. The overall decrease follows in a same way as it was shown for SN. In all samples the biggest decrease was detected at a mashing temperature between 55 °C and 62 °C (sample decreased by [%]: A: 32.56, B: 32.25, C: 33.47, D: 33.6, E: 35.19, F: 33.73) were proteolytic enzymes shows their optima. These decreases are mainly caused by endopeptidase [23, 33]. They have their optima at pH values between 3.9 and 5.5 and temperatures between 45 ° and 50 °C. Although peptidases are mainly inactivated at higher temperatures, proteins are still solubilised between 55 ° to 65 °C.

3.4 Changes of the protein profile during mashing

To gain further technological knowledge of the mashing process and the influences on the proteolytic profile sample A (wheat/barley malt ratio [%]: 100/0), D (wheat/barley malt ratio [%]: 50/50) and F (wheat/barley malt ratio [%]: 0/100) were analysed using the Lab-on-a-Chip technique. Their deviation and changes of the protein profile during mashing are illustrated in figure 5 to 8. Due to the fact, that each cereal grain is characterized by a specific protein profile, specific cereal peaks for wheat and barley were detected in the analysed mashes. Several protein peaks and peak areas could be detected for sample A (Fig. 5), which are classified into peak numbers 1 to 9 (6 kDa, 7kDa, 9 kDa, 14 kDa, 21 kDa, 25 kDa, 40 kDa, 55 and 63 kDa respectively). Proteins of sample A (wheat/barley malt ratio [%]: 100/0) consist mainly of 14 kDa peaks, which are known as α-amylase/trypsin inhibitors. Moreover low molecular weight fractions which can be dedicated as lipid transfer proteins (LTP) with a

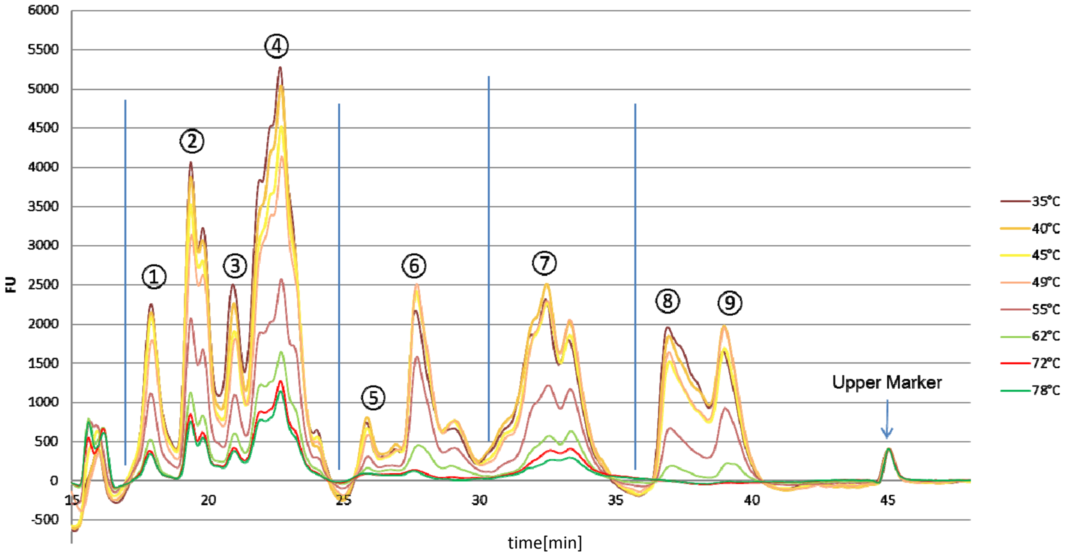

Fig. 5 **Moleculare wheigt distribution of 100 % wheat malt (sample A) during mashing process; 1 = 6 kDa, 2 = 7 kDa, 3 = 9 kDa, 4 = 14 kDa, 5 = 21 kDa, 6 = 25 kDa, 7 = 40 kDa, 8 = 55 kDa, 9 = 63 kDa**

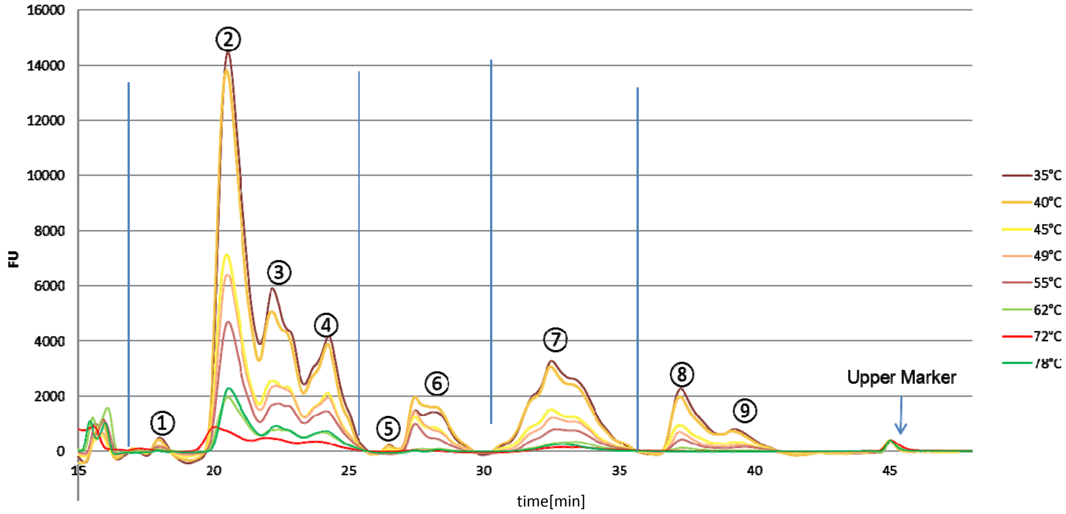

Fig. 6 **Moleculare wheigt distribution of 100 % barleymalt (sample E) during mashing process; 1 = 6 kDa, 2 = 9 kDa, 3 = 14 kDa, 4 = 17 kDa, 5 = 21 kDa, 6 = 25 kDa, 7 = 40 kDa, 8 = 55kDa, 9 = 63 kDa**

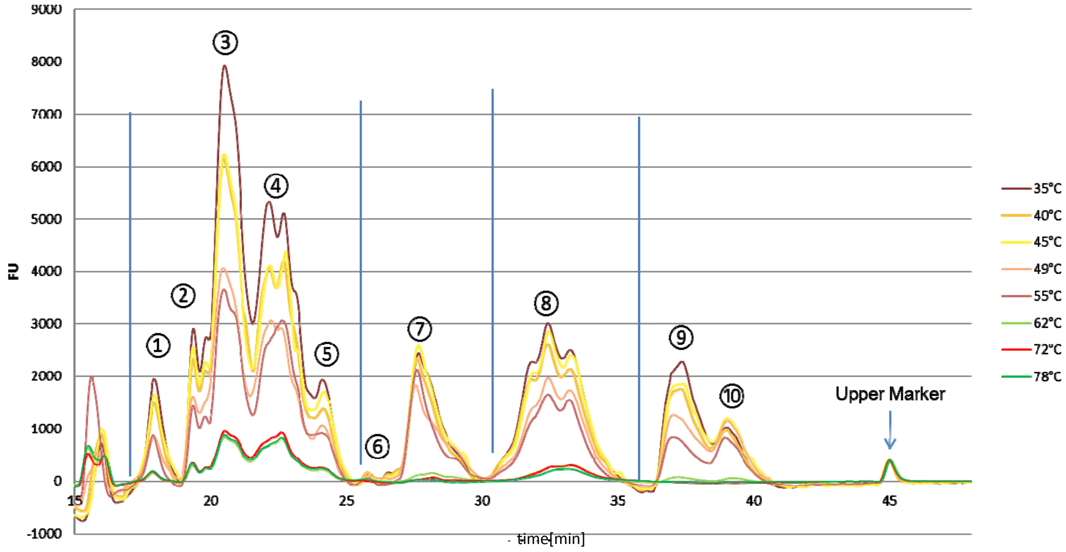

Fig. 7 **moleculare wheigt distribution of 50 %wheat malt and 50 % barley malt (sample C) during mashing process; 1 = 6 kDa, 2 = 7 kDa, 3 = 9 kDa, 4 = 14 kDa, 5 = 17 kDa, 6 = 21 kDa, 7 = 25 kDa, 8 = 40 kDa, 9 = 55 kDa, 10 = 63 kDa**

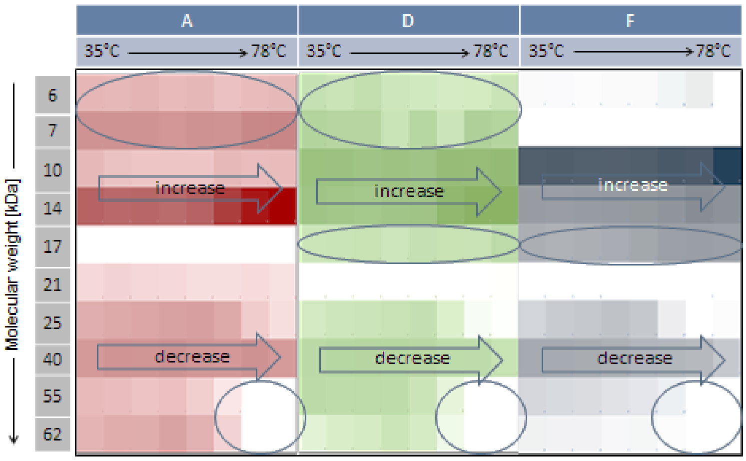

Fig. 8 **Protein interactions of different grist compositions during mashing process**

molecular weight of 6, 7 (LTP2) and 9 kDa (LTP1) were detected. Also peaks of 21 and 25 kDa (haze active proteins), 40 kDa (Protein Z), 55 and 63 kDa (dedicated as β-amylases [36]) were detected at the beginning of the mashing process. These proteins are described as albumin and globuline fractions due to the findings of *Faltermaier* et al. [17], where an overall degradation of storage proteins during the malting process was investigated. Regarding to the total percentage of the proteins found during mashing an increase of the LTP 2 as well as the Protein Z could be detected (16.6 to 20.5 % total protein and 31.6 to 45.4 % total protein, respectively, data not shown). During mashing the LTP 1 protein undergoes chemical changes, like glycolisation processes. The molecular weight is increasing and LTP1 is transferred to a foam positive form [15, 22]. Protein peaks of 55 and 62 kDa are completely degraded at the end of the mashing process, which can be argued that β-amylases are inactivated at temperatures around 70 °C [32]. LTP1 as well as protein Z are rich in disulfid bonds, why they are protected against enzymatic degradation and are heat stable [22]. Based on the degradation of higher molecular fractions, the relative proportion of low molecular proteins like LTPs and the protein 14 kDa peak are increasing.

Barley malt samples differ during the mashing process first in their protein constitution as well as in their percentage of total protein. For sample F also 9 different peaks could be detected (Fig. 6). However, no 7 kDa peak for barley malt mashes, but additionally a 17 kDa peak was investigated, which is referred as haze positive ε-1-hordein/glycoprotein [9, 33, 42]. The relative concentration of the 9 kDa peak is higher for barley malt mashes than for wheat malt mashes (7.4 and 43.9 % total at 35 °C, respectively, data not shown). An increase in the lower molecular protein peaks (9, 14 and 17 kDa) can be detected during the evaluation of barley malt mashes, which is caused by proteolytic degradation of high molecular weight proteins to low molecular ones. Compared to mashes of sample A where the relative concentration stays almost constant, the protein Z in mashes of sample F is degraded from 13.8 to 6.7 % of total protein. This has to be investigated more detailed in further studies.

Figure 7 show the protein changes of sample D (consisting of 50 % wheat malt and 50 % barley malt) during mashing. For sample D 10 peaks were identified in this study. Hence, both cereal specific protein peaks 7 kDa peak (specific for wheat) and 17 kDa peak (specific for barley) were detected in the mash. The higher molecular weight proteins were comparable degraded as it was shown for wheat and barley malt mashes. Only slight increases in low molecular protein fractions were measured. LTP 1 was increased during mashing from 28.3 to 33 % of total protein whereas the 14 kDa peak increased from 26.1 to 39.1 % of total protein. Again, protein Z was degraded from 14.5 to 10.6 % of total protein. With increasing grist composition regardless which cereal has the highest amount, the cereal specific protein fraction is increasing (data not shown).

Differences in specific protein peaks and profile were detected for all samples during mashing. Moreover, for all samples increases in low molecular weight fractions as well as an increase of foam positive proteins during mashing were shown (Fig. 8). However, a decrease of haze relevant proteins was evaluated. With higher barley malt grist more foam positive proteins (LTP1 and ε-1-hordein) are transferred to the final wort. However, with higher proportion of wheat malt more middle molecular and haze relevant protein fractions (21 and 25 kDa) are released in the wort. Considering these findings for processability and quality aspects haze and foam stability can be influenced positively in the final beer with the right grist composition of wheat and barley malt.

4 Conclusion

The grist composition of wheat and barley malt has a high influence on the resulting processability and final wort quality. It was shown, that extract, viscosity, soluble nitrogen, and AX increased with increasing wheat malt ratio. However, marginal differences between sample B, C and D as well as E and F were identified. In contrast, with higher wheat ratio protease activity, final attenuation as well as β-glucan declined.

During the mashing process extract, final attenuation, viscosity, soluble nitrogen and β-glucan increased, whereas arabinoxylan (AX), FAN, pH and protease activity decreased. Between 55 ° and 62 °C the amylolytic mash values extract and final attenuation rose most considerably. Concerning the processability and wort quality it can be stated that only marginal differences between sample B, C and D as well as E and F for amylolytic attributes could be detected. In sense of extract and AAL, a wheat malt ratio higher than 50 % shows no benefit for brewing purpose. Due to the similar deviation of the extract graph it can be assumed that amylolytic enzymes and their changes during mashing of wheat malt are comparable to those of barley malt. Regarding mash temperature between 55 and 62 °C the cytolytic mash attributes viscosity and β-glucan denoted the biggest increase, whereas AX showed the greatest decline. A relationship between viscosity and mash attribute AX and β-glucan couldn't be detected at a sufficiently extent. Proteolytic parameter declined strongly during the first mashing stages (35 ° to 55 °C) remained almost constant from 55 ° to 78 °C. In consideration of processability and wort quality for wheat beer production insufficient SN values of barley malt can be compensated by good wheat malts as well as wheat malt ratio.

In the second part of this study a protein profiling was performed to evaluate changes during the mashing process as well as the influence of grist composition. Differences in specific protein peaks and profile were detected for all samples during mashing. Moreover, all samples increased in low-molecular foam promoting proteins during mashing, whereas middle-molecular, mainly turbidity active proteins, were mostly reduced. High-molecular enzyme active proteins between 55 and 63 kDa were completely degraded. However, a decrease of haze relevant proteins was evaluated. With higher barley malt grist more foam positive proteins (LTP1 and ε-1-hordein) are transferred to the final wort. In contrast, with higher proportion of wheat malt more middle molecular and haze relevant protein fractions (21 and 25 kDa) are released in the wort. Considering these findings for processability and quality aspects haze and foam stability can be influenced positively in the final beer with the right grist composition of wheat and barley malt.

In this study the wheat variety Elixer and barley variety Marthe was used. However, further research has to be done, due to the varying constitutions of wheat and barley malt, which may also influenced by growing area, weather conditions as well as malting regimes [38].

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