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Title	Fundamental studies of the application of wheat for malting and brewing
Authors	Faltermaier, Andrea E.
Publication date	2015
Original Citation	Faltermaier, A. E. 2015. Fundamental studies of the application of wheat for malting and brewing. PhD Thesis, University College Cork.
Type of publication	Doctoral thesis
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Download date	2026-11-16 09:41:47
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UNIVERSITY COLLEGE CORK

DEPARTMENT OF FOOD AND NUTRITIONAL SCIENCES



Fundamental studies on the application of wheat for malting and brewing

Thesis presented by

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Univ. Dipl.-Ing. Food Technology

For the degree of

Doctor of Philosophy

(PhD in Food Science and Technology)

Under the supervision of

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September 2015

Abstract

Wheat (Triticum aestivum L.) has a long tradition as a raw material for the production of malt and beer. While breeding and cultivation efforts for barley have been highly successful in creating agronomically and brew- technical optimal specialty cultivars that have become well established as brewing barley varieties, the picture is completely different for brewing wheat. An increasing wheat beer demand results in a rising amount of raw material. Wheat has been - and still is - grown almost exclusively for the baking industry. It is this high demand that defines most of the wheat breeding objectives; and these objectives are generally not favourable in brewing industry. It is of major interest to screen wheat varieties for brewing processability and to give more focus to wheat as a brewing cereal. To obtain fast and reliable predications about the suitability of wheat cultivars a new mathematical method was developed in this work. The method allows a selection based on generally accepted quality characteristics. As selection criteria the parameters raw protein, soluble nitrogen, Kolbach index, extract and viscosity were chosen. During a triannual cultivation series, wheat varieties were evaluated on their suitability for brewing as well as stability to environmental conditions. To gain a fundamental understanding of the complex malting process, microstructural changes were evaluated and visualized by confocal laser scanning and scanning electron microscopy. Furthermore, changes observed in the micrographs were verified and endorsed by metabolic changes using established malt attributes. The degradation and formation of proteins during malting is essential for the final beer quality. To visualise fundamental protein changes taking place during malting, samples of each single process step were analysed and fractioned according their solubility. Protein fractions were analysed using a Lab-on-a-chip technique as well as OFFgel analysis. In general, a different protein distribution of wheat compared to barley or oat could be confirmed. During the malting process a degradation of proteins to small peptides and amino acids could be observed in all four Osborn fractions. Furthermore, in 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. This study investigated the suitability of wheat for malting and brewing industry and closed the scientifical gap of amylolytic, cytolytic and proteolytic changes during malting and mashing.

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Acknowledgements

Several people contributed in many different ways to my work. Without your support, challenges, opening gaps as well as bringing me out of my comfort zone the completion of this thesis would not have been possible.

First and foremost, I would like to thank Professor Dr. Dr. Elke K. Arendt, Professor Dr.-Ing. Thomas Becker and Dr.-Ing. Martina Gastl for supervising this work. I am very thankful for their guidance, friendship as well as their encouragement during the last years.

A special thanks goes to the InBev Bailett Latour committee for funding this doctoral thesis. It is an honor for me to be part of the IBL family.

Furthermore, I take the chance to thank Dr. Martin Zarnkow. Martin, without you I still would be the little Bavarian girl from next door. Thanks for your support and your trust in me.

Many thanks to all of my student colleagues in Cork for the outstanding Irish multi culti way of living. Special thanks to Markus, Sophie and Outi - and sorry for occupying your living room that often.

Many thanks to my colleagues in Weihenstephan, the old and new working Group raw materials, Andreas Maier (Mein Lieblingsmälzer), the brewers: Tony Pichlmeier, Michi Ammer, Manfred Wallenwein and Andi Stürtzer, Konrad Lackermeier, the LAB Crew: Christoph Heimerl , Monika Braasch, Kerstin Holtz and Heike Wolf. Also a special thanks to my Post Docs: Dr. Frithjof Thiele, Dr. Elisabeth Wiesn, Dr. Jean Titze, Dr. Deborah Waters and Dr. Emanuele Zannini. Not to forget my students: MSc Judith Stigler and Dipl.-Ing. Johannes Negele, many thanks for your great work and help.

Further on I would like to thank those colleagues who become friends during this special time:

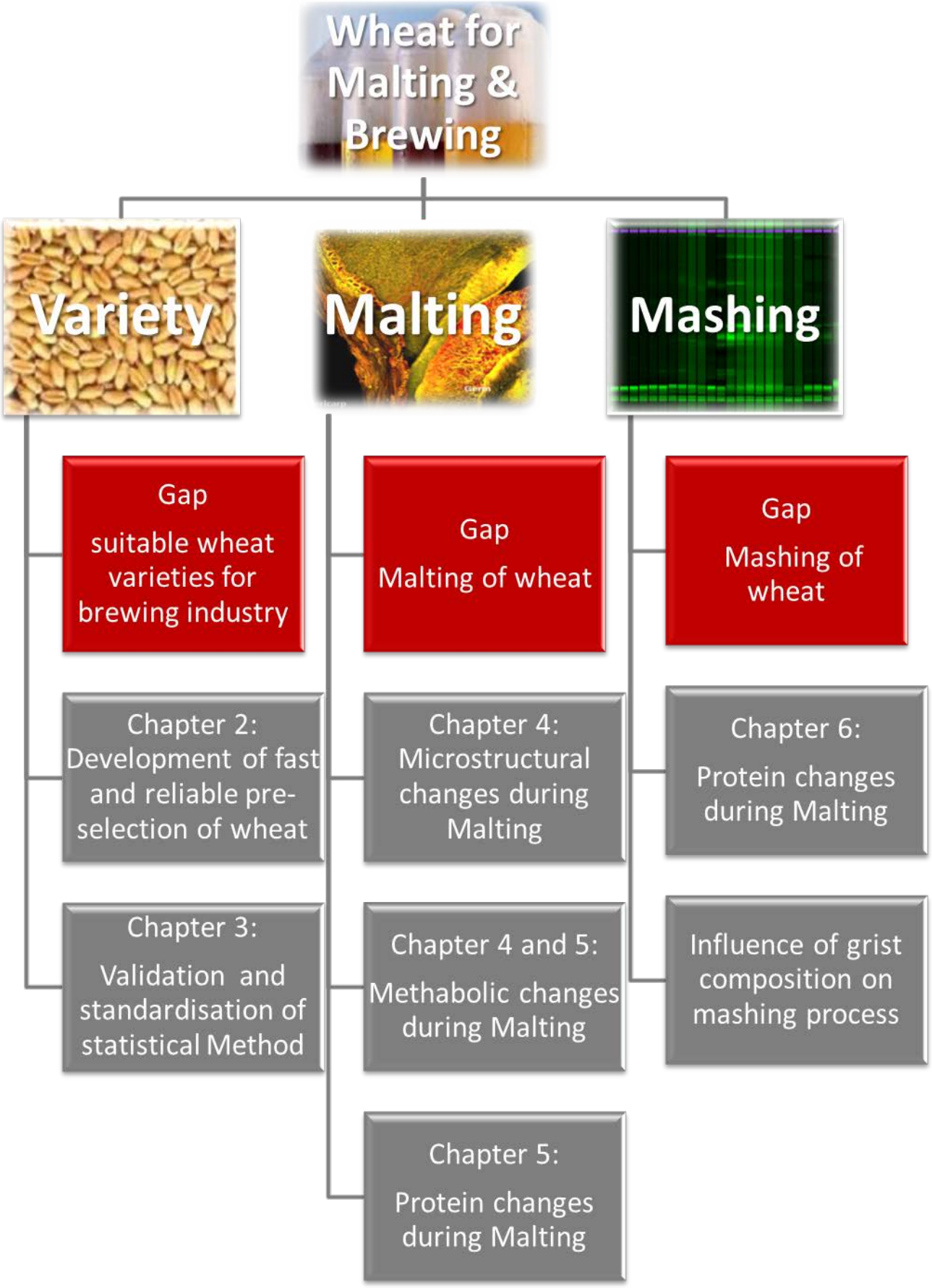
Dr. Stefan Hanke and Sebastian (Kappi) Kappler: Guys - thanks for bringing me into the science of beer. Christopher Holtz, Alicia Munoz Insa, Cajetan Geißinger, Christoph Neugrodda, and Christoph Föhr: special thanks for the great inspiring roof top discussions. Without you, my work live would not have been balanced. Thanks for your honesty and your critical point of view. Cynthia Almaguer: Cucu – thanks for the fact that you are as you are.

Above all, I would like to thank my family – my parents Elfriede and Andreas, my brother Oli, my sister in law Anja, my nephew Korbinian - and Dani for their unconditional support and encouragement throughout all my studding years. Thank you for always believing in me. You all made me to the person I am now.

Finally, I would like to thank Sebastian – my best friend – my everything. Thank you for your help, your understanding and your support throughout all these years. To meet you was and is the best thing ever happened to my live – I love you.

“Hinfallen. Aufstehen. Krone richten. Weitermachen”

AAL	Apparent attenuation limit
ASBC	American Society of Brewing Chemists
AX	Arabinoxylan
CLSM	Confocal laser scanning microscopy
E	Extract
EBC	European Brewing Convention
FAN	Free amino nitrogen
FU	Fluorescence unit
HMW	High molecular weight
ICC	Internationale Gesellschaft für Getreidewissenschaft und -technologie
IEF	Isoelectric focusing
KI	Kolbach index
LMW	Low molecular weight
LTP	Lipid transfer protein
MEBAK	Mitteleuropäische Brau- und Analysenkommision
MW	Molecular weight
RP	Raw protein
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
SN	Soluble nitrogen
TP	Total protein
Visc.	Viscosity
WEAX	Water extractable arabinoxylan


Wheat for Malting & Brewing
Variety
Malting
Mashing
Gap
suitable wheat varieties for brewing industry
Gap
Malting of wheat
Gap
Mashing of wheat
Chapter 2: Development of fast and reliable pre-selection of wheat
Chapter 4: Microstructural changes during Malting
Chapter 6: Protein changes during Malting
Chapter 3: Validation and standardisation of statistical Method
Chapter 4 and 5: Methabolic changes during Malting
Influence of grist composition on mashing process
Chapter 5: Protein changes during Malting

Für Sebastian

CHAPTER 1

REVIEW

COMMON WHEAT (*Triticum aestivum* L.)

AND IT'S USE AS A BREWING CEREAL

1.1 History, diversity and the current wheat beer situation

Wheat, one of the oldest cultivated crops that has been grown as food at least since 8.000-10.000 BC (1, 2), has a long tradition as a raw material for the production of malt and beer. Nevertheless, it has been studied to a much lesser extent than barley. Wheat beer is a specialty beer that evolved in Bavaria, in the late Middle Ages. Historically, the key difference between what was then labelled "brown beer" and "white beer," was the type of fermentation - a distinction that has since been included in the modern version of the German Beer Purity Law (*Vorläufiges Biergesetz*). According to that prescription, all wheat beer brewed in Germany must be top- fermented, made with at least 50 percent malted wheat, and have an original gravity of at least 11 °Plato. For optimal brew-house results, within the confines of this law, efforts have been made in recent years to breed and cultivate wheat varieties that are suitable for wheat beer-making. Before the feudal purity law (*Reinheitsgebot*) took effect in 1516, brewers used just about any available raw material for fermentation and flavouring. Mashes consisted of barley, or wheat, and/or oats and the brews were flavoured with ingredients such as herbs, willow bark, even oxen bile. However, such unusual ingredients, often resulted in completely undrinkable concoctions. As far as wheat beer is concerned, today's feudal purity law, Germany's "provisional beer law," has evolved from that very description. It now stipulates, and is also stated the *World Beer Cup Style Guidelines*, that "white beer" must be made from at least 50 % malted wheat and be top-fermented. Also an original gravity (ºPlato) 1.047-1.056 (11.8-14 ºPlato), apparent extract/final gravity (ºPlato) 1.008- 1.016 (2-4 ºPlato), alcohol by weight (volume) 3.9-4.4% (4.9-5.5%), bitterness (IBU) 10-15 and colour between SRM

(EBC) 3-9 (6-18 EBC) comprises a south German- style Hefeweizen. It must also have a “typical” flavour and aroma (3).

Throughout its long history, the popularity of wheat beer has fluctuated greatly. Its market share reached a low ebb at the beginning of the 20th century of only a few percentage points of the total annual Bavarian beer output. Still from the early 1960s to the 1980s, German wheat beer consumption was confined almost entirely to the southern regions of the country. However, in recent years that situation has changed, with Weissbier production almost doubling between 1990 and 2009 (Figure 1.1), in spite of a concomitant overall decline in German beer consumption. Non-alcoholic wheat beer has in particular experienced a steep increase in popularity. As a result, roughly one out of every 10 beers sold in Germany today is a wheat beer, as indicated in a recent survey by GlobalMalt (4). In other countries, wheat beers are also gaining market share. This includes the classic Belgian wheat beer style called “wit” in Flemish and “bière blanche” in French. Belgian wheat beers tend to be brewed with plenty of unmalted wheat. They may also exhibit slightly sour and spicy notes from the addition of coriander and bitter orange peel.

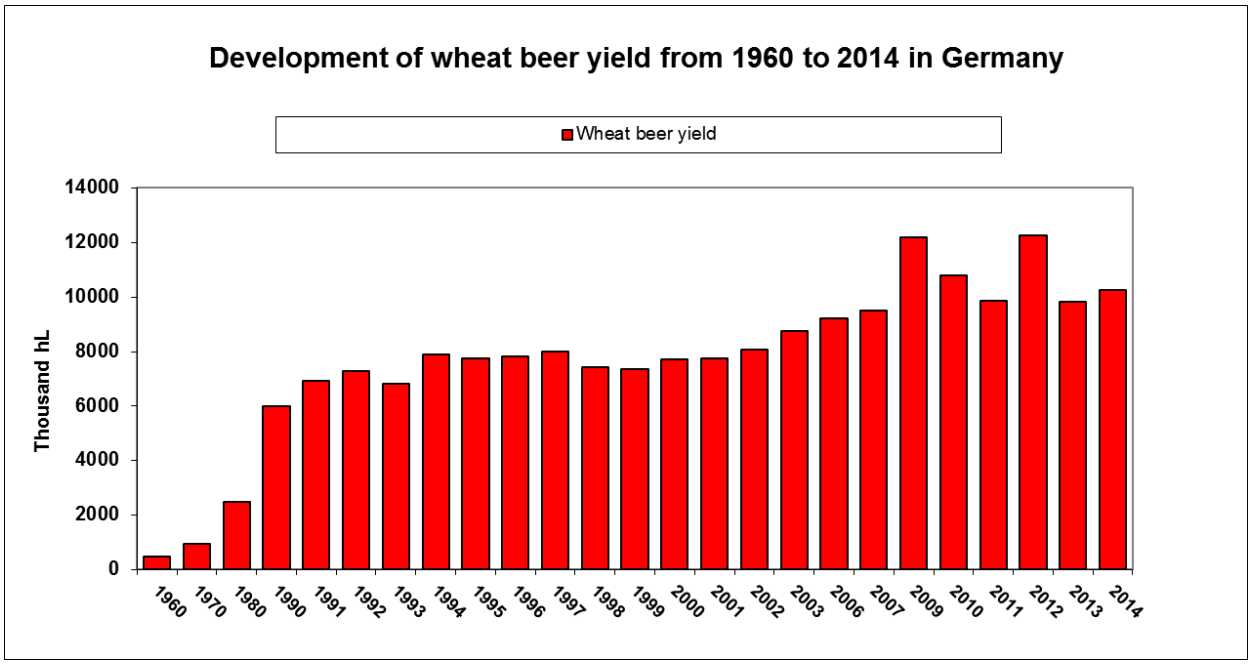


Figure 1.1: Development of wheat beer yield from 1960 to 2014 in Germany (modified by Faltermaier)

As the production of wheat beer increases, so does the demand for suitable brewing wheat. While breeding and cultivation efforts for barley have been highly successful in creating agronomically and brew-technical optimal specialty cultivars that have become well established as brewing barley varieties, the picture is completely different for brewing wheat, even though wheat is the world's second-most planted grain crop after corn. An increasing wheat beer demand results in a rising amount of raw material. Wheat has been - and still is - grown almost exclusively for the baking industry. It is this high demand that defines most of the wheat breeding objectives; and these objectives are generally not favourable to brewers. Baking wheat should be as high in protein as possible; and it should have stable Hagberg Falling Numbers (high numbers indicate low levels of enzyme activity and vice versa). A high protein content, which may be a boon for farmers and bakers, is a bane for brewers, since it can cause long lautering times, filtration difficulties, and fermentation problems in the brewery, as well as decreased flavour stability in the finished beer. Table 1.1 (5) and 1.2 (6) show quality criteria for wheat malt suitable for brewing purposes (7). Thus, wheat in brewing has been somewhat

overlooked from a scientific perspective. Table 1.1: Recommended values for wheat malt and barley malt (5)

Table 1.2: Recommended values for wheat malt and barley malt (5)

Analytica attributes	units	Recommended wheat malt values	Recommended barley malt values
Water content	%	4.5-5.0	4.5-5.0
Protein	%	11.0-13.0	9.5-10.5
Extract	% (d.m.)	>83	>81
Viscosity	mPa*s	<1.800	>1.560
Final attenuation	%, app.	>79	>80
Soluble N	mg/100g malt (d.m.)	650-780	600-700
Kolbach Index	%	37-40	38-42
FAN	mg/100g malt (d.m.)	90-120	120-160

Table 1.3: Limiting values for quality parameters of wheat malt suitable for brewing purpose (6)

	not acceptable		acceptable		good		acceptable		not acceptable	
	min	max	min	max	min	max	min	max	min	max
RP [%,d.m.]	-	9.9	10	10.9	11	12.5	12.6	13.4	13.5	-
SN [g/100g malt, d.m.	-	0.649	0.66	0.669	0.700	0.780	0.781	0.900	0.901	-
KI [%]	-	34.9	35	36.9	37	40	40.1	45	45.1	-
E [%, d.m.]	not acceptable			<84	84					
Viscosity [mPas]	1.800						>1.800 not acceptable			

Wheat beer shows a big variation in its production and brewing process. Overviews of the absolute values, without the amount of production of each surveyed brewery of the different producing techniques used for wheat beers, are listed in Table 1.3 (8).

Table 1.3: Variation of the production technique for wheat beer brewing (8)

Determining Parameter	Field/Allocation
Bulk value (wheat)	50 – 100%
Kolbach Index	35 – 45%
Mash temperature	30 – 57 °C
Proteolysis rest	45 – 58 °C, 10 – 26 min
Maltose rest	59 – 69 °C, 10 – 120 min
Infusion/Decoction	60% Infusion, 40% Decoction
Boiling	50 – 120 min
Fermentation vessels	25%: Keg 19%: vertical tank 25%: abandoned tank 31%: ZKG
Fermentation temperature	18 – 25 °C
Fermentation time	2 – 7.5 days

Determining Parameter	Field/Allocation
"Speisegabe"	42%: final wort 8%: sweet wort 23% bottom fermented "Kräuse" 7% mixture of top- and bottom fermented "Kräuse"
Maturation	75% Bottle Maturation 25% Tank Maturation
Duration of warm storage	16 – 22 °C, 3 – 7 days
Duration of cold storage	4 – 10 °C, 6 - 18 days
Yeast donation for after-ripening	40%: no yeast 7%: top fermenting yeast 25%: bottom fermenting yeast 7%: mixture
Pasteurizer (KZE)	47%: yes 53%: no

Beer flavour occurs due to the interactions of diverse flavour compounds such as alcohols, esters, carbonyl compounds, organic acids, phenolic substances and sulphuric compounds. Back et al. (9) attempted to categorize wheat beers in terms of their dominant flavour orientations. He divided them into four main groups: (a) ester- like, (b) phenolic-like, (c) neutral and (d) yeast type. Esters have an enormous influence on the flavour of wheat beers. Many wheat beers also have a strong "malty" component. With the exception of the “neutral” type, wheat beers tend to be much more aromatic than bottom-fermented brews. Chemically, the characteristic phenolic, clove-like flavour stems from 4-vinylguaiacol (4-VG); the fruity, aromatic, banana-like flavour from isoamyl acetate; and the estery component from ethyl acetate. The sensorial threshold is very low and can be characterized as aromatic in marginal concentrations. Furthermore, wheat beer can also have a typically “malty” component (maltol, furaneol). Table 1.4 (7) shows some typical concentrations of aroma compounds and their relevance in wheat and lager beers.

Table 1.4: Aroma compounds in "Weißbier" and Lager beer (7)

Aroma compound	units	Average in Weißbier	Average in Lager
Propanol-1	mg/L	15.0-30.0	5.0-20.0
Isoamy alcohol	mg/L	40.0-100.0	30.0-50.0
Hexanol-1	µg/L	15.0-50.0	10.0-30.0
Octanol-1	µg/L	10.0-40.0	20.0-40.0
2-Phenyl ethanol	mg/L	15.0-45.0	10.0-30.0
Ethyl acetate	mg/L	10.0-50.0	5.0-20.0
Isobutyl acetate	mg/L	0.05-0.8	0.05-0.1
Isoamyl acetate	mg/L	0.5-0.8	0.5-2.0
Hexyl acetat	µg/L	3.0-15.0	3.0-15.0
Isovaleric acid	mg/L	0.2-1.0	0.2-1.0
Hexanoic acid	mg/L	1.0-4.0	0.5-2.0
Caprylic acid	mg/L	2.0-10.0	2.0-10.0
gamma-nonalacton	µg/L	20.0-50.0	20.0-40.0
4-Vinylguaiacol	mg/L	0.5-3.5	0.1-1.0

Wheat beer has more unique aromatic compounds compared to lager beer. Essential for the phenolic aroma is 4-VG, and for the estery flavour isoamyl acetate

and ethyl acetate (10), whereas middle chain fatty acids are responsible for off-flavours. Herrmann (8) stated, that the influence of malt on the aroma compounds depends primarily on the malt technology. A correlation between the aroma relevant esters (3- methylbutyl acetate and 2-methylbutyl acetate) and the content of the corresponding amino acids, such as leucine and isoleucine has been found. It should be noted that amino acids are not only dependent on the wheat variety, but also on agronomical conditions and environmental influences. For the formation of sufficient 4-VG, the amount of ferulic acid in the sweet wort is essential. The author also reported that no differences in aroma compounds were found between decoction and infusion processes. In this review, a detailed look at specific characteristics such as carbohydrates, pentosans, protein fractions and enzymes and their influence on the brewing process are reviewed.

1.2. Wheat taxonomy and morphology

In 2011/2012 the worldwide wheat acreage was about 222.614 million hectare (ha) with a production of 691.503 million metric tons. Compared to 2007, this was an increase of ~3%. After maize, wheat is the main grain amongst the cereal crops (U.S. Department of Agriculture, 2012). Although wheat is a staple food crop, a small proportion of humans cannot tolerate wheat products, since they are suffering from celiac disease. Celiac disease is an autoimmune disorder that restricts an individual from consuming food products containing gluten (i.e. wheat, spelt, barley, rye and oats) (11).

Wheat (*Triticum spp*.) includes diploid (Einkorn), tetraploid (Emmer and Durum) and hexaploid species (12). Common wheat (*Triticum aestivum* L.) is a hexaploid caryopsis that belongs to the grass family *Poaceae* (13). Wheat is an annual grass

and can be classified by the terms of the growing season (winter or spring wheat), by the gluten content, or by grain colour (red, white or amber). Depending on the cultivar and location, the size of the kernel varies widely. The kernels average around 8 mm in length and around 35 mg in weight (14). The grains are naked, ovoid, generally rounded and have deep furrows or creases. Without the husks, the grains lack rigidity, which can cause compaction and handling problems during malting when the grain is wet (12, 13). Figure 1.2, shows the typical morphological structure of the wheat grain. The chemical composition of wheat is shown in Table 1.5 (10).

Table 1.5: Chemical composition of wheat (10)

Wheat	% of kernel weight	% of total starch	% of total protein
Pericarp	8.9	0	4.5
Aleurone	7.0	0	15.5
Endosperm	82.5	100	72.0
Scutellum	1.5	0	4.5
Embryo	1.0	0	3.5

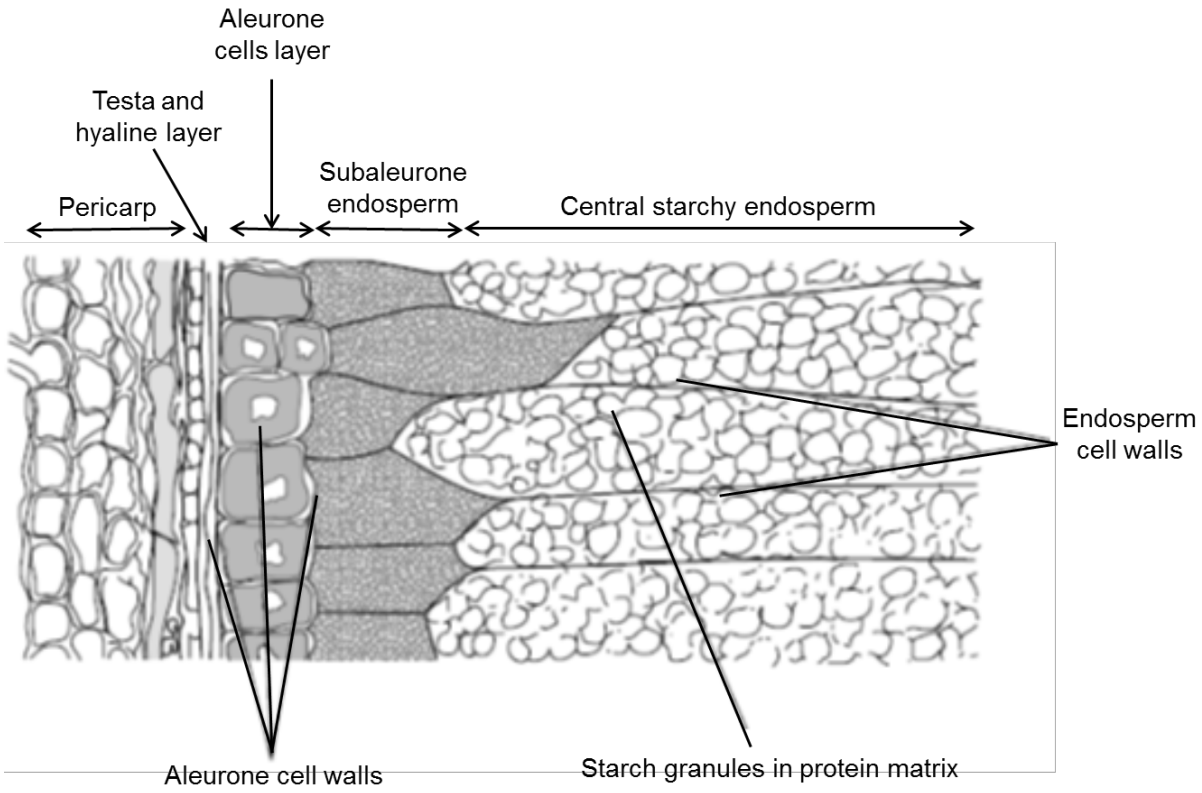


Figure 1.2: Transverse section of wheat grain (modified by Faltermaier)

The pericarp, which surrounds the whole seed, is composed of several layers (14), the outer epidermis, hypodermis, parenchyma, intermediate cells, cross cells, and tube cells (2). The total pericarp has been reported to compromise 5% of the kernel and consist of approximately 6% protein, 2% ash, 20% cellulose and 0.5% fat, with the residual being non-starch polysaccharides.

The seed coat and the pigment strand are not the same tissue, but together they provide a complete covering for the seed. The seed coat of wheat is the outermost layer of the true seed and is fused to the pericarp (2).

The aleurone layer, which surrounds the kernel, is generally one cell thick and covers the starchy endosperm and the germ. The aleurone cells are thick-walled (6-8 µm), essentially cubical, typically block shaped (37-65 µm by 25-75 µm) and free of starch at maturity (2, 13). Aleurone cells contain a large nucleus and a large number of aleurone granules. They are relatively high in ash, protein, total

phosphorus, phytate phosphorus, fat, and niacin. Furthermore, the enzyme activity in the aleurone cells is the highest of the entire grain (13). Over the embryo, the aleurone cells are modified, becoming thin-walled cells that do not contain aleurone granules (2, 13). The aleurone cells are also a storage reserve for lipid droplets (2). The germ, also called the embryo of wheat, comprises 2.5-3.5% of the kernel and lies on the lower dorsal side of the caryopsis (Figure 1.3). It is composed of two major parts, the embryonic axis, and the scutellum that functions as a storage organ (2, 13, 14). The germ is relatively high in protein (25%), sugar (18%) which is mainly sucrose and raffinose, oil (16%), and ash (5%). It contains no starch, but contains many enzymes and is rather high in B vitamins, as well as in vitamin E with values ranging up to 500 ppm (14).

The starchy endosperm (Figure 1.4) is composed of peripheral (60 μm in diameter), prismatic (130-200 μm long, 40-60 μm wide) and central cells (2.6 μm thick, 72-144 μm length, 70-120 μm wide) (2, 15, 16). These are composed of pentosans, other hemicelluloses, and β-glucan, but no cellulose. The principal content of the endosperm cells is starch and proteins, but the proportional contribution varies according to the location of the cells (14). Throughout the endosperm, all cells contain approximately the same amount of protein (17). Hence, the peripheral cells, which have the lowest starch content, contain the highest protein percentage (2). Starch and proteins, two major storage reserves, make up the bulk of the endosperm. These cells are packed with starch granules and embedded in a protein matrix. The protein is mostly gluten, the storage protein of wheat (14).

1.3. Specific characteristics of wheat and their influence on brewing

1.3.1 Carbohydrates

Carbohydrates constitute about 80% of the dry matter of the wheat kernels, which includes dietary fibre. The majority occurs in the wheat as polysaccharides. Starch is the predominant polysaccharide, in addition to dietary fibres such as cellulose and hemicelluloses, which are present in smaller amounts (Table 1.6) (10). Small amounts of soluble carbohydrates have been identified in wheat, composed of mono- , di-, tri- and oligosaccharides (2).

Table 1.6: Distribution of carbohydrates in wheat [%] (10)

	Endosperm	Germ	Bran
Starch	95.8	31.5	14.1
Pentosans and hemicelluloses	2.4	15.3	43.1
Cellulose	0.3	16.8	35.2
Sugars	1.5	36.4	7.6

1.3.1.1 Starch

Starch is the most abundant carbohydrate in wheat and wheat flour and occurs exclusively in the endosperm. It is found as discrete granules within the cells of the endosperm (2). In the wheat endosperm, two types of starch granules can be found (A- and B-type), as illustrated in Figures 1.4-1.9. They differ in size and shape. The large granules (10-40 µm in size and disc) have a lenticular shape and the small granules (2-8 µm in diameter) are near-spherical and polygonal (2, 14, 18, 19). Granules of each size between these extremes can be found, but the two sizes and shapes mentioned above are predominant. The large granules constitute 3-4% of the total number of granules in the wheat starch at maturity (20), and they contribute 50-75% of the total weight of the starch (21). In an aqueous solution,

wheat starch is birefringent in polarized light and shows the typical Maltese cross pattern (Figure 1.8) in a centric location (2). Large granules have a groove around the equatorial surface (Figure 1.6) (17, 21), which is the point of action for enzyme activity and attack by hydrolases (i.e. glucoamylase and α-amylase) (22). Thereby, raw starch is subjected to enzyme action either *in vitro* or during germination. A number of minor non-carbohydrate components, such as lipid, protein and phosphorus, are also found in wheat starch. These occur in such small amounts that it is not clear whether they are trace constituents of the starch sample, or granules, or are contaminants that are not completely removed during the isolation of the starch. Although they are present in small amounts, they can affect the properties and behaviors of the starch (2). Yamamori and Endo (23) investigated the starch granules of hexaploid wheat containing a group of three proteins, known as SGP-1 (starch granule protein-1) proteins, which have apparent molecular masses of 100, 108, and 115 kDa. Li *et al*. (24) demonstrated in their research that these polypeptides are starch synthases, which are present in both the starch

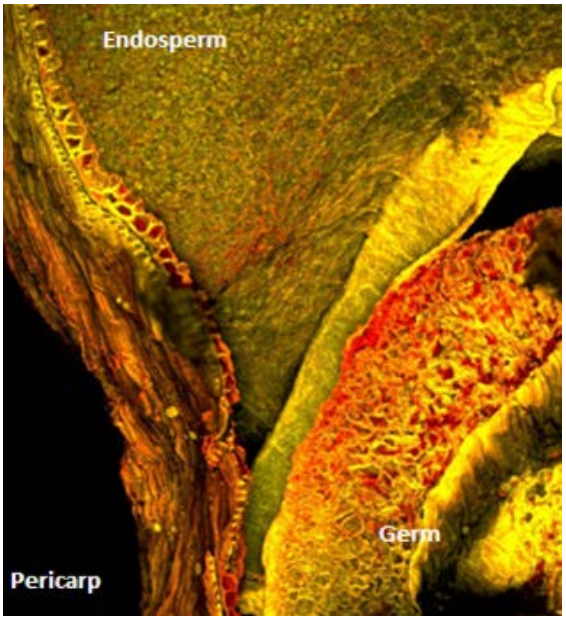


Figure 1.1: Aleurone layer, endosperm, germ and pericarp of unmalted common wheat (stained with three fluorescence dyes: red: proteins, yellow: cell walls, green: starch granules; magnification = 40x)

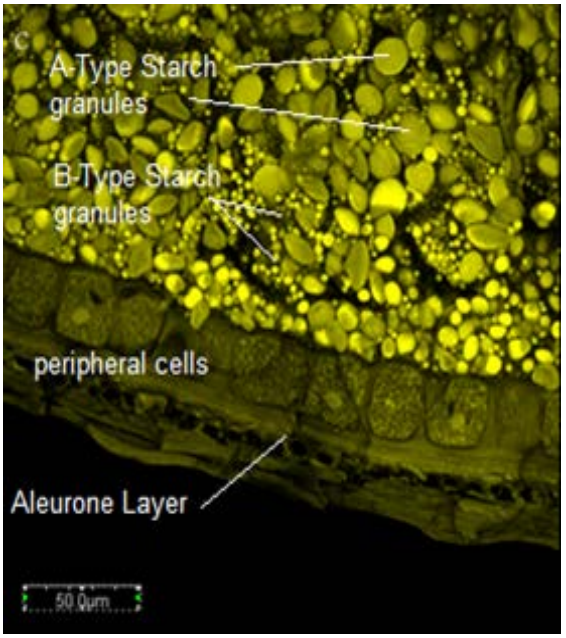


Figure 1.3: Aleurone layer and endosperm from unmalted Wheat kernel: large (A-Type) and small (B-Type) starch granules (magnification = 40x)

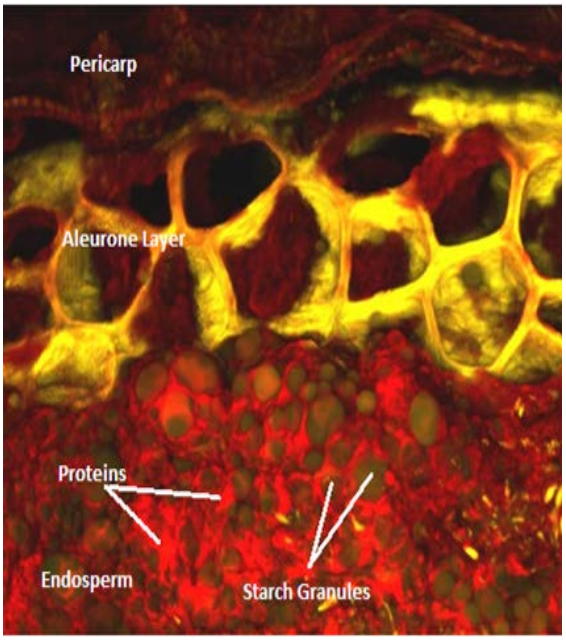


Figure 1.2: Aleurone layer and endosperm of unmalted wheat (stained with three fluorescence dyes: red: proteins, yellow: cell walls, green: starch granules; magnification = 40x)

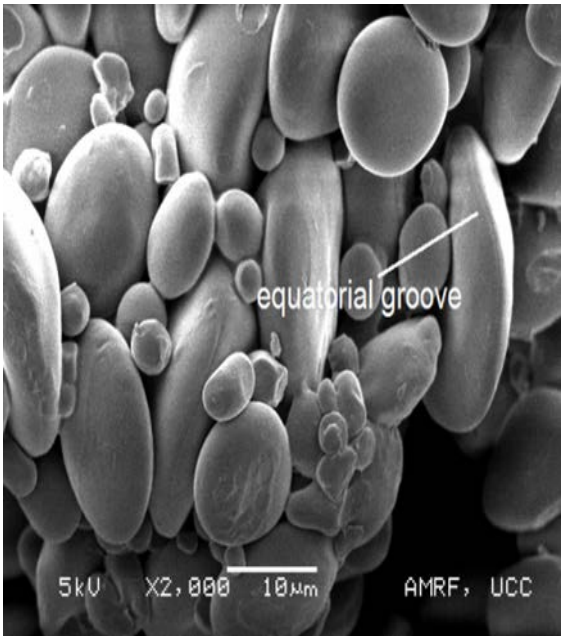


Figure 1.4: SEM Endosperm of unmalted common wheat (magnification = 2000x)

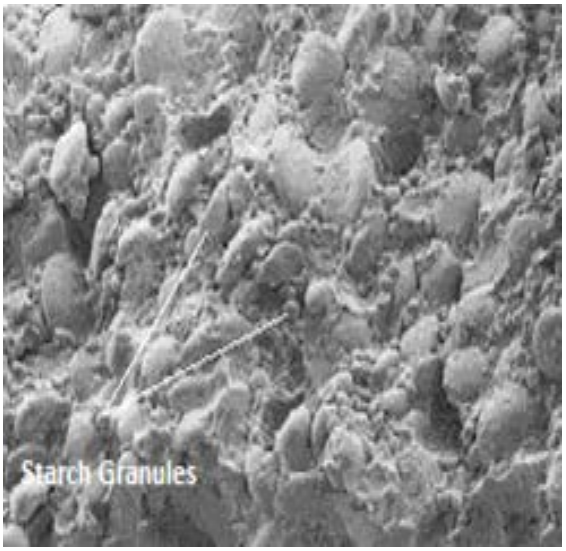


Figure 1.5: SEM: Endosperm of unmalted wheat starch granules (magnification = 500x)

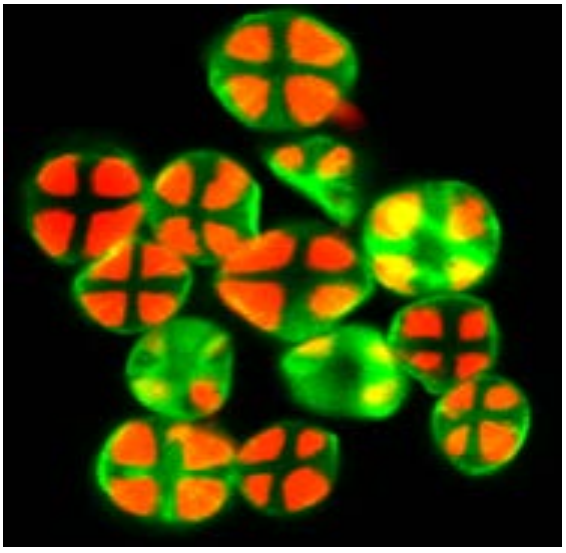


Figure 1.6: CLSM: Maltese cross of wheat starch granules (magnification = 200x)

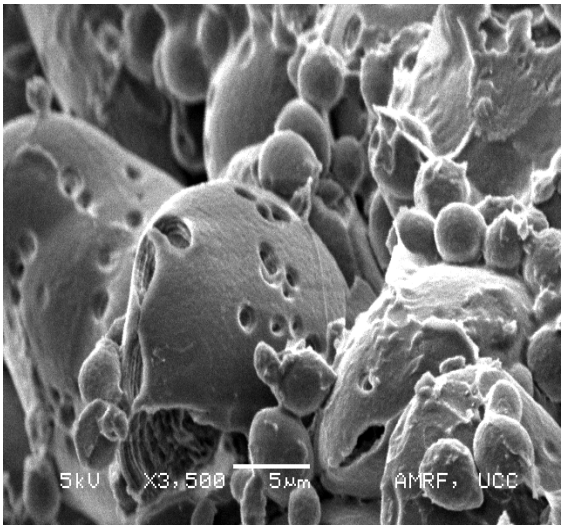


Figure 1.7: SEM: Endosperm of malted common wheat (magnification = 500x)

granuel and the soluble fraction at the early stage of wheat endosperm development, but that they are exclusively granule bound at mid and late endosperm development. The features of pores, channels and cavities of wheat starch are less understood than the ones found in barley and rye. However, several studies have described the existence of these channels and cavities in wheat starch granules. Patterns of enzymatic attack on them have been observed to produce large pin holes at the surface along the equatorial groove (Figure 9). Han *et al*. (25) observed a network of channels in the wheat starch granules, similar to protein structures, suggesting that these channels possess protein components. More recently, Glaring *et al.* (26) observed channels traversing the growth rings within the large lenticular granules. Wheat starch is not uniform and consists of granules, which may vary not only in size, but also in physical, chemical and functional properties (27). Dronzek *et al.* (28) suggested differences between the small and large granules in their physical structure based on the differences observed in the extent of degradation through amylases. Kulp (27) concluded that the small starch granules differ from the large ones in the following attributes. (a) They have a lower iodine affinity, relatively lower amylase levels or some intrinsic structural differences; (b) they swell more and become less soluble at high temperatures than large starch granules; (c) they are attacked more readily by amylases and (d) they have a higher water-binding capacity. Another difference between small and large wheat starch granules is that the small granules contain more lipid (29, 30). The smaller granules usually also have a lower amylose content (18, 30). Furthermore, large wheat starch granules show a large proportion of long amylopectin chains (degree of polymerization [DP] 24-30). Starch α-amylolysis also depends on the particle size of the starch granules. It has been shown that starch α-amylolysis does not occur evenly for small and large wheat starch granules (31). Particularly for barley, it was found that at a low temperature of around 35 °C, small

starch granules are hydrolysed faster than the larger ones (32). Large barley starch granule hydrolysis proceeds at low temperatures, through pinholes from the inside (32). However, at higher temperature of 65 °C, large granules were found to be more susceptible to α-amylase degradation, since large starch granules gelatinise earlier, than the smaller ones (31). On a higher structural level, large wheat starches are more crystalline (33). Also small and large starch

granules show different behaviors during the brewing (32) and baking process (30, 34). Divergence in functional properties can reasonably be expected to be provoked by differences in gelatinization, a characteristic for both granule sizes (18, 30, 34). In the literature, high gelatinization enthalpies have been related to high crystallinity (35, 36). The gelatinization temperature of wheat starch ranges from 58 to 64 °C. Vermeylen et al. (37) compared the gelatinization properties of starches and starch fractions. They found that isoamylase debranching revealed systematic, but small, differences between amylopectin chain lengths of the small and large granule starch fractions. Wide-angle-X-ray diffraction showed predominant A-type crystallinity for wheat starches, and that large granules had systematically higher B-types. Total crystallinity was lower for the small starch granules. Small-angle-X-ray scattering indicated structural differences between amylopectins of distinct granule classes (37).

1.3.1.2 Starch and its influence on brewing process

The starch fraction is the main component found in wheat. It is an important indicator for malsters and brewers, since it contributes to the extract and final attenuation of worts. Jin *et al.* (38) stated in their study that with an increasing protein content of the wheat, an increase in the storage proteins and a decrease in the soluble proteins were found. With an increase in protein, a decrease in starch content is recognised, and therefore a decrease in extract and final attenuation (39). High protein levels in the

endosperm, leading to starch/protein compacting, limit endosperm hydration and enzyme modification during malting. Furthermore, the agricultural conditions, such as climate, fertilization, and soil properties have an influence on the starch and protein content (14, 39). Therefore, malsters and brewers favour brewing cereals that are high in starch content and low in protein content. During malting, amylolytic enzymes are formed that break starch down into small and soluble sugar compounds. The extract yield is one of the most important amylolytic malt quality attributes (39). It is one of the values that is 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 (40). Increased proteolytic activity increases starch availability and can also produce, given the circumstances, higher extract values. A potential extract of more than 80% is expected for a barley malt used for brewing purposes. The extract values for wheat are higher and are around the range of 83%.

A certain part of extract, the fermentability percentage of the wort that can be fermented, is referred as the apparent attenuation limit (AAL) (39). The attenuation limit depends upon the availability of the fermentable sugars and on the yeast remaining in contact with wort (12). 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%. Wheat malt recorded AAL values vary between 75.7 and 82.2%. (39, 41). During mashing, starch breakdown is very important (42). 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.

Milling is an important stage in brewing because it affects the later stages of the brewing process (e.g. production of sugars, speed of wort separation) and hence it affects the quality of the final product (5). Previous studies on starch damage during milling, report that parameters such as mill roll speed and gap of rollers affect the extent of starch damage (43). Mousia *et al*. (44) performed a study on the influence of milling parameters on starch hydrolysis. They stated that reduced roller gap and/or increased roller speed and/or high differential speed enhanced starch hydrolysis, resulting into higher sugar concentrations in the wort, which resulted in reducing the mashing time required for the maximum sugar concentration. Six-roll milling gave a higher final sugar concentration in the wort when compared to four-roll milling.

1.3.2 Pentosans

In wheat, pentosans are only a minor constituent compared to rye, which is very rich in pentosans at 6 - 8% (10). Unlike barley and oats, wheat endosperm is rich in arabinoxylans and very poor in β-D-glucans (45).

1.3.2.1 Arabinoxylans

Arabinoxylans (AX) are the predominant hemicelluloses based on the whole wheat kernel (6 – 7%) (46). From 1.5 to 2.5% of the AX can be found in the endosperm, where they constitute 66% of the endosperm cell wall (47). The cell wall material of the aleurone layer is richer in β-D-glucan than the starchy endosperm tissue, but arabinoxylan still remains as the principal constituent (2).

Both the soluble and the non-soluble arabinoxylans consist of β-1,4-linked D-xylopyranosyl residues, with monomeric α–L-arabinofuranose substituted at the C(O)-3 and C(O)-2 position. Ferulic acid can be coupled through an ester linkage to the

C(O)-5 position. The arabinose-to-xylose ratio is an important parameter for arabinoxylans and is 0.5 – 0.6 in the wheat endosperm. Arabinoxylans from different areas of the wheat kernel differ in their structure. The AX found in the outer layer of the grain are less substituted with arabinose than those found in the inner endosperm (47). Up to 30% of wheat bran consists of glucuronoarabinoxylans incorporating methylated glucuronic acid into the arabinoxylans structure.

Arabinoxylans also known to increase viscosity during brewing. Beers contain from 0.5-4.2 g/L arabinoxylan, with high levels found in German wheat beers made from malted wheat (48). Incomplete degradation of the endosperm cell walls reduces the amount of extract yield during mashing. The concentration of arabinoxylans released into the wort is controlled by the mashing temperature (49). Since wheat endosperm contains more arabinoxylans than barley (45), increased amounts of wheat used for brewing result in higher viscosities and reduced filtration volumes. It has been reported that the effects of arabinoxylans on viscosity and filterability are at least as important as the effects of β-glucan (50). Furthermore, arabinoxylans have been described as prebiotics (51, 52) and the concentration of soluble arabinoxylans in wort can be increased directly using certain malting conditions (53).

1.3.2.2 β-Glucan

Compared to other cereals such as barley, (1→3)(1→4)-β-D-glucan is only found in small quantities in the wheat grain. Dependent on the variety, the amount of β-glucan ranges from 0.31 to 6.7% (54-56). Unlike the cereals that are rich in β-glucan, such as oat and barley, the highest concentrations of β-glucan are found in the inner aleurone cell walls and subaleurone endosperm cell walls (57, 58). As described by Cui *et al*. (54), the predominant molar proportion of trisaccharide units in β-glucan wheat (72.4%) is higher than in all other known cereal β-glucans e.g., oat β-glucan

(55.0%) and barley (62.1%). Although the distribution of these trisaccharide units is random (59), the repetition of the cellotriosyl units gives wheat β-glucans a higher ordered confirmation, which might even promote self-association. This would also explain the greater gelling capacity and the poorer solubility compared to other cereal β-glucans (56, 60).

1.3.2.3 Cell wall substances and their influence on brewing process

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 (61). As mentioned above, 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 pentosane 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. A steeping degree of 44%, a rather low germination temperature with an average of 14-15 °C, and a steeping and germination period of 7 days in total, results in good malting conditions for wheat. Wheats with higher wort viscosities have to be treated with higher water contents, up to 47%, as well as with a falling malt temperature regime from 19 °C to 15 °C (39). Wheat has a low β-glucan content but a high pentosan content, depending mainly on rabinoxylans (AX). These arabinoxylans occur naturally in plants. Arabinoxylans are partly cross-linked by diferulic acid bridges and possibly other condensation products of ferulic acid and therefore there can be differences in solubility (62). During malting, the cell walls are degraded and the water-extractable arabinoxylan (WEAX) content increases (63). A higher degree of arabinose substitutions or a shorter backbone chain length of xylan increases the solubility of AX (64). In literature, it is stated that increased WEAX cause problems during the

brewing process. Complications in the filtration and lautering processes (25, 65), as well as contributions to premature yeast flocculation (66) can occur. However, Burberg *et al*. (67) could not find any correlation between WEAX and the resulting wort viscosity when they analysed 40 malted wheat samples harvested between 2006 and 2008. Krahl *et al*. (53) performed a study to determine the influence of malting parameters on the WEAX content of wheat and other cereals. The influence of germination temperature, germination time and moisture content on the resulting WEAX content of the produced wheat malt was investigated. Krahl *et al*. (53) stated that the WEAX content could be influenced in cereal grains by modifying the malting regime. and suggested the following. (1) Increasing the WEAX content with higher moisture content at low temperatures, and decreasing it if higher temperatures are used. (2) Constant moisture content and higher germination temperatures result in higher WEAX levels. (3) WEAX levels increase significantly from 5 to 6 days of germination, but decrease if a longer germination time is chosen. Lu *et al*. (49) studied the effect of arabinoxylan 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 arabinoxylan 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 (68). A further conclusion of this study was that when more arabinoxylans are solubilised, it leads to an increase in wort viscosity and therefore retards wort filtration. Finscher and Stone (69) reported that, arabinoxylans can form highly viscous solutions, especially in the presence of β-glucan. 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. However,

the more finely ground the grist was, the more arabinoxylans were released into the wort and an increasing wort viscosity could be recognized.

Grant and Briggs (70) investigated the histochemical location of arabinosidase and xylosidase in germinating wheat grains. The α-L-arabinofuranosidase (EC 3.2.1.55) and the β-D-xylanopyranosidase (EC 3.2.1.37) activity were present in the embryo, the scutellum, and the aleurone layer, as well as in the starchy endosperm. The majority of these enzymes were found in the aleurone layer, whereas in ungerminated wheat, these enzymes were not detected. The researchers used different histochemical detecting methods and investigated the method's detection limitations. They did not measure enzymes in the other tissues, where these enzymes were also present. More specific, histochemical or enzymatic methods still need to be developed, for measuring arabinosidase and xylosidase. These enzymes and especially their amount may be a new attribute to specify the malt quality of brewing relevant wheat.

1.3.3 Proteins

Wheat proteins influence brewing and the beer's characteristics. During malting, a significant amount of the cereal proteins are hydrolysed and therefore become water-soluble. While wheat does not generally have a higher protein content than barley, wheat does contain higher molecular weight proteins (71). Moreover, high molecular weight proteins, especially the wheat glycoproteins, have been associated with the improving the foam characteristics of wheat beers (71-73) and to contributing to haze formation (71, 74-76). Malting is the initial step in traditional beer production. The malt defines and influences the resulting beer type and quality. The main purpose of malting is for the production of enzymes and for the degradation of specific substances, as starch granules, cell walls and proteins. During the malting process,

high molecular weight storage proteins are degraded by proteolytic enzymes to various degrees, from mid-size chains to amino acids. In Figure 10 electropherograms of the wheat proteins, in different malting stages, are shown. The Lab-on-a-Chip technique was used to investigate the differences in protein content and distribution. The degradation and formation of proteins during the malting process is shown. This particular technique has already been applied and published in many malting research publications (77-79).

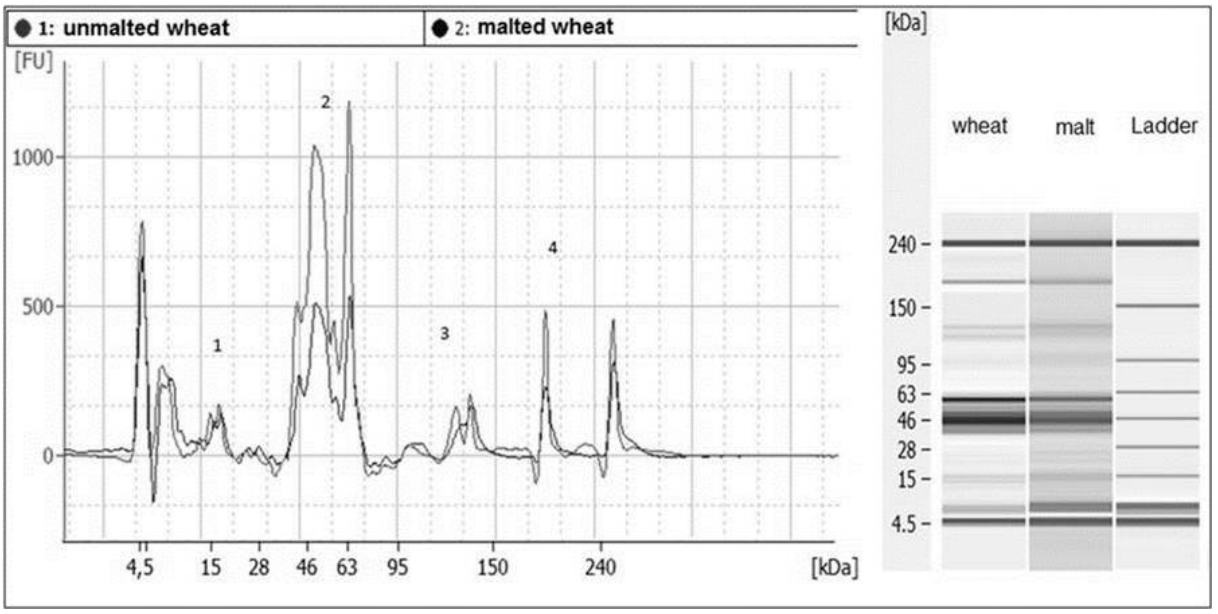


Figure 1.10: Electropherogram of the Protein fractions in different malting stages (80)

Faltermaier *et al*. (80) investigated the changes in the content and composition of the protein fractions of the unmalted wheat during the steeping, germination and kilning processes. Pilot scale standard malting regimes were employed and frequent sampling ensured comparable analyses of the proteolytic hydrolysis reactions throughout. The wheat protein was fractionated and analysed using Lab-on-a-Chip capillary electrophoresis (molecular weight based separation) and OFFGEL-capillary electrophoresis (2-D separation combining isoelectric focusing and molecular weight separation) thus providing a deeper insight into the proteolysis process associated with the wheat. The primary finding of this research on wheat protein modification

was that wheat proteolysis during malting is comparable to barley malt proteolysis. Both malts contained similar protein peaks when examined using 2D capillary gel electrophoresis. In barley malt, these are known to have foam and haze stabilising functions.

Due to the major impact of gluten proteins on bread baking processes, much work has been performed to understand the composition, structure and behaviour of the prolamin and glutelin fractions of the wheat. Nevertheless, there is still a lack of knowledge and further work has to be performed to obtain a full characterisation of the wheat proteins.

The two main groups of protein in wheat are the storage proteins and cytoplasmic proteins and each of these groups consists of hundreds of different proteins.

According to their solubility proteins can be divided into different fractions (81). Albumins are soluble in water and coagulate when heated. Globulins are soluble in dilute salt solutions. Wheat prolamins (gliadins) are alcohol soluble proteins, while wheat glutenins are soluble in acid or base solutions. Albumins and globulins account for 15 to 20% of the total protein content. These are mainly physiologically active proteins and contain enzymes and inhibitors. Wheat storage proteins are gliadins and glutenins, which are known as the gluten proteins.

1.3.3.1 Storage proteins

Wheat storage (gluten) proteins constitute up to 80% of the total wheat grain protein content (82). Gluten is composed of gliadins (monomeric proteins) and glutenins (polymeric proteins) (83) and occur mainly in the endosperm of wheat grains. Monomeric gliadins (soluble in alcohol), which in baking processes contribute to dough viscosity and extensibility, can be classified into four groups based on their electrophoretic mobility at low pH: the slowest moving group - ω-gliadins (S-poor, ~40-

75 kDa) and the three faster groups – α-, β-, and γ-gliadins (S-rich, ~30-45 kDa) (84). Polymeric glutenins (extractable in acetic acid) consist of HMW glutenins (HMWG) and LMW glutenins (LMWG) (~30-40 kDa). In the literature, several authors describe HMWG ranging from ~80-120 kDa (82), whereas D'OVIDIO AND MASCI (84) indicate a MW range of ~65-90 kDa.

The glutenins have two main characteristics: they are not soluble in diluted salt solutions and 70% ethanol, and the macromolecule is composed of polypeptide chains bound by disulfide bonds. They are multichained and vary in molecular weight from about 100,000 to several million (14). Glutenin is formed from different subunits bound together by a network of disulfide bonds. Different subunits may differ in their tendency to polymerize, and once polymerized, may have different capacities for interaction with other constituents (85).

1.3.3.2 Proteins with physiological functions

Proteins, which are not gliadin or glutenin polypeptides, are often called soluble cytoplasmic or non-gluten proteins, and have physiological functions in the wheat kernel. They primarily consist of albumin (molecular weight 17,000 – 28,000) and globulin (molecular weight up to 60,000). A large number of these metabolically active proteins have been found and identified as enzymes, regulatory proteins, transport proteins, etc. The majority of soluble proteins are located in the embryo and in the aleurone layers. Little is distributed throughout the endosperm (2, 86). PAYNE AND RHODES (86) noted that soluble proteins are complex mixtures, containing metabolic enzymes that survive dehydration. Hydrolytic enzymes necessary for germination, enzyme inhibitors, and proteins that clot red blood cells, are amongst these proteins. The most important group of metabolically active proteins in wheat

are the enzymes. Both biosynthetic enzymes and degradative enzymes are known to be present in wheat, but only the degradative enzymes have a major impact on food processing.

1.3.3.3 Starch-degrading enzymes

In the late fifties and sixties, studies were performed regarding the behaviour of α-amylase, β-amylase and proteases during the germination and malting of wheat. Differences in the enzyme activities were observed in the different varieties and classes of wheat. In the early studies, it was reported that increasing the degree of steeping, germination time and temperature resulted in a higher α-amylase content. Malt enzymes however are more susceptible to heat when the moisture content of the malt is high (87-90). It was also reported that the autolytic protease activity of malted wheat flour increased with longer germination periods, higher degrees of steeping and with higher protein levels (91). In contrast to α-amylase, β-amylase is not produced during germination, since it is present in an insoluble or latent form in the ungerminated grain (92). The β-amylase is than solubilised during germination.

Malting losses are caused by leaching, respiration, and root and shoot growth. These losses depend largely on the degree of enzyme formation. Research has focused on the minimisation of these losses by “optimising” the malting process and thus reaching enhanced malting yields. Therefore, the impact of gibberellic acid (GA) on growth and on the enzyme activity of wheat has been investigated in many studies (87, 93, 94). Gibberellic acid is a plant hormone that regulates growth and influences various development processes in cereals. The studies have all reported increased enzyme activities of cereal grains and malts during germination caused by GA, and that α-amylase was also increased to a great extent. Compared to conventionally produced malts, the treated malts had the same enzyme activity level, however a

shorter germination period was needed (one up to three days). Shorter steeping and germination times, as well as the inhibition of the growth of rootlets, can yield major advantages (more capacity of malting yields and more bulk volume, respectively) in the malting process (87, 93, 94).

Amylolytic enzyme activity during brewing is measured as diastatic power. Enzymes degrade the starch granules during malting and especially during the mashing process. Diastatic power is a parameter that measures the malt's capacity to hydrolyse starch into fermentable sugars. The α-amylase degrades the native starch granules by hydrolysing the α-1,4-linked glucose polymers. The β-amylase, limit dextrinase, β-glucosidase and α-amylase can debranch and degrade maltodextrins and soluble polymers (95).

1.3.3.4 The α-amylases (EC 3.2.1.1)

Amylases are technologically the most important enzymes in wheat and wheat flour processing. The α-amylase enzyme is an endo-amylase, which hydrolyses more or less randomly the α-1,4 linkages of starch. Amylopectin and amylose are attacked by α-amylase from the inside. The α-amylase degrades the amylose to dextrins of about 6 glucose monomers. In addition, the amylopectin is reduced to dextrins. However, α-amylase cannot degrade the α-1→ 6 linkages, but it can attack the α-1→ 4 linkages between the branches. The residual side chains can be degraded by the β-amylases. The α-amylase enzyme is a calcium metalloenzyme that is activated by the calcium cation. Ungerminated wheat contains a minimum and variable level of α-amylase. In germinating wheat grains, an increase in the α-amylase activity may be observed due to its synthesis in the aleurone cells (10, 47). During wheat grain development, the α-amylase is located mainly in the pericarp, and decreases in activity throughout the kernel's development, becoming largely undetectable at maturity. Small amounts of

α-amylase are also present in the native grains. Levels of α-amylase are different among the wheat cultivars. Grain size and α-amylase activity are correlated with each other (96).

Beside limited dextrinases and endopeptidases, α–amylase is an enzyme that is formed de novo, which means that the α-amylase is not available in the dormant grain, but is formed during germination in the aleurone layer. The α-amylase content is affected and influenced by many factors, for example the wheat variety, environmental influence, germination conditions, steeping degree, germination time, and oxygen content. The nzymes are also influenced during the kilning stage, where the loss of α-amylase is much lower than the loss of β-amylase, which is more heat sensitive. During mashing, as 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 α-amylase enzymes during mashing are at a pH level of 5.6-5.8 and a temperature of 70-75 °C. The α-amylase is inactivated at 80°C in a relatively short time (39).

1.3.3.5 The β-amylases (EC 3.2.1.2)

The β-amylase of wheat is located mainly in the endosperm and it is an exo-active enzyme that attacks starch from the non-reducing end, and breaks every second α-1,4 linkage of the molecule starch. Hence, the enzyme releases maltose; but it cannot pass the branch points (α-1,6) of amylopectin. Most of the β-amylase in the endosperm of maturing grains is inactive, but it can be activated by reducing agents. Rowsell and Goad (97) concluded that the latent β-amylase is bound to glutenin via disulfide linkages, and proteases release the enzyme still bound to the glutenin polypeptides. Amongst the starch degrading enzymes, β-amylase is the main

contributor of diastatic power in the malted grains (95). However, β-amylase is not built de novo during germination, as are the other amylolytic enzymes (39, 98, 99). During grain development, the β-amylase is built up in two main forms: an insoluble protein complex and a soluble form. The bound form becomes active when released by proteolytic activity (39, 99). The optimal conditions for β-amylases during mashing are at a pH of 5.4-5.6 and a temperature of 60-65 °C. The β-amylase is inactivated at temperatures above 70°C (5, 7).

1.3.3.6 Protein-degrading enzymes

Proteolytic enzymes are classified according to their catalytic mechanisms and can be distinguished as endoproteases or exoproteases. Serine protease activity (EC 3.4.21) is responsible for most of the proteolytic activity during the early stages of the wheat's grain development, decreasing at later stages of kernel maturation. The major role of the proteases is a physiological one, rather than one of degrading the storage proteins (14). Cysteine proteases (EC 3.4.22) have a similar catalytic mechanism using a sulphydryl group of a cysteine residue, instead of the hydroxyl group, which is required in the serine proteases. Cysteine proteases occur mainly in the germinated wheat and they have the same pH optimum as aspartic proteases (EC 3.4.23) below pH 7.0.

Nitrogenous substances in cereal grains are mainly presented as high molecular protein bodies. In cereal grains, proteins occur in the aleurone layer and in the endosperm (mainly storage proteins), as well as in starch granules. Storage proteins are attacked mostly during malting. The malting conditions can be varied to influence protein degradation. Amino acids are important for yeast growth during fermentation, while polypeptides are claimed to provide foam stability and to give fullness to the beer (39). To evaluate the protein degradation during malting, the amount of total

nitrogen (%), called Kolbach Index, is employed.

The loss of enzymes is more extensive when wet malt reaches higher temperatures. Enzymes suffer less during dry heat than during wet heat. Consequently, pale malts are more enzyme active than darker malts. The most important protein degradation products in malt, produced by proteolytic enzymes during germination, are macro peptides, polypeptides, peptides and amino acids (39). 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). The optimal conditions for the protease activity are listed in Table 1.7 (5).

Table 1.7: Optimal conditions of proteases (5)

	pH	Temperature (°C)
Endopeptidase	5.0	40-50
Carboxypeptidase	5.2	50-(60)
Dipeptidase	8.2	40-45
Aminopeptidase	7.2	40-45

1.3.3.7 Amino acid composition and nutritional quality

The amino acid composition of the wheat depends on the wheat variety. In general, cytoplasmic proteins have a higher nutritional value compared to storage proteins, due to a higher lysine content and a lower glutamine content (85).

All gliadin components have extremely high glutamic acid content. In some ω-gliadins, the glutamic acid content is higher than 50%. Most of the glutamic acid

content of the gliadins, which are present as glutamine, provides a concentrated source of nitrogen that can be used readily during germination. The aspartic acid and asparagine content of all gliadins is relatively low. The gliadins have a high proline content, which has an effect on the secondary structure of the gliadin of the polypeptides. This is due to the formation of α-helices, which are hindered by the presence of proline side chains. Gliadins are low in basic amino acids, especially in lysine. The ω-gliadins have relatively high levels of phenylalanine, in addition to their high content of glutamine and proline, and they comprise up to 10% of the total residue. Also typical for the ω-gliadins is the low amount of cysteine and methionine (14, 85).

The amino acid composition of the HMW storage proteins found in the wheat endosperm is similar to those of the LMW proteins. A slightly higher content of basic amino acids and a lower amount of glutamic acid and proline may be observed in the endosperm. In addition, the average content of the amino acids having hydrophobic side chains is smaller (85). Table 1.8 (10) shows the differences in the amino acid composition between the different protein fractions.

Table 1.8: Amino acid composition of wheat protein fractions (10)

Amino acid	Protein Fraction				
	Albumin	Globulin	Gliadin	Glutenin 1[b]	Glutenin 2[c]
Lys	4.8	5.1	0.7	1.2	2.1
His	2.2	3.1	1.8	0.9	0.6
Arg	5.2	10.7	2.0	3.5	4.3
Asp + Asn	7.7	8.0	2.6	4.0	5.4
Thr	3.8	3.8	1.7	2.5	3.0
Ser	4.0	4.5	2.9	4.4	3.4
Glu + Gln	24.6	19.2	42.3	41.7	35.0
Pro	9.4	4.8	15.0	12.0	9.9
Gly	4.2	5.4	1.6	3.8	4.9
Ala	5.1	5.2	1.8	2.4	3.6
Cys	2.8	2.2	2.4	1.3	1.0
Val	6.1	6.5	4.2	3.5	4.7
Met	1.9	2.0	1.3	1.3	1.8
Ile	3.3	3.9	3.8	3.4	3.7
Leu	7.2	7.4	6.6	6.6	7.9
Tyr	2.6	3.2	2.8	3.2	4.4
Phe	4.9	4.6	6.0	5.4	4.8

[a] mole percent on an ammonia-free basis; adapted from Dubetz et al (1979)

[b] Extracted with 0.05 N acetic acid

[c] Extracted with 0.05 N NaOH

1.3.3.8 Proteins and their influence on brewing process

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 (39, 100, 101). 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. Furthermore, some proteins contribute to mouthfeel, flavour, texture, body, colour, and to the nutritional value of the beer (102, 103). 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 a 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 (71, 74, 79, 104-106).Important attributes for malsters and brewers include not only the proteolytic activity, but also the cytolytic and amylolytic activity. Malt proteins have a high impact on the brewing process and the resulting beer quality. Back et al. (9) have pointed out that wheat malt with raised proteolytic attributes, independent of the variety or malting regime, shows a decline in sensory characteristics. 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.

Kolbach index (KI) represents the proportion of total nitrogen present in the malt that is soluble, related to the total protein content in the malt. The amount of soluble nitrogen depends on the malt and on the way it has been mashed (107). During germination, in the cell storage proteins are hydrolyzed (reduction) and proteins are synthesized (assembly). If a balance between reduction and assembly is reached, the KI does not alter during germination despite progressive proteolysis in the endosperm. However, a negative correlation between foam stability and KI has been found (108). Evans and Sheehan (109) assumed that a low-malt modification (low KI) would improve foam quality, and Narziß et al. (110) stated that there was a significant decrease in foam stability when overmodified malt was used.

Generally, wheat and wheat malt show greater proportions of high molecular protein fractions in wort than barley, although they are lower in free amino nitrogen content (111, 112). The low molecular weight nitrogen substances are referred to as free amino nitrogen (FAN). They represent about 20% of the soluble nitrogen content in the malt. Low molecular nitrogen compounds, especially amino acids in wort, impact

fermentation performance and the development of fermentation by-products (107). If the FAN concentration is high during beer production, the yeast has more nutrients available, and therefore fermentation can take place more rapidly and alcohol production is higher. Therefore, FAN values must be sufficiently high to ensure that a lack of nitrogenous yeast nutrients does not limit the fermentation (113). Low molecular weight nitrogen compounds play a central role in the colour and flavour development of malt and they follow a Strecker reaction (107). Also a high FAN concentration can result in the development of undesired off-flavours due to Maillard reactions (7, 41). Appropriate barley malt standard values to assure yeast nutrition suggest a FAN content between 120 and 150 mg/100g. Based on these values, some wheat malts present lower levels (79-139 mg/100g) than recommended, which can consequently result in a problematic and limited fermentation.

Proteolytic solubility (soluble nitrogen) characterizes the amount of nitrogen in wort and this depends on the raw protein content of the malt and the dissolving ability of malting and mashing procedure. Soluble nitrogen (SN) influences beer quality as well as the brewing process. 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. The brewing parameter for the amount of nitrogen degradation products, which are solubilised during congress mashing, is referred to as the soluble nitrogen (SN) level. The SN value of the wheat, as determined using a congress mashing wort, should lie in the range of 650-780 mg/100g.

Haze intensity and stability are important quality characteristics for wheat beers. Delvaux (74) conducted a fundamental study on hazes in white beers. He stated that wheat malting had a positive effect on beer haze (114). The major compounds involved in wheat beer haze are proteins and polyphenols (115, 116). Wheat gluten

proteins interact with polyphenols and with protein-polyphenol complexes (104). This lead to the conclusion that wheat gluten proteins are haze-active. High gluten levels form insoluble complexes that are too large to stay in suspension and precipitate, whereas with low gluten levels, a haze is formed (74, 117). Wheat varieties vary greatly in their protein composition, especially in their gluten levels, and the levels are an important contributor to haze stability. Depraetere et al. (105) studied haze stability by using different wheat and barley varieties and confirmed the results reported by Delvaux et al. (117). Moreover, they stated that not only wheat gluten levels influence haze intensity, but also barley malt properties. This result was confirmed by Schwarz (118). Asano et al. (119) showed that the gliadins were quite haze active. By altering the protein composition using proteolytic enzymes, a strong influence on permanent haze characteristics was determined. Delvaux (74) stated that partial hydrolysis resulted in smaller haze particles and a more stable haze. Haze intensity in wheat beers depends largely on the wheat gluten level and on the molecular weight of these proteins. During the malting of wheat, haze-active wheat proteins are degraded to lower molecular weights; this results in smaller particles and thus in a more stable haze. Furthermore, less high- and more low-molecular weight proteins are found in the worts made using wheat malt. Depraetere et al. (105) also observed more haze stabile wheat beers when overmodified malts were used. When higher proteolytic activity in malt occurs, then wheat proteins are more degraded during brewing. The use of wheat malt significantly increases the permanent haze of wheat beers, due to the proteolysis of the haze-active wheat gluten proteins.

1.4. Conclusions

Malting barley is the most researched grain type in the brewing world, while relatively little is known about the brewing wheat grain. The average beer can contain more than 450 different substances, all of which can affect flavour, aroma, colour, stability, and the overall beer quality. This makes beer one of the world's most complex foods. Since there are no specific analytical data and methods established for wheat and wheat malt as a brewing cereal, analytical methods that are approved for barley have to be used and transferred to wheat for use in brewing. Furthermore, not all established methods are appropriate for selecting and analyzing wheat and wheat malt, and as a consequence other criteria must be used (5, 7, 111). When selecting wheat varieties for brewing purposes, the most important criteria for the malsters and the brewer are viscosity, protein content, soluble nitrogen, extract (final attenuation) and the Kolbach index.

With the rising popularity of wheat beers all over the world, much more research will be needed before our understanding of wheat will catch up with our understanding of barley.

CHAPTER 2

COMMON WHEAT (*Triticum aestivum* L.)

THEORETICAL STUDY ON A STATISTICAL METHOD FOR THE SIMPLE AND RELIABLE PRE-SELECTION OF WHEAT MALT TYPES FOR BREWING PURPOSES BASED ON GENERALLY ACCEPTED QUALITY CHARACTERISTICS

2.1. Introduction

From the early 1960s to the 1980s, the German wheat beer consumption was still confined almost entirely to the southern regions of the country. In recent years, however, that situation has changed dramatically: The popularity of wheat beer is generally increasing so that the wheat beer output and consumption in Germany doubled from 1990 to 2009 (120, 121), in contrast to an overall decline in the per-capita consumption of beer. Especially non-alcoholic wheat beer has experienced a steep increase in popularity. As a result, roughly one of every ten beers sold in Germany nowadays is a wheat beer, as a recent survey by GlobalMalt indicates (4). In fact, wheat beer has acquired almost a cult status, not just in Germany but also worldwide. Especially in Germany, the production of wheat malt increased from 85,000 to almost 100,000 tons in 2010 (+15%). However, the proportion of wheat (Triticum aestivum L.) used in brewing is very low with regard to the total wheat production. Therefore, wheat breeding programs aimed at brewing quality are very limited to date. Furthermore, very little attention has been given to malting of wheat in comparison to malting of barley and other cereals. Since the baking industry is the largest consumer of wheat, breeders and farmers focus on baking quality to optimize their profit. So wheat has been – and still is – grown almost exclusively for the baking industry. This leads to a problem for maltsters and brewers because no specific wheat cultivars are available for brewing wheat beer. However, some wheat cultivars available on the market are suitable for beer production, but most of them show only a few optimum parameters, whereas other parameters are out of the optimal range. Therefore, wheat cultivars have to be screened for their suitability for malting and brewing purposes to ensure processability.

Although wheat has a long tradition as a raw material for the production of malt and

beer, only a few publications exist dealing with limiting values or quality parameters for wheat regarding brewing technology. However, the authors agree that most malt characteristics are owed to the cultivar-induced variability providing an opportunity for breeding wheat exhibiting brewing quality (111, 122-124). In Europe, the evaluation of malt quality is carried out according to the malt quality characteristics described by Mitteleuropäische Brautechnische Analysenkommision (MEBAK) or European Brewery Convention (EBC) (112, 125, 126). In this connection, especially viscosity (Visc), extract (E) and nitrogen compounds – such as raw protein (RP), soluble nitrogen (SN), Kolbach index (KI) – play an important role. To date valid and accepted criteria for wheat malt used in brewing are: RP 11-12.5%, d.m.; SN 0.65-0.78 g/100 g malt, d.m.; KI 37-40%; Visc <1.8 mPas and E >83%, d.m. (7). These wheat malt characteristics primarily focus on the three main biochemical processes cytolysis, proteolysis and amylolysis.

Another parameter often mentioned in the literature as an analysis criterion for wheat malt is α-amylase activity and β-amylase activity (diastatic power) or fermentability. Levels of α- and β-amylase are fair meaningless as the contents of this enzymes are normally in vast excess of what is required (12, 127). Fermentability testing is very dependent on the yeast used (127). For example, if the brewery experiences a problem with the premature flocculation of the yeast with certain malt batches, it makes no sense to perform this test with some other yeast strains (127). Therefore, all three parameters, α-amylase, β-amylase and fermentability, will not be taken into account in this study.

In general, malt specifications were introduced to protect the brewer by ensuring a good brewhouse yield, minimum process problems and the production of a good quality beer (128). However, dissatisfaction with malt analyses is certainly no new phenomenon (128), after many years of highlighting the limitations of malt analyses, processing

problems do still occur (127).

Annually, with the help of small-scale malting trials a wheat cultivar catalogue is established containing the characteristic specifications of wheat malts of a particular period, usually several harvest years (129). This catalogue serves as basis for the selection of wheat cultivars currently available on the market. The pre-selection eventually involves whether a wheat cultivar is accepted as being suitable for brewing or not. Thus, over the long term, it is useful in determining if a wheat cultivar is suitable for brewing. For this reason, the interpretation of analytical data is of highest importance.

A proper interpretation of results achieved using any analytical procedure requires that the analyst such as maltster or brewer considers carefully the diverse sources of error associated with the data obtained. One of the major factors, the random error, will be discussed in this paper. Formal concepts and terminology for measurement uncertainty were brought together with the publication of the Guide to the Expression of Uncertainty in Measurement (GUM) (130).

In order to not derive false conclusions from an analysis, the understanding of sample size, measurement uncertainty and error propagation is of decisive importance (131). Both, the correct expression of measurand and its measurement uncertainty are central elements. The Eurachem definition of uncertainty of a measurement is „a parameter with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand” (132). This parameter is defined as a standard deviation, a confidence interval or some other quantitative measure of variability. The measurand is defined as the quantity of an analyte or other measurable quantity such as extract. Unlike uncertainty, error is defined as the difference between the individual result and the true value of the measurand. However, error is an idealized concept and cannot be known exactly. In

contrast, uncertainty describes the most likely limits associated with a measurement value. Uncertainty values must not be used to “correct” a measurement result.

In Chapter 1 it was found, that a gap in specific and suitable wheat cultivars for malting and brewing occur. Moreover with rising wheat beer popularity, the demand of suitable wheat cultivars for malting and brewing purposes increased. To obtain fast and reliable predications about the suitability of wheat cultivars a new mathematical method was developed in this chapter 2. The method allows a selection based on generally accepted quality characteristics. As selection criteria the attributes raw protein, soluble nitrogen, Kolbach index, extract and viscosity were chosen. The approach is explained by means of one wheat cultivar used in brewing.

The matrix developed in this chapter is validated and the method is standardized in chapter 3.

2.2. Material and Method

2.2.1 Malting

Wheat (Triticum aestivum L.), cultivar Herrmann, harvested in 2010 in Buxheim (Germany) was used for this study. The standard MEBAK method 1.5.3 (122) for micromalting (1 kg) was used and slightly modified for wheat as follows. Steeping schedule (water and air temperature 14±0.1 °C): 1st day 5 h steep, 19 h air rest; 2nd day 4 h steep, 20 h air rest; 3rd day steep until a degree of steeping of 45% is reached. Keep an air rest for the remainder of the day. After steeping and air rests for a total of 48 h, the degree of steeping can be increased to 45% through spraying. The germination condition was 4 days at 15 °C. Germination occurred in tempered climate chambers, which guaranteed a constant air humidity of >95%. The moisture content of the green malt must be 45.0-45.5% at the start of kilning process. The

kilning (withering) schedule was 16 h 50 °C (H2O < 10%) followed by 1 h at 60 °C, 1 h at 70 °C and 5 h at 80 °C. Finally, a malt cleaning step was performed in such way that all rootlets are removed without damaging the husks. The total malting time was around 191 h (8 days). In the above mentioned MEBAK method for micromalting of barley the steeping and germination take only 6 days in total. According to Sacher's extensive work (111), an optimized malting procedure for wheat with regard to a higher extract yield and better modification the following growing period conditions of 45%, 15 °C and 7 days are recommended. Therefore the standard method was extended by one day of germination.

2.2.2 Analytical Methods

Analyses were performed according to the approved methods described by MEBAK and EBC using congress mash regime (112, 122, 125, 126). The measurand, its abbreviation, the used method and equipment for each malt parameter are as follows (16): soluble nitrogen (SN) acc. to MEBAK 3.1.4.5.2.1 and EBC 4.9.1 analyzed with a Kjeltec, FOSS, Hillerød, Denmark; total nitrogen (TN) acc. to MEBAK 3.1.4.5.1.1 and EBC 4.3.1 analyzed a with Kjeltec, FOSS, Hillerød, Denmark; raw protein (RP) acc. to MEBAK 1.5.2.1 and EBC 3.3.1; Kolbach index (KI) acc. to MEBAK 3.1.4.5.3 and EBC 4.9.1; extract (E) acc. to MEBAK 3.1.4.2.2 and EBC 4.5.1 with DMA, Anton Paar, Graz, Austria; viscosity (Visc) acc. to MEBAK 3.1.4.4.1 and EBC 4.8 measured with an AMVn-Automated Micro Viscometer, Anton Paar, Graz, Austria.

2.2.3 Limiting Values for Quality Attributes

A detailed subdivision of the above mentioned characteristic quality attributes for wheat malt (see 2.1 Introduction), which are traditional accepted by industry, has been carried out on the basis of empirical values gained in practice and has been proven reasonable. The limiting values for the malt quality attributes measured are given in Table 2.1.

Table 2.1: Traditional and accepted limiting values for quality attributes of wheat malt suitable for brewing purposes based on MEBAK

	not acceptable		acceptable		good		acceptable		not acceptable	
	min	max	min	max	min	max	min	max	min	max
RP [%,d.m.]	-	9.9	10	10.9	11	12.5	12.6	13.4	13.5	-
SN [g/100g malt,	-	0.649	0.66	0.669	0.700	0.780	0.781	0.900	0.901	-
KI [%]	-	34.9	35	36.9	37	40	40.1	45	45.1	-
E [%, d.m.]	not acceptable			<84	84					
Viscosity [mPas]	1.800						>1.800 not acceptable			

2.2.4 Mathematical Method

For a better understanding, the applied mathematical methods are mentioned and described in the following section “Results and discussion”.

2.3. Results and discussion

The proposed mathematical model for predicting wheat malt quality is presented in a step by step manner to best interpret the results of this study.

2.3.1 Critical Evaluation of Measurement Results

Analytical experiments can be brought into a general scheme, where the end results will be obtained by performing a certain analysis. These quantities, which have been determined in the analysis, are named measurands. Their respective values are

described as measured values which are always afflicted with measurement uncertainties. In most cases, the measurand is already the end result of the experiment.

If the end result is based on different measurable quantities, e.g. which have to be used for the calculation of the end result, the error propagation has to be taken into consideration. The calculation of errors shows how exactly the end result is. For example, the quantity of the Kolbach index is dependent on two measurands: soluble nitrogen and total nitrogen. Both measured values are already uncertain. Thus, each measurement corresponds to the final error of the end result of the experiment.

As a measure of uncertainty the standard deviation σ is used. The end result is expressed with an uncertainty value which also has probability character. Within this uncertainty range the true – but always unknown – value will be found with a definite probability, in most cases 95%.

2.3.2 Measurement Uncertainty

In Table 2.2, the measured values for soluble nitrogen, total nitrogen and Kolbach index for one single wheat malt are shown which have been repeatedly determined (n = 10).

Table 2.2: Measured values xi for the quality parameters soluble nitrogen (SN), total nitrogen (TN), raw protein (RP), Kolbach index (KI), extract (E) and viscosity (Visc) obtained by repeated measurement (n = 10) as well as arithmetic mean $\bar{x}$, standard deviation s, standard deviation of the arithmetic mean $s_{\bar{x}}$ and uncertainty Δ of the mean. The series of measurements were tested for both normal distribution using the David test (133) as well as chi-square test (134) and outliers according to Grubbs and Beck (135).

i (n = 10)	SN [g/100 g malt, d.m.]	TN [%, d.m.]	RP [%, d.m.]	KI [%]	E [%, d.m.]	Visc (based on 8.6%) [mPa·s]
1	0.690	1.888	11.8	36.5	84.8	1.72
2	0.695	1.872	11.7	37.1	84.7	1.73
3	0.695	1.888	11.8	36.8	84.0	1.73
4	0.695	1.888	11.8	36.8	84.6	1.71
5	0.695	1.872	11.7	37.1	84.8	1.73
6	0.702	1.904	11.9	36.9	84.7	1.75
7	0.695	1.888	11.8	36.8	84.5	1.77
8	0.701	1.888	11.8	37.1	84.2	1.75
9	0.702	1.904	11.9	36.9	84.4	1.74
10	0.689	1.872	11.7	36.8	84.6	1.77
$\bar{x}$	0.6959	1.8864	11.79	36.9	84.5	1.74
s[a]	0.0046	0.0118	0.074	0.187	0.263	0.020
$s_{\bar{x}}$[b]	0.0014	0.0037	0.023	0.059	0.083	0.006
David test[c]	true[1-5]	true[4-5]	true[2-5]	true[1-5]	true[1-5]	true[1-5]
χ^2 test[d]	true[2]	true[2]	true[2]	true[2]	true[2]	true[2]
Outlier[e]	0	0	0	0	0	0
Δ[f]	0.0033	0.0084	0.0528	0.1334	0.1879	0.0143

[a] According to equation 4
[b] According to equation 5
[c] Confidence level for David test: [1] = 90%, [2] = 95%, [3] = 97.5%, [4] = 99%, [5] = 99.5%
[d] Confidence level for χ^2 test: [6] = 95%
[e] According to Grubbs and Beck (135)
[f] According to equation 8.

The corresponding frequency distributions are represented in which the absolute frequency of a measured value is plotted against the measurand x (class) (Fig. 2.1).

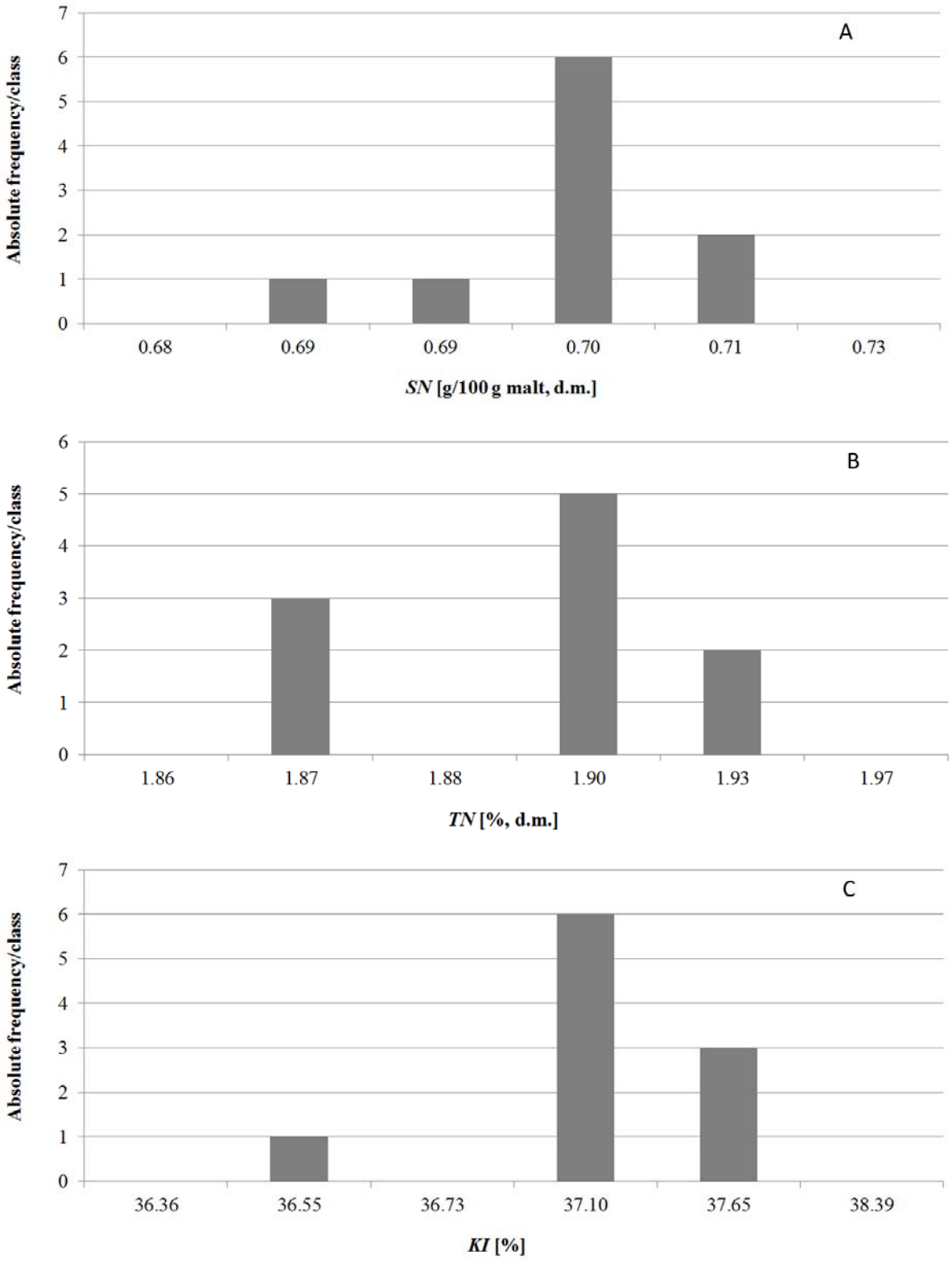


Figure 2.1: Frequency distributions (histograms) of the measured values (see Table 2.2) for soluble nitrogen SN (A), total nitrogen TN (B) and Kolbach index KI (C), plotted as absolute frequency per class

The individual measured values of the measurand x are x1, x2, x3,…, xn. The most probable value for x is the arithmetic mean $\bar{x}$ of n individual values.

If many measurements are performed, the distribution function often represents a Gaussian bell curve, whose maximum lies at the true arithmetic mean μ:

$$\mu = \lim_{n\to\infty} \frac{1}{n} \sum_{i=1}^{n} x_i. \qquad \text{(eq. 1)}$$

In the case of these analytical measurements, μ is always unknown since infinitely many measurements can never be made, which is why in practice the value of μ is estimated by means of the best value meaning the arithmetic mean $\bar{x}$. By performing an individual measurement, the measured value obtained is one out of n probable values. With the help of the corresponding Gaussian distribution curve, the probability with which this value will occur can be determined.

This implies that the measured values obtained are descended from a normally distributed population. Data obtained using analytical methods can be mostly regarded as approximately normally distributed. This assumption, however, should be tested as a matter of principle and not only in case of doubt by applying appropriate testing procedures. For this purpose, different tests are available. In this study, all data from Table II were tested by applying both the rapid test according to David (133) for five levels of significance and the chi-squared test of goodness of fit for one level of significance (134). In addition, all data were tested for outliers according to Grubbs (135) and found to be without any outliers.

In the following, the aim is to give a measure for measurement uncertainty which characteristically describes the deviation of the individual value xi from the arithmetic mean $\bar{x}$ and can be used for all measured values. However, the measurement uncertainty is the parameter for the consistency of the raw material – particularly malt (127).

At first, the mean error is calculated using $\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})$. Since the term $(x_i - \bar{x})$ is equiprobable positive or negative, this mean error gives always zero which is why the "mean square error" s2, the so-called empirical variance, is used instead:

$$s^2 = \frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2. \qquad \text{(eq. 2)}$$

As a characteristic measure of error (measurement uncertainty) for an individual measured value the standard deviation is given:

$$\sigma = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2}. \qquad \text{(eq. 3)}$$

By calculating the standard deviation of replicate measurements of a single sample, the precision of a procedure is often assessed (136).

It has to be noted that the standard deviation given in equation 3 is defined as the true standard deviation of the Gaussian distribution that is the population of all theoretically possible measurements. In practice, it is not applied to finite samples. Instead the empirical standard deviation is used:

$$s = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2}. \qquad \text{(eq. 4)}$$

For a small number of measurements, the empirical standard deviation is a better estimate of the true standard deviation (σ).

Thus, the standard deviation s is a measure for indicating that an individual value out of n measured values lies with a certain probability within a certain interval about the arithmetic mean $\bar{x}$. For the determination of s according to equation 4, n measurements are necessary and also that $n \geq 2$. Furthermore, these n measurements result in an arithmetic mean $\bar{x}$, whose deviation from the true arithmetic mean μ is certainly smaller than that of the individual measured value. But even this arithmetic mean will deviate from the true arithmetic mean μ. Hence, there is a distribution of means which is a

distribution of means which is a Gaussian distribution but not as broad as the one of the individual measured values.

The standard deviation of the arithmetic mean $s_{\bar{x}}$ is connected with the standard deviation of the individual measured value s (134) by

$$s_{\bar{x}} = \frac{s}{\sqrt{n}} = \sqrt{\frac{1}{n\cdot(n-1)}\sum_{i=1}^{n}(x_i - \bar{x})^2}. \qquad \text{(eq. 5)}$$

In Table 2.2, the standard deviations of the arithmetic mean $s_{\bar{x}}$ are given. If a measurement result is determined as mean of several measurements, the measurement uncertainty is determined by means of $s_{\bar{x}}$. For a comprehensive evaluation, the number n of performed measurements must additionally be given.

However, if the end result and its measurement uncertainty Δ should be given independent of n, the following question needs to be clarified: „What is the maximum deviation of the sample mean $\bar{x}$ from the expected true value μ of the population distribution, if the significance level α is given as 5%“ (134). In addition, it has to be taken into account that not only the true value μ is unknown but also the true variance of population σ2.

2.3.3 Confidence Limits for the Mean with Unknown Standard Deviation σ

In order to give a secure measurement uncertainty of the mean $\bar{x}$, the relation between the standard deviation of the arithmetic mean $s_{\bar{x}}$ and the unknown standard deviation of the population σ will be considered in the following with the help of a simulation calculation.

For that purpose, plausible assumptions for the values of both the unknown standard deviation σ and the unknown true arithmetic mean μ have to be made. According to

MEBAK, a repeatability

$$r = 6.7–0.12 \cdot m \qquad \text{(eq. 6)}$$

for method 3.1.4.5.3 (Kolbach index) has been determined by means of interlaboratory tests (125), in which m can be equated with the respective mean $\bar{x}$.

The repeatability r represents the absolute value which can be expected as difference between two individual results (under repetitive conditions) with a given probability of 95% (137).

According to DIN ISO 5725, the repeatability is calculated using the equation

$$r = 2.83 \cdot sr, \qquad \text{(eq. 7)}$$

in which sr is the standard deviation of repeatability (137). The factor 2.83 in equation 7 is recommended as norm independent of the number of measurement results. Precision and accuracy, like the repeatability, are discussed in much greater depth by Massart et al. (138). Default values for the Kolbach index lie between 35% and 45% according to MEBAK (125).

For a theoretical analysis, the following assumptions were made on the basis of data given by MEBAK. ***1.*** The measurement of the Kolbach index for one wheat malt is performed an almost infinite number of times (population of n = 1 million). ***2.*** The true mean μ of the Kolbach index concerning this population of analysis results for wheat malt is 40%. ***3.*** The true standard deviation σ of the population (calculated from sr using equations 6 and 7) is 0.67%, in the following rounded to 0.7%.

With the help of a simulation calculation, a normally distributed population has been calculated by means of random numbers using the parameters μ = 40% and σ = 0.7%.

The frequency distribution, which has been calculated from n = 1 million measurement results (Fig. 2.2). The mean μ lies almost exactly at x = 40%. The inflection points of the

function are at x1/2 = μ ± σ (x1 = 39.3% and x2 = 40.7%) and mark the interval including 68% of all possible measurement results. In order to cover 95% of all measurement results, an interval from μ – 1.96·σ to μ + 1.96·σ is adequate.

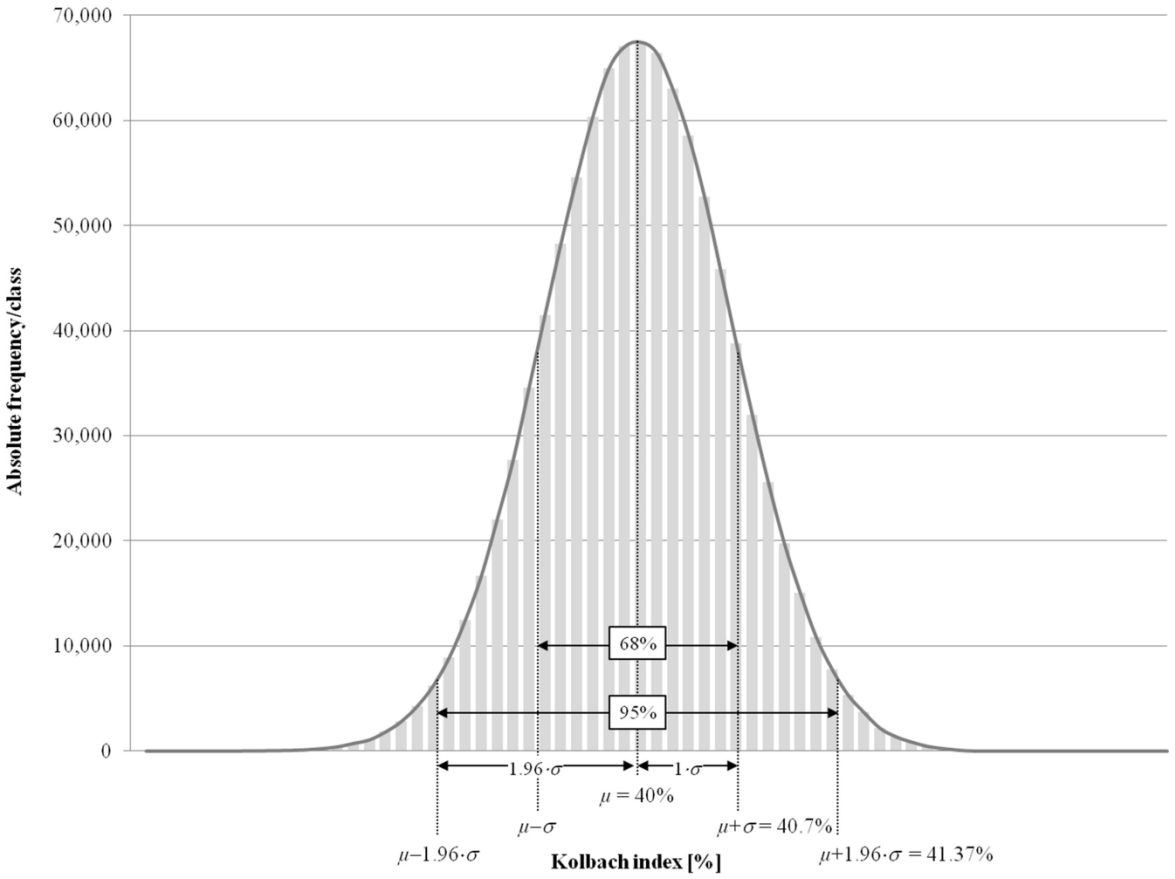


Figure 2.2: Frequency distributions (histograms) of the measured values for Kolbach index (KI) regarding one wheat malt, plotted as absolute frequency per class.

How does an estimated value s behave with regard to the true value σ, if one sample is taken from a number n of the population? In order to test the dependence of the estimate s from the number of samples n, a certain number n (e.g., n = 100) has been taken from the population and the empirical standard deviation s calculated out of it in each case. Samples were taken until the number of calculated standard deviations ns reached 10,000. This means that 10,000 times 100 values were randomly taken from 1 million measured values (population) in order to calculate the respective standard

deviations s out of it. In Figure 2.3, the frequency distribution of the standard deviations for sample sizes n = 100, n = 25, n = 5 and n = 3 are shown.

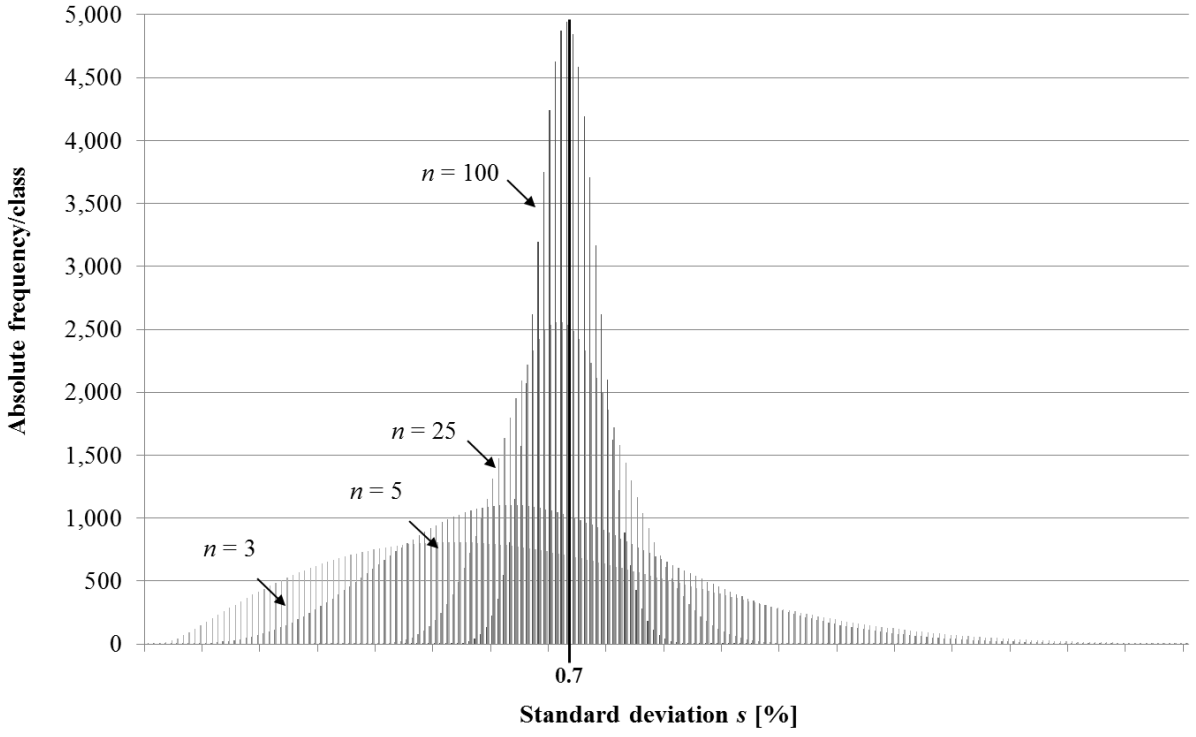


Figure 2.3: Frequency distributions (histograms) of the standard deviations for Kolbach index with a sample size of n = 100, n = 25, n = 5 and n = 3, plotted as absolute frequency per class.

With a decreasing number of samples, the frequency distribution becomes broader and is shifting more and more to the left. The estimates s for the true standard deviation σ (mathematically the distribution of the variances $\sigma 2$) follow a chi-square distribution. For $n \geq 100$, it is approximately transformed into a normal distribution.

Due to the left shift of the distribution, the proportion of standard deviations that are smaller than the true standard deviation ($\sigma = 0.7\%$) increases. This means that it is more likely to calculate with a small number of samples n a smaller standard deviation s in comparison to the real (true) standard deviation σ of the population.

In many publications, only the standard deviation is used to express the measurement error, however, this can lead to momentous misinterpretations of measured data.

In order to avoid this error, the measurement uncertainty of the mean (see equation 5) must be multiplied by a factor t. This factor originates from the t-distribution according to Gosset (139) and is tabulated for different confidence levels (e.g., P = 95%). This means that the measurement uncertainty Δ of the mean is independent of the sample size n as follows:

$$\Delta = \frac{t \cdot s}{\sqrt{n}}. \qquad \text{(eq. 8)}$$

Thus, the factor t of the measurement uncertainty is a function of the number of samples n and therefore of the degree of freedom of the measurement repetitions and decreases with an increasing number of measurements.

In Table 2.2, the measurement uncertainties Δ for all measurands calculated using equation 8 are given. The respective measurement result has to be interpreted so that $\bar{x}$ is the best estimate of the respective value of the measurand x and that $\bar{x}$–Δ to $\bar{x}$+Δ represents an interval within which a large proportion of the distribution of values can be expected, that can be assigned to $\bar{x}$ in a reasonable way (130).

2.3.4 Uncertainty of the End Result (Error Propagation)

In the case of Kolbach index, the end result KI is composed of two measurands soluble nitrogen (SN) and total nitrogen (TN). The measurement uncertainties of the individual measurands (ΔSN and ΔTN), which are propagated to the end result, can be expressed as uncertainty of the end result ΔKI. This uncertainty indicates that the true value can be found with a certain probability within ΔKI. For example, is SN afflicted with the uncertainty ΔSN according to equation 8, then that is passed on to the end result KI in the form of KI ± ΔKI. The respective uncertainty ΔKI of the end result is calculated as follows:

$$\Delta \mathrm{KI} = \frac{\mathrm{dKI}}{\mathrm{dSN}} \cdot \Delta \mathrm{SN}. \qquad \text{(eq. 9)}$$

However, for the determination of ΔKI, equation 9 has to be extended by all relevant measurands and is therefore composed of two parts in the form

$$\Delta \mathrm{KI} = \sqrt{\left(\frac{\partial \mathrm{KI}}{\partial \mathrm{SN}} \cdot \Delta \mathrm{SN}\right)^2 + \left(\frac{\partial \mathrm{KI}}{\partial \mathrm{TN}} \cdot \Delta \mathrm{TN}\right)^2}. \qquad \text{(eq. 10)}$$

Because of the statistical character of the individual components, those cannot be simply summed up but must be "added up geometrically". ∂KI/∂SN and ∂KI/∂TN are partial derivatives of the function KI with respect to the individual measurands (variables). It is valid:

$$\mathrm{KI}(\mathrm{SN}, \mathrm{TN}) = \frac{\mathrm{SN}}{\mathrm{TN}} \cdot 100 \qquad \text{(eq. 11)}$$

and

$$\frac{\Delta \mathrm{KI}}{\mathrm{KI}} = \sqrt{\left(\frac{\Delta \mathrm{SN}}{\mathrm{SN}}\right)^2 + \left(\frac{-\Delta \mathrm{TN}}{\mathrm{TN}}\right)^2}. \qquad \text{(eq. 12)}$$

By means of equation 12, the actual measurement uncertainty of Kolbach index can be calculated using the measured values given in Table 2.2:

$$\Delta \mathrm{KI} = \sqrt{\left(\frac{\Delta \mathrm{SN}}{\mathrm{SN}}\right)^2 + \left(\frac{-\Delta \mathrm{TN}}{\mathrm{TN}}\right)^2} \cdot \mathrm{KI} = \sqrt{\left(\frac{0.0033 \frac{\mathrm{g}}{100\ \mathrm{g\ malt}}}{0.6959 \frac{\mathrm{g}}{100\ \mathrm{g\ malt}}}\right)^2 + \left(\frac{-0.0084\%}{1.8864\%}\right)^2} \cdot 36.9\% = 0.239\%. \qquad \text{(eq. 13)}$$

It can be summarized that by determining the uncertainty ΔKI for the parameter KI (function of the measurands SN and TN) a mathematical function in the general form of equation 12 is received representing the dependence of the uncertainty of the result from the measurement uncertainties of the individual measurands. Using the example of equation 13, it can be found that ΔSN has a stronger effect on the uncertainty of the end result than ΔTN.

It has been found that the measurement uncertainty ΔKI calculated according to equation 13 is higher than that calculated according to equation 8 as given in Table 2.2. Thus, the influence of error propagation due to individual measurands has always to be taken into account in view of the evaluation of results.

On the one hand, the measurement uncertainty shows the uncertainty of the analyses. On the other hand, the uncertainty is a good indication of the consistency of the raw material. Results of malt analyses are mostly given as single average values (means). However, it is possible to get two malts with identical mean values of a specification that perform totally differently in a brewing operation (127). Whilst the average for the hypothetical parameter of two malt samples are the same, the distribution can be very different, so that the latter may well include a percentage of problematic material (127). One explanation for this is the inherent biological variability of the wheat. Another area of concern could be the blending to obtain average results. With the help of the measurement uncertainty the brewer can easily check if the brewers concern that the malster has dumped some dubious quality malt into the blend is right. On the other side, the malster can prove that the brewer might only blame the malt for some other problems in the brewery (127).

2.3.5 Selection on the Basis of Nitrogen Related Quality Parameters

The functional relation of equation 11 can be represented by a two-dimensional matrix as shown in Table 2.3. Here, the dark gray fields index not acceptable, the pale gray fields still acceptable and the white fields good measured values (see Table 2.1).

Table 2.3: Matrix 1 representing the relation between KI, SN and TN (RP). Column A (A2:A36) contains possible values for RP, column B (B2:B36) possible values for TN. Both parameters are connected with each other by the factor 6.25. In row 1 (C1:T1), the values for SN are given. In field C2:T36, the values for the corresponding KI are tabulated (see equation 11).

	A	B	C	D	E	G	I	J	K	L	M	N	O	P	Q	R	S	T
	RP	*TN*	*SN* [g/100 g malt, d.m.]															
1	[%, d.m.]	[%, d.m.]	0.640	0.645	0.650	0.670	0.690	**0.693**	**0.696**	**0.699**	0.700	0.750	0.770	0.780	0.800	0.890	0.900	1.000
2	9	1.440	44.44	44.79	45.14	46.53	47.92	48.13	48.33	48.54	48.61	52.08	53.47	54.17	55.56	61.81	62.50	69.44
3	9.2	1.472	43.48	43.82	44.16	45.52	46.88	47.08	47.28	47.49	47.55	50.95	52.31	52.99	54.35	60.46	61.14	67.93
4	9.4	1.504	42.55	42.89	43.22	44.55	45.88	46.08	46.28	46.48	46.54	49.87	51.20	51.86	53.19	59.18	59.84	66.49
5	9.6	1.536	41.67	41.99	42.32	43.62	44.92	45.12	45.31	45.51	45.57	48.83	50.13	50.78	52.08	57.94	58.59	65.10
6	9.8	1.568	40.82	41.14	41.45	42.73	44.01	44.20	44.39	44.58	44.64	47.83	49.11	49.74	51.02	56.76	57.40	63.78
7	10	1.600	40.00	40.31	40.63	41.88	43.13	43.31	43.50	43.69	43.75	46.88	48.13	48.75	50.00	55.63	56.25	62.50
8	10.2	1.632	39.22	39.52	39.83	41.05	42.28	42.46	42.65	42.83	42.89	45.96	47.18	47.79	49.02	54.53	55.15	61.27
9	10.4	1.664	38.46	38.76	39.06	40.26	41.47	41.65	41.83	42.01	42.07	45.07	46.27	46.88	48.08	53.49	54.09	60.10
10	10.6	1.696	37.74	38.03	38.33	39.50	40.68	40.86	41.04	41.21	41.27	44.22	45.40	45.99	47.17	52.48	53.07	58.96
11	10.8	1.728	37.04	37.33	37.62	38.77	39.93	40.10	40.28	40.45	40.51	43.40	44.56	45.14	46.30	51.50	52.08	57.87
12	11	1.760	36.36	36.65	36.93	38.07	39.20	39.38	39.55	39.72	39.77	42.61	43.75	44.32	45.45	50.57	51.14	56.82
13	11.1	1.776	36.04	36.32	36.60	37.73	38.85	39.02	39.19	39.36	39.41	42.23	43.36	43.92	45.05	50.11	50.68	56.31
14	11.2	1.792	35.71	35.99	36.27	37.39	38.50	38.67	38.84	39.01	39.06	41.85	42.97	43.53	44.64	49.67	50.22	55.80
15	11.3	1.808	35.40	35.67	35.95	37.06	38.16	38.33	38.50	38.66	38.72	41.48	42.59	43.14	44.25	49.23	49.78	55.31
16	11.4	1.824	35.09	35.36	35.64	36.73	37.83	37.99	38.16	38.32	38.38	41.12	42.21	42.76	43.86	48.79	49.34	54.82
17	11.5	1.840	34.78	35.05	35.33	36.41	37.50	37.66	37.83	37.99	38.04	40.76	41.85	42.39	43.48	48.37	48.91	54.35
18	11.6	1.856	34.48	34.75	35.02	36.10	37.18	37.34	37.50	37.66	37.72	40.41	41.49	42.03	43.10	47.95	48.49	53.88
19	11.7	1.872	34.19	34.46	34.72	35.79	36.86	37.02	37.18	37.34	37.39	40.06	41.13	41.67	42.74	47.54	48.08	53.42
20	**11.74**	**1.878**	34.07	34.34	34.60	35.67	36.73	**36.89**	**37.05**	37.21	37.27	39.93	40.99	41.52	42.59	47.38	47.91	53.24
21	**11.79**	**1.886**	33.93	34.19	34.46	35.52	36.58	**36.74**	**36.90**	**37.05**	37.11	39.76	40.82	41.35	42.41	47.18	47.71	53.01
22	**11.84**	**1.894**	33.78	34.05	34.31	35.37	36.42	36.58	**36.74**	**36.90**	36.95	39.59	40.65	41.17	42.23	46.98	47.51	52.79
23	11.9	1.904	33.61	33.88	34.14	35.19	36.24	36.40	36.55	36.71	36.76	39.39	40.44	40.97	42.02	46.74	47.27	52.52
24	12	1.920	33.33	33.59	33.85	34.90	35.94	36.09	36.25	36.41	36.46	39.06	40.10	40.63	41.67	46.35	46.88	52.08
25	12.1	1.936	33.06	33.32	33.57	34.61	35.64	35.80	35.95	36.11	36.16	38.74	39.77	40.29	41.32	45.97	46.49	51.65
26	12.2	1.952	32.79	33.04	33.30	34.32	35.35	35.50	35.66	35.81	35.86	38.42	39.45	39.96	40.98	45.59	46.11	51.23
27	12.3	1.968	32.52	32.77	33.03	34.04	35.06	35.21	35.37	35.52	35.57	38.11	39.13	39.63	40.65	45.22	45.73	50.81
28	12.4	1.984	32.26	32.51	32.76	33.77	34.78	34.93	35.08	35.23	35.28	37.80	38.81	39.31	40.32	44.86	45.36	50.40
29	12.5	2.000	32.00	32.25	32.50	33.50	34.50	34.65	34.80	34.95	35.00	37.50	38.50	39.00	40.00	44.50	45.00	50.00
30	12.6	2.016	31.75	31.99	32.24	33.23	34.23	34.38	34.52	34.67	34.72	37.20	38.19	38.69	39.68	44.15	44.64	49.60
31	12.7	2.032	31.50	31.74	31.99	32.97	33.96	34.10	34.25	34.40	34.45	36.91	37.89	38.39	39.37	43.80	44.29	49.21
32	12.8	2.048	31.25	31.49	31.74	32.71	33.69	33.84	33.98	34.13	34.18	36.62	37.60	38.09	39.06	43.46	43.95	48.83
33	12.9	2.064	31.01	31.25	31.49	32.46	33.43	33.58	33.72	33.87	33.91	36.34	37.31	37.79	38.76	43.12	43.60	48.45
34	13	2.080	30.77	31.01	31.25	32.21	33.17	33.32	33.46	33.61	33.65	36.06	37.02	37.50	38.46	42.79	43.27	48.08
35	13.5	2.160	29.63	29.86	30.09	31.02	31.94	32.08	32.22	32.36	32.41	34.72	35.65	36.11	37.04	41.20	41.67	46.30
36	14	2.240	28.57	28.79	29.02	29.91	30.80	30.94	31.07	31.21	31.25	33.48	34.38	34.82	35.71	39.73	40.18	44.64
			KI [%]															

By means of the correct determination of measurement uncertainties of individual parameters, the appropriate area for the wheat cultivar can be marked (numbers in bold). The intersection is represented by the fields J20:K20; J21:L21 and K22:L22. The sample space shows that the wheat malt lies with regard to the parameters RP in the good, SN in the acceptable and KI in both the acceptable and good range. If a condition

for the acceptance of wheat malt for brewing purposes would be that at least two parameters must lie in the good range, then the consideration of only the mean would lead to an error in the selection, since the actual measured value can be assumed to lie with a probability of 95% within the sample space.

2.3.6 Selection on the Basis of Extract and Viscosity

After selecting the wheat malt based on nitrogen related parameters by means of matrix 1 (Table 2.3), another two quality parameters, extract and viscosity, have to be considered. Those can also be graphically represented in a matrix, in which white fields show the range for suitable cultivars and dark gray fields the range for non-suitable cultivars. In Table 2.4 (matrix 2), the wheat malt (see Table 2.2) is represented as cultivar 1. In addition, another two theoretical cultivars have been included in the matrix. Here, the cultivar 2 meets the specifications for the parameter viscosity and non-significant the specifications for the parameter extract. However, cultivar 3 lies significantly above the limit for viscosity. Both, cultivar 2 and cultivar 3 would not be selected for brewing purposes.

The relation between matrix 1 and matrix 2 is illustrated in Figure 2.4. It has been found that the selection criteria can be extended by other parameters, for example by means of a z-axis. The relevant area for another parameter is marked by z1 and z2 in Figure 2.4. Here, it is clearly shown that the selection of cultivars is a question of a sample space which has to be interpreted as a multidimensional room that has been attributed to the different selection criteria.

Table 2.4: Matrix 2 representing the relation between extract (E) and viscosity (Visc). The dark gray fields define the rejection region for wheat malt cultivars

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
	Visc	*E* [%, d.m.]														
1	[mPa·s]	83.60	83.70	83.80	83.90	84.00	84.10	84.20	84.30	**84.43**	**84.50**	**84.72**	84.80	84.90	85.00	85.10
2	1.50															
3	1.51															
4	1.52															
5	1.53															
6	1.54															
7	1.55															
8	1.56															
9	1.57															
10	1.58															
11	1.59															
12	1.60															
13	1.61															
14	1.62															
15	1.63															
16	1.64															
17	1.65															
18	1.66															
19	1.67															
20	1.68															
21	1.69															
22	1.70															
23	1.71															
24	1.72															
25	**1.73**															
26	**1.74**															
27	**1.75**															
28	1.76															
29	1.77															
30	1.78															
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35	1.83															
36	1.84															

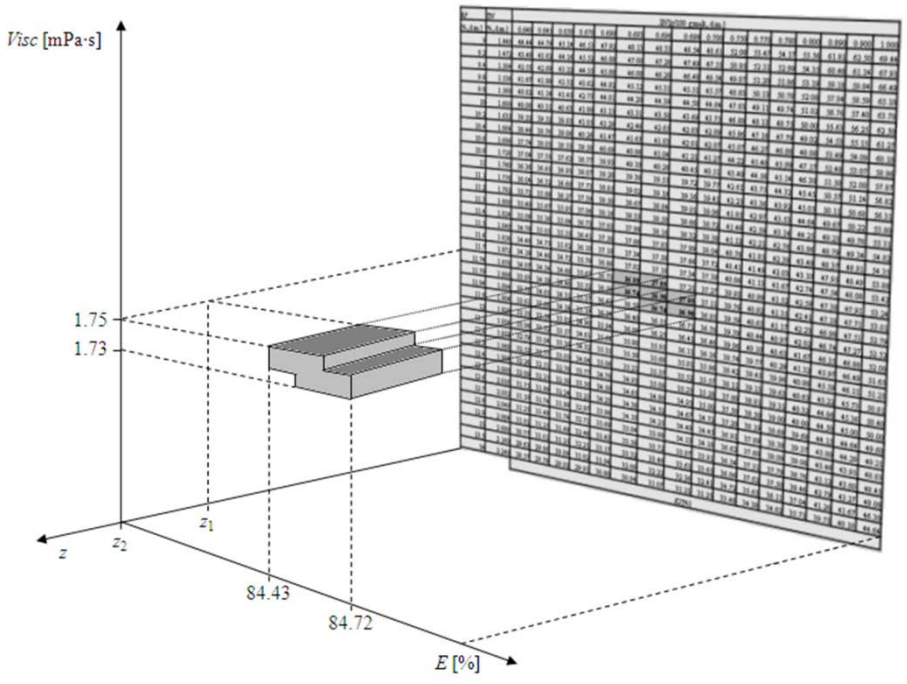


Figure 2.4: Sample space represented as a multidimensional room which has been attributed to the different selection criteria

2.4 Conclusions

It can be concluded that by means of the presented mathematical method a simple and reliable pre-selection of wheat malt types for brewing purposes based on generally accepted quality characteristics is possible. In particular, extract, viscosity and nitrogen related quality parameters (raw protein, soluble nitrogen, Kolbach index) have been chosen. Using small-scale malting trials and brewing technological methods, the properties of one wheat cultivar have been determined and discussed as an example.

With the help of a theoretical study on the indication of measurement uncertainty, it could be shown that the correct interpretation of the measured analytical results is of special significance. Here, the measurement result has to be interpreted so that it is always the best estimate of the corresponding measurand value and that the mean plus or minus its measurement uncertainty gives an interval in which a large proportion of the distribution of values can be expected that can be assigned to the measurand in a reasonable manner. Further, these intervals or sample spaces could be represented by means of a multidimensional room. Thus, it was possible to decide quickly, whether a cultivar has actually fulfilled the quality specifications or not.

Finally, it should be mentioned that the presented study and selection of brewing wheat cultivars deals solely with named test criteria. Market-based aspects such as competitiveness, market acceptance of cultivars and value added (profitability due to high grain yield) were ignored here.

CHAPTER 3

COMMON WHEAT (*Triticum aestivum* L.)

PRE-SELECTION OF WHEAT MALT TYPES FOR BREWING PURPOSES BASED ON GENERALLY ACCEPTED QUALITY CHARACTERISTICS

3.1. Introduction

Beer contains more than 450 different substances, all of which can affect flavour, aroma, colour, stability, and overall beer quality (113). This makes beer one of the world's most complex foods, even though only four ingredients are required in the production of beer: malt, water, yeast, and hops. Malting is the initial step in traditional beer production, whereby the brewing process and the resulting beer type and quality are strongly determined by the quality of malt. Crucial parameters which lead to the required malt quality attributes are: a, the variety itself with its specific constitution b, the malt modification and c, the growing region as well as the harvest year. Barley and wheat are cereals which are used for malting and brewing, both with a long historically background. However, they differ in many aspects – agronomically, chemically, morphologically a as well as producing quantity and yield (2, 85, 140). The proportion of common wheat (*Triticum aestivum* L.) used in brewing is very low with the regard of the total wheat production. In the case of Germany only 0.6% of the total wheat harvest is used for brewing. On the contrary, barley is mainly produced for brewing and is still the most used cereal in the brewing industry.

For brewing, wheat varieties are favored, which are low in protein content but high in starch. As of now, no specific varieties, only suitable ones, are available and established for brewing purpose. It is of major interest, screening wheat varieties on brewing suitability and focusing more on wheat as a brewing cereal. Although wheat has a long tradition as a raw material for the production of malt and beer, only a few publications exist dealing with limiting values or quality attributes for wheat regarding brewing technology. The authors agree that most malt characteristics are owed to the cultivar-induced variability providing an opportunity for breeding wheat exhibiting brewing quality (6, 80, 111, 128).

Cereals selected for the use in brewing industry must have special quality requirements for malt and beer production. Breeding and cultivation efforts for barley have been highly successful in creating agronomically and brew-technically optimal high quality value barley cultivars for malting and brewing industries. Nevertheless, due to its optimisation for the baking industry, wheat 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.

The goal of wheat breeders is to develop varieties with high protein contents and attributes which are fundamental for good quality flours, considering exclusively milling and baking quality (141, 142). Moreover, wheat varieties are classified in to different quality groups. They are divided based on their amount of gluten content, starch content or other substances of interest in food and feed industry. The classes are explained and listed in Table 3.1.

Table 3.1: Wheat cultivar classification based on minimum requirements and quality attributes as defined by industry

Wheat quality groupe					
attribute	units	E	A	B	C
moisture	%	< 14.5	< 14.5	< 14.5	< 14.5
protein	%, d.m.	> 14.0	> 13.0	> 12.0	< 11.0
falling number	sec	> 275.0	> 250.0	> 220.0	> 220.0
hectolitre	kg	> 78.0	76.0-77.0	> 76	> 76
sedimentation	mL	50.0-60.0	35.0-40.0	20.0-25.0	n.a
DON	ppm	< 750.0	< 750.0	< 750.0	< 750.0

The cultivar classification to each quality group is based on selected minimum requirements of the important quality attributes, e.g. protein content, gluten index, falling number and value of sedimentation. The groups are divided into 4 qualities: E, A, B and C (elite wheat, quality wheat, bread wheat, and other wheat respectively). Wheat varieties which are particularly used for cookie production are indicated with

index K. This classification depends and occurs mainly in milling and baking industry to evaluate the baking performance of cereals.

Concerning the fact, that no specific analytical data and methods are given and established for wheat and wheat malt, analytical methods which are approved for barley have to be used. Moreover, not all established methods are appropriate for selecting and analysing wheat and wheat malt, which consequently leads to a gap of knowledge (5, 7, 111). When selecting wheat varieties for brewing purpose, the most important criteria for malsters and brewers are viscosity (Visc), protein content (TN), soluble nitrogen (SN), extract (E) and Kolbach index (KI) (values shown in chapter 2). Too high or too low contents, of the above mentioned attributes, can lead to problems during the brew.

In this chapter 26 different wheat varieties (with different quality groups) are evaluated on their quality malt potential. These wheat varieties were grown and harvested in 7 growing areas in Germany in 2012 (listed in Table 3 . 3). The high amount of samples (164) is necessary, because wheat quality can also be influenced by fertilisation, soil, climate conditions and growing regions (2, 7, 12, 39, 143). The mathematically model, described in chapter 2, was used to evaluate and select wheat varieties on their malting and brewing purpose as well as their stability on environmental impacts. Furthermore, to get additional information about the stability to climate conditions, varieties where analysed during a triannual cultivation series. Proteolytic, amylolytic and cytolytic malt values are crucial criteria and where chosen for selecting wheat malt varieties.

3.2. Materials and Methods

3.2.1 Materials

At least 22 different wheat varieties (with different quality groups listed in Table 3.2) were grown and harvested per growing area in Germany in 2012 (in [region/#of varieties]: Kirchseon/25, Reith/23, Feisteneich/25, Köfering/24, Buxheim 24, Greimersdorf/22, Günzburg/24). The standard MEBAK (Mitteleuropäische Brau- und Analysenkommision) (112) malting method 2.5.3.1 (15° vegetation temperature, 45% steeping degree and 7 day of vegetation) was carried out in a pilot scale malting facility (Chair of Brewing and Beverage Technology, Weihenstephan, Germany). Seeds were germinated in tempered climate chambers with a constant air humidity of >95%. The initial kilning temperature for the green malt was set to 50 °C and gradually increased, over 24 h, to 80 °C. All analyses are based on methods described in the EBC (145) ASBC or MEBAK handbooks (112).

Table 3.2: Wheat varieties grown and analysed on malt quality in 2012

quality groupe	variety	# of growing regions	quality groupe	variety	# of growing regions
A	**Atomic**	**7**	B	**Colonia**	**7**
A	**Cubus**	**7**	B	**Manager**	**7**
A	**Forum**	**7**	B	**Mentor**	**7**
A	**Impression**	**7**	B	**Orkas**	**2**
A	**JB Asano**	**7**	B	**Sophytra**	**5**
A	**Joker**	**7**	B	**Tobak**	**3**
A	**Julius**	**7**	C	**Bombus**	**7**
A	**Kometus**	**7**	C	**Elixer**	**7**
A	**Meister**	**7**	C	**Muskat**	**7**
A	**Opal**	**7**	C_K	**Hermann**	**7**
A	**Pamier**	**7**	C_K	**Tabasco**	**4**
A	**Patras**	**7**	E	**Famulus**	**5**
A	**Akratos**	**6**	E	**Kerubino EU**	**6**

3.2.2 Analytical Methods

Analytical procedures were carried out in triplicate, at least (n ≥ 3), and the means of all results were calculated with standard deviations. Biological replications were done in duplicate. Statistical analyses were performed according to ANOVA (analysis of variance) with p-values < 0.005. All concentrations are based on dry matter (d.m.) unless otherwise stated.

Analyses of malted wheat varieties were performed according to the approved methods of the EBC and MEBAK (Mitteleuropäische Brau- und Analysenkommision) 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 (112). The apparent attenuation (amount of fermentable sugars) was detected by MEBAK method 4.1.4.10 (112). Total protein (TP) content was analysed according to MEBAK method 1.5.2.1 and calculated as total nitrogen. The protein content was measured according 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 (111, 146, 147) and approved methods according AACC (method 46.18) and ICC, wheat varieties which are used for feed purpose are multiplied with 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 soluble nitrogen (SN) content was determined by using an automatic Kjeltec system, according to MEBAK method 3.1.4.5.2.1 (112). The Kolbach Index (KI) was calculated from the formula according to MEBAK method 4.1.4.5.3 (112). α-Amino nitrogen, also

referred to a free amino nitrogen (FAN), was determined using a Skalar working station (Skalar, Breda, Netherlands), following MEBAK method 3.1.4.5.5 (112). Wort viscosity was measured using a falling ball viscometer (AMVn-Automated Micro Viscometer Anton Paar, Graz, Austria).

3.3. Results and discussions

The evaluation of malt quality is carried out according to the malt quality characteristics described by MEBAK or EBC (112). In this connection, especially viscosity (Visc), extract (E) and nitrogen compounds – such as raw protein (RP), soluble nitrogen (SN), Kolbach index (KI) – play an important role. To date valid and accepted criteria for wheat malt used in brewing are: RP 11-12.5%, d.m.; SN 650-780 mg/100 g malt, d.m.; KI 37-40%; Visc <1.8 mPas and E >83%, d.m. (7). These wheat malt characteristics primarily focus on the three main biochemical processes cytolysis, proteolysis and amylolysis. According to the detailed subdivision of the above mentioned quality attributes for wheat malt stated in chapter 2, a first quality selection of evaluated varieties can be conducted (Table 3.3). In average, the crop year 2012 show relatively good and high extract and AAL yields. However the protein contents of all analysed varieties are out of the accepted criteria. Only two varieties result in not acceptable Kolbach Indexes (Opal and Patras) and soluble nitrogen values (Colonia and Famulus). The analysed viscosity values lie all below the maximum limit of 1.800mPas.

Table 3.3: Average results of 7 growing regions in 2012; according limiting values for quality attributes of wheat malt (Chapter 2, Table 2.1): dark gray = not acceptable, gray = acceptable, white = good

			extract	AAL	protein	Kolbach Index	solble N	viscosity
	variety	# growing region	%, malt d.m.	%	N*6,25 , %	%	mg/100g malt d.m.	(8,8%) mPas
quality groupe	Crop Year 2012 (average results of 7 growing regions)							
A	Atomic	7	82.5	80.1	14.4	35	768	1.739
A	Cubus	7	83.7	82.9	14.8	37	820	1.688
A	Forum	7	83.7	82.4	14.5	40	890	1.595
A	Impression	7	83.2	80.4	14.6	40	877	1.700
A	JB Asano	7	83.7	81.2	14.6	40	868	1.626
A	Joker	7	83.1	80.5	14.4	37	795	1.737
A	Julius	7	82.7	82.3	14.5	36	778	1.633
A	Kometus	7	82.9	81.4	14.8	35	787	1.697
A	Meister	7	82.8	80.2	15.1	36	802	1.712
A	Opal	7	82.4	82.0	15.1	31	710	1.825
A	Pamier	7	83.5	80.2	14.8	37	826	1.815
A	Patras	7	83.5	79.7	14.8	34	758	1.805
A	Akratos	6	84.5	82.3	13.6	41	836	1.653
B	Colonia	7	82.7	80.9	14.7	40	900	1.618
B	Manager	7	82.7	81.0	14.3	39	841	1.713
B	Mentor	7	84.2	80.7	14.1	37	794	1.618
B	Orkas	2	82.8	83.2	14.9	36	737	1.836
B	Sophytra	5	83.2	80.9	14.7	39	877	1.643
B	Tobak	3	82.9	80.8	13.9	37	772	1.608
C	Bombus	7	84.6	81.3	14.1	37	783	1.670
C	Elixer	7	84.0	82.3	13.9	37	782	1.589
C	Muskat	7	83.6	80.9	14.2	38	807	1.654
C_K	Hermann	7	84.0	81.3	13.7	40	820	1.602
C_K	Tabasco	4	84.1	80.8	13.7	42	874	1.527
E	Famulus	5	82.6	81.3	15.7	40	904	1.581
E	Kerubino EU	6	82.9	82.5	14.6	37	799	1.664
	average		83.3	81.3	14.5	38	816	1.675

These values only show the average of each variety over all growing regions. To gain a deeper insight on the influence of the variety itself and the growing region amylolytic, proteolytic, and cytolytic malt values are discussed separately for selecting wheat malt varieties. In the following Figures minimum and maximum values of each variety per growing region are indicated with intervals.

3.3.1 Amylolytic malt quality attributes

The extract yield, one of the most important quality attributes of any cereal malt (39), represents the fermentable sugars generated during mashing, through amylase hydrolysis of starch to saccharides, and is essential for a successful fermentation. Extract allows drawing direct conclusions about the content of mostly soluble sugar

substances, and indicates later alcohol percentage (148). Increased proteolytic solution increases the starch availability and may also result in higher extract values. Final attenuation depends on the availability of fermentable sugars and on the yeast remaining in contact with wort (12), which means a complete hydrolysis of starch and a sufficient amino acid supply for yeast. The extract values and final attenuation for wheat, determined in congress mashing wort, should lie over 83% dm and over 79% respectively, according to literature (39, 111, 112). Malsters and brewers favour brewing cereals that are high in starch content and low in protein content. With an increase in protein, a decrease in starch content is recognised, and therefore a decrease in extract and final attenuation (39). In Figure 3.1 the extract and AAL values for all analysed varieties are shown. The maximum achieved values for all varieties lie above 83% - the recommended extract criteria. The varieties Elixer (88.19±0.06), Bombus (88.33±0.10) and Mentor (87.99±0.12), grown in Kirchseeon, show the highest extract values of all analysed varieties. Wheat varieties from the quality group C result in high and constant extract contents, on average 84 %. However, the wheat varieties Cubus, Forum, JB Asano, Pamier, Patras, Akratos (quality group A) and Mentor (quality group B) lay in average over the recommended extract value of 83%. In average the varieties Atomic, Impression, Julius, Kometus, Meister, Opal (quality group A), Colonia, Manager, Orkas, Tobak (quality group B) Famulus and Kerubino (quality group E) do not reach the requested 83% of extract content. Therefore, these varieties seem to be not acceptable for brewing. Conspicuously, a homogenous distribution of the mean extract is obtained in all varieties.

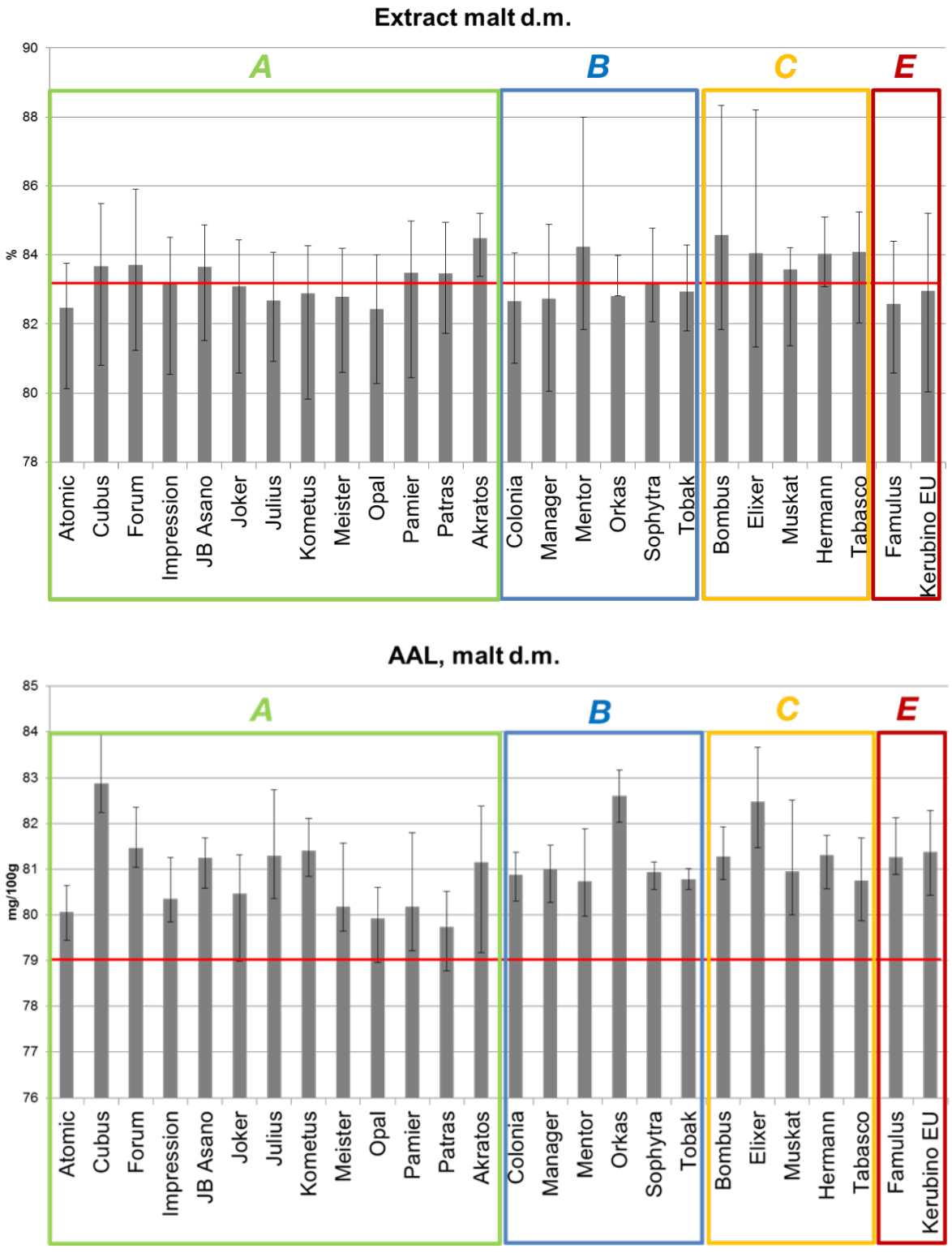


Figure 3.1:Minimum and maximum amylolytic values for analysed varieties in 2012; wheat quality groups according Table 3.1.

However, significant differences of extract value in quality groups as well as growing region can be detected as it is shown in Figure 3.2. The extract value of quality group C is the highest in all growing regions. No significant differences in each growing

region between quality group A and B could be detected, accept growing region 5. The quality group E shows the lowest extract values in all regions. However, significant differences in growing regions of all quality groups were detected. Kirchseeon (5) resulted in the highest whereas Greimerdorf (3) showed the lowest, extract values for all quality groups. The growing regions 1 to 3 resulted in lower valued than the growing regions 4 to 7. This leads to the conclusion that extract content depends on variety and is also influenced by growing region.

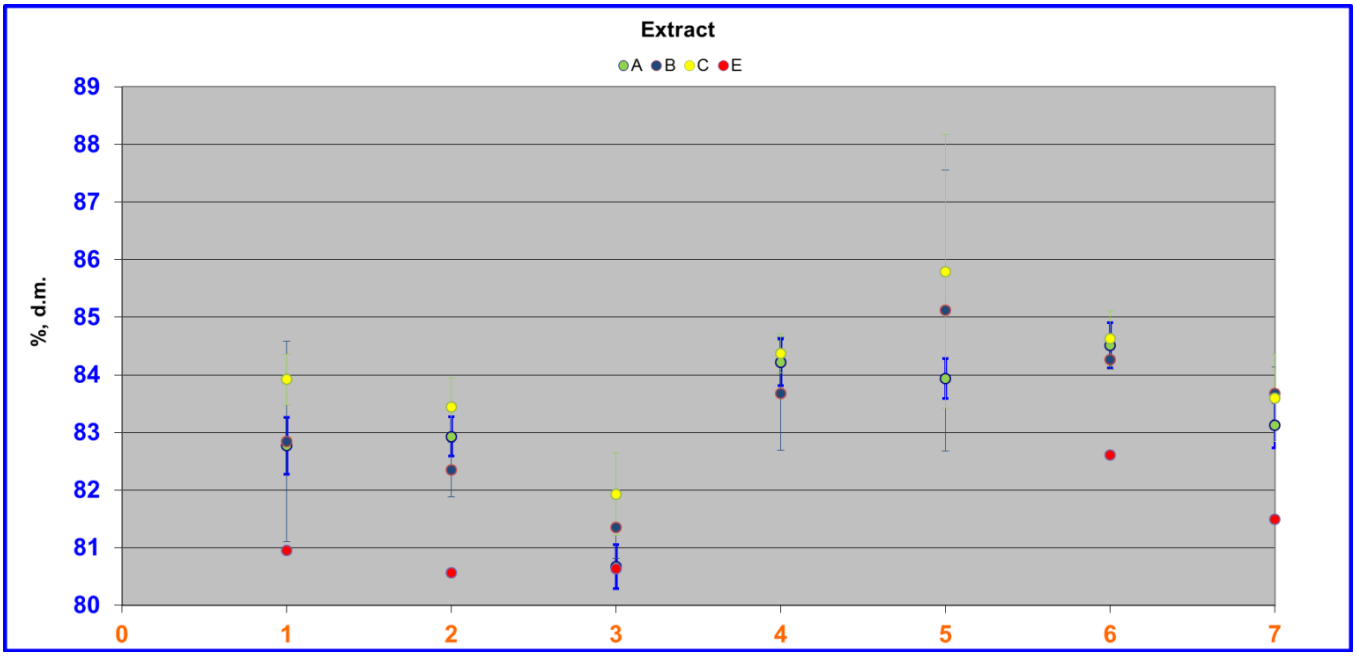


Figure 3.2: T-test distribution of extract values of different quality groups and growing region with a confidence interval of 95%, wheat quality groups according Table 3.1.

The final attenuation results reinforce the overall good amylolytic attributes for the crop year 2012. All varieties lie over the minimum required value for AAL. Furthermore, the agricultural conditions, such as climate, fertilization, and soil properties have an influence on the starch and protein content (14, 39). This goes along with the evaluated extract and AAL contents.

All varieties from quality group C as well as Cubus, Forum, JB Asano, Pamier, Patras, Akratos (quality group A) and Mentor (quality group B) show good and

acceptable amylolytic malt quality attributes and seems to be suitable for brewing. Greimersdorf (growing region 3) seems to be not suitable for growing wheat.

3.3.2 Proteolytic malt quality attributes

Nitrogen content of brewing relevant cereals is essential for beer production. With increasing protein content decreasing extract content is recognized (7, 12, 111, 112, 149). Raw protein, soluble nitrogen and Kolbach index are important selection attributes for brewing cereals. Figure 3.3 show the deviation of protein content of all analysed varieties. All samples have relatively high protein contents (in average over 14%, d.m.). The lowest raw protein contents show the varieties Hermann (11.38±0.14), Elixer (12.90±0.0.08) (quality group C, grown in Köfering), Tabasco (12.67±0.20, quality group C, grown in Kirchseeon) as well as Julius (12.79±0.17, quality group A, grown in Günzburg). Famulus (quality group E) is not suitable for brewing, due to the highest proteolytic malt attributes in all growing regions as well as low amylolytic values. With increasing protein content, a decrease in extract values goes along. Concerning the raw protein content the varieties Mentor (quality group B), Bombus, Elixer, Tabasco Hermann (quality group C) as well as Akratos, Julius and Atomic (quality group A) seems to be interesting for brewing, due to low raw protein contents. Additionally, all these varieties show good amylolytic quality attributes. Significant differences in quality groups as well as growing regions could be detected (show in Figure 3.4) for raw protein as it was already found for extract values. The lowest raw protein contents were detected for quality group C in all growing regions. No significant differences were found for quality group A and B in the growing region itself. However, growing regions 1, 2, 3 and 7 show significant higher raw protein contents than 4, 5 and 6. With increasing raw protein content, a decrease in amylolytic attributes can be recognized. Concluding these results raw protein content depends on variety and is also influenced by growing region.

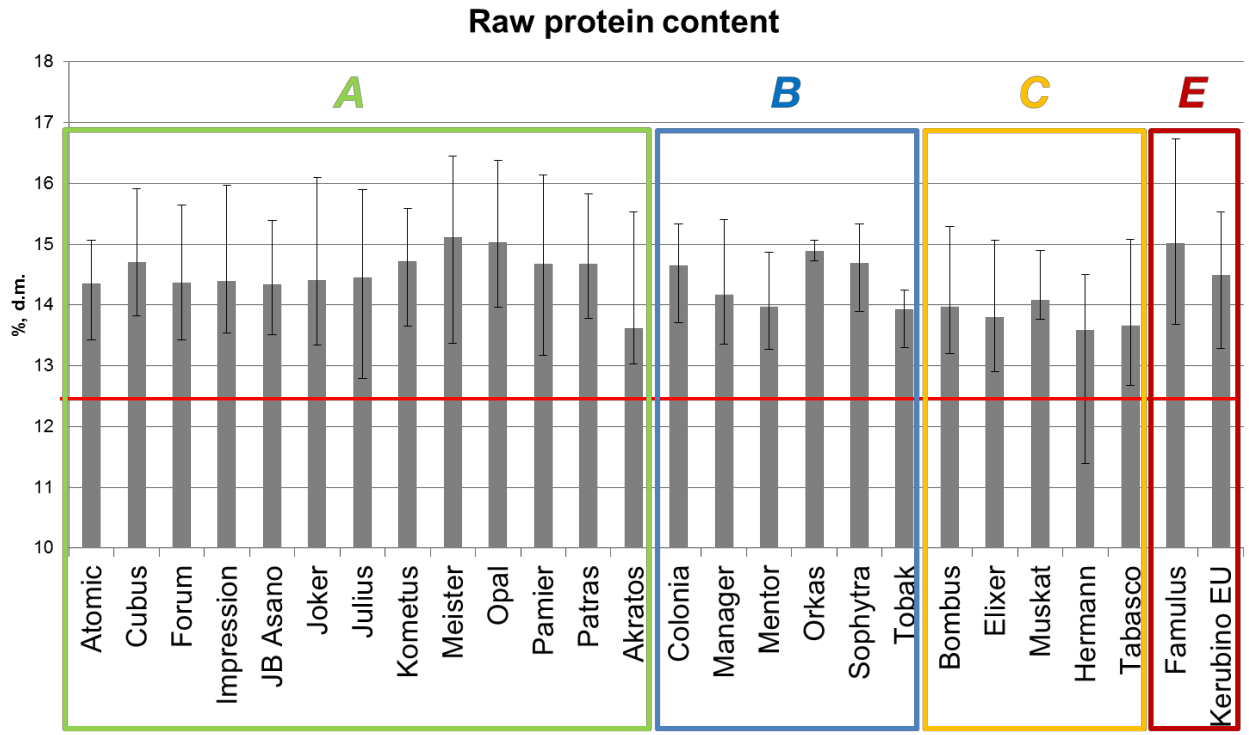


Figure 3.3: Minimum and maximum –raw protein values for analysed varieties in 2012, wheat quality groups according Table 3.1.

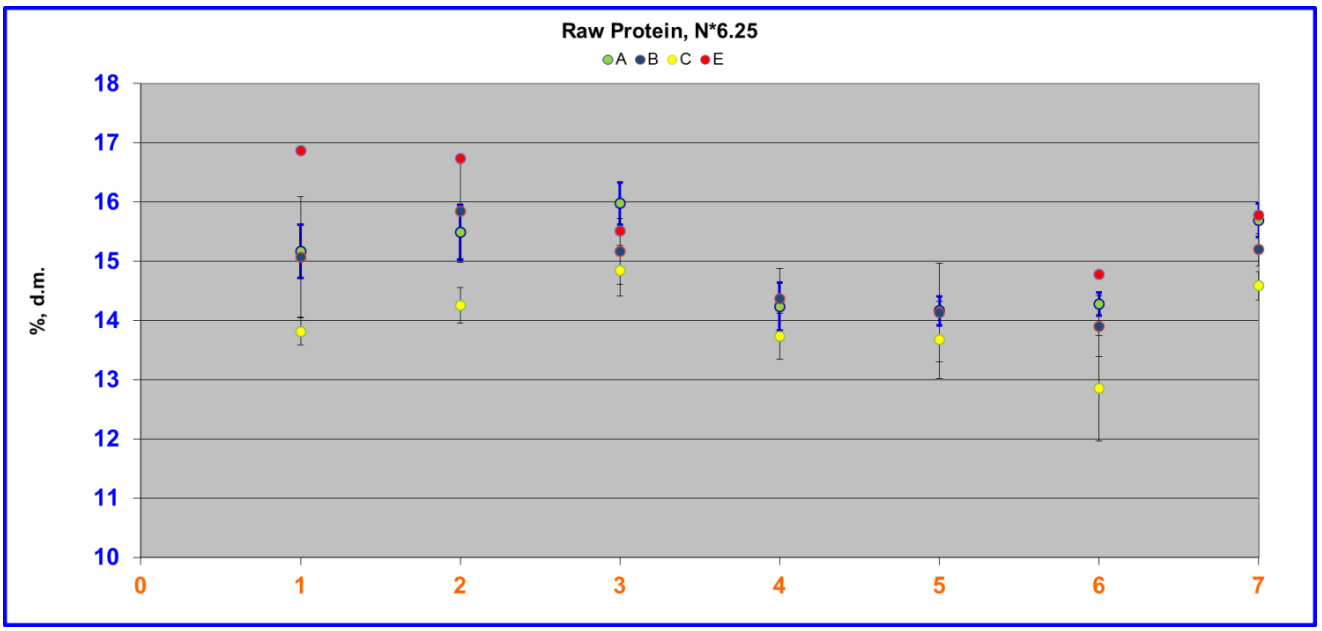


Figure 3.4: T-test distribution of raw protein values of different quality groups and growing region with a confidence interval of 95%, wheat quality groups according Table 3.1.

For the evaluation and interaction of the proteolytic malt quality attributes matrix 1, established in chapter 2, was used. The resulting matrix provide a fast overview, if a variety has good, acceptable and poor (appears as white, gray and black fields respectively) attributes for malting. The matrix is composed of total protein (horizontal axis), Kolbach index (vertical axis) and soluble nitrogen (matrix field). The used settings, modified according to MEBAK, EBC and ASBC standard methods, are listed in chapter 2 Table 2.1 (Limiting values for quality parameters of wheat malt suitable for brewing purposes). The different growing regions are number and colour coded (**Buxheim: 1, Feisteneich: 2, Greimersdorf: 3, Günzburg: 4, Kirchseeon: 5, Köfering: 6, Reith: 7)**

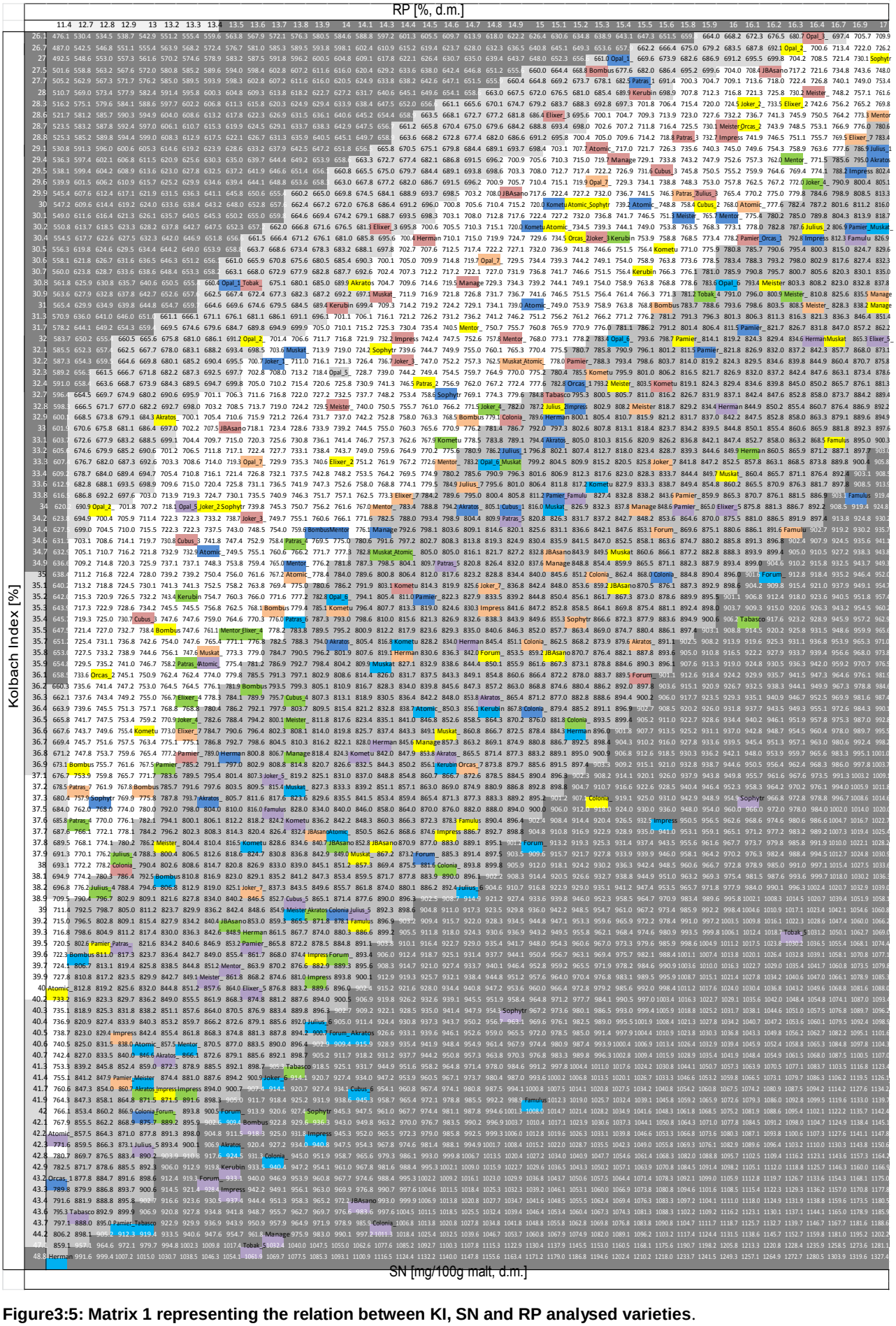


Figure3:5: Matrix 1 representing the relation between KI, SN and RP analysed varieties.

Furthermore, considering this matrix (Figure 3.5), a first visual tendency is given for the cultivars suitable for malting based on the proteolytic attributes. According to HERRMANN (2005) (8) nitrogen fractions are more influenced by the malting intensity (over 50 %), followed by the variety itself and environmental attributes (growing region, climate conditions, soil, etc.). Moreover, this matrix shows that not all analyzed and evaluated varieties lay in good or acceptable fields and that not every growing region is optimal for every variety. Mainly all samples which appear in the dark gray field (not acceptable) were grown in Kirchseeon (A: Forum, Impression, JB Asano, B: Manager, Colonia, Sophytra, E: Famulus, Kerobinio) and Köfering (A: Forum, Impression, Cubus, Pamier, Akratos, Joker, B: Tobak, Colonia, C: Tabasco, Bombus). Although these samples and growing regions show relatively low protein content (Figure 3.4), they result in too high soluble N content. In contrast, the wheat variety group A and C grown in Greimersdorf (3) are high in crude protein content and result in good soluble N values. This goes along with the findings for extract content and AAL value, mentioned above. Wheat samples, grown in Reith, result in acceptable to good proteolytic attributes. The varieties Famulus (quality group A) and Forum (quality group E) for example, appear more often as black and gray fields, which are described as not suitable or acceptable attributes for malting and brewing purpose. Variety Hermann an Elixer appear mainly in the white fields, which represent good quality attributes. Again, a dependency and influence of growing region on varieties was investigated. The varieties Akratos, Kometus, Opal, Patras (quality group A), Mentor, Orkas, Tobak (quality group B) as well as Elixer und Hermann (quality group C) show good and acceptable proteolytic as well as amylolytic malt quality attributes.

3.3.3 Cytoloytic malt quality attributes

Evaluating the cytolytic specifications for barley malt, many analytical methods and attributes are established and approved by EBC, MEBAK and ASBC (e.g. friability, steely kernels, homogeneity and modification, viscosity and β-glucan). However, up till know viscosity is the only decisive cytolytic attribute to characterize wheat and its suitability for malting and brewing. Viscosity value for wheat malt should lay below 1.80 mPas. 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 (61). As mentioned above, 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%). Hemicelluloses of wheat are therefore mainly responsible for the high wort viscosity and the pentosans have the biggest impact. It has been reported that the effects of arabinoxylans on viscosity and filterability are at least as important as the effects of β-glucan (50). In Figure 3.6 all varieties show good viscosity values. In the harvest year 2012/2013 only 6 out of 166 varieties lay over 1.800 mPas - the maximum values for good malt quality. Figure 3.7 show the deviation of quality groups and growing regions. No significant differences between quality group and growing region can be detected. However, significant differences in quality groups were evaluated. The quality group A results in the highest viscosity values in each growing region. In contrast, quality group E followed by C have the lowest viscosity values. These results goes along with the findings of HERRMANN 2005 (8), that viscosity depends mainly on the variety and can marginal be influenced by malting as well as environmental conditions.

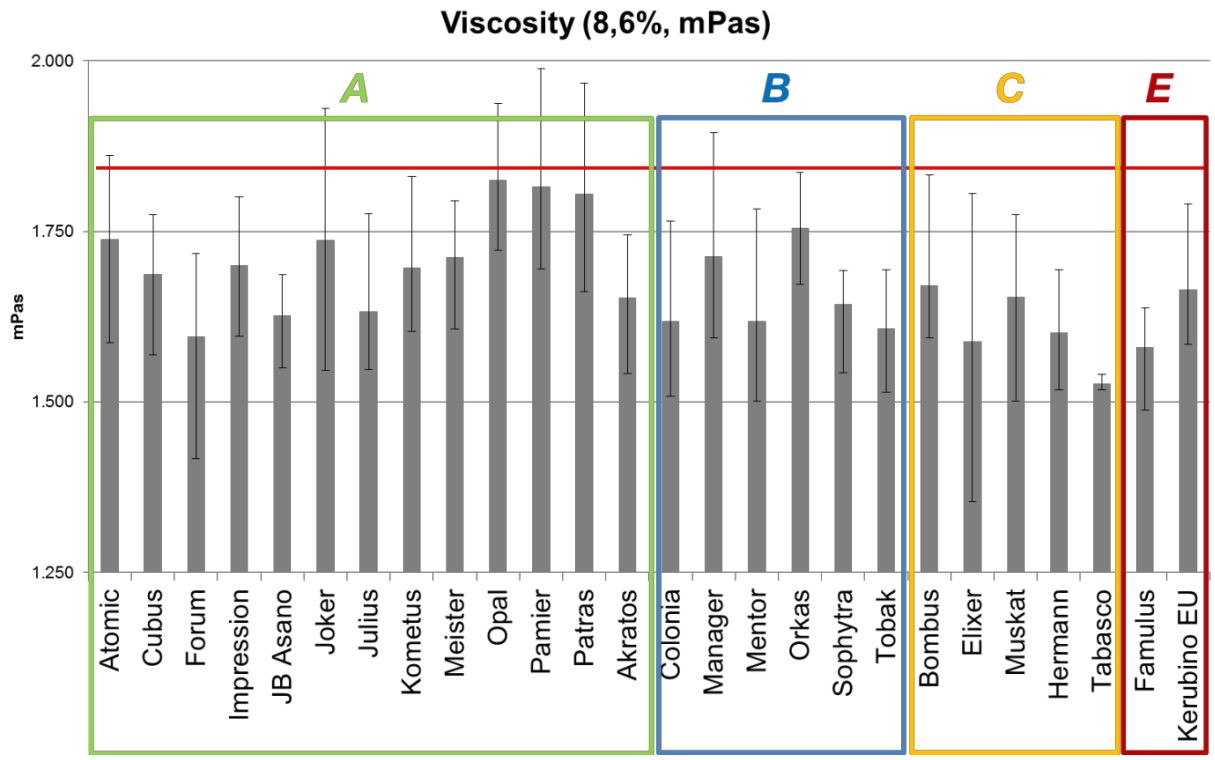


Figure 3.6: Minimum and maximum –viscosity values for analysed varieties in 2012, wheat quality groups according Table 3.1.

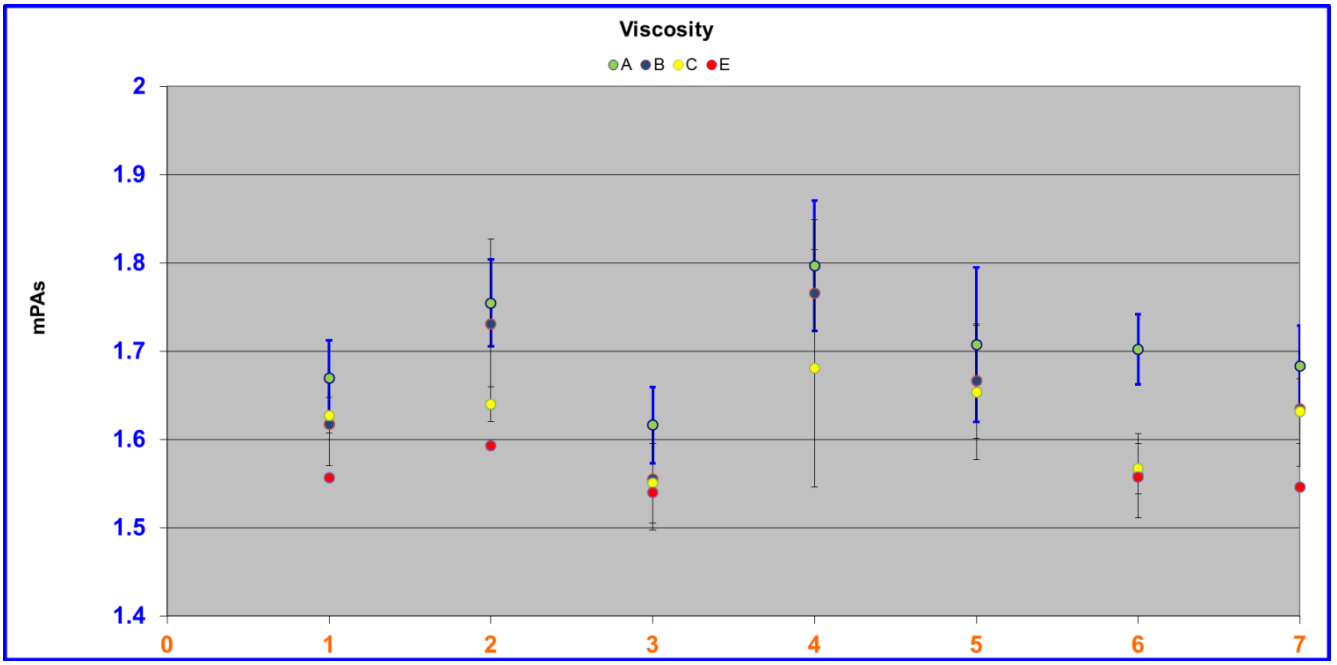


Figure 3.7: T-test distribution of viscosity values of different quality groups and growing region with a confidence interval of 95%, wheat quality groups according Table 3.1.

3.3.4 Triannual cultivation series

To get additional information about the stability to climate conditions, varieties where analysed during a triannual cultivation series. Raw protein (RP), soluble nitrogen (SN), Kolbach Index (KI), extract (E) and viscosity (Visc) are crucial criteria for total malt quality and where chosen for selecting wheat malt varieties. Regarding the finding above it was evaluated, that raw protein, extract and viscosity are variety attributes. It was found that raw protein and therefore soluble nitrogen is also strongly affected by growing region and environmental conditions. All analysed varieties where grown at least in 4 different growing areas, and at least for 2 crop years. Table 3.4 shows the overall results of the triannual cultivation series. The weighing of these values was according to the limiting values for quality parameters of wheat malt suitable for brewing purposes (chapter 2 Table 2.1) and indicated as following:

not acceptable: (●) = 3 points; acceptable: (◍) = 2 points, good: (○)=1 point.

According this weighing: the lowest sum a variety can achieve is in total are 5 points. The lower the total sum of the critical quality malt attributes is, the more suitable and stable the variety is over the years, and vice verses.

It can clearly be seen, that all varieties in average result in good viscosity values (○) and not acceptable raw protein contents (●). As it was shown in chapter 3.2., not acceptable raw protein content does not result directly in a not acceptable SN content. Moreover, high RP does not always result in low extract contents, neither in not acceptable KIs. During the triannual cultivation series an overall increase of raw protein content was recognized. This leads to the suggestion, that raw protein values which are recommended for wheat malt should be adjusted to the given.

Table 3.4: Average results of triannual cultivation series (not acceptable: (●) = 3 points; acceptable: (●) = 2 points, good: (○)=1 point)

	2010-2012																					growing area									crop year			Total weight
	RP [%, d.m.]					*SN* [g/100g, d.m.]					*KI* [%]					*E* [%, d.m.]					*Visc* [mPas, 8.6%]														
Cultivar	∅	±	*a*	*n*	☑	∅	±	*a*	*n*	☑	∅	±	*a*	*n*	☑	∅	±	*a*	*n*	☑	∅	±	*a*	*n*	☑	BH	KF	FN	GD	GB	KS	R	GS	WD	2010	2011	2012	
Akratos (A)	13.96	±	0.54	12	●	0.84	±	0.05	12	●	38.25	±	3.57	12	○	84.75	±	0.67	12	○	1.65	±	0.05	12	○	1	1	1	0	1	1	1	0	0	1	1	1	7
Akteur (A)	15.18	±	0.87	9	●	0.78	±	0.08	9	○	32.13	±	4.08	9	●	83.12	±	0.72	9	○	1.57	±	0.06	9	○	1	1	1	1	0	1	1	1	1	1	1	0	8
Atomic (A)	14.30	±	0.76	8	●	0.78	±	0.05	8	○	35.66	±	3.81	8	●	83.05	±	2.11	8	○	1.71	±	0.12	8	○	1	1	1	1	1	1	1	0	0	0	1	1	7
Batis (C)	14.06	±	0.60	8	●	0.83	±	0.09	8	●	36.75	±	3.95	8	●	84.41	±	2.38	8	○	1.59	±	0.06	8	○	0	1	0	1	1	1	0	1	1	1	1	0	8
Bombus (C)	13.96	±	0.73	8	●	0.80	±	0.09	8	●	37.59	±	5.25	8	○	84.83	±	2.03	8	○	1.65	±	0.09	8	○	1	1	1	0	1	1	1	0	0	0	1	1	8
Colonia (B)	14.15	±	1.36	14	●	0.86	±	0.08	14	●	39.45	±	5.82	14	○	83.38	±	1.13	14	○	1.58	±	0.08	14	○	1	1	1	1	1	1	1	1	1	1	1	1	8
Cubus (A)	14.20	±	0.87	19	●	0.81	±	0.11	19	●	36.54	±	5.24	19	●	84.40	±	1.62	19	○	1.68	±	0.10	19	○	1	1	1	1	1	1	1	1	1	1	1	1	9
Elixer (C)	13.61	±	0.83	9	●	0.78	±	0.17	9	○	39.39	±	7.41	9	○	84.61	±	2.30	9	○	1.62	±	0.08	9	○	1	1	1	1	1	1	1	0	0	0	1	1	7
Event (E)	14.57	±	0.84	6	●	0.79	±	0.09	6	●	32.27	±	3.18	6	●	83.67	±	1.66	6	○	1.63	±	0.04	6	○	1	0	1	1	1	0	0	1	1	1	1	0	10
Famulus (E)	15.58	±	1.21	13	●	0.88	±	0.07	13	●	35.76	±	4.49	13	●	82.53	±	1.52	13	●	1.56	±	0.05	13	○	1	1	1	1	1	1	1	1	1	1	1	1	11
Forum (A)	14.33	±	0.83	9	●	0.89	±	0.03	9	●	40.67	±	3.07	9	○	84.41	±	2.16	9	○	1.59	±	0.11	9	○	1	1	1	1	1	1	1	0	0	0	1	1	8
Hermann (C)	13.27	±	0.87	17	●	0.81	±	0.08	17	●	39.08	±	5.16	17	○	85.00	±	1.17	17	○	1.61	±	0.07	17	○	1	1	1	1	1	1	1	1	1	1	1	1	7
Impression (A)	14.14	±	0.79	16	●	0.79	±	0.10	16	●	35.65	±	5.79	16	●	83.87	±	1.26	16	○	1.72	±	0.13	16	○	1	1	1	1	1	1	1	1	1	1	1	1	9
JB Asano (A)	14.31	±	0.76	18	●	0.83	±	0.10	18	●	36.82	±	5.13	18	●	84.47	±	1.16	18	○	1.62	±	0.09	18	○	1	1	1	1	1	1	1	1	1	1	1	1	9
Joker (A)	14.23	±	0.92	9	●	0.80	±	0.08	9	●	36.86	±	4.67	9	●	83.68	±	1.67	9	○	1.71	±	0.13	9	○	1	1	1	1	1	1	1	0	0	0	1	1	9
Julius (A)	14.19	±	0.79	19	●	0.81	±	0.09	19	●	36.03	±	4.39	19	●	83.41	±	1.26	19	○	1.64	±	0.09	19	○	1	1	1	1	1	1	1	1	1	1	1	1	9
Kerubino (E)	14.22	±	0.87	15	●	0.76	±	0.11	15	○	34.44	±	6.11	15	●	83.58	±	1.78	15	○	1.66	±	0.09	15	○	1	1	1	1	1	1	1	1	1	1	1	1	8
Komet (A)	14.69	±	0.71	13	●	0.79	±	0.07	13	●	34.96	±	3.95	13	●	83.76	±	1.62	13	○	1.67	±	0.08	13	○	1	1	1	1	1	1	1	1	0	0	1	1	10
Manager (B)	13.82	±	0.73	16	●	0.79	±	0.12	16	●	36.62	±	5.61	16	●	83.77	±	1.53	16	○	1.71	±	0.12	16	○	1	1	1	1	1	1	1	1	1	1	1	1	9
Meister (A)	14.79	±	1.17	19	●	0.80	±	0.07	19	●	34.02	±	4.32	19	●	83.84	±	1.52	19	○	1.70	±	0.10	19	○	1	1	1	1	1	1	1	1	1	1	1	1	9
Mentor (B)	14.01	±	0.63	8	●	0.80	±	0.05	8	●	37.35	±	3.57	8	○	84.35	±	1.80	8	○	1.62	±	0.08	8	○	1	1	1	1	1	1	1	0	0	0	1	1	8
Muskat (C)	13.67	±	0.70	19	●	0.78	±	0.10	19	○	35.49	±	4.47	19	●	85.04	±	1.79	19	○	1.68	±	0.12	19	○	1	1	1	1	1	1	1	1	1	1	1	1	8
Orcas (B)	14.17	±	0.70	15	●	0.81	±	0.08	15	●	35.57	±	3.99	15	●	85.17	±	1.32	15	○	1.73	±	0.12	15	○	1	1	1	1	1	1	1	1	1	1	1	1	9
Pamier (A)	14.80	±	0.84	19	●	0.83	±	0.07	19	●	35.61	±	3.97	19	●	84.08	±	1.20	19	○	1.74	±	0.14	19	○	1	1	1	1	1	1	1	1	1	1	1	1	9
Patras (A)	14.74	±	0.86	8	●	0.78	±	0.07	8	○	34.80	±	4.15	8	●	83.79	±	1.27	8	○	1.76	±	0.15	8	○	1	1	1	1	1	1	1	0	0	0	1	1	9
Sophytra(B)	14.18	±	1.08	13	●	0.86	±	0.09	13	●	37.74	±	5.20	13	○	84.53	±	1.53	13	○	1.63	±	0.08	13	○	1	1	1	1	1	1	1	1	1	1	1	1	8
Tabasco (C)	13.70	±	0.99	10	●	0.81	±	0.08	10	●	37.99	±	5.76	10	○	84.75	±	1.44	10	○	1.60	±	0.07	10	○	1	1	1	1	1	1	1	0	0	1	1	1	8
Tobak (C)	13.92	±	0.46	5	●	0.83	±	0.13	5	●	38.98	±	7.21	5	○	83.49	±	1.46	5	○	1.61	±	0.06	5	○	0	1	0	1	1	1	0	0	0	1	0	1	8

- Raw protein (RP), soluble nitrogen (SN), Kolbach Index (KI), extract (E) and viscosity (Visc) are crucial criteria for total malt quality and where chosen for selecting wheat malt varieties
- Raw protein, extract and viscosity are variety attributes
- Raw protein and therefore soluble nitrogen is also strongly affected by growing region and environmental conditions

The varieties Akratos, Akteur (quality group A), Elixer and Hermann (quality group C) show the lowest sum in total weight, and are indicated therefore as the most suitable and stable wheat varieties for malting and brewing. In contrast, Event, Famulus (quality group E) and Komet (quality group A) show the highest total weight, and seems to be less suitable for malting and brewing.

3.4. Conclusion

When selecting wheat varieties for brewing purpose, the most important criteria for malsters and brewers are viscosity (Visc), protein content (TN), soluble nitrogen (SN), extract (E) and Kolbach index (KI). Too high or too low contents, of the above mentioned attributes, can lead to problems during the brew. Crucial parameters which lead to the required malt quality attributes are: a, the variety itself with its specific constitution b, the malt modification and c, the growing region as well as the harvest year. In the harvest year 2012 significant differences of extract value in quality groups as well as growing region were detected. No significant differences in each growing region between quality group A and B could be detected, accept growing region 5. The quality group E showed the lowest extract values in all regions.

Significant differences in quality groups as well as growing regions could be detected for raw protein as it was already found for extract values. The lowest raw protein contents were detected for quality group C in all growing regions. No significant differences were found for quality group A and B in the growing region itself. With increasing raw protein content, a decrease in amylolytic attributes can be recognized. Concluding these results raw protein content depends on variety and is also influenced by growing region. The nitrogen fractions are more influenced by the

malting intensity (over 50 %), followed by the variety itself and environmental attributes (growing region, climate conditions, soil, etc.). Not acceptable raw protein content does not result directly in a not acceptable SN content. Moreover, high raw protein content do not always result in low extract contents, neither in not acceptable KIs. A dependency and influence of growing region on varieties and proteolytic quality malt attributes were investigated.

Significant differences for the quality malt attribute viscosity in each quality group were evaluated. The quality group A results in the highest viscosity values in each growing region. In contrast, quality group E followed by C have the lowest viscosity values. Based on these results, viscosity depends mainly on the variety and is marginal influenced by the growing region and environmental conditions.

During the triannual cultivation series an overall increase of raw protein content was recognized. This leads to the suggestion, that raw protein values which are recommended for wheat malt should be adjusted to the given The so far opinion, that only wheat varieties from quality group C are suitable for malting and brewing was refused by the findings in this chapter. The varieties Akratos, Akteur (quality group A), Elixer and Hermann (quality group C) show the lowest sum in total weight, and are indicated therefore as the most suitable and stable wheat varieties for malting and brewing.

Potential quality malt attributes for selecting and validating wheat varieties are established. However, the malt quality attribute viscosity shows still a gap in evaluating wheat varieties for the brewing industry and has to be analysed in further studies.

CHAPTER 4

COMMON WHEAT (*Triticum aestivum* L.)

EVALUATING MICROSTRUCTURAL CHANGES DURING THE MALTING PROCESS BY USING CONFOCAL LASER SCANNING MICROSCOPY AND SCANNING ELECTRON MICROSCOPY

4.1. Introduction

Optical techniques, like confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM), are used in science to visualize biological, structural or chemical processes, which are commonly investigated by analytical methods. These optical tools are techniques which have found wide application in biological and medical science. Moreover, CLSM and SEM becomes into a more important technique for food material, which can be seen in the increased amount of publications in this area (40, 150-155). The key feature of CLSM is fluorescence staining. These dyes are polyaromatic hydrocarbons or heterocyclic compounds, which are able to detect photons, after light stimulation with specific wavelengths. All fluorescence dyes have a specific affinity to stain and visualize different substances like proteins, lipids or cell walls. In addition, every fluorescence dye has a certain spectrum of excitation and emission wavelength. Specific parts, regions and structures of specimens can be detected and lasers convert them into signals. These signals are transferred into information and are visualized with specific software as 3-D images (156). It is a powerful tool for visualizing the structure of biopolymer mixtures and food products on the micrometre scale and to produce optical sections through a three-dimensional (3-D) specimen. SEM and CLSM have different merits and optical benefits. Combining both optical techniques and comparing the obtained images can provide an in-depth understanding of the food microstructure. CLSM achieves an overview of single compounds in the grain, due to specific staining dyes. On the other hand SEM has a higher magnification where a deeper insight in the kernel structure can be obtained; hence compounds cannot be clearly distinguished from each other.

Malting is defined as a limited germination under controlled conditions and is regarded as the first step in beer production. It is induced by many complex biochemical processes, like degradation and modification of cell walls, proteins and starch granules in cereal grains (7, 41, 149), which can be visualised by using CLSM and SEM.

A traditional malting process can be divided in three key steps: steeping, germination and kilning. The increased water content stimulates respiration in the embryo and hydrates the stored starch in the endosperm. As the embryo activity increases, gibberellins are produced. These are natural plant hormones, which diffuse into the aleurone, where they stimulate the production of hydrolytic enzymes during germination (12, 14, 42, 149). The germinating process can be divided into the *growth process* (rootlets and acrospires develop), enzyme formation (starch degrading enzymes, cytolytic enzymes, protein degrading enzymes and fat degrading enzymes develop), and metabolic changes (e.g. proteins are degraded into smaller molecules such as amino acids and peptides). During the germination, enzyme synthesis and kernel modifications take place under strictly controlled conditions, such as temperature, moisture and aeration. During the kilning process the kernel is dried to a moisture content of 3–5% to ensure microbiological stability of the product, to stop or retard the biochemical reactions, color and shelf life, and to produce aroma and flavor compounds (39, 41).

The main purpose of malting is the production of enzymes where the main enzyme activities are (a) amylolysis, (b) cytolysis and (c) proteolysis (12, 149). These enzymes are absolutely essential for the degradation of large molecules during mashing. One of the most obvious physicochemical changes, that occur during malting is enzymatic degradation of the cereal endosperm and its conversion into soluble peptides and amino acids, providing substrates for the synthesis of proteins and the growing

embryo (149). The enzymes cause changes, which results in the degradation of high molecular weight to low molecular weight compounds.

Figure 4.1 monitors a wheat kernel during the single malting stages. The growth and the changing of the outer surface of the kernel are clearly visible, where the development of the acrospires is an attribute of the germination progress.

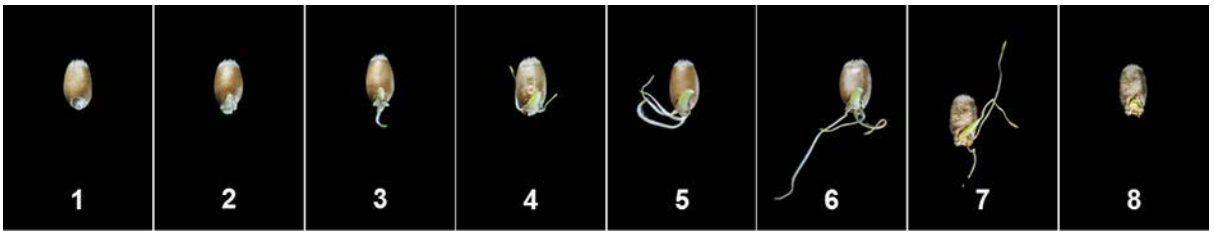


Figure 4.1: Wheat kernel during the malting process: 1. unmalted wheat; 2. steeping; 3-6. germination; 7. kilning; 8. malted wheat kernel (149)

In this chapter microstructural changes during the complex malting process were evaluated and visualized by CLSM and SEM. Furthermore, changes observed in the micrographs were verified and endorsed by metabolic changes using established malt attributes.

4.2. CLSM – evaluation of staining dyes

Critical factors in achieving optimal visualization by CLSM are the pretreatment of samples, the specific fluorescence dye for the substance of interest, as well as the dye concentration. All fluorescence dyes have a specific affinity to stain and visualize defined substances for analyzing specimens. In addition, every fluorescence dye has a certain spectrum of excitation and emission wavelength which makes it more difficult for the multi-labeling of samples. In literature, many different fluorescence dyes and methods are published for staining proteins, β-glucan and starch. However, unlike this study, almost all specimens were soft or liquid and the samples which had to be analyzed were also separated as well as dispersed in easy accessible mediums. Another task of this research is to stain protein and starch

separately. Staining dyes which are recommended and specific for protein matrix show also an affinity to starch.

For staining the protein in specimens the most common and frequently used fluorescence dyes are Acid Fuchsin, Fluorescine isothiocyante (FITC), Texas red and Rhodamine B. However, Rhodamine B (40, 157, 158), FITC (151, 159-161) and Safranin-O (162-165) are fluorescence staining dyes for starch. Therefore, the starch staining dyes were tested, to find the best for staining the whole cereal grain. All three mentioned staining dyes have an affinity to proteins, too, and are only useful staining dyes for starch, in the absence of protein (160, 164). DÜRRENBERGER et al. (163) mentioned that Safranin-O does not show fluorescence in the absence of starch. Nevertheless, it is in evidence, that Safranin-O stains the protein matrix, too. WIJNGAARD et al. (157) and ZARNKOW et al. (40) observed already the microstructural changes of buckwheat, barley and proso millet throughout the malting process using SEM and CLSM respectively. They used a single staining procedure, and visualized only one substance in the grain. In their study they used Rhodamine B for starch and Acid Fuchsin for protein. ZARNKOW et al. (40) mentioned that Rhodamine B has an affinity to protein, if the concentration of the protein is high. In other researches Rhodamin B and FITC were directly used and mentioned as a fluorescence dye for staining proteins (159, 161). Due to these reasons and published papers mentioned above, the use of Rhodamine B, FITC and Safranin-O are not suitable to stain a complex cereal grain.

4.2.1 Staining proteins

Acid Fuchsin, which binds covalently on the proteins with disulfide bridges, was used as a staining dye for proteins. The chemical structure of Acid Fuchsin dye belongs to the triarylmethan family (166). It is specific to proteins and underwent no photo bleaching over time in the CLSM. Therefore, it is well known and published in numerous papers as a specific fluorescence staining dye for proteins (40, 151, 157, 162, 163, 166, 167). To ensure that only the protein is stained, the specimen is pretreated with 2,4-DNPH and periodic acid as described by HOLOPAINEN et al.(167).

4.2.2 Staining starch

The *Periodic Acid Schiff`s* (*PAS*) reaction was used as it was found as a more specific staining dye for starch in many papers and literature (154, 165, 168). Only the starch granules are visualized obviously. *PAS* is a method for the demonstration of carbohydrates in tissue sections. The function of the periodic acid is to oxidize some of the tissue carbohydrates. The aldehyde groups develop when carbohydrates are treated with the periodic acid. They can then form, condense with the Schiff´s reagent a bright red coloration. The fundamental point is that the treatment of the tissue with periodic acid must be able to produce an aldehyde on the carbohydrate component.

4.2.3 Staining Cell walls

For cell walls, especially β-glucan, the fluorescence dye *Calcofluor white* was selected. This stain is an optical brightener with a high affinity to β-1,4-glucans such cellulose or chitin (167). *Calcofluor white* stains only the cell walls of the cereal grains.

As a result of the previous trials, the staining dyes *Acid Fuchsin* (for proteins), *Calcofluor white* (for β-glucan), and *PAS* (for starch) were selected. The proteins and

β-glucan could be stained at the same time, but no other staining dye combination worked satisfactory for staining the starch. Therefore, starch was stained alone.

4.3. Materials and Methods

4.3.1 Malting process and standard analysis

The wheat variety “Hermann” was grown and harvested in 2010 in Germany. The standard MEBAK (Mitteleuropäische Brau- und Analysenkommision) (112) malting method 2.5.3.1 (15° C germination temperature, 45% steeping degree and 7 day of vegetation) was carried out in a pilot scale malting facility (Chair of Brewing and Beverage Technology, Freising Weihenstephan, Germany). Seeds were germinated in tempered climate chambers with a constant air humidity of >95%. The initial kilning temperature for the green malt was set to 50 °C and gradually increased, over 24 h, to 80 °C. Samples were taken daily throughout the malting process. All analyses are based on methods described in MEBAK handbooks (112). Analyses of unmalted wheat, grains during malting, and final wheat malt were performed according to the approved methods of MEBAK (Mitteleuropäische Brau- und Analysenkommision) 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 (30). The apparent attenuation (amount of fermentable sugars) was detected by MEBAK method 4.1.4.10 (112). Total protein (TP) content was analysed according to MEBAK method 1.5.2.1 and calculated as total nitrogen. The protein content was measured according 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 (111, 147, 169) and approved methods according AACC (method 46.18)

and ICC, wheat varieties which are used for feed purpose are multiplied with 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 soluble nitrogen (SN) content was determined by using an automatic Kjeltec system, according to MEBAK method 3.1.4.5.2.1 (112). The Kolbach Index (KI) was calculated from the formula according to MEBAK method 4.1.4.5.3 (112). α-Amino nitrogen, also referred to as free amino nitrogen (FAN), was determined using a Skalar working station (Skalar, Breda, Netherlands), following MEBAK method 3.1.4.5.5 (112). The free amino acid composition was analyzed using HPLC according MEBAK method 2.6.4.1.2. 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 (186), where haemoglobin was used as a substrate. Analytical procedures were carried out in triplicate, at least ($n \geq 3$), and the means of all results were calculated with standard deviations reported in Table 4.1. Biological replications were done in duplicate. Statistical analyses were performed according to ANOVA (analysis of variance) with p-values < 0.005. All concentrations are based on dry matter (d.m.) unless otherwise stated.

The malting process was divided for this research according to Table 4.1. Confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) were used to investigate the microstructural changes in cereal grains during the malting process. All samples were packaged under vacuum and frozen at – 80 ºC. A minimum of five kernels per sample stage were investigated.

Table 4.1: Malt quality analysis throughout malting and typical pale barley and pale wheat malt attributes

Samples (n=4)	Day	Malt Extract (%)	Final Attenuatio (%)	Viscosity (mPas)	Soluble Nitrogen (mg/100g malt or mg/100g grain*)	Free Amino Nitrogen (mg/100g malt or mg/100g grain*)	Kolbach Index (%)	Protease activity (U/g)
Wheat	1	48.73 ± 1.29	51.20 ± 0.50	2.05 ± 0.12	281.00 ± 5.66	24.50 ± 0.71	15.20 ± 0.42	2.16 ± 0.005
Steeping	2	56.05 ± 2.33	58.93 ± 1.99	2.09 ± 0.09	290.00 ± 2.63	29.75 ± 1.26	15.65 ± 0.13	2.55 ± 0.005
Steeping	3	72.80 ± 1.24	64.90 ± 1.22	2.05 ± 0.10	368.00 ± 4.99	37.25 ± 2.99	16.60 ± 0.25	2.94 ± 0.058
Germination	4	80.43 ± 0.43	71.90 ± 0.83	1.84 ± 0.02	372.00 ± 10.78	46.75 ± 1.50	19.86 ± 0.59	3.41 ± 0.010
Germination	5	83.88 ± 0.15	71.30 ± 0.42	1.90 ± 0.03	444.00 ± 9.070	56.25 ± 1.89	24.43 ± 0.47	5.95 ± 0.036
Germination	6	85.48 ± 0.25	75.18 ± 0.44	1.88 ± 0.01	545.00 ± 7.040	70.75 ± 4.11	30.53 ± 0.78	7.28 ± 0.008
Germination	7	86.33 ± 0.22	76.80 ± 0.42	1.85 ± 0.01	627.00 ± 10.13	93.50 ± 4.80	35.25 ± 1.03	8.81 ± 0.014
Green Malt	8	87.18 ± 0.10	77.34 ± 1.33	1.75 ± 0.01	697.00 ± 14.31	116.25 ± 0.50	39.15 ± 0.70	14.81 ± 0.008
Wheat malt	9	86.89 ± 0.15	79.15 ± 0.57	1.78 ± 0.01	700.00 ± 6.85	121.00 ± 0.52	38.93 ± 0.32	14.30 ± 0.019
Typical pale barley malt	N/A	80.00 – 83.50	80.00 – 83.00	1.50 – 1.57	650.00 – 750.00	135.00 – 155.00	38.00 – 42.00	N/A
Typical pale wheat malt	N/A	81.00 – 86.00	78.00 – 82.00	1.55 – 1.80	600.00 – 800.00	100.00 – 140.00	35.00 – 45.00	N/A

* mg/100g grain is used instead of mg/100g malt to signify measurement units for the unmalted wheat grains.
N/A means not applicable

4.3.2 CLSM

4.3.2.1 Specimen preparation

For preparing the specimens a fixation and a dehydration of the grains is necessary. The fixation method of IRVING (162) was used. Therefore, each grain was fixed for a minimum of one week with 2% glutaraldehyde in a 0.05M phosphate buffer (pH 7.2) to plasticize the structure of the grains. After the fixation the probes were dehydrated in an ethanol graded series (deonised H_2O, 50%, 70%, and 96% ethanol, respectively 3 times for 30 minutes).

After the dehydration procedure the specimens were embedded in a methacrylate resin (*Technovit 7100*, Heaeus Kulzer GmbH, Wertheim, Germany) as recommended by the manufacture. The polymerized samples were sectioned (50 µm sections) in a rotary microtome HM 355s (ThermoScientific) using a tungsten carbide diamond coated blade (ThermoScientific).

4.3.2.2 Staining proteins and cell walls

Sections were stained using the methods which are published and described by DÜRRENBERGER et al. (163) and HOLOPAINEN et al. (167). The 5µm thin specimens were pretreated for staining in 2,4-dinitrophenylhydrazin for 20 minutes and washed well in tap water for 30 minutes, and in 0.5% periodic acid for 20 minutes and washed well in tap water for 30 minutes again. This is a modification of the *PAS* method. The periodic acid is used to block preexisting aldehydes, so that the staining dye *Acid Fuchsin* is specific for proteins and does not bind on other polysaccharides, like starch.

Protein was stained with aqueous 0.1% (w/v) *Acid Fuchsin* (Sigma Aldrich, Germany) for one minute, and β-glucan was stained with 10% (w/v) *Calcofluor white* for 1 minute (fluorescence brightener 28, Sigma Aldrich, Germany). To visualize the fluorescence dyes, a Laser LD 405 with a Filter BA 430-480 F was used for *Calcofluor white*, and a Laser 548 with a Filter BA 660 F was used for *Acid Fuchsin*. Due to different excitation wavelength, both staining dyes could be used at the same time, so that a multiple labeling was possible. In exciting light, intact cell walls stained with *Calcofluor white* appear blue and proteins stained with *Acid Fuchsin* appear red. Because of the pretreating of the probes, starch is unstained and appears black.

4.3.2.3 Staining the starch

To detect the starch granules and to convert the digital signal in a fluorescence image, a *Periodic Acid Schiff`s* (*PAS*) Kit (Sigma-aldrich, Germany) was used. Samples were treated with the periodic acid for 10 minutes, rinsed and washed with tap water followed by distilled water. The sections were then treated with the Schiff`s reagent for 20 minutes, rinsed and washed with tap water and distilled water as before. The sections were placed two times for each five minutes in acid solution

(3 ml HCl 37%, 5 ml distilled H_2O, 92 ml ethanol 96%), washed with tap water and put on a cover glass for microscopy. A blue excitation light with the Laser 488 nm and a Filter BF 510 FF were used to detect the starch granules stained with the PAS.

4.3.2.4 Visualize the stained substances with CLSM

A FV300 confocal laser-scanning system (Olympus, Germany) mounted on an Olympus IX80 inverted microscope with a 40x dry objective was used. Fluorescence images of a number of optical sections were acquired by scanning the sample along the optical axis using a 488 nm excitation line for starch and a 543 nm excitation line for proteins. Fluorescence images of a number of optical sections were acquired by scanning the sample along the optical axis in 1 µm steps. To obtain 3-D images, Volocity 5.4 (Improvision Limited, Coventry, UK) was used.

4.3.3 SEM

All samples used were freeze-dried beforehand. Longitudinal sections were prepared by cracking the seeds with a razor blade. Samples were mounted on circular specimen holders (Agar Scientific, Plain stubs for JEOL 10×10 mm Dia) with double carbon tape (Agar Scientific, Carbon Tabs 9mm) and gold labled. A JEOL scanning electron microscope type 5510 (JEOL, Tokyo, Japan) at 3 to 5 kV at a working distances of 10 mm (close-up view of specific regions in the kernel e.g. endosperm) and 40 mm (whole kernel) respectively was used. Furthermore images were taken by using a Hitachi TM-1000 Tabletop Microscope (Hitachi, Tokyo, Japan). Sizes of starch granules were determined with the measuring tool of the image producing software.

4.4 Results and Discussion

The germination process of a wheat grain and its transformation from wheat to malt is pictured in Figure 4.1. The growth of the acrospires is an easy detectable attribute to assess the progress of germination. After the first steeping day the acrospires appeared. Rootlets were built at the beginning of germination. However, to gain deeper insight in the germinating grain and visualizing the analysed metabolic changes during the malting process, CLSM and SEM were used.

4.4.1 Structure of a wheat kernel

The microstructure of unmalted wheat is presented in Figure 4.2 and 4.3. The pericarp (Fig. 4.2C), which surrounds the whole seed, is composed of several layers (14), the outer epidermis, hypodermis, parenchyma, intermediate cells, cross cells, and tube cells (2). The cross cells measure 100-150 µm long by 15-20 µm wide and 10-15 µm thick and have their long axis perpendicular to the long axis of the kernel. The tube cells (elongated and knobby in outline, 120-130 µm length, 12-15 µm wide, 5-10 µm thick), which form an incomplete layer, represent the inner epidermis of the pericarp. The total pericarp has been reported to compromise 5% of the kernel and consist of approximately 20% cellulose, 6% protein, 2% ash and 0.5% fat, with the residual being non-starch polysaccharides (2).

The aleurone layer (Fig. 4.2A-D), which surrounds the kernel, is generally one cell thick and covers the starchy endosperm and the germ. The aleurone cells are thick-walled (6-8 µm), essentially cubical, typically block shaped (37-65 µm by 25-75 µm) and free of starch at maturity (2, 13). Aleurone cells contain a large nucleus and a large number of aleurone granules. They are relatively high in ash, protein, total phosphorus, phytate phosphorus, fat, and niacin. Furthermore, the enzyme activity in

the aleurone cells is the highest of the entire grain (13). Over the embryo, the aleurone cells are modified, becoming thin-walled cells that do not contain aleurone granules (2, 13). The aleurone cells are also a storage reserve for lipid droplets (2). Compared to other cereals such as barley, (1→3)(1→4)-β-D-glucan is only found in small quantities in the wheat grain. Dependent on the variety, the amount of β-glucan ranges from 0.31 to 6.7% (55-57, 60).

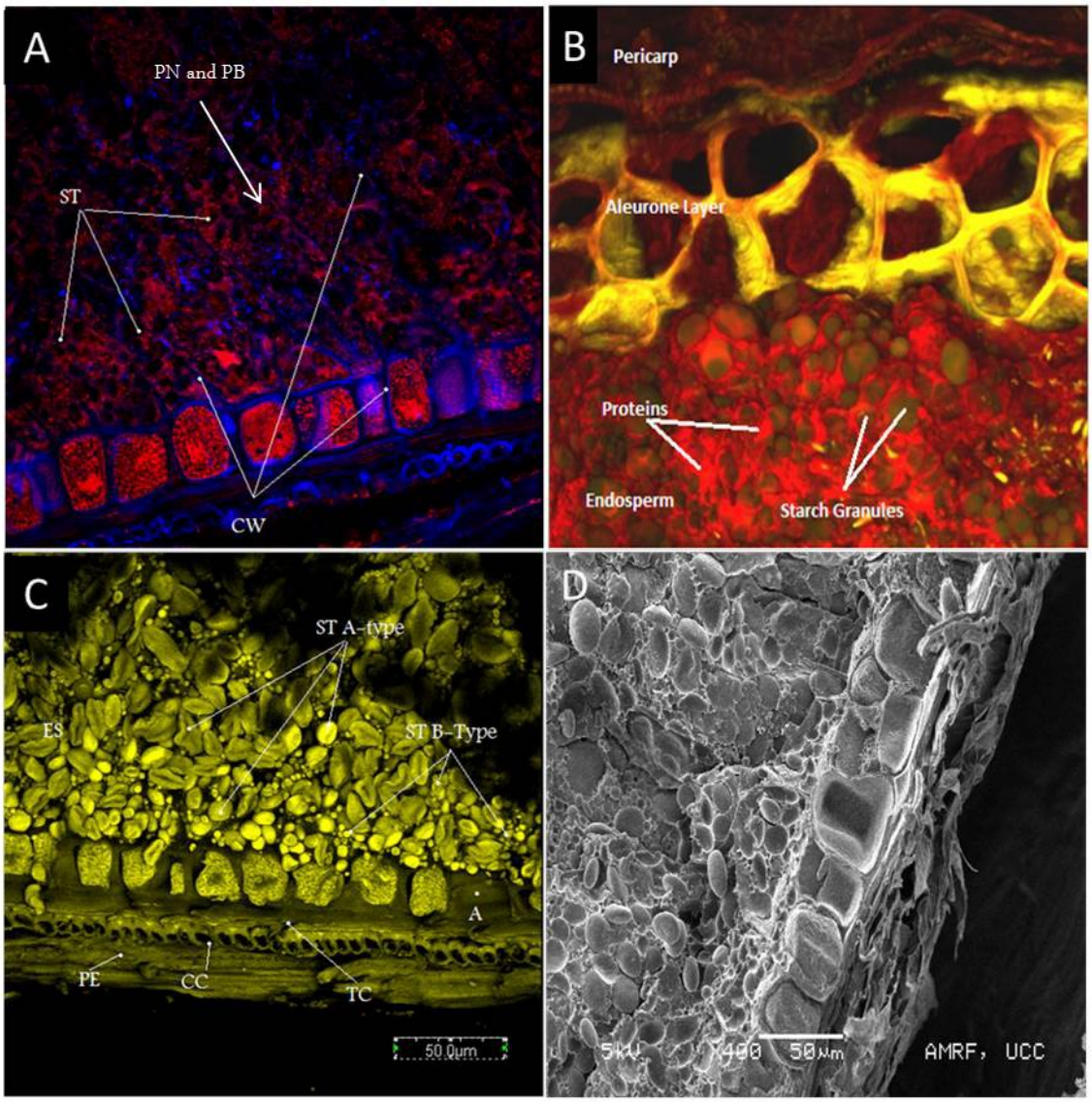


Figure 4.2: Aleuron layer-unmalted wheat (magnification = ×40); *CW* cell wall, *ST* starch granule, *PN* protein network, *PB* protein body, *PE* pericarp, *CC* cross cell, *TC* tube cell, *AL* aleuron layer, *ES* endosperm, a, b *red* stained = proteins; *blue* stained = cell walls; starch is unstained and appears black; c *yellow* stained = starch granules; cell walls and proteins are not stained; d SEM picture

Unlike the cereals that are rich in β-glucan, such as oat and barley, the highest concentrations of β-glucan are found in the inner aleurone cell walls and subaleurone endosperm cell walls (57, 58). The cell walls, consisting of β-glucans, are clearly apparent throughout the whole grain. β-glucan (Fig. 4.2A and B) is mainly found in the inner aleurone cell walls and subaleurone endosperm cell walls (57). They surround and separate the protein network, the aleurone cells, as well as the endosperm and germ cells from each other (Figure 4.3) and appear to be fully intact.

The germ (Fig. 4.3B), also called the embryo of wheat, comprises 2.5-3.5% of the

kernel and lies on the lower dorsal side of the caryopsis (Figure 4.3B). It is composed of two major parts, the embryonic axis, and the scutellum that functions as a storage organ (2, 13, 14). The germ is relatively high in protein (25%), sugar (18%) which is mainly sucrose and raffinose, oil (16%), and ash (5%). It contains no starch, but contains many enzymes and is rather high in B vitamins, as well as in vitamin E with values ranging up to 500 ppm (2).

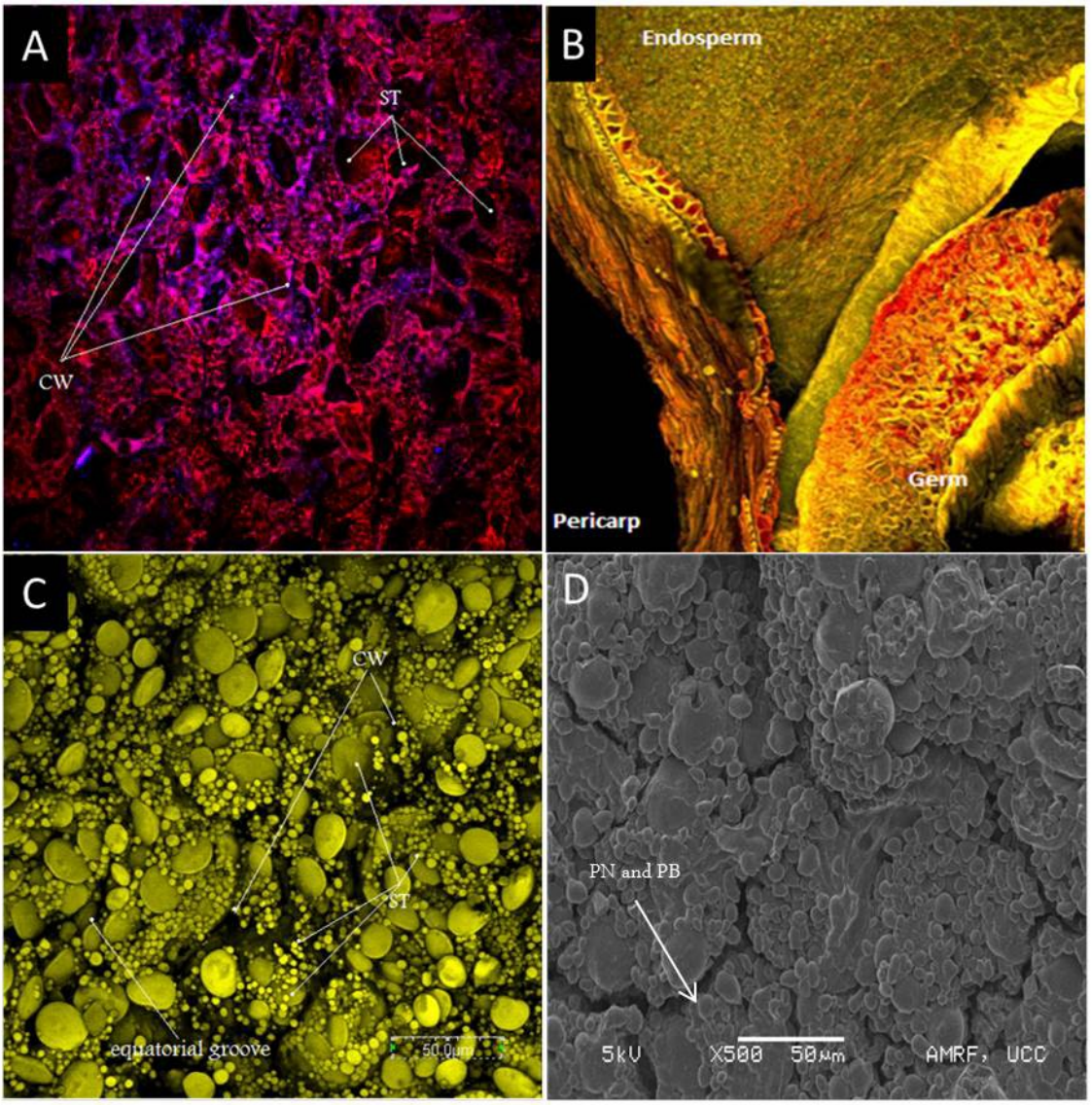


Figure 4.3: Endosperm-unmalted wheat (magnification = ×40); *CW* cell wall, *ST* starch granule, *PN* protein network, *PB* protein body, *PE* pericarp, *CC* cross cell, *TC* tube cell, *AL* aleuron layer, *ES* endosperm; A, B *red* stained = proteins; *blue* stained = cell walls; starch is unstained and appears black; C *yellow* stained = starch granules; cell walls and proteins are not stained; D SEM picture

The starchy endosperm (Fig. 4.3A, C and D) is composed of peripheral (60 µm in diameter), prismatic (130-200 µm long, 40-60 µm wide) and central cells (2.6 µm

thick, 72-144 µm length, 70-120 µm wide) (2, 15, 16). These are composed of pentosans, other hemicelluloses, and β-glucan, but no cellulose. The principal content of the endosperm cells is starch and proteins, but the proportional contribution varies according to the location of the cells (14). Throughout the endosperm, all cells contain approximately the same amount of protein (17). Hence, the peripheral cells, which have the lowest starch level, contain the highest protein percentage (2). Starch granules of two types occur in all except peripheral cells. They differ in size and shape. The large granules (A-type; up to 40 µm) are lens-shaped and the small granules (B-type; 2-8 µm in diameter) are near-spherical (2, 13). Starch and proteins, two major storage reserves, make up the bulk of the endosperm. These cells are packed with starch granules and embedded in a protein matrix. The protein is mostly gluten, the storage protein of wheat (14). In the ungerminated grain the starch granules are embedded in the protein matrix, which consist of protein bodies (Fig. 4.2 and 4.3). This matrix seems to be cementric and fully intact.

4.4.2 Metabolic and microstructural changes during malting of common wheat (Triticum aestivum L.)

In this research amylolytic, cytolytic and proteolytic changes which occur during wheat malting were investigated (see Table 4.1).

4.4.2.1 Amylolytic changes

The starch fraction is the main component found in wheat. It is an important indicator for malsters and brewers, since it contributes to the extract and final attenuation of worts. Starch is degraded mainly due to the action of β-amylases as well as α-amylases and α-glucosidases. During malting, amylolytic enzymes are formed and activated that break starch down into small and soluble sugar compounds. Steeping is traditionally used to increase the moisture content of cereal grain which will induce

germination. Therefore, hydrolytic pre-existing enzymes are released and new enzymes are produced in the aleurone layer which increases enzymatic activities. During the steeping period, the grain imbibes water and gets swollen. The B-granules of wheat starch are associated with a higher absorption, earlier hydration and more swelling than the A-granules, because of a higher proportion of amorphous zones they are more accessible to water (170). In the first wet steeping stage, the water diffuse first through pericarp, before in the second wet steeping stage the endosperm get more fluffy and swollen (pictures not shown). Although hydrolysis of small wheat starch granules was faster than that of large granules, by the reason of higher surface area per unit weight, other anatomical features such as appearance of pores on the large granules, might have an influence as well (27, 171). However, amlyolytic activity and starch degradation can be detected during steeping. Malt extract as well as final attenuation, both most important amylolytic malt quality attributes (39), increases during steeping from initial yields 48.73 to 72.80% and 51.20 to 64.90%, respectively. Using scanning electron microscopy, the large granules are attacked at the groove and at the localized sites of the surface. Some pinholes were already showing on the surfaces of starch granules in the floury endosperm after the first steeping and a large number of pinholes appeared after the steeping process. After one day of germination (figure not shown) the size of the holes increased significantly. Once the surface is eroded, the degradation seems to move through the layers of the granules toward the center. The center is completely digested, whereas only portions of the radial starch are broken down. The erosion of the small granules proceeds differently. In these granules small circular spots, randomly distributed over the surface, are attacked. However, before starch can be degraded to smaller molecules cytolytic and/or proteolytic degradation have to proceed. This can be confirmed by evaluating e.g. viscosity and SN, which show the highest value changes from the beginning of

steeping to the end of germination, shown in Table 4.1 (viscosity is decreasing by 0.24 mPas and SN is increasing by 337 mg/100 g malt). During germination, the protein network, a cementric material in which the starch granules are embedded, is degraded and loses the compact and dense structure, but cell wall fragments were occasionally observed. Loss of this structure allows the release of starch granules making them available to attack by α-amylases. These amylases break down the starch compounds to lower molecular structures (sugars) which are important for the fermentability of the malt in the brewing process. Differences of the degree of degradation of starch granules throughout the kernel could be detected. It seems that starch granules are more degraded near the aleurone layer and germ region (Figure 4.4D), than in the inner endosperm (Figure 4.5D). The increasing degradation of the vitreous endosperm might be caused by the enhanced amount of hydrolytic enzymes produced in the aleurone layer. It can clearly be seen that starch granules show enzymatic erosion on the surface and along the equatorial groove (Figure 4.5D).

The degradation process of starch was also measured and investigated by analyzing malting parameters. During the individual malting stages extract increased by ~30% from an initial value of 48.73% in unmalted wheat showing the importance of the malting process. The final attenuation of the wheat malt is also a critical characteristics in malt quality and in this study, it increased by ~20% to 79.15% in the final malted wheat.

4.4.2.2 Proteolytic changes

An increase in the proteolytic values; Kolbach index (KI), soluble nitrogen (SN) and free amino nitrogen (FAN) occurs as malting proceeds. The KI is an indicator of proteolytic modification of the malt and reflects protein solubilised during malting and preparation of the congress mash. Combined, FAN, free amino acid composition and

KI describe protein degradation during the malting process. These attributes are increased from 2.5 to 5-fold in unmalted wheat to malt (Table 4.1). During germination, metabolic changes cause protein degradation forming smaller molecules. Moreover, storage protein modification in germinating cereal grains are divided in three phases: a, the contents of protein bodies in the embryo, scutellum and aleurone layer are hydrolized; b, storage proteins accumulated in the starchy endosperm are mobilized by proteases that are produced and secreted from the aleurone layer and scutellum to produce small peptides; c, and these peptides are hydrolized to amino acids (172). During steeping no visible changes in the protein structure, either in the cell walls could be detected by CLSM or SEM. However, a slight increase of SN as well as FAN could be detected, due to uptake of water, solubilisation during steeping and hydrolyzing of protein bodies. Wheat proteins are divided into two major categories: gluten; including gliadins and glutenins, and non- gluten; consisting of water-soluble albumins and salt-soluble globulins. The non- gluten proteins are generally present in the embryo and aleurone, representing about 15 to 20 % of the wheat seed proteins (173). They include biological active enzymes and protease inhibitors such as α-amylase-inhibitor protein, β-amylases-inhibitor protein, lipid transfer proteins, polyphenol oxidases, peroxidases (173-175), all of which play various important roles during the malting and brewing processes. Some high molecular weight albumins (HMW albumins) and certain globulins (triticins) also have functions as storage proteins. They form part of the gluten protein complex through disulphide bonds, and are degraded and/or accumulated during germination and grain development, respectively (176). Wheat storage (gluten) proteins make up to 80% of the total wheat grain protein content (177). Gluten is composed of gliadins (monomeric proteins) and glutenins (polymeric proteins) (83) and mainly occur in the endosperm of wheat grains. During germination, cell storage proteins are hydrolysed and proteolytic and other enzymes are

synthesised throughout malting (39). It was found that glutenins are strongly influenced by the uptake of water and increasing temperature during steeping and is followed by a decrease during germination and a complete levelling off in the malt. It can be assumed that glutenins are totally degraded during kilning (80). The microstructural results correlate with the analysed attributes. However, CLSM and SEM do not provide detailed and obvious proteolytic changes. A degradation of the cementric protein matrix, as well as a dense protein structure can be observed (Figure 4.4AB and 4.5AB).

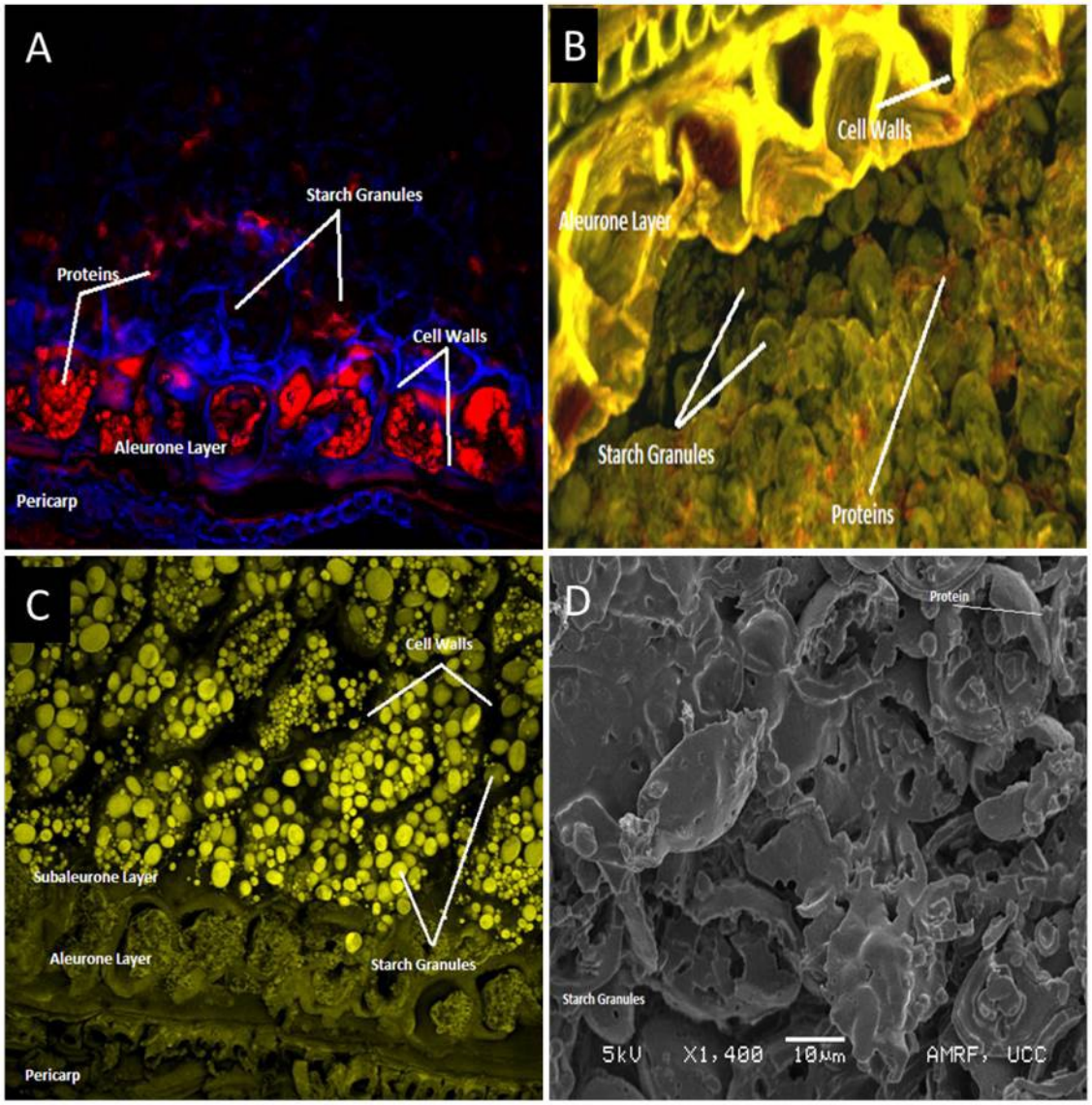


Figure 4.4: Aleuron layer-malted wheat (magnification = ×40); A, B red stained = proteins; blue stained = cell walls; starch is unstained and appears black; C Yellow stained = starch granules; cell walls and proteins are not stained; D SEM picture

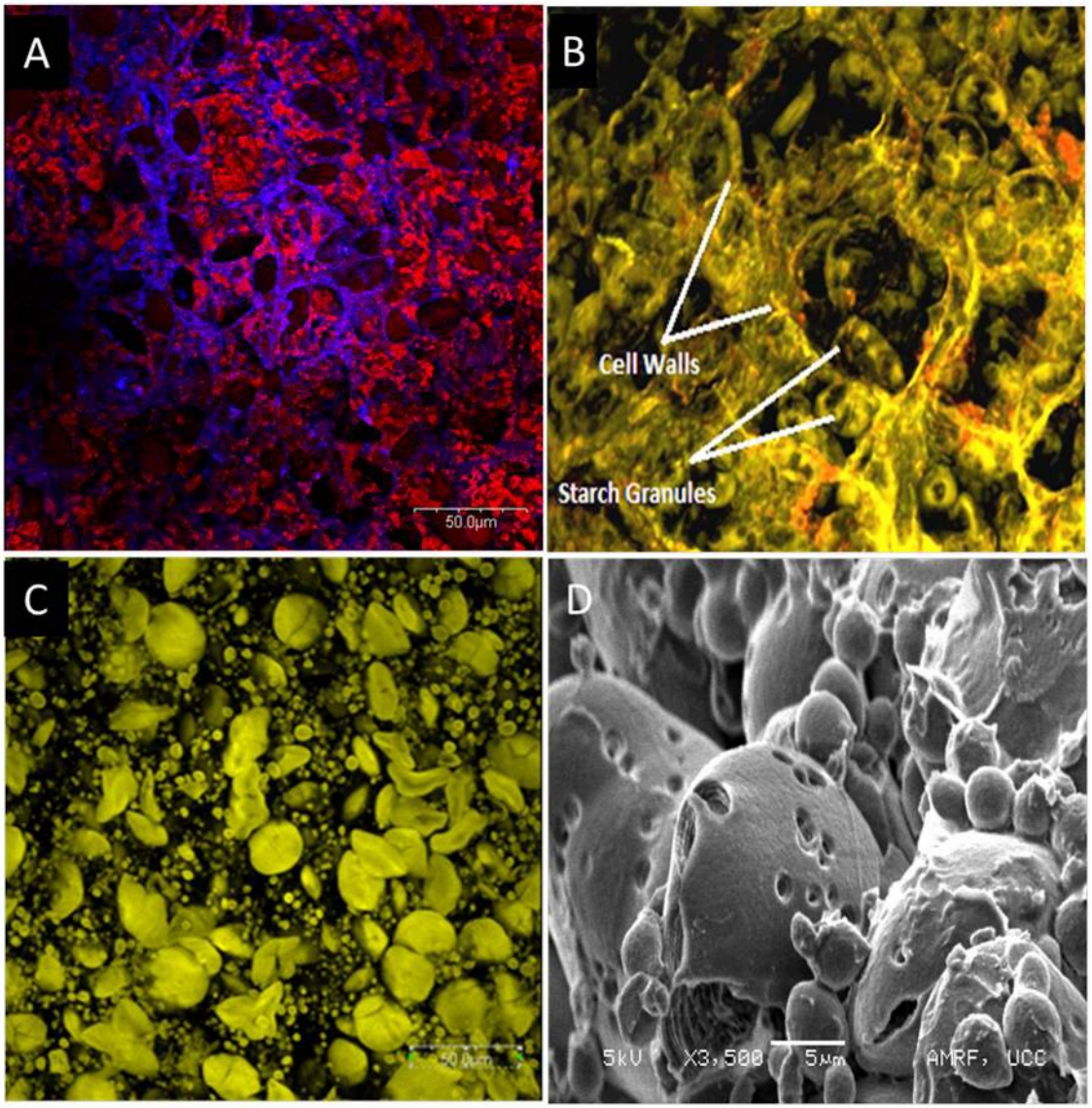


Figure 4.5: Endosperm-malted wheat (magnification = ×40); A, B red stained = proteins; blue stained = cell walls; starch is unstained and appears black; C Yellow stained = starch granules; cell walls and proteins are not stained; D SEM picture

In the final malt the protein matrix seems to be more degraded close to the outer layers and embryo, as it was already detected for starch granules. Furthermore, during the malting process an almost 7-fold increase in proteolytic activity was analysed. The activity increased as germination proceeded, to reach a maximum 5-7 days after the start of imbibition (from 2.16 to 14.30 mg of L-leucine/h/g, respectively: unmalted wheat to wheat malt) (172, 179, 180). With an increase of protease activity, an increase of proteolytic charactersitics goes along. These values nearly double in the

germination stage. Due to the hydrolysis of the native protein, which gives both high molecular weight protein breakdown products and low molecular weight proteolysis products (peptides and amino acids) (149), the total amount of amino acids increase 3.8-fold with the highest quantity found on the last day of germination (1368.2 mg/100 mL). The majorities of amino acids are heat sensitive and are thus degraded during kilning; however, this excludes alanine, arginine, tryptophan and phenylalanine, which are actually present in increased amounts after kilning (see Table 4.2).

Table 4.2: Mean values of free amino acid composition (mg/100 mL) throughout the malting process (n=3; $p < 0.005$)

Amino acids (mg/100 mL)	Unmalted Wheat	Day 1 Steeping	Day 2 Steeping	Day 4 Germination	Day 5 Germination	Day 6 Germination	Malted Wheat
Aspartic Acid	4.197	5.045	4.425	4.367	6.747	7.308	5.155
Glutamic Acid	2.759	2.575	4.704	7.247	7.911	9.532	3.242
Asparagine	4.046	6.561	5.326	6.536	18.047	22.452	14.357
Serine	0.461	0.542	1.325	4.010	7.059	7.053	5.365
Histidine	0.188	0.274	0.549	2.597	6.727	5.274	3.745
Glutamine	0.429	0.890	1.692	13.273	26.266	23.496	13.830
Glycine	0.605	0.756	0.827	1.615	2.478	2.431	1.970
Threonine	0.412	0.522	0.978	2.675	4.613	4.569	3.948
Alanine	0.561	0.859	1.118	2.240	4.024	4.126	4.664
Arginine	2.294	3.409	6.579	10.273	11.446	11.963	11.535
Tyrosine	0.2277	0.206	0.366	2.227	4.691	5.147	4.573
Valine	0.5678	0.772	1.216	3.419	6.125	6.313	5.762
Methionine	0.1297	0.170	0.276	0.774	1.340	1.294	1.095
Tryptophan	2.36	2.443	2.834	3.407	4.019	4.098	4.587
Isoleucine	0.3731	0.473	0.735	2.898	5.531	5.589	4.524
Phenylalanine	0.3671	0.457	0.830	3.423	6.613	6.861	7.321
Leucine	0.7238	0.975	1.654	6.266	10.985	10.409	7.568
Lysine	0.5307	0.734	0.977	2.204	3.646	4.075	3.518
Total amino acids	218.6	265.3	364.3	773.9	1334.5	1368.2	1015.6
Free amino nitrogen	26.9	32.7	44.9	95.3	164.2	168.5	125

These increases can be attributed to Strecker aldehydes reactions and decreases are due to the aminocarbonyl reaction (149). The most abundant amino acids were asparagines (Asn) and glutamine (Gln) whose levels increase either due to proteolytic cleavage from pre-existing proteinaceous material or are formed de novo through the Asn synthase (E.C. 6.3.5.4) and Gln synthetase (E.C. 6.3.1.2) activities, respectively. The degradation of the proteinoceus matrix causes a net increase in

smaller peptides and amino acids which are essential for brewing and maturation. Lineback and Ponpipom (181) found in their investigation, that during later stages of germination amorphous material covers the surface of wheat starch granules. This result correlates nicely with the results found in the later stages of kilning in the present study. At the end of the kilning steps, the protein seems to be fully degraded throughout the kernel. When staining the specimen at this stage with the protein specific dye *Acid Fuchsin*, only starch granules are seen, and detected by the fluorescence signal. It has previously been reported, that starch granules also include proteins moieties internally. HAN et al (182) observed a network of channel-like protein structures within commercial wheat starch granules using a protein specific dye, suggesting that channels in wheat starch granules posses a protein component. Also Yamamori and Endo (183) suggested that wheat starch granules contain a group of proteins known as the SGP-1 (starch granule protein-1) proteins, which have molecular masses of approximately 100, 108 and 115 kD. It can be suggested, that at this time of the malting process the proteins in the wheat grain must be significantly degraded, so that the specific protein staining dye reacts with the proteins inside the starch granule and make them visible.

4.4.2.3 Cytolytic changes

The viscosity of congress worts from malts depends on the total amount of solubilized material and the composition of the extract, as well the activity of enzymes during the mashing process. Hydrocolloids, like β-glucans and arabinoxylans, starch residues (not degraded by amylases) as well as proteins have a large impact on viscosity. Viscosity is the primary malt quality characteristic for wheat as it gives an indication of its processability during beer production, especially lautering and filtration. During malting, cytolytic enzymes degrade cell wall substances into smaller

molecules. Thus, they are initiating a breakdown and solubilisation of components which results in lower viscosity values. Wort and beer viscosity is influenced by the macromolecules which are present. For instance, Sadosky et al. (50) found that arabinoxylan, β-glucans and dextrins increase liquid viscosity. Additionally, hydrolytic enzymes are increasingly active and play a major role in breaking down the cell wall structure of cereal grains. Wheat has a lower β-glucan content than barley, but has more arabinoxylans, which may be a causative factor in the increased viscosities of wheat malt. Because enzymatic hydrolysis is continuous during all stages of malting, a 13% decrease in viscosity can be observed from the initial stages of processing to the final malt (unmalted wheat: 2.048 mPas, malted wheat: 1.779 mPas).

In wheat, pentosans are only a minor constituent compared to rye, which is very rich in pentosans at 6 - 8% (10). Unlike barley and oats, wheat endosperm is rich in arabinoxylans and very poor in β-D-glucans (45). Arabinoxylans (AX) are the predominant hemicelluloses based on the whole wheat kernel (6 – 7%) (46). From 1.5 to 2.5% of the AX can be found in the endosperm, where they constitute 66% of the endosperm cell wall (47). The cell wall material of the aleurone layer is richer in β-D-glucan than the starchy endosperm tissue, but AX still remains as the principal constituent (2). The main AX polysaccharides are modified by certain glycoside hydrolyses which include arabinosidase, endo-xylanase and β-xylosidase. Grant and Briggs (70) investigated the histochemical location of arabinosidase and xylosidase in germinating wheat grains. The α-L-arabinofuranosidase (EC 3.2.1.55) and the β-D-xylanopyranosidase (EC 3.2.1.37) activity were present in the embryo, the scutellum, and the aleurone layer, as well as in the starchy endosperm. The majority of these enzymes were found in the aleurone layer, whereas in ungerminated wheat, these enzymes were not detected.

The cell walls are clearly apparent throughout the whole grain. β-glucan is mainly found in the inner aleurone cell walls and subaleurone endosperm cell walls (57). They surround and separate the protein network, the aleurone cells, as well as the endosperm and germ cells from each other (Figure 4.2 and 4.3) and appear to be fully intact in the unmalted wheat grain. Cell wall modification and degradation, the cytolysis, is caused by hemicellulase and protein degrading enzymes. During steeping no significant differences in viscosity as well as in the microscopic pictures (Figure not shown) can be seen, due to the fact that cell wall degrading enzymes have to be activated. These finding can be compared to the results of DeBacker et al. (184). Moreover, the increasing of cell wall degrading enzymes during germination and decreasing after kilning can be compared to other cereals, e.g. barley (149) or oats (185).

However, during germination the AX in the cell walls of the starch containing endosperm is broken down to allow access of hydrolytic enzymes to the starch granules and protein stored within the cells. However, the decrease in viscosity of wheat worts coincides with the breakdown of soluble proteins and starch. This can be detected in the increase in the proteolytic as well as amylolytic attributes. The microstructural changes of cell walls can be clearly seen in CLSM and SEM pictures of malted wheat (Figure 4.4A-C and 4.5A-B). As it was detected for proteins and starch, cell walls are more degraded and eroded near the embryo as well as the aleuron layer than the upper endosperm.

4.5. Conclusions

Starch, cell walls and proteins are degraded during the malting of common wheat (*Triticum aestivum* L.). Changes taking place during malting process were detected and evaluated using physico-chemically methods as well as two optical tools. Both microscopes were necessary for the investigation and the evaluation of the microstructural changes, because SEM and CLSM have different merits when studying cereal grains. With the CLSM an overview of the single compounds of the grain was achieved which can be quantified then. On the other hand SEM has a higher magnification where you get a deeper insight in the kernel structure. Especially little bites on the surface of the starch kernels, when they are degraded by the enzymes, and also a detailed starch structure can be monitored with the SEM.

CLSM and SEM visualized the single stages of the malting process. The imbibing of water and the swelling of the proteins and starches, as well as the degradation of them and the cell walls could be detected. CLSM visualized and identified the single compounds in the grain with the help of the specific staining dyes which is an advantage to the SEM. Moreover, protein, starch and cell walls could be examined independently. Hence, the organization of the microstructural compounds is easier to detect with CLSM than SEM. CLSM is an excellent tool for studying the overall change in the structure during processing grains. The changes of wheat during the individual malting stages were shown by images, visualizing 3D structures. It proved that wheat starch is surrounded by a protein matrix, which is broken down throughout the germination process. Also differences in the overall starch and protein structure between unmalted and malted wheat became evident. With the SEM, higher magnification can be achieved; nevertheless it is more difficult to see differences between starch and proteins and to visualize cell walls. Otherwise SEM revealed that

wheat starch is degraded by enzymes, which results in bites and erosion of the surface.

To get a better understanding of the changes taking place during the malting process of cereals SEM and CLSM are supporting optical tools for standard analytic, which should be used in combination, to monitor the microstructural changes.

The gap of microstructural changes during malting of wheat is closed. As it was previously mentioned, proteins have a significant impact on the processability of wheat regarding brewing. Chapter 5 reveals a closer look on proteolytic changes and protein modification during malting.

CHAPTER 5

PROTEIN MODIFICATIONS AND METABOLIC CHANGES TAKING PLACE DURING THE MALTING OF COMMON WHEAT (*Triticum aestivum* L.)

5.1. Introduction

Wheat (Triticum aestivum L.) is the most widely consumed cereal in the world and has a long history of use as a raw material for the traditional production of conventional malts and beers. Nevertheless, due to its optimisation for the baking industry, wheat 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. Beer is a complex fermented cereal malt extract mixture with hundreds of constituents including macromolecules such as proteins, nucleic acids, polysaccharides and lipids (12). Malting is the first step in beer production and is one of the main beverage quality defining parameters. Malting is used to produce enzymes and to break down starch granules for enzymatic hydrolysis at a later stage (149). For the purpose of this research, we will focus on the cereal proteins which are well known to have a high impact on the brewing process and thus, the resultant beer qualities. 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 (100, 101, 149).

In beer production, the protein type, quantity, and size distributions are of particular importance in terms of filtration, foam and haze stability, and fermentability. Barley malt protein characterisation has been the focus of many research articles and, in particular, their influence on and relationship with these specific beer qualities. To contrast wheat with barley, an obvious starting point is to look at the differences in the protein content which is higher in wheat. Wheat also has an increased proportion of high molecular weight (HMW) proteins, a characteristic which has been correlated to superior foam characteristics and enhanced haze formation, when compared to barley malt beer (104-106, 187).

In the mature grain, wheat proteins are divided into two major categories: gluten; including gliadins and glutenins, and non-gluten; consisting of water-soluble albumins and salt-soluble globulins. The non-gluten proteins (molecular weights mainly between 12 to 60 kDa) are generally present in the embryo and aleurone, representing about 15 to 20 % of the wheat seed proteins, respectively (94). They include biological active enzymes and protease inhibitors such as α-amylase-inhibitor protein, β-amylases-inhibitor protein, lipid transfer proteins, polyphenol oxidases, peroxidases (94, 174, 175), all of which play various important roles during the malting and brewing processes. Some high molecular weight albumins (HMW albumins) and certain globulins (triticins) also have functions as storage proteins. They form part of the gluten protein complex through disulphide bonds, and are degraded and/or accumulated during germination and grain development, respectively (176).

Wheat storage (gluten) proteins make up to 80% of the total wheat grain protein content (177). Gluten is composed of gliadins (monomeric proteins) and glutenins (polymeric proteins) (83) and mainly occur in the endosperm of wheat grains. Monomeric gliadins (soluble in alcohol) which, in baking processes, contribute to dough viscosity and extensibility can be classified into four groups based on their electrophoretic mobility at low pH; the slowest moving group -ω-gliadins (S-poor, ~40-75 kDa) and three faster groups – α-, β-, and γ-gliadins (S-rich, ~30-45 kDa) [16]. Polymeric glutenins (extractable in acetic acid) consist of HMW glutenins (HMWG) and low molecular weight (LMW) glutenins (LMWG) (~30-40 kDa). In literature, several authors describe HMWG ranging from ~80-120 kDa (85, 177), whereas D'Ovidio and Masci (84) indicate a MW range of ~65-90 kDa.

The degradation and formation of proteins during malting is essential for the final beer quality. In malting, a limited germination procedure is the initial step in traditional beer production and strongly defines the type and quality of the resulting product. One of

the most obvious physicochemical changes that occur during malting is enzymatic degradation of the cereal endosperm and its conversion into soluble peptides and amino acids, providing substrates for the synthesis of proteins and the growing embryo (149). Compared to the commonly used malting cereal, barley, relatively less is known about the proteolytic, amylolytic and cytolytic impacts of wheat on malting and brewing; although wheat proteins are most extensively investigated, among cereal grains. The first purpose of this study was to determine the effect of germination on chemical compositions, especially on malt relevant attributes.

In spite of the comprehensive scientific publications on wheat grain proteins, in particular from a baking perspective, 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 from the raw wheat grain during germination to the final malted wheat, with comparison to the well-established barley malt protein changes, and in doing so to develop and utilise novel protein analyses methods.

5.2. Materials and Method

5.2.1 Materials

The wheat variety “Hermann” was grown and harvested in 2010 in Germany. The standard MEBAK (Mitteleuropäische Brau- und Analysenkommision) (112) malting method 2.5.3.1 (15° vegetation temperature, 45% steeping degree and 7 day of vegetation) was carried out in a pilot scale malting facility (Chair of Brewing and Beverage Technology, Weihenstephan, Germany). Seeds were germinated in tempered climate chambers with a constant air humidity of >95%. The initial kilning temperature for the green malt was set to 50 °C and gradually increased, over 24 h, to

80 °C. All analyses are based on methods described in the EBC (145) ASBC or MEBAK handbooks (112). Unmalted, steeped, 3-day-germinated and kilned (malted) wheat was sampled and freeze-dried (VirTus Benchtop 4k, Biopharma Process Systems, Winchester, UK). Roots were removed (grain cleaner machine SNL 3, Pfeuffer GmbH, Kitzingen, Germany) and sample were milled with a disc mil (Bühler GmbH, Braunschweig, Germany) set at a fine setting of 0.05 mm and stored at - 80 °C.

5.2.2 Metabolic changes during malting of common wheat (Triticum aestivum L.)

Analytical procedures were carried out in triplicate, at least ($n \geq 3$), and the means of all results were calculated with standard deviations reported in Table 5.1. Biological replications were done in duplicate. Statistical analyses were performed according to ANOVA (analysis of variance) with p-values < 0.005. All concentrations are based on dry matter (d.m.) unless otherwise stated.

Analyses of unmalted wheat, grains during malting, and final wheat malt were performed according to approved methods of the EBC, MEBAK (Mitteleuropäische Brau- und Analysenkommision) using congress mash programs (MEBAK method 3.1.4.4.1), which are the standard methodologies used in industry. Extract was calculated using an Anton Paar Alcolyzer (Anton Paar, Graz, Austria), following MEBAK method 4.1.4.2.2 (112). The apparent attenuation (amount of fermentable sugars) was detected by MEBAK method 4.1.4.10 (112). Total protein (TP) content was analysed according to MEBAK method 1.5.2.1 and calculated as total nitrogen. The protein content was measured according 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 (111, 147, 169) and approved methods according AACC (method 46.18) and ICC, wheat varieties which are used for feed purpose are multiplied with 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 soluble nitrogen (SN) content was determined by using an automatic Kjeltec system, according to MEBAK method 3.1.4.5.2.1 (112). The Kolbach Index (KI) was calculated from the formula according to MEBAK method 4.1.4.5.3 (112). α-Amino nitrogen, also referred to as free amino nitrogen (FAN), was determined using a Skalar working station (Skalar, Breda, Netherlands), following MEBAK method 3.1.4.5.5 (112). Wort viscosity was measured using a falling ball viscometer (AMVn-Automated Micro Viscometer Anton Paar, Graz, Austria).

5.2.3 Total protein analysis of wheat

Samples were taken daily throughout the malting process and initial total protein analyses of all samples were done using the Lab-on-a-chip system (Agilent 2100 Bioanalyzer, 230+-Chip, Agilent Technologies, Palo Alto, CA). Unmalted, steeped, 3-day-germinated and kilned (malted) wheat was sampled and processed (Section 2.1). The proteolytic enzyme activity (U) was measured according to the method of Brijs (186), 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.

5.2.3.1 Modified Osborne fractionation of wheat proteins

Unmalted, steeped, germinating and malted wheat were analysed and extracted by the modified Osborne (standard and reference method for proetins since decades) fractionation of wheat proteins as reported by Klose et al. (77). Briefly, the samples were extracted twice with 100 ml of distilled water (albumin fraction) followed by two extractions with 100 mL of 0.5 % NaCl (globulin fractions). The remaining flour was then extracted three times with 150 mL of 55% 1-propanol +1 % Dithiothreitol (DTT) (prolamin fraction) and then extracted three times with a solvent containing 6 M urea, 2 % sodium dodecyl sulphate (SDS) and 1% DTT (glutelin fraction and residue). Each extraction was carried out at room temperature for 10 min. and centrifuged afterwards at 10,000 rpm for 10 min.

5.2.3.2 Lab-on-a-Chip analyses of the total protein extracted during individual malting stages

To evaluate protein changes during the malting process, a Lab-on-a-Chip capillary gel electrophoresis system was used (Agilent 2100 Bioanalyzer, 230+-Chip, Agilent Technologies, Palo Alto, CA). Each run included a ladder comprising reference proteins of 7, 15, 28, 46, 63, 95, 150 kDa plus an upper marker of 240 kDa and a lower marker of 4.5 kDa. Each sample contained an internal standard comprising the upper and lower marker as well. Any peak detected below 14 kDa is termed a system peak and is not included in 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 230+ chip (Agilent Technologies).

5.2.3.3 OFFGEL fractionator analyses of proteins extracted throughout malting

To evaluate protein changes during the malting process, a second novel protein analysis technique called OFFGEL fractionation was used (Agilent 3100 OFFGEL fractionation, Agilent Technologies, Palo Alto, CA) and compared to 2DGel (validated data not shown). OFFGEL is a 2-D protein capillary separation method related to the Lab-on-a-chip (Section 2.2.4). In contrast to Lab-on-a-chip, OFFGEL uses isoelectric focusing (IEF) of samples as the first dimension to separate proteins on an Immobilized pH Gradient (IPG) gel strip. The 3100 OFFGEL electrophoresis system separates proteins according to their isoelectric points, and the individual fractions are recovered in the liquid phase, as per user manual (Agilent Technologies). Briefly, the first step of OFFGEL analysis is rehydration of the gel strips (pH 3-10). The diluted sample is then distributed across all wells in the strip and placed in the OFFGEL rig. When a voltage is applied the same effect occurs as in conventional IEF with individual proteins stopping where the pH equals its pI. The fractionated samples are

easily recovered using a pipette and processed for downstream experiments, as per user manual (Agilent Technologies).

For analysis of the total protein content, 80 mg of milled wheat samples were extracted during the malting process, as described by the manufacturers (Agilent Technologies). The samples were then analysed by Lab-on-a-chip (Section 2.2.4) without any further preparation using the Agilent 2100 Bioanalyzer.

5.2.3.4 2D-PAGE

Before 2D-PAGE analysis, samples were dissolved in a solubilisation buffer containing 9 M urea, 4% CHAPS, 0.05% Triton X100 and 65 mM DTT. 125 µl of protein solution were applied to each 7 cm IPG 3-10 strip (ReadyStripTM, Bio-Rad). IEF was carried out using a Bio-Rad Protean IEF cell with a controlled temperature of 20 °C. The running conditions were as follows: Passive rehydration; 8 h, active rehydration (50 V); 8 h, rapid 300 V; 30 min, linear 4000 V; 20000 V-h, rapid 300 V; 99 h. After IEF was completed, the IPG strips were equilibrated for 15 min. in a buffer containing 50 mM Tris-HCl pH 8.8, 6 M urea, 30% glycerol, 2% SDS and 130 mM DTT. Following this, the strips were equilibrated for a further 15 min. in the same solution, except DTT was replaced with 130 mM iodoacetamide and 5 µL bromophenol blue. SDS-PAGE was performed with 12.5% polyacrylamide gels, at 20 °C, in Tris/glycine/SDS running buffer according manufacturer`s instructions (Bio-Rad Criterion Dodeca cell manufacturer's manual). The gels were run at 100 V until the tracking dye reached the bottom of the gel. To visualize the proteins, the gels were first incubated in a fixing solution, containing ethanol and phosphoric acid and then in an incubation solution containing methanol, phosphoric acid and ammonium sulphate dissolved in distilled water. Coomassie brilliant blue was used to stain the gels for 4 h (77).

5.2.3.5 Free amino acid analysis

The free amino acid profile of the malting samples over time was measured using reverse phase HPLC with pre-column derivatisation (Spherisorb ODS II, 5µm, 20 * 4.6 mm; separation column, Spherisorb ODS II, 5 µm, 250 * 4.6 mm) and fluorescence detection, according MEBAK method 3.1.4.5.5.2.

5.3. Results and discussion

5.3.1 Metabolic changes during malting of common wheat (Triticum aestivum L.)

Samples were taken throughout the malting process and then analysed according EBC and MEBAK standard methods.

Steeping is traditionally used to increase the moisture content of the wheat (or more commonly barley) which will induce germination. This increases enzymatic activities and finally kilning the metabolically active grains to further increase flavour and colour attributes. In this research amylolytic, cytolytic and proteolytic changes which occur during wheat malting were investigated (see Table 5.1).

Table 5.1: Malt quality analysis throughout malting and typical pale barley and pale wheat malt attributes

Samples (n=4)	Day	Malt Extract (%)	Final Attenuation (%)	Viscosity (mPas)	Soluble Nitrogen (mg/100g malt or mg/100g grain*)	Free Amino Nitrogen (mg/100g malt or mg/100g grain*)	Kolbach Index (%)	Protease activity (U/g)
Wheat	1	48.73 ± 1.29	51.20 ± 0.50	2.05 ± 0.12	281.00 ± 5.66	24.50 ± 0.71	15.20 ± 0.42	2.16 ± 0.005
Steeping	2	56.05 ± 2.33	58.93 ± 1.99	2.09 ± 0.09	290.00 ± 2.63	29.75 ± 1.26	15.65 ± 0.13	2.55 ± 0.005
Steeping	3	72.80 ± 1.24	64.90 ± 1.22	2.05 ± 0.10	368.00 ± 4.99	37.25 ± 2.99	16.60 ± 0.25	2.94 ± 0.058
Germination	4	80.43 ± 0.43	71.90 ± 0.83	1.84 ± 0.02	372.00 ± 10.78	46.75 ± 1.50	19.86 ± 0.59	3.41 ± 0.010
Germination	5	83.88 ± 0.15	71.30 ± 0.42	1.90 ± 0.03	444.00 ± 9.070	56.25 ± 1.89	24.43 ± 0.47	5.95 ± 0.036
Germination	6	85.48 ± 0.25	75.18 ± 0.44	1.88 ± 0.01	545.00 ± 7.040	70.75 ± 4.11	30.53 ± 0.78	7.28 ± 0.008
Germination	7	86.33 ± 0.22	76.80 ± 0.42	1.85 ± 0.01	627.00 ± 10.13	93.50 ± 4.80	35.25 ± 1.03	8.81 ± 0.014
Green Malt	8	87.18 ± 0.10	77.34 ± 1.33	1.75 ± 0.01	697.00 ± 14.31	116.25 ± 0.50	39.15 ± 0.70	14.81 ± 0.008
Wheat malt	9	86.89 ± 0.15	79.15 ± 0.57	1.78 ± 0.01	700.00 ± 6.85	121.00 ± 0.52	38.93 ± 0.32	14.30 ± 0.019
Typical pale barley malt	N/A	80.00 – 83.50	80.00 – 83.00	1.50 – 1.57	650.00 – 750.00	135.00 – 155.00	38.00 – 42.00	
Typical pale wheat malt	N/A	81.00 – 86.00	78.00 – 82.00	1.55 – 1.80	600.00 – 800.00	100.00 – 140.00	35.00 – 45.00	

* mg/100g grain is used instead of mg/100g malt to signify measurement units for the unmalted wheat grains.
N/A means not applicable

Typical malting parameters and quality values for barley and wheat are compared with the results of the wheat malt characterised in this study (Table 5.1). The malt extract yield is one of the most important quality attributes of any cereal malt (149). It represents the fermentable sugars generated during mashing, through amylase hydrolysis of starch to saccharides, and is essential for a successful fermentation. In Table 5.1, the continuous increases in extract yield during the malting process are illustrated, with a final maximum value of 86.89% which is 3-6% higher than the typical minimum values for barley malts. During the individual malting stages extract increased by ~30% from an initial value of 48.73% in unmalted wheat showing the importance of the malting process in ameliorating the fermentability of wheat. The final attenuation of the wheat malt is also a critical parameter in malt quality and in this study, it increased by ~20% to 79.15 % in the final malted wheat. These values fall within the typical range (Table 5.1) and this, in combination with the high extract values, indicate production of good quality malt.

Viscosity is the primary malt quality parameter for wheat as it gives an indication of its processability during beer production, especially lautering and filtration. During malting, cytolytic enzymes degrade cell wall substances into smaller molecules thus initiating a breakdown and solubilisation of components decreasing the viscosity. After malting the viscosity had decreased by 13% (unmalted wheat: 2.048 mPas, malted wheat: 1.779 mPas). Wort and beer viscosity is influenced by the macromolecules present. For instance, Sadosky et al. (50) found that arabinoxylan, β-glucans and dextrins increase liquid viscosity. Additionally, hydrolytic enzymes are increasingly active and play a major role in breaking down the cell wall structure of cereal grains. Wheat has a lower β-glucan content than barley, but has more arabinoxylans, which may be a causative factor in the increased viscosities of wheat malt. Because enzymatic hydrolysis is continuous during all stages of malting, a 13% decrease in

viscosity can be observed from the initial stages of processing to the final malt. Due to the huge influence of wheat gluten proteins on the viscoelastic nature of bread dough (187a), it can be assumed that gluten proteins also have an impact on the viscosity of wheat malt.

An increase in the proteolytic values; Kolbach index (KI), soluble nitrogen (SN) and free amino nitrogen (FAN) occurs as malting proceeds. The KI is an indicator of proteolytic modification of the malt and reflects protein solubilised during malting and preparation of the congress mash. Combined, FAN, free amino acid composition and KI describe protein degradation during the malting process. These parameters are increased from 2.5 to 5-fold in unmalted wheat to malt (Table 5.1). During germination, cell storage proteins are hydrolysed and proteolytic and other enzymes are synthesised throughout malting. This results in the degradation of the proteinoceus matrix causing a net increase in smaller peptides and amino acids which are essential for brewing and maturation. LMW nitrogen compounds, especially amino acids in wort, impact the fermentation performance and the development of fermentation by-products, thus playing a central role in the colour and flavour development of malt following the Strecker and Maillard reactions (149). Additionally, small proteinaceous matter contributes positively to the desirable haze characteristics of wheat beer and stabilises the foam (106). In summary, proteolytic activity increases during malting and proteins are degraded into smaller nitrogenous substances.

Furthermore, total proteolytic enzyme activity was analysed using haemoglobin as substrate and absorption was ensured against L-leucine as standard. During the malting process a 6.9-fold increase in proteolytic activity could be observed. The results revealed an increase in the proteolytic activity levels from 2.16 to 2.94 mg of L-leucine/h/g during steeping, and 3.41 to 8.81 mg of L-leucine/h/g during germination, reaching a maximum of 8.81 to 14.30 mg of L-leucine/h/g during the last steps of

malting. The highest increase was observed during germination and in green malt when the levels hit a 4.3-fold increase in proteolytic activity. These results agree with previous findings (179). There the activity increased as germination proceeded, to reach a maximum 5-7 days after the start of imbibitions (179, 180, 188).

During malting, especially in the germination stage, metabolic changes cause protein degradation forming smaller molecules. Moreover, storage protein modification in germinating cereal grains are divided in three phases; a, the contents of protein bodies in the embryo scutellum and aleurone layer are hydrolized; b, storage proteins accumulated in the starchy endosperm are mobilized by proteases that are produced and secreted from the aleurone layer and scutellum to produce small peptides; c, and these peptides are hydrolized to amino acids (188). A comparison of the free amino acid content in unmalted and malted wheat (Table 5.2) revealed an increase in all amino acids during the malting process. This is due to the hydrolysis of the native protein, which gives both HMW protein breakdown products and LMW proteolysis products (peptides and amino acids) (149).

Table 5.2: Mean values of free amino acid composition (mg/100 mL) throughout the malting process (n=3; p < 0.005)

Amino acids (mg/100 mL)	Unmalted Wheat	Day 1 Steeping	Day 2 Steeping	Day 4 Germination	Day 5 Germination	Day 6 Germination	Malted Wheat
Aspartic Acid	4.197	5.045	4.425	4.367	6.747	7.308	5.155
Glutamic Acid	2.759	2.575	4.704	7.247	7.911	9.532	3.242
Asparagine	4.046	6.561	5.326	6.536	18.047	22.452	14.357
Serine	0.461	0.542	1.325	4.010	7.059	7.053	5.365
Histidine	0.188	0.274	0.549	2.597	6.727	5.274	3.745
Glutamine	0.429	0.890	1.692	13.273	26.266	23.496	13.830
Glycine	0.605	0.756	0.827	1.615	2.478	2.431	1.970
Threonine	0.412	0.522	0.978	2.675	4.613	4.569	3.948
Alanine	0.561	0.859	1.118	2.240	4.024	4.126	4.664
Arginine	2.294	3.409	6.579	10.273	11.446	11.963	11.535
Tyrosine	0.2277	0.206	0.366	2.227	4.691	5.147	4.573
Valine	0.5678	0.772	1.216	3.419	6.125	6.313	5.762
Methionine	0.1297	0.170	0.276	0.774	1.340	1.294	1.095
Tryptophan	2.36	2.443	2.834	3.407	4.019	4.098	4.587
Isoleucine	0.3731	0.473	0.735	2.898	5.531	5.589	4.524
Phenylalanine	0.3671	0.457	0.830	3.423	6.613	6.861	7.321
Leucine	0.7238	0.975	1.654	6.266	10.985	10.409	7.568
Lysine	0.5307	0.734	0.977	2.204	3.646	4.075	3.518
Total amino acids	218.6	265.3	364.3	773.9	1334.5	1368.2	1015.6
Free amino nitrogen	26.9	32.7	44.9	95.3	164.2	168.5	125

The majority of amino acids are heat sensitive and are thus degraded during kilning; however, this excludes alanine, arginine, tryptophan and phenylalanine, which are actually present in increased amounts after kilning (see Table 5.2). The total amount of amino acids increase 3.8-fold with the highest quantity found on the last day of germination (1368.2 mg/100 mL). During kilning, most amino acids are degraded, with the exception of alanine, arginine, tryptophan and leucine. These increases can be attributed to Strecker aldehydes reactions and decreases are due to the aminocarbonyl reaction (149). The most abundant amino acids were asparagines (Asn) and glutamine (Gln) whose levels increase either due to proteolytic cleavage from pre-existing proteinaceous material or are formed de novo through the Asn synthase (E.C. 6.3.5.4) and Gln synthetase (E.C. 6.3.1.2) activities, respectively. Klose et al. (78) found different results in oat where aspartic acid and glutamic acid were higher in unmalted than malted oat.

Total protein analysis, using the Protein 230+ Lab-on-a-chip, showed several protein peaks in four separate areas; 14-30 kDa (area 1), 38-63 kDa (area 2), 100-140 kDa

(area 3) and, 170-180 kDa (area 4), in all four samples (unmalted wheat, during steeping, during germination and malted wheat). The results of unmalted and malted wheat are shown, Figure 5.1.

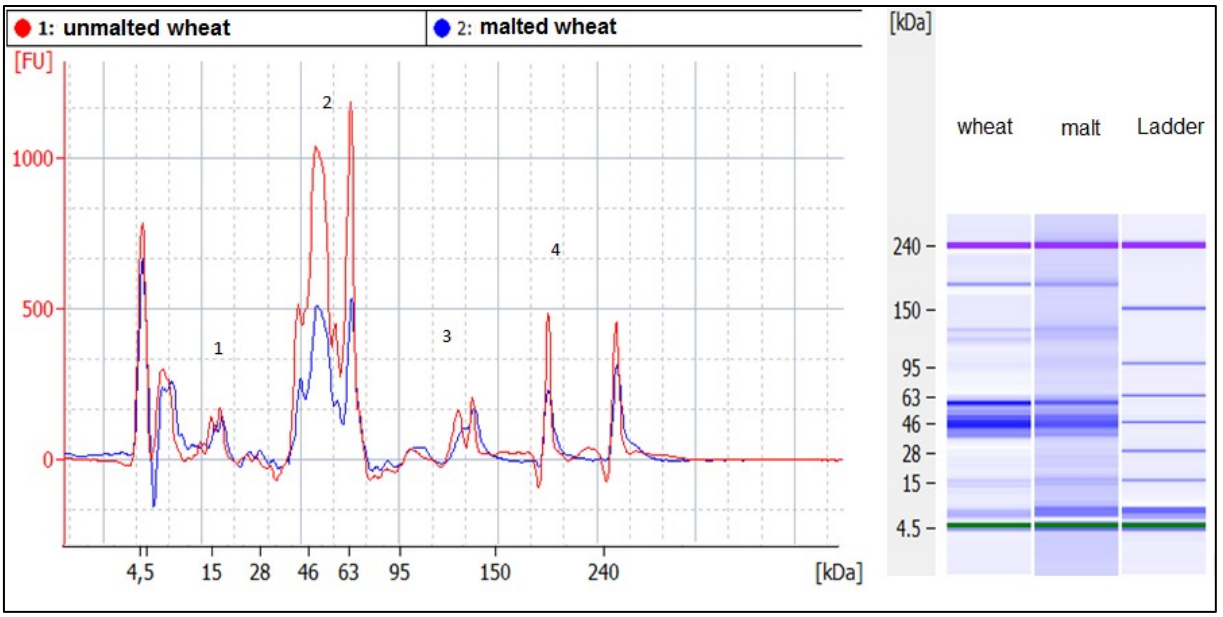


Figure 5.1: Electropherogram and SDS-like image showing total protein content of unmalted and malted wheat showing area 1-4. Area 1 is referred to lipid transfer proteins; in area 2 refers to Protein Z (~40kDa).

Relative peak concentrations in area 1 demonstrate that these proteins were degraded by 27% during malting. In area 2, peak heights and concentration decreased by 58% after malting from about 1000 FU (Fluorescence Units) to about 480 FU and in areas 3 and 4 a decrease of 37% can be detected when unmalted and malted wheat are compared (calculated based on the peak area; Figure 5.1). A breakdown of all proteins occurs at each stage of malting and an overall decrease was shown. Jin et al. (38) reported that a higher wheat protein content was correlated with an increase in storage proteins and a decrease in soluble proteins.

In addition, the 2D (IEF and MW separation) capillary gel electrophoresis technique, OFFGEL fractionation, was used to further separate total wheat protein throughout malting. Results are comparable to the conventional slab-based 2D-PAGE, as shown

in Figure 5.2.

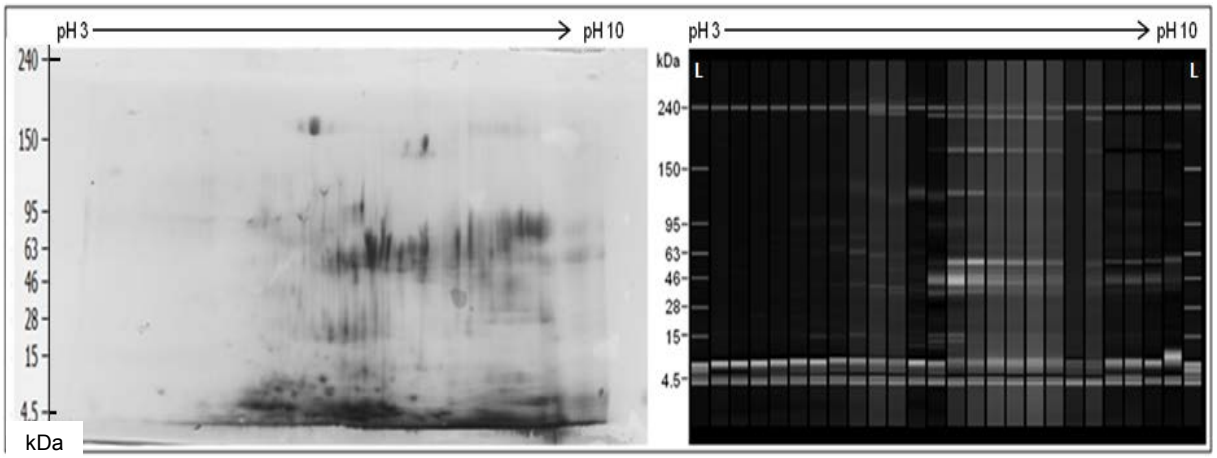


Figure 5.2: 2D-PAGE gel image of the same sample with pH3-10 and OFFGEL simulated gel-like image of malted wheat protein between pH3-10, with an upper marker at 240kDa and a lower marker at 4.5kDa (the upper and lower markers are system peaks and not actual detected proteins). (L = Ladder: 240, 150, 85, 63, 28, 15 and 4.5kDa)

There are very similar protein patterns detected in both the 2D-PAGE gel (Figure 5.2) and the OFFGEL simulated "gel-like" images (Figure 3.2), showing the parallel nature of the two techniques. In contrast to traditional isoelectric focusing (IEF), the fractionated sample is delivered in liquid phase (OFFGEL mode). Moreover, the "gel-like" images results in clearer spots, than the 2D-PAGE does. The bulk of proteins are detected in neutral and higher pH ranges and also, the highest relative protein concentrations and degradation levels (pH 6.3: 88 %, pH 6.6: 86%, pH 6.9: 70%, pH 7.2: 85%, pH 7.4: 81%, pH 7.7: 85%, pH 8: 89%) are found in the region from pH 6.5 to 8. In addition, degradation of individual proteins, and their relative concentrations during the malting process, is clearly visible using the new OFFGEL system (results not shown). At lower pH ranges, proteins during steeping, germination and in malt have higher relative concentrations than in wheat, but are still low when compared to the proportion of proteins above pH 5. On evaluation of the wheat and malt protein images it can be seen that a degradation of proteins occurs and there is a noticeable molecular weight movement of proteins.

Protein Z and LTP1 (Lipid Transfer Protein) are barley albumin proteins which are found both in the malt and beer (105, 106, 187, 189). A peak (electropherogram) corresponding to these proteins was observed in our wheat malt study (Figure 5.1). They could be detected in wheat as well as the deriving malt. These well characterised proteins impart a positive effect on foam formation and stability, and beer haze (190). Malt proteins have a huge impact on the brewing process and the resulting beer quality. Protein content and size distribution are of particular interest in terms of filtration, fermentability, and foam and haze stability. Total protein analysis gives an overall net view of protein changes during malting. However, for a more detailed analysis, the proteins were fractioned and sampled at each stage from raw wheat to the final kilned malt.

5.3.2 Protein fractions

To provide a fundamental understanding of how wheat proteins are affected by the increasing proteolytic activity during malting, the four protein fractions; albumins, globulins, gliadins and glutelins, were separated according to Osborne (81) fractionation and analysed using the Lab-on-a-Chip technique. The quantities and relative amounts of the soluble protein fractions change substantially during malting. Nitrogenous substances are lost by leaching during steeping, but there are no significant net changes during malting.

Albumins: This fraction which makes up 14.7% of wheat protein and only 12.1% of barley protein has a typical MW from 12 to 60 kDa. Several protein peaks and peak areas could be detected in unmalted and malted wheat, which were classified into areas 1 to 4 (14-23kDa, 25-30 kDa, 38-50 kDa, 55-63 kDa, respectively), see Figure 5.3. The degradation pattern of proteins is evident in all areas. Merlino *et al.* (191)

made a proteomic analysis of albumins and globulins in hexaploid wheat kernels and found that α-amylase-inhibitors are present in LMW albumins (16 kDa), whereas β-amylase is found a ~63 kDa. When comparing raw and malted wheat, it can be hypothesised that decreases in the malt albumin fraction are possibly due to hydrolysis of storage proteins. Jin *et al.* (38) found that glutenin and gliadins are decomposed during malting and are transformed to the albumin fraction. Proteins with MW between 55 to 63 kDa in the albumin fraction increased during malting resulting in a broader peak area, probably due to protein synthesis, the release of some latent proteins and/or the hydrolysis of insoluble hordeins (190). It seems that these proteins are more vulnerable to proteolytic attack and the hydrolysates become water-soluble. As in other studies on barley and malt (77, 187, 190), the 40 kDa protein Z peak was found in unmalted wheat and in smaller amounts in the malt. In wheat LTP1 are also found, in the albumin fraction, and are of great technological importance because of their involvement in beer foam formation. It can be assumed that LTP1 is also degraded during malting as it was shown for barley (77). This decrease is probably due to Maillard-reactions that occur during kilning (192).

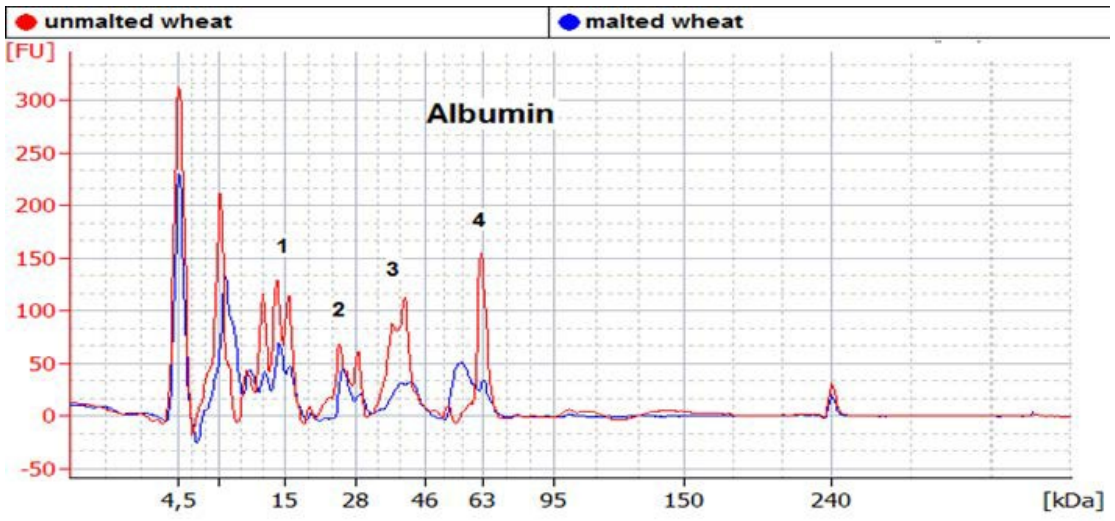


Figure 5.3: Electropherogram of Albumin fractions of unmalted and malted wheat protein sample as separated using the Protein 230^{+}LabChip.

Globulin: The globulin fraction has the lowest concentration of all fractions (as indicated in Figure 5.4) and consists of metabolically active and storage proteins (85). They are present in the starchy endosperm of wheat and consist of large (approximately 40 kDa) and small (22-23 kDa) polypeptides. In Figure 5.4, the electropherogram of the salt-soluble wheat fractions, the globulins, of unmalted and malted wheat, are shown. The globulin fractions, like albumins, can also be divided into 4 protein peak area groups (area 1: 14-20 kDa, area 2: 20-26 kDa, area 3: 28-40 kDa and area 4: 46-60 kDa). In the range of 22 to 58 kDa, more specific wheat globulin storage proteins called triticins are found. When we compare unmalted wheat with steeped wheat, there are no detectable protein peaks greater than 40 kDa, showing extensive degradation of the HMW proteins during malting. Wheat globulins, in area 4, are totally degraded during the malting process leading to the assumption that these proteins are hydrolysed for embryo germination.

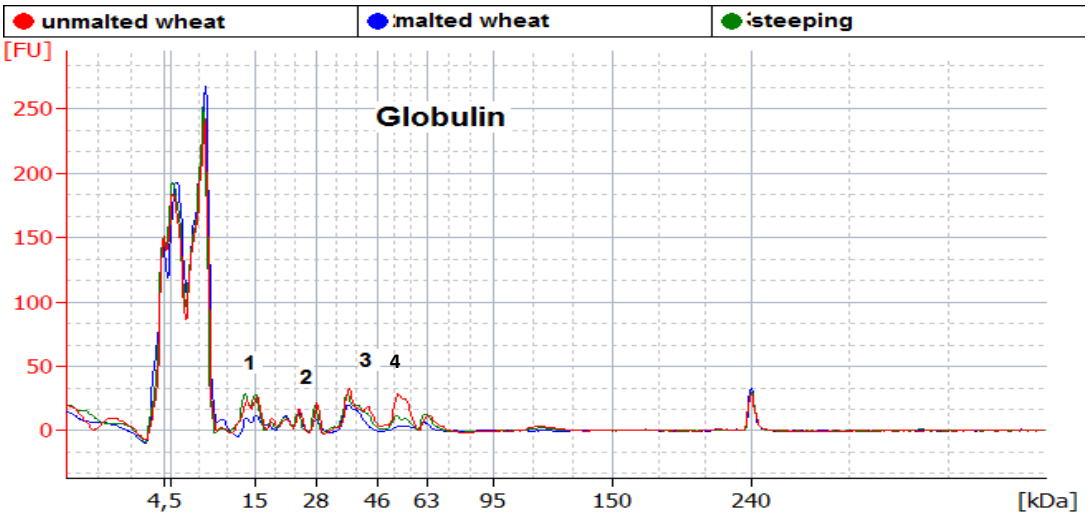


Figure 5.4: Electropherogram of Globulin fractions of unmalted and malted wheat protein sample as separated using the Protein 230^{+}LabChip

Gliadin: The alcohol soluble protein fraction of wheat (~32% of wheat protein) has a typical MW from 30-80 kDa. Gliadins can be more specifically classified into ω-gliadins (40-75 kDa) and α-, β-, and γ-gliadins (30-45 kDa). Figure 5.5 shows the electropherogram of unmalted and malted wheat gliadin fractions. Three characteristic peaks areas can be found ranging from 38-60 kDa (area 1), 88-120 kDa (area 2) and 170-200 kDa (area 3). No significant changes can be detected between wheat and steeping, and thus we can deduce that gliadins are unaffected by imbibing water. The most dramatic degradations can be seen when raw wheat is compared to malt. A previously unreported and unidentified protein peak in area 3, which has a higher MW than typical gliadins, is detected in all stages of malting. This needs to be further characterised for identification and to elucidate its functionality in malting and brewing processes.

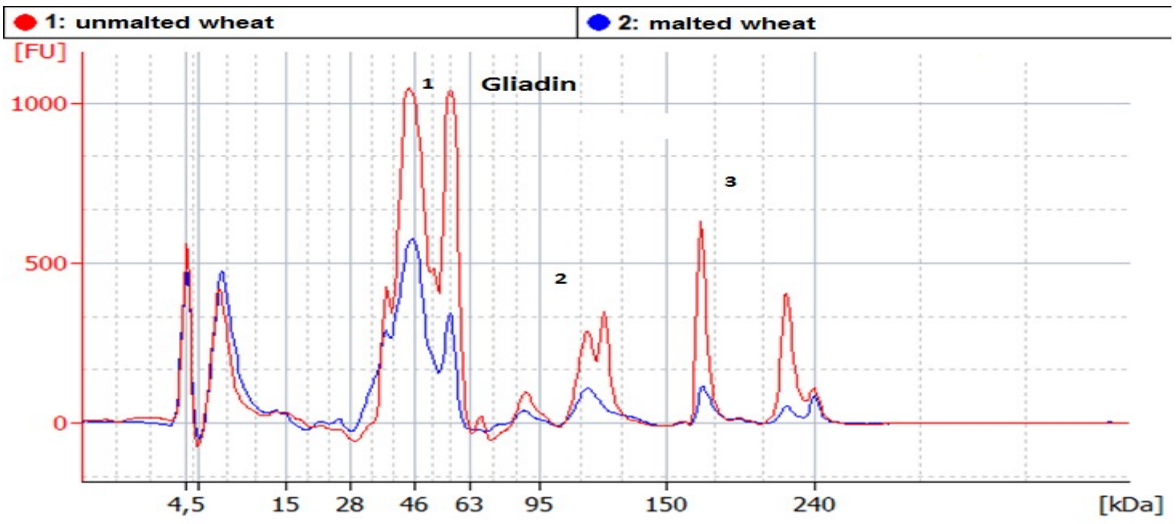


Figure 5.5: Electropherogram of Gliadin fractions of unmalted and malted wheat protein sample as separated using the Protein 230^{+}LabChip

During barley malting, a decrease of the prolamin fraction (corresponding to gliadins in wheat) was also detected (77). It can be assumed that wheat gliadins also function as storage proteins, which supply the embryo with peptides and amino acids during germination (85). Wheat gluten, especially gliadins, were found to be

involved in haze formation and stabilisation (114). Moreover, the permanent haze intensity increases when wheat malt is used because of proteolysis of wheat gluten proteins (114). Jin et al. (38) also deduced that with an increase in total protein content gliadins and glutenins also increased, and albumins and globulins decrease. Because storage proteins in wheat are known to contribute to the viscoelastic properties of wheat dough (187a), it can also be assumed that they influence the viscosity in wheat malt. It is well known that increased protein content is negatively correlated with the starch content and therefore decreases the malt extract. Sacher and Narziss (193) stated that wheat varieties for brewing purposes should have a low protein content to avoid problems during filtration and lautering and possibly decrease viscosity. This is in contrast to the wheat varieties for baking purposes which have high gluten (protein) contents.

Glutenin: These storage proteins are extractable in dilute acetic acid and have a LMW portion in the range of ~30-40 kDa (area 1) and a HMW portion in the range of ~80-120 kDa (area 2). Glutenins differ not only in their MW from the other Osborne fractions of unmalted wheat, but also in their changes during the malting process. Figure 5.6 shows the electropherogram of unmalted and malted wheat. It can be assumed, that glutenins are strongly influenced by the uptake of water and increasing temperature during steeping because a 2-fold increase is found in the electropherogram peaks detected at this time. This is followed by a decrease during germination and a complete levelling off of peaks in the malt fraction. It can be assumed that glutenins are totally degraded during kilning (Figure 5.6). This is in agreement with Klose et al. (77) as well as Weiss et al. (194), who found rapid decreases in glutenin during oat and barley malting, respectively, in the first five days of the malting process.

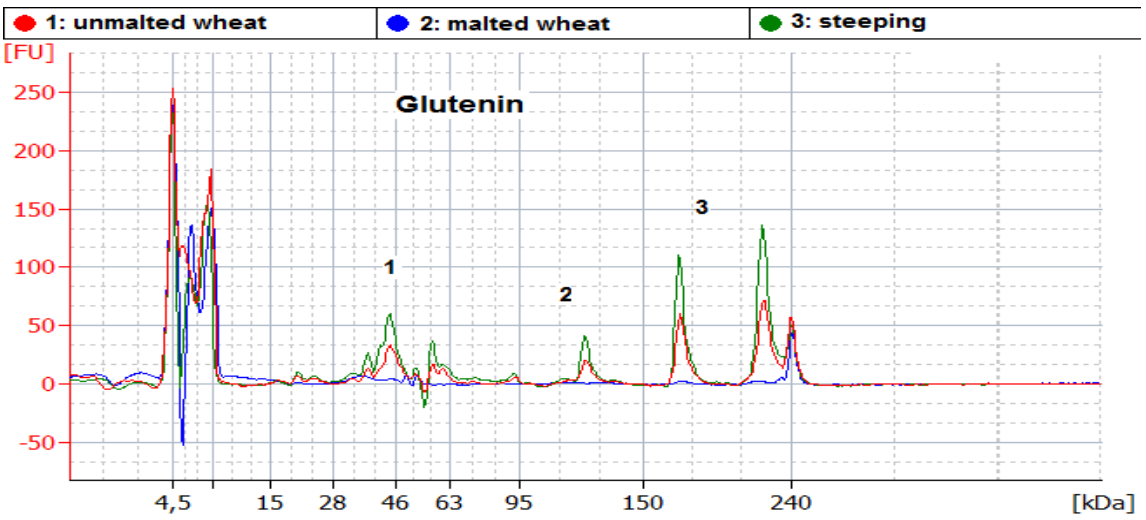


Figure 5.6: Electropherogram of Glutenin fractions of unmalted, steeped and malted wheat protein sample as separated using the Protein 230^{+}LabChip

5.4. Conclusion

In summary, this study exposes the total protein changes taking place in each of the four Osborne fractions during wheat malting. In this study, we have shown that enzymatic hydrolysis modified wheat during the malting process. Parallels could be drawn between barley and wheat malt proteins, such as the identification of Protein Z, which contributes to haze and foam, as well as LTP1, which is also related to foam stability. In general, a degradation of proteins to small peptides and amino acids could be observed over the entire grain processing time. Albumins and globulins show a slight decrease in the concentrations during malting due to the degradation of HMW proteins, which become water soluble upon degradation. Also, during steeping, a protein peak increase was observed in the gliadin fraction, which contributes to haze stability.

To conclude, further research needs to be undertaken to identify the protein peaks which appear during malting. In particular, novel wheat malt-specific proteins which are present in the final malt (and indeed wheat beer) must be characterized. This could potentially reveal functions for these proteins in haze and foam formation/stability or expose their contribution to flavor and viscosity. This evaluation is of particular interest to bridge the gap in current knowledge of the protein influences in wheat malting and brewing.

CHAPTER 6

EVALUATION OF MASHING ATTRIBUTES AND PROTEIN PROFILE USING DIFFERENT GRIST COMPOSITION OF BARLEY AND COMMON WHEAT (*Triticum aestivum* L.)

6.1. 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 (111) as well as the determination of quality related aroma compounds for wheat beer production (8). Sacher (111) 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 enzymatic degradation of high and low molecular substances of cereal grains, started during the malting process (195). 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 (42). Starch is solubilized in three steps: a, *mechanical*: swelling of starch granules; b, *chemical*: gelatinisation of starch granules; c, *enzymatical*: degradation of starch granules (12, 149). 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 (42, 149). 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) (5).

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 (61). 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%) (10). 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 (195). 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 (100, 101, 149). 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. (102, 103). 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 (71, 74, 79, 104-106). 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 (128, 149). 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 chapter 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.

6.2. Materials and Method

6.2.1 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 (112). All analyses are based on approved methods for malting and brewing as described in the EBC (145) or MEBAK handbooks (112).

6.2.2 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 6.1. 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.

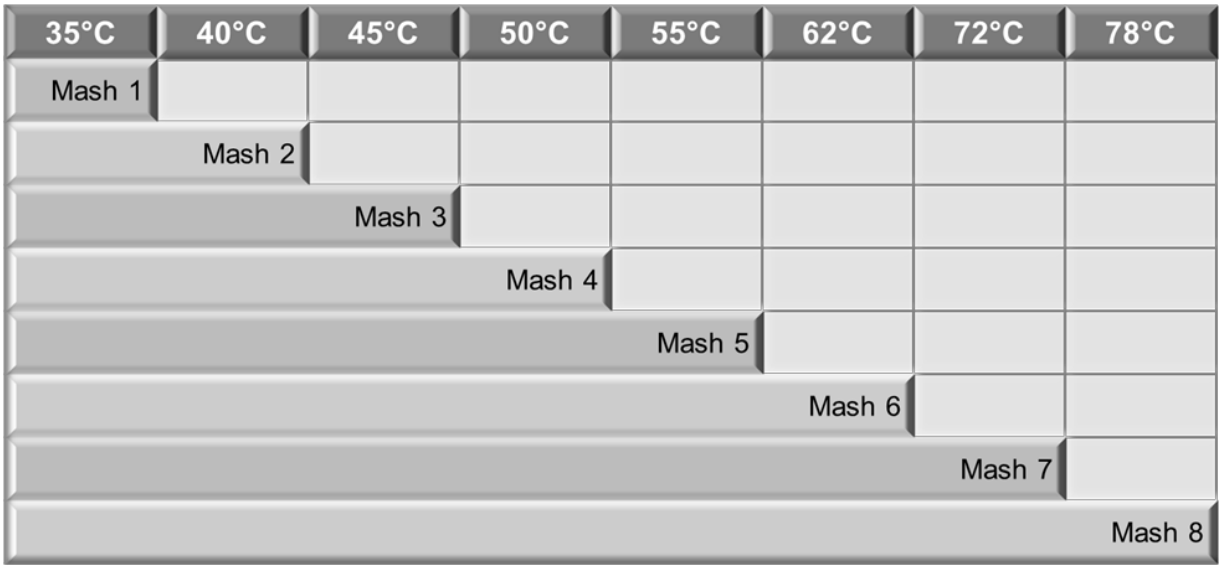


Figure 6.1: Isothermal mashing - sampling plan

6.2.3 Malt and mash analysis

Analytical procedures were carried out at least in triplicate (n ≥ 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 (112, 145, 196) 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 (112). The

apparent attenuation (amount of fermentable sugars) was detected by MEBAK method 4.1.4.10 (112). 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 (112). 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 (111, 147, 169) 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 (112). 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 (112). Wort viscosity was measured using a falling ball viscometer (AMVn-Automated Micro Viscometer Anton Paar, Graz, Austria). β-glucan was quantified using a Skalar working station (Skalar, Breda, Netherlands), following MEBAK method 4.1.4.9.2 The proteolytic enzyme activity (U) was measured according to the method of Brijs (186), 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 (197) and Kiszonas et al. (198). 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.

6.2.4 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).

6.3. 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.

6.3.1 Amylolysis

Figure 6.2 and Table 6.1 shows the devolution of extract yield during mashing and represents one of the most important amylolytic malt quality attributes (5), which are very important for the brew house yield.

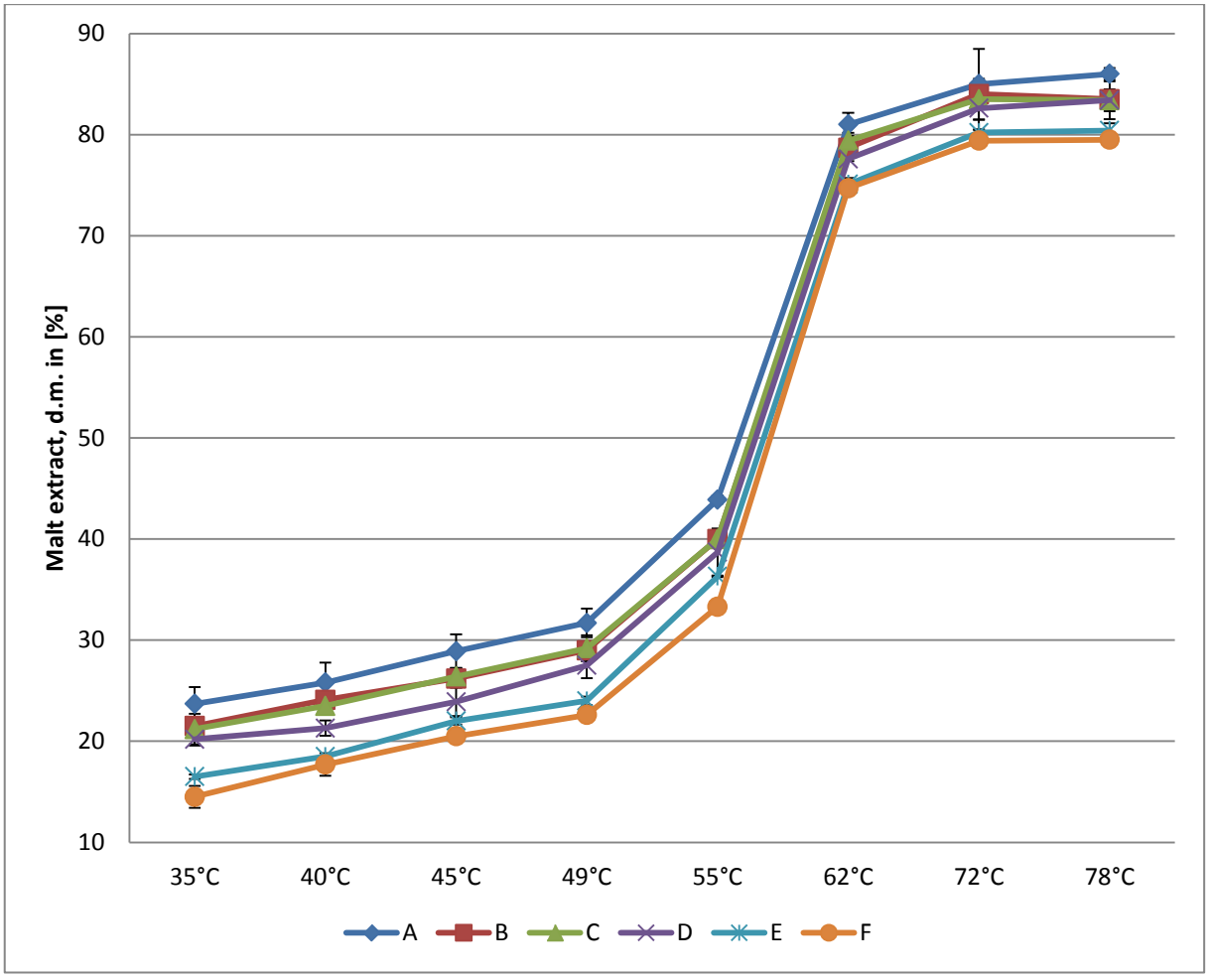


Figure 6.2: Modification of extract yield during mashing process; wheat/barley ratio [%]: A (100/0), B (75/25), C (60/40), D (50/50), E (25/75), F (0/100)

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 (40). 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 (111, 149, 198). 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 (12, 149). In this study a generally increase of the extract values of each grist composition throughout the mashing process can be detected (Figure 6.2). 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 6.1).

Table 6.1: Amylolytic results during different mashing stages; wheat/barley ratio [%]: A (100/0), B (75/25), C (60/40), D (50/50), E (25/75), F (0/100)

Sample	Malt extract in % d.m. (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	23,7	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

Sample	AAL % (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	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

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 maybe 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 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 α-amylase enzymes during mashing are at a pH level of 5.6-5.8 and a temperature of 65-75 °C (149). α-amylase attacks the starch granule from the inside and possess substrates for β-amylase, 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% (149). 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 (149). The AAL depends upon the availability of the fermentable sugars and on the yeast remaining in contact with wort (12). 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 %. (41, 149). The evaluated values (Table 6.1) 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 (149, 198). 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.

6.3.2 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 (61). 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%). Hemicelluloses of wheat are therefore mainly responsible for the high wort viscosity and the pentosans have the biggest impact. Table 6.2 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 6.2). 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 (36, 149).

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 (149).

Table 6.2: Cytolytic results during different mashing stages; wheat/barley ratio [%]: A (100/0), B (75/25), C (60/40), D (50/50), E (25/75), F (0/100)

Sample	Viscosity mPas (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	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

Sample	Arabinoxylan content mg/L (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	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

Moreover, in literature is stated that increased water extractable arabinoxylans (WEAX) cause problems during the brewing process. Complications in the filtration and lautering processes (25, 65), as well as contributions to premature yeast flocculation (66) can occur. Lu et al. (49) 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 (69).

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-L-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 6.2). 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 (69) 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 (49) 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 6.3. 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.

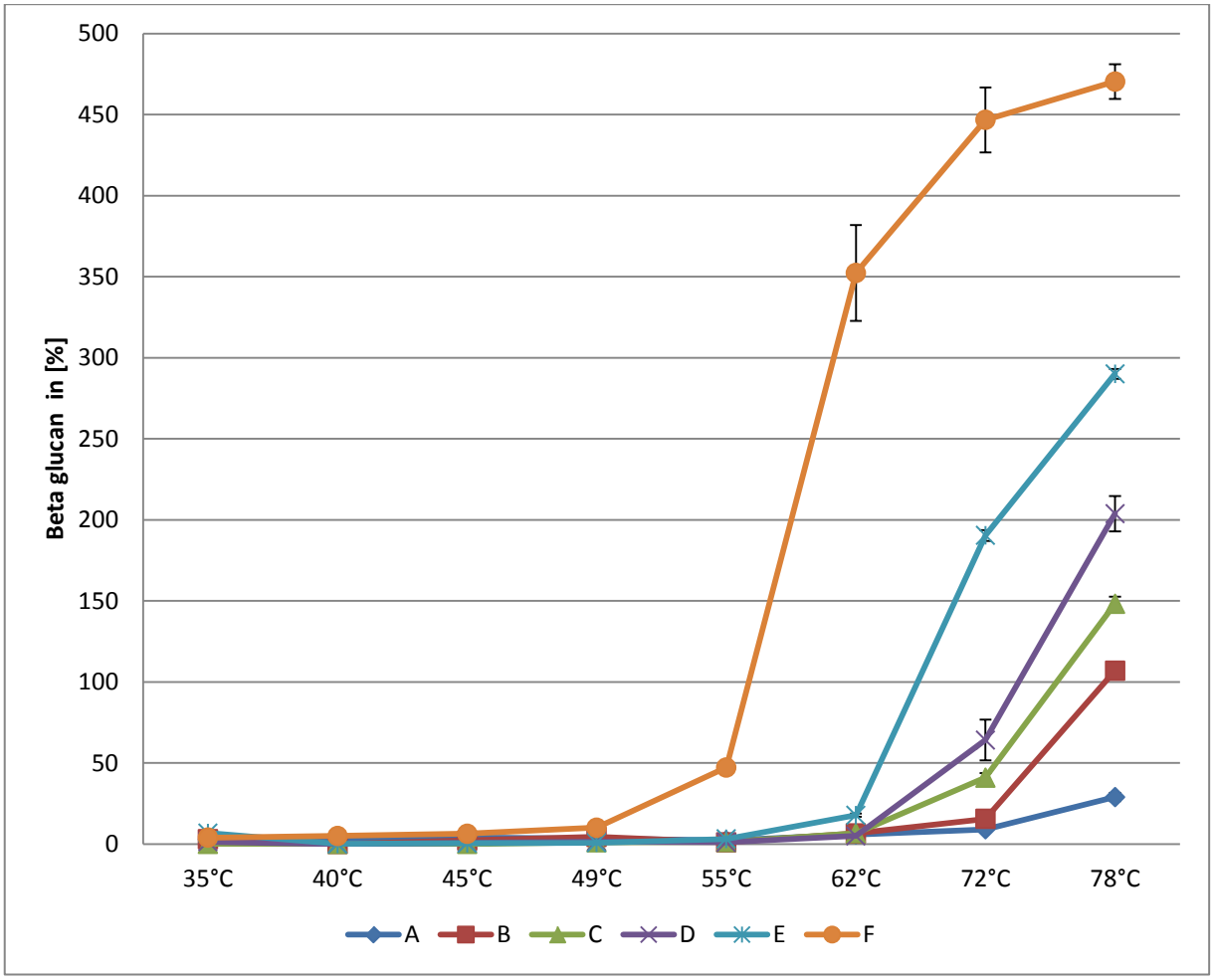


Figure 6.3: Modification of β-glucan during mashing process; wheat/barley ratio [%]: A (100/0), B (75/25), C (60/40), D (50/50), E (25/75), F (0/100)

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 (149). 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.

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.

6.3.3 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 (12) and should lie in the range of 600-800 mg/100g for wheat malt and 650-750 mg/100g for barley malt (111, 112). At the beginning of mashing, soluble nitrogenous substances pass directly into the mash. This can also be clearly seen in Table 6.3, where continuous increases of soluble nitrogen (SN) as well as an overall increase of 130 mg/100mg 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/100g 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/100g 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 (111). Optimal protein degradation depends on solubility, malt enzyme activity and the mashing conditions (i.e., temperature, time and pH level of the mash) (5, 41, 42). 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 (42). This can also be seen in Table 6.3, 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.

Table 6.3: Proteolytic results during different mashing stages; wheat/barley ratio [%]: A (100/0), B (75/25), C (60/40), D (50/50), E (25/75), F (0/100)

Sample	SN in [mg/100g 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

Sample	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

Furthermore, total proteolytic enzyme activity was analysed using haemoglobin as substrate and absorption was ensured against L-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 6.3 and Figure 6.4).

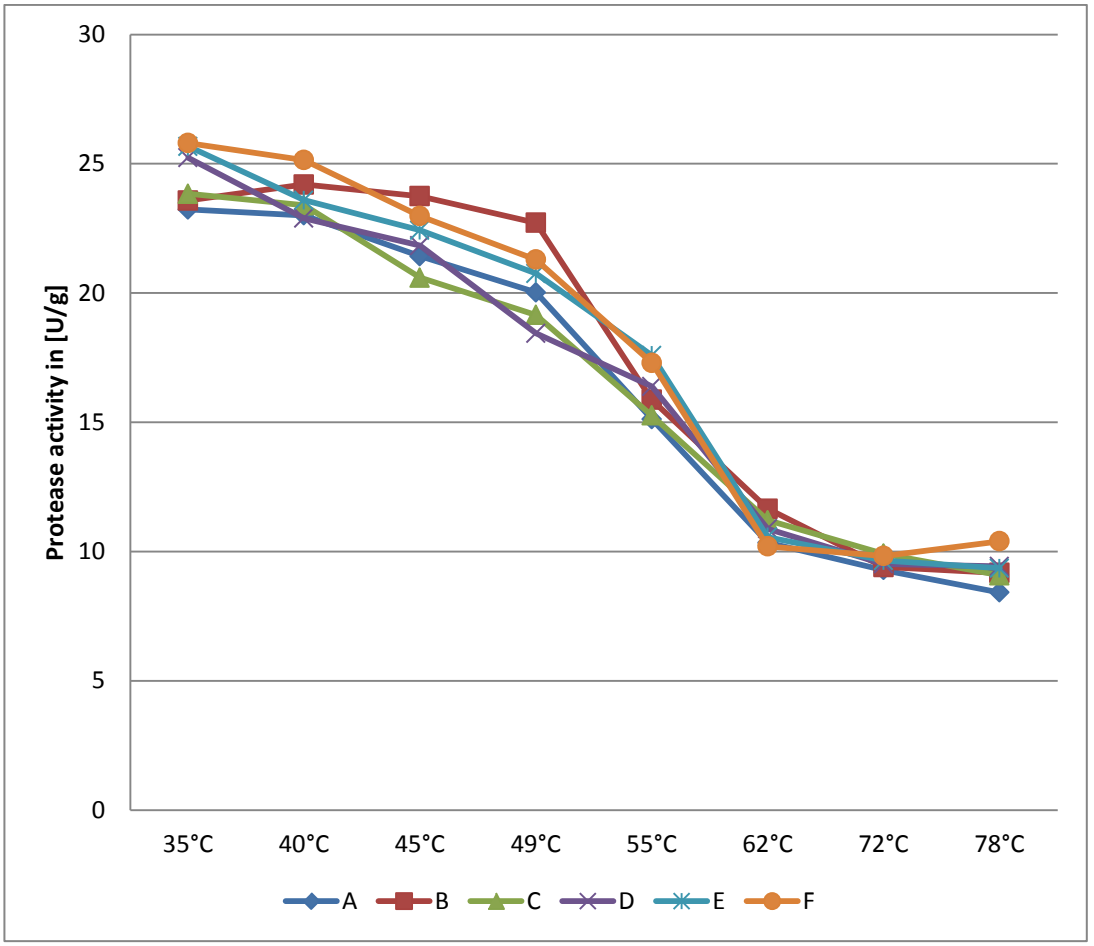


Figure 6.4: Protease activity during different mashing stages; wheat/barley ratio [%]: A (100/0), B (75/25), C (60/40), D (50/50), E (25/75), F (0/100)

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 (80, 112, 197). 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 (77, 149). 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.

6.3.4 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 6.5 to 6.8. 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 (Figure 4.5), 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).

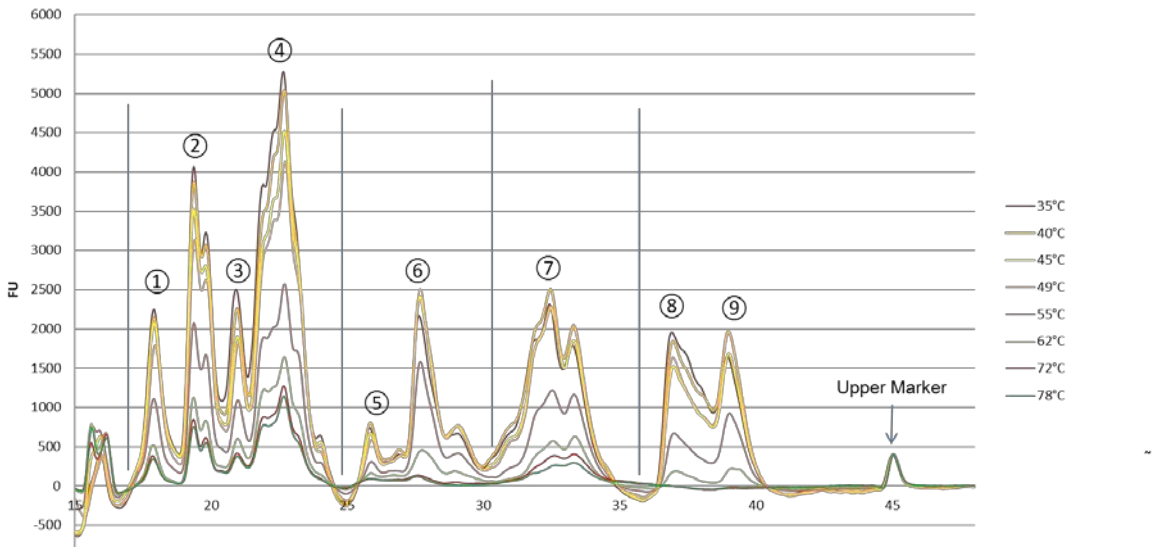


Figure 6.5: Moleculare wheigt distribution of 100% wheat malt (sample A) during mashing process; 1= 6kDa, 2= 7kDa, 3= 9kDa, 4= 14kDa, 5= 21kDa, 6= 25kDa, 7= 40kDa, 8= 55kDa, 9= 63kDa

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 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 (199)) 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 (80), 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 (195, 200). 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 (5). LTP1 as well as protein Z are rich in disulfid bonds, why they are protected against enzymatic degradation and are heat stable (195). 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 (Figure 6.6). 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 (74, 79, 149). 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.

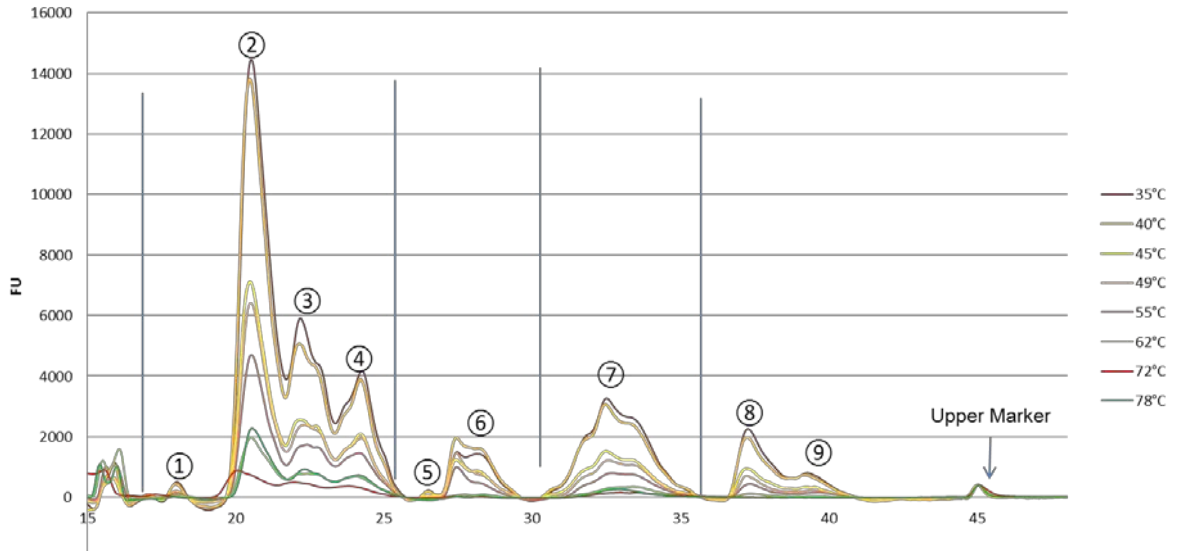


Figure 6.6: Moleculare wheigt distribution of 100% barleymalt (sample E) during mashing process; 1= 6kDa, 2= 9kDa, 3= 14kDa, 4= 17kDa, 5= 21kDa, 6= 25kDa, 7= 40kDa, 8= 55kDa, 9= 63kDa

Figure 6.7 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).

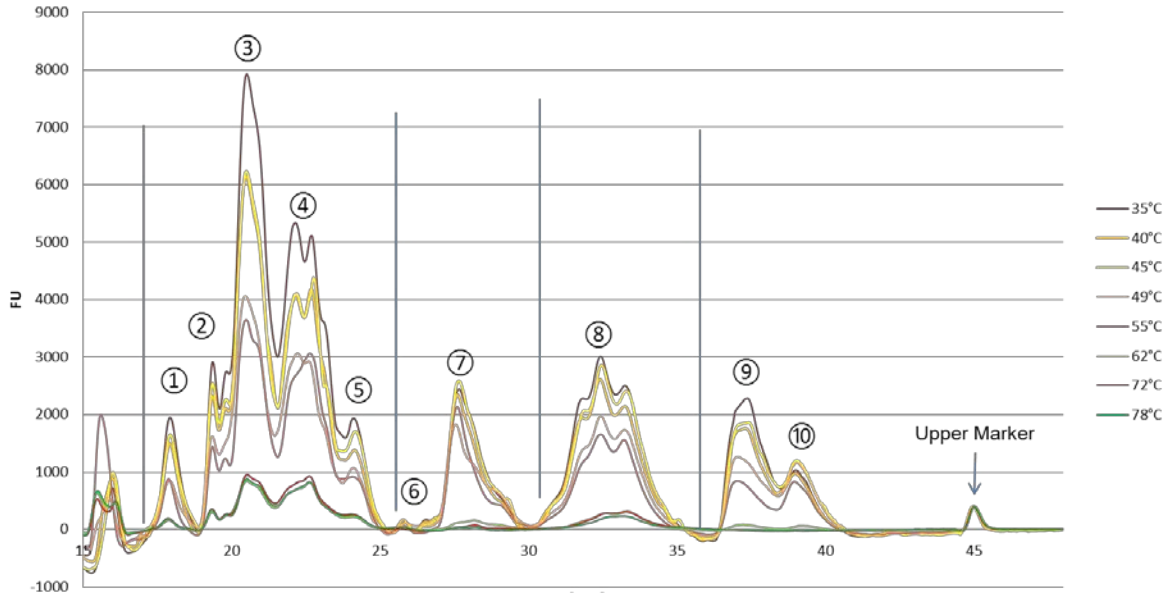


Figure 6.7: Moleculare wheigt distribution of 50%wheat malt and 50% barley malt (sample C) during mashing process; 1= 6kDa, 2= 7kDa, 3= 9kDa, 4= 14kDa, 5= 17kDa, 6= 21kDa, 7= 25kDa, 8= 40kDa, 9= 55kDa, 10= 63kDa

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 (Figure 6.8). 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.

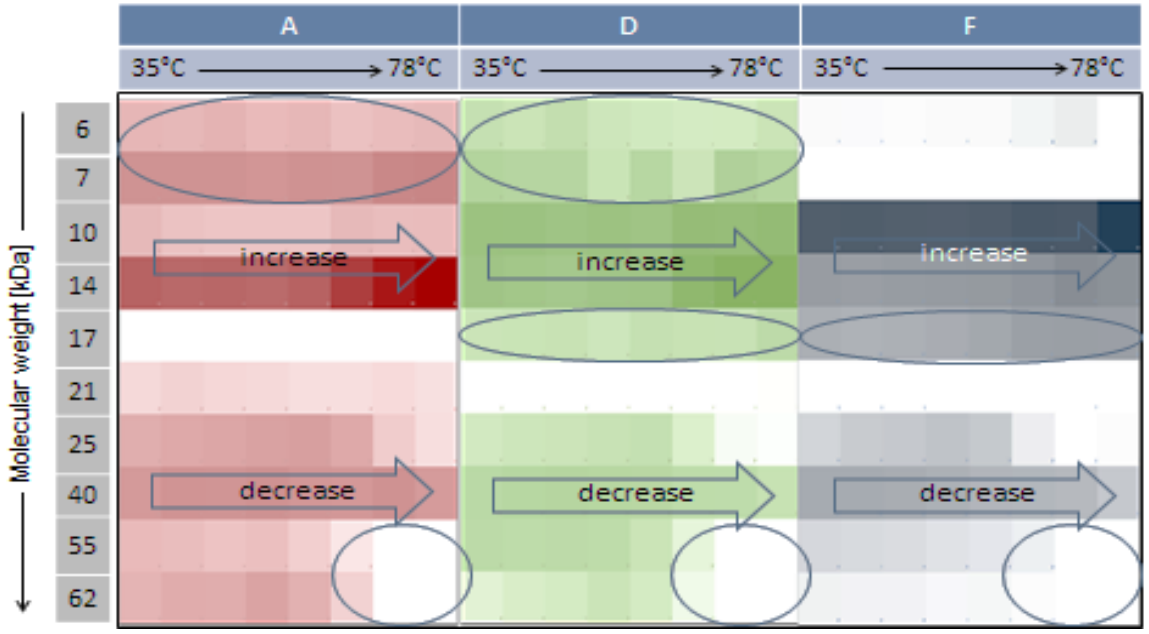


Figure 6.8: Protein interactions of different grist compositions during mashing process wheat/barley ratio [%]: A (100/0), D (50/50), F (0/100)

6.4. 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 chapter 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 (111).

CHAPTER 7

COMMON WHEAT (*Triticum aestivum* L.)

OVERALL DISCUSSION and FUTURE WORK

7.1 Introduction

Beer contains more than 450 different substances, all of which can affect flavour, aroma, colour, stability, and overall beer quality (12). This makes beer one of the world's most complex foods, even though only four ingredients are required in the production of beer: malt, water, yeast, and hops. Malting is the initial step in traditional beer production, whereby the brewing process and the resulting beer type and quality are strongly determined by the quality of malt. Crucial parameters which lead to the required malt quality attributes are: a, the variety itself with its specific constitution b, the malt modification and c, the growing region as well as the harvest year. Compared to the commonly used malting cereal, barley, relatively less is known about the proteolytic, amylolytic and cytolytic impacts of wheat malt during malting and mashing; although wheat proteins and enzymes are most extensively investigated, among cereal grains. Malting is defined as a limited germination under controlled conditions and is regarded as the first step in beer production. It is induced by many complex biochemical processes, like degradation and modification of cell walls, proteins and starch granules in cereal grains (7, 41, 149). The degradation and formation of proteins during malting is essential for the final beer quality and defines the type and quality of the resulting product. One of the most obvious physicochemical changes that occur during malting is enzymatic degradation of the cereal endosperm and its conversion into soluble peptides and amino acids, providing substrates for the synthesis of proteins and the growing embryo (149). Furthermore, mashing is the first biochemical process step of brewing and completes the enzymatic degradation of high and low molecular substances of cereal grains, started during the malting process (38). Specific enzymes are required, similar to the germination process, to solubilize insoluble to soluble malt fractions 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. In these thesis fundamental studies on the selecting of wheat varieties, structural, metabolic and protein changes during malting and mashing were conducted.

7.2 Selecting wheat varieties for malting and brewing purpose

As of now, no specific varieties, only suitable ones, are available and established for brewing purpose. It is of major interest, screening wheat varieties on brewing suitability and focusing more on wheat as a brewing cereal. Although wheat has a long tradition as a raw material for the production of malt and beer, only a few publications exist dealing with limiting values or quality attributes for wheat regarding brewing technology. The authors agree that most malt characteristics are owed to the cultivar-induced variability providing an opportunity for breeding wheat exhibiting brewing quality (6, 80, 111, 128). Cereals selected for the use in brewing industry must have special quality requirements for malt and beer production. Breeding and cultivation efforts for barley have been highly successful in creating agronomically and brew-technically optimal high quality value barley cultivars for malting and brewing industries. Nevertheless, due to its optimisation for the baking industry, wheat 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. Malt specifications were introduced to protect the brewer by ensuring a good brewhouse yield, minimum process problems and the production of a good quality

beer (120). When selecting wheat varieties for brewing purpose, the most important criteria for malsters and brewers are viscosity (Visc), protein content (TN), soluble nitrogen (SN), extract (E) and Kolbach index (KI) (values shown in chapter 2). Too high or too low contents, of the above mentioned attributes, can lead to problems during the brew. However, some wheat varieties available on the market which are suitable for beer production, but most of them show only a few optimum parameters, whereas other parameters are out of the optimal range. Therefore, wheat varieties have to be screened for their suitability for malting and brewing purposes to ensure processability. Annually, with the help of small-scale malting trials a wheat variety catalog is established containing the characteristic specifications of wheat malts of a particular period, usually several harvest years (131). This catalog serves as basis for the selection of wheat varieties currently available on the market. The pre-selection eventually involves whether a wheat variety is accepted as being suitable for brewing or not. Thus, it decides if wheat breeding is successful in the long term. For this reason, the interpretation of analytical data is of highest importance. A proper interpretation of results achieved using any analytical procedure requires that the analyst such as maltster or brewer considers carefully the diverse sources of error associated with the data obtained. The mathematically model, developed and described in chapter 2, was used to evaluate and select wheat varieties on their malting and brewing purpose as well as their stability on environmental impacts.

It was found in chapter 3, that crucial parameters which lead to the required malt quality attributes are: **I)** the variety itself with its specific constitution **II)** the malt modification and **III)** the growing region as well as the harvest year. In the harvest year 2012 significant differences of extract value in quality groups as well as growing region were detected. No significant differences in each growing region between quality group A and B could be detected, accept growing region 5. The quality group

E showed the lowest extract values in all regions. Significant differences in quality groups as well as growing regions could be detected for raw protein as it was already found for extract values. The lowest raw protein contents were detected for quality group C in all growing regions. No significant differences were found for quality group A and B in the growing region itself. With increasing raw protein content, a decrease in amylolytic attributes can be recognized. Concluding these results raw protein content depends on variety and is also influenced by growing region. The nitrogen fractions are more influenced by the malting intensity (over 50 %), followed by the variety itself and environmental attributes (growing region, climate conditions, soil, etc.). Not acceptable raw protein content does not result directly in a not acceptable SN content. Moreover, high raw protein content do not always result in low extract contents, neither in not acceptable KIs. A dependency and influence of growing region on varieties and proteolytic quality malt attributes were investigated.

Significant differences for the quality malt attribute viscosity in each quality group were evaluated. The quality group A results in the highest viscosity values in each growing region. In contrast, quality group E followed by C have the lowest viscosity values. Based on these results, viscosity depends mainly on the variety and is marginal influenced by the growing region and environmental conditions.

During the triannual cultivation series an overall increase of raw protein content was recognized. This leads to the suggestion, that raw protein values which are recommended for wheat malt should be adjusted to the given. The so far opinion, that only wheat varieties from quality group C are suitable for malting and brewing was refused by the findings in this chapter. The varieties Akratos, Akteur (quality group A), Elixer and Hermann (quality group C) show the best quality attributes, and are indicated therefore as the most suitable and stable wheat varieties for malting

and brewing. Due to this finding, variety Hermann and Elixer were used for further studies in this thesis.

7.3 Metabolic and microstructural changes during malting

Confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) were used to visualise the analysed metabolic as well as to investigate the microstructural changes in wheat (*Triticum aestivum* L.) during the malting process. With the help of these powerful optical tools, the organization of starch, proteins and cell walls of cereals could be investigated separately. CLSM and SEM visualized the single stages of the malting process. The imbibing of water and the swelling of the proteins and starches as well as the degradation of them and the cell walls could be detected. CLSM visualized and identified the single compounds in the grain with the help of the specific staining dyes which is an advantage to the SEM.

7.3.1 Amylolytic changes

The starch fraction is the main component found in wheat (10). It is an important indicator for malsters and brewers, since it contributes to the extract and final attenuation of worts (149). Starch is degraded mainly due to the action of β-amylases as well as α-amylases and α-glucosidases. During malting, amylolytic enzymes are formed and activated that break starch down into small and soluble sugar compounds (149). Steeping is traditionally used to increase the moisture content of cereal grain which will induce germination (7, 41). Therefore, hydrolytic pre-existing enzymes are released and new enzymes are produced in the aleurone layer which increases enzymatic activities.

During the steeping period, the grain imbibes water and gets swollen. The B-granules of wheat starch are associated with a higher absorption, earlier hydration and more swelling than the A-granules. Due to of a higher proportion of amorphous zones they are more accessible to water (131). In the first wet steeping stage, the water diffuses first through the pericarp, before in the second wet steeping stage the endosperm get more fluffy and swollen. Although hydrolysis of small wheat starch granules was faster than that of large granules, by the reason of higher surface area per unit weight, other anatomical features such as appearance of pores on the large granules, might have an influence as well 27, 171). However, amlyolytic activity and starch degradation can be detected during steeping. Malt extract as well as final attenuation, both most important amylolytic malt quality attributes (39), increases during steeping from initial yields 48.73 to 72.80% and 51.20 to 64.90%, respectively. Using scanning electron microscopy, the large granules are attacked at the groove and at the localized sites of the surface. Some pinholes were already showing on the surfaces of starch granules in the floury endosperm after the first steeping. An increased number of pinholes were recognized after the end of steeping. After one day of germination the size of the holes increased significantly. Once the surface is eroded, the degradation seems to move through the layers of the granules toward the center. The center is completely digested, whereas only portions of the radial starch are broken down. The erosion of the small granules proceeds differently. In these granules small circular spots, randomly distributed over the surface, are attacked. However, before starch can be degraded to smaller molecules cytolytic and/or proteolytic degradation have to proceed. This can be confirmed by evaluating e.g. viscosity and SN, which show the highest value changes from the beginning of steeping to the end of germination, shown in chapter 4 and 5 (viscosity is decreasing by 0.24 mPas and SN is increasing by 337 mg/100 g malt). During germination, the protein network, a cementric material in which

the starch granules are embedded, is degraded and loses the compact and dense structure, but cell wall fragments were occasionally observed. Loss of this structure allows the release of starch granules making them available to attack by α-amylases. These amylases break down the starch compounds to lower molecular structures (sugars) which are important for the fermentability of the malt in the brewing process. Differences of the degree of degradation of starch granules throughout the kernel could be detected. It seems that starch granules are more degraded near the aleurone layer and germ region than in the inner endosperm. The increasing degradation of the vitreous endosperm might be caused by the enhanced amount of hydrolytic enzymes produced in the aleurone layer. It can clearly be seen that starch granules show enzymatic erosion on the surface and along the equatorial groove.

The degradation process of starch was also measured and investigated by analyzing malting parameters. During the individual malting stages extract increased by ~30% from an initial value of 48.73% in unmalted wheat showing the importance of the malting process. The final attenuation of the wheat malt is also a critical characteristic in malt quality and in this study, it increased by ~20% to 79.15% in the final malted wheat.

7.3.2 Proteolytic changes

In Chapter 3 and 4 an increase in the proteolytic values; Kolbach index (KI), soluble nitrogen (SN) and free amino nitrogen (FAN) could be detected. The KI is an indicator of proteolytic modification of the malt and reflects protein solubilised during malting and preparation of the congress mash. Combined, FAN, free amino acid composition and KI describe protein degradation during the malting process. These attributes are increased from 2.5 to 5-fold in unmalted wheat to malt. During germination, metabolic

changes cause protein degradation forming smaller molecules. Moreover, storage protein modification in germinating cereal grains are divided in three phases: a, the contents of protein bodies in the embryo, scutellum and aleurone layer are hydrolized; b, storage proteins accumulated in the starchy endosperm are mobilized by proteases that are produced and secreted from the aleurone layer and scutellum to produce small peptides; c, and these peptides are hydrolized to amino acids (172). During steeping no visible changes in the protein structure, either in the cell walls could be detected by CLSM or SEM. However, a slight increase of SN as well as FAN could be detected, due to uptake of water, solubilisation during steeping and hydrolyzing of protein bodies. A degradation of the cementric protein matrix as well as a dense protein structure can be observed. In the final malt the protein matrix seems to be more degraded close to the outer layers and embryo, as it was already detected for starch granules. Furthermore, during the malting process an almost 7-fold increase in proteolytic activity was analysed. The activity increased as germination proceeded, to reach a maximum 5-7 days after the start of imbibition (from 2.16 to 14.30 mg of L-leucine/h/g, respectively: unmalted wheat to wheat malt) (172, 179, 180). With an increase of protease activity, an increase of proteolytic charactersitics goes along. These values nearly double in the germination stage. Due to the hydrolysis of the native protein, which gives both high molecular weight protein breakdown products and low molecular weight proteolysis products (peptides and amino acids) (149), the total amount of amino acids increase 3.8-fold with the highest quantity found on the last day of germination (1368.2 mg/100 mL). The majorities of amino acids are heat sensitive and are thus degraded during kilning; however, this excludes alanine, arginine, tryptophan and phenylalanine, which are actually present in increased amounts after kilning.

7.3.3 Cytoloytic changes

Viscosity is the primary malt quality characteristic for wheat as it gives an indication of its processability during beer production, especially lautering and filtration. During malting, cytolytic enzymes degrade cell wall substances into smaller molecules. Thus, they are initiating a breakdown and solubilisation of components which results in lower viscosity values. Wort and beer viscosity is influenced by the macromolecules which are present. For instance, Sadosky et al. (50) found that arabinoxylan, β-glucans and dextrins increase liquid viscosity. Additionally, hydrolytic enzymes are increasingly active and play a major role in breaking down the cell wall structure of cereal grains. Wheat has a lower β-glucan content than barley, but has more arabinoxylans, which may be a causative factor in the increased viscosities of wheat malt. Because enzymatic hydrolysis is continuous during all stages of malting, a 13% decrease in viscosity can be observed from the initial stages of processing to the final malt (unmalted wheat: 2.048 mPas, malted wheat: 1.779 mPas).

The cell walls are clearly apparent throughout the whole grain. β-glucan is mainly found in the inner aleurone cell walls and subaleurone endosperm cell walls (57). They surround and separate the protein network, the aleurone cells, as well as the endosperm and germ cells from each other and appear to be fully intact in the unmalted wheat grain. Cell wall modification and degradation, the cytolysis, is caused by hemicellulase and protein degrading enzymes. During steeping no significant differences in viscosity as well as in the microscopic pictures can be seen, due to the fact that cell wall degrading enzymes have to be activated. These finding can be compared to the results of DeBacker et al. (184). Moreover, the increasing of cell wall degrading enzymes during germination and decreasing after kilning can be compared to other cereals, e.g. barley (149) or oats (185). However, during germination the AX in the cell walls of the starch containing endosperm is broken

down to allow access of hydrolytic enzymes to the starch granules and protein stored within the cells. However, the decrease in viscosity of wheat worts coincides with the breakdown of soluble proteins and starch. This can be detected in the increase in the proteolytic as well as amylolytic attributes (chapter 3 and 4). As it was detected for proteins and starch, cell walls are more degraded and eroded near the embryo as well as the aleuron layer than the upper endosperm.

7.4 Protein changes during malting

In beer production, the protein type, quantity, and size distributions are of particular importance in terms of filtration, foam and haze stability, and fermentability. Barley malt protein characterisation has been the focus of many research articles and, in particular, their influence on and relationship with these specific beer qualities. To contrast wheat with barley, an obvious starting point is to look at the differences in the protein content which is higher in wheat. Wheat also has an increased proportion of high molecular weight (HMW) proteins, a characteristic which has been correlated to superior foam characteristics and enhanced haze formation, when compared to barley malt beer (104-106, 187). To provide a fundamental understanding of how wheat proteins are affected by the increasing proteolytic activity during malting, the four protein fractions; albumins, globulins, gliadins and glutelins, were separated according to Osborne (81) fractionation and analysed using the Lab-on-a-Chip technique. The quantities and relative amounts of the soluble protein fractions change substantially during malting. Nitrogenous substances are lost by leaching during steeping, but there are no significant net changes during malting.

7.4.1 Albumins

This fraction which makes up 14.7% of wheat protein and only 12.1% of barley protein has a typical MW from 12 to 60 kDa. Several protein peaks and peak areas could be detected in unmalted and malted wheat, which were classified into areas 1 to 4 (14-23kDa, 25-30 kDa, 38-50 kDa, 55-63 kDa, respectively). The degradation pattern of proteins is evident in all areas. Merlino *et al.* (191) made a proteomic analysis of albumins and globulins in hexaploid wheat kernels and found that α-amylase-inhibitors are present in LMW albumins (16 kDa), whereas β-amylase is found at ~63 kDa. When comparing raw and malted wheat, it can be hypothesised that decreases in the malt albumin fraction are possibly due to hydrolysis of storage proteins. Jin *et al.* (38) found that glutenin and gliadins are decomposed during malting and are transformed to the albumin fraction. Proteins with MW between 55 to 63 kDa in the albumin fraction increased during malting resulting in a broader peak area, probably due to protein synthesis, the release of some latent proteins and/or the hydrolysis of insoluble hordeins (190). It seems that these proteins are more vulnerable to proteolytic attack and the hydrolysates become water-soluble. As in other studies on barley and malt (77, 187, 190), the 40 kDa protein Z peak was found in unmalted wheat and in smaller amounts in the malt. In wheat LTP1 are also found, in the albumin fraction, and are of great technological importance because of their involvement in beer foam formation. It can be assumed that LTP1 is also degraded during malting as it was shown for barley (77). This decrease is probably due to Maillard-reactions that occur during kilning (192).

7.4.2 Globulins

The globulin fraction has the lowest concentration of all fractions (as indicated in Figure 5.4) and consists of metabolically active and storage proteins (85). They are present in the starchy endosperm of wheat and consist of large (approximately 40 kDa) and small (22-23 kDa) polypeptides. The electropherogram of the salt-soluble wheat fractions, the globulins, of unmalted and malted wheat, are shown. The globulin fractions, like albumins, can also be divided into 4 protein peak area groups (area 1: 14-20 kDa, area 2: 20-26 kDa, area 3: 28-40 kDa and area 4: 46-60 kDa). In the range of 22 to 58 kDa, more specific wheat globulin storage proteins called triticins are found. When we compare unmalted wheat with steeped wheat, there are no detectable protein peaks greater than 40 kDa, showing extensive degradation of the HMW proteins during malting. Wheat globulins, in area 4, are totally degraded during the malting process leading to the assumption that these proteins are hydrolysed for embryo germination.

7.4.3 Gliadins

The alcohol soluble protein fraction of wheat (~32% of wheat protein) has a typical MW from 30-80 kDa. Gliadins can be more specifically classified into ω-gliadins (40-75 kDa) and α-, β-, and γ-gliadins (30-45 kDa). Figure 5.5 shows the electropherogram of unmalted and malted wheat gliadin fractions. Three characteristic peaks areas can be found ranging from 38-60 kDa (area 1), 88- 120 kDa (area 2) and 170-200 kDa (area 3). No significant changes can be detected between wheat and steeping, and thus we can deduce that gliadins are unaffected by imbibing water. The most dramatic degradations can be seen when raw wheat is compared to malt. A previously unreported and unidentified protein peak in area 3, which has a higher MW than typical

gliadins, is detected in all stages of malting. This needs to be further characterised for identification and to elucidate its functionality in malting and brewing processes. During barley malting, a decrease of the prolamin fraction (corresponding to gliadins in wheat) was also detected (77). It can be assumed that wheat gliadins also function as storage proteins, which supply the embryo with peptides and amino acids during germination (85). Wheat gluten, especially gliadins, were found to be involved in haze formation and stabilisation (114). Moreover, the permanent haze intensity increases when wheat malt is used because of proteolysis of wheat gluten proteins (114). Jin et al. (38) also deduced that with an increase in total protein content gliadins and glutenins also increased, and albumins and globulins decrease. Because storage proteins in wheat are known to contribute to the viscoelastic properties of wheat dough (82), it can also be assumed that they influence the viscosity in wheat malt. It is well known that increased protein content is negatively correlated with the starch content and therefore decreases the malt extract. Sacher and Narziss (193) stated that wheat varieties for brewing purposes should have a low protein content to alleviate problems during filtration and lautering and possibly decrease viscosity. This is in contrast to the wheat varieties for baking purposes which have high gluten (protein) contents.

7.4.4 Glutenin

These storage proteins are extractable in dilute acetic acid and have a LMW portion in the range of ~30-40 kDa (area 1) and a HMW portion in the range of ~80-120 kDa (area 2). Glutenins differ not only in their MW from the other Osborne fractions of unmalted wheat, but also in their changes during the malting process. Figure 5.6 shows the electropherogram of unmalted and malted wheat. It can be assumed, that glutenins are strongly influenced by the uptake of water and increasing temperature

during steeping because a 2-fold increase is found in the electropherogram peaks detected at this time. This is followed by a decrease during germination and a complete levelling off of peaks in the malt fraction. It can be assumed that glutenins are totally degraded during kilning (Figure 5.6). This is in agreement with Klose et al. (77) as well as Weiss et al. (194), who found rapid decreases in glutenin during oat and barley malting, respectively, in the first five days of the malting process.

7.5 Metabolic changes during mashing

Mashing is the first biochemical process step of brewing and completes the enzymatic degradation of high and low molecular substances of cereal grains, started during the malting process (195). 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. The aim of chapter 6 is primarily gain a fundamental knowledge of wheat malt during the mashing process. 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). 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.

7.6 Protein changes during mashing

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) (5). 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. 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. 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 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) 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 (80), 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). 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 (108, 195). 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 (5). LTP1 as well as

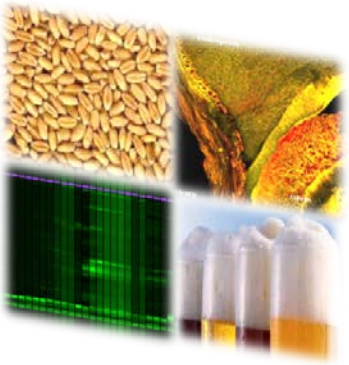
protein Z are rich in disulfid bonds, why they are protected against enzymatic degradation and are heat stable (195). Based on the degradation of higher molecular fractions, the relative proportion of low molecular proteins like LTPs and the protein 14 kDa peak are increasing. Barley malt samples differ during the mashing process first in their protein constitution as well as in their percentage of total protein. For sample F (wheat/barley malt ratio [%]: 0/100) also 9 different peaks could be detected. 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 (39, 74, 79). 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. 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.

7.7 Future Work

In conclusion this thesis reveals an understanding of the importance of wheat variety selection on malt quality attributes and their influence on malting and brewing. A pre-selection as well as long-term growing trial is crucial to identify specific brewing wheat varieties. However, further studies on the suitability of wheat varieties have to be done, in case of optimization using adequate malting regimes to achieve good quality malts. Furthermore, the gap of scientific knowledge was closed regarding metabolic and protein changes during malting and mashing. In general, a degradation of proteins during malting and mashing could be observed. Similarities to the degradation of barley malt proteins during malting were analysed. However, wheat and barley differ widely in their protein profile. This was investigated during the mashing trials, where various grist composition of barley and wheat malt were evaluated. 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. Further studies on enzymatic changes as well as viscosity causing substances during the whole brewing process should be provoked, to increase the processability and quality of the final wheat beer.

Wheat for the application in malting and brewing

- Further studies on the suitability of wheat varieties have to be done, using optimized
  - malting regimes to achieve good quality malts
  - mashing regimes to achieve good quality worts
  - brewing regimes to achieve good quality beers
- Further studies should be provoked to increase the processability and quality of the final wheat beer on
  - optimisation of enzymes and enzymatic degradation in wheat
  - viscosity causing substances during the whole brewing process
- Further studies on wheat malt
  - analytical methods and
  - quality attributes



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CHAPTER 8

APPENDIX

List of Publications

2010: *Evaluation of different yeast strains on the quality of beer produced from malted proso millet (Panicum miliaceum L.)*; Zarnkow, Martin; Faltermaier, Andrea; Back, Werner; Gastl, Martina; Arendt, Elke K. *European Food Research and Technology* vol. 231 issue 2 June 2010, p. 287 - 295

2012: *Bread. Chapter Sixteen;* E. Zannini, A. Moroni, M. Belz, A. Faltermaier, and E. K. Arendt. *In: Bread and other baked goods*. Bamforth C. (Ed). Oxford University Press.

A case of insufficient research - A boon for wheat farmers, a bane for wheat brewers; Andrea Faltermaier and Martina Gastl, Journal of BBII 4/2012, p.44 - 46

2013: *Theoretical treatise on a statistical method for the simple and reliable preselection of wheat malt types for brewing purposes based on generally accepted quality characteristics*; Jean Titze, Andrea Faltermaier, Thomas Becker, Elke Arendt, Martina Gastl, Journal of ASBC

Protein modifications and metabolic changes taking place during the malting of common wheat (Triticum aestivum L.), Andrea Faltermaier, Deborah Waters, Thomas Becker, Elke K. Arendt, Martina Gastl, Journal of ASBC

2014 *Common wheat (Triticum aestivum L.) and ist use as a brewing cereal – a review*; Faltermaier, A., Waters, D., Becker, T., Arendt, E., Gastl, M.: JIBD Volume 120 (2014) 1.

2015 *Evaluating microstructural changes during the malting process by using confocal laser scanning microscopy and scanning electron microscopy.* Faltermaier, A. E., Becker, T., Arendt, E. K., Zarnkow, M., Gastl, M.: European Food Research and Technology (2015). DOI 10.1007/s00217-015-2450-x.

Evaluation of mashing attributes and protein profile using different grist composition of barley and wheat malt. Faltermaier, A., Negele, J., Becker, T., Gastl, M., Arendt, E.:. Brewing Science 68 (2015), 67-77.

List of Oral and Poster Presentations

2010: Faltermaier A., Gastl M., Arendt E.K. and Becker T.; Impact of wheat varieties on the quality of malt, Young Scientists and Technologists in Malting, Brewing and Distilling, Freising, Weihenstephan, Germany

Faltermaier, A., M. Gastl, and T. Becker, Selecting criteria for brewing relevant wheat varieties, in 35th Brewing and Malting Conference 2010: Pilsen, Czech Republic.

Faltermaier A., Gastl M., Arendt E.K. and Becker T.; Microstructural Changes in Wheat Grain (Triticum aestivum L.) during the Malting Process by Using Confocal Laser Scanning Microscopy and Scanning Electron Microscopy, in Master Brewers Association of America, 123rd anniversary convention, Brewing Summit 2010: Providence, Rhode Island, USA.

2011: Faltermaier A., Gastl M.,. Arendt E.K. und Becker T.; Vermälzungs-eigenschaften unterschiedlicher Brauweizensorten, in 44. Technologisches Seminar 2011: Freising, Germany.

Faltermaier A., Gastl M., Arendt E.K. und Becker T.; Einfluss der Keimparameter auf die Malzqualität von Brauweizen, in 8. Rohstoffseminar 2011: Freising, Germany.

Faltermaier A., Gastl M., and Becker T., Changes in protein compositions during malting of common wheat (Triticum aestivum L.) in ASBC 74th Annual Meeting 2011: Ft. Myers, Florida, USA.

Faltermaier A., Gastl M., Arendt E.K. and Becker T.; Modifying the malting conditions of common wheat (Triticum aestivum L.) by using Response Surface Methodology to ensure processability for brewing purpose, in ASBC 74th Annual Meeting 2011: Ft. Myers, Florida, USA.

2012: Faltermaier A., Gastl M., Arendt E.K. und Becker T.; Veränderungen der Proteinzusammensetzung während des Mälzens, in 45. Technologisches Seminar 2012: Freising, Germany

Faltermaier A., Gastl M., Arendt E.K. und Becker T.; Statistische Methode zur einfachen und sicheren Vorselektion von Weizensorten für Brauzwecke auf Basis allgemein anerkannter Qualitätsmerkmale - Anwendungsbeispiel zur Evaluierung von Brauweizensorten, in 9. Rohstoffseminar 2012: Freising, Germany

2013: Faltermaier A., Gastl M., Arendt E.K. und Becker T.; Veränderungen der Proteinzusammensetzung während des Brauens mit 100% Weizenmalz , in 46. Technologisches Seminar 2013: Freising, Germany

Faltermaier A., Gastl M., Arendt E.K. und Becker T.; Evaluierung von Brauweizensorten, in 10. Rohstoffseminar 2013: Freising, Germany

Faltermaier A., Gastl M., Arendt E.K. und Becker T.; Changes in protein profile and enzymatic activity during mashing when using different grist compositions of barley and wheat malt and their influence to beer quality, in EBC 2013, Luxembourg.