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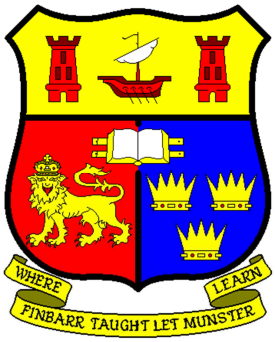
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School of Food and Nutritional Sciences



Fundamental studies on the application of enzymes when brewing with unmalted oats and sorghum

Thesis presented by

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Prof. D.Sc. Dr. Elke K. Arendt

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Abstract

Brewing with up to 40% unmalted cereals such as barley, wheat, rice, and maize is allowed as well as practiced in many European countries (e.g. Belgium, the Netherlands, Luxembourg, France). The use of unmalted oats or sorghum in brewing has great potential for creating new beer types/flavors and saving costs. However, the substitution of barley malt with unmalted oats or sorghum, also referred to as adjuncts, is not only innovative but also challenging. In general, oats have high contents of husk, β-glucan, protein, as well as fat, and thus low extract contents. Sorghum, lesser known in Europe, is gluten-free and usually exhibits high polyphenol concentrations as well as a high starch gelatinization temperature. The overall objectives of this Ph.D. project were: 1) to get a better understanding of the impact of various types and levels of unmalted oats and sorghum on the quality and processability of mashes, worts, and beers; 2) to provide solutions in terms of the application of industrial enzymes to overcome potential problems. For these purposes, a highly precise rheological method using a controlled stress rheometer (Physica MCR 301) was developed and successfully applied as a tool for optimizing process parameters, exogenous enzyme additions, and product quality. Furthermore, eight different oat cultivars were compared in terms of their suitability as brewing adjuncts and two very promising types (husked/naked oats) identified. In another study, the limitations of barley malt enzymes and the benefits of the application of industrial enzymes in high-gravity brewing with oats were investigated. It is recommended to add exogenous enzymes to high-gravity mashes when substituting 30% or more barley malt with oat adjunct in order to prevent filtration and fermentation problems. Pilot-scale brewing trials (60 L) using 10–40% unmalted oats revealed that the sensory quality of oat beers improved with increasing adjunct level. In addition, commercially available oat and sorghum flours were implemented into brewing. It has been found that the use of up to 70% oat flour and up to 50% sorghum flour, respectively, is not only technically feasible but also economically beneficial. In a further study on sorghum was demonstrated that the optimization of industrial mashing enzymes has great potential for reducing production costs. A comparison of the brewing performance of red Italian and white Nigerian sorghum clearly showed that European grown sorghum is suitable for brewing purposes; 40% red sorghum beers were even found to be very low in gluten (<100 ppm).

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Chapter 1

Introduction

Introduction

In recent years, the interest in oats (*Avena sativa*) and sorghum (*Sorghum bicolor*) for food and beverage production has increased considerably due to their potential health benefits. Oat β-glucan has cholesterol-lowering properties and can reduce the risk of coronary heart disease; sorghum grain is gluten-free and thus suitable for people suffering from celiac disease *(1–3)*. Oats represented the most widespread brewing cereal in the Middle Ages *(4)* but lost their significance in beer production since barley proved to be more suitable for malting and brewing purposes *(5)*. Today, they are only used in some specialty beers such as oatmeal stouts to enhance flavor and mouthfeel *(6)*. As a result, very few brewing-related studies on malted oats *(6–15)* and even fewer on unmalted oats *(16–18)* exist at present, with the exception of the studies conducted within this Ph.D. project. More than 70 years ago, Hopkins *(16)*, Thompson *(17)*, and Moritz *(18)* looked into the use of up to 20% flaked oats (including husks) in brewing to overcome shortages in the supply of barley. They arrived at the conclusion that the substitution of malted or flaked barley with unmalted oats should be limited to 10–15% of the total grist in order to maintain product quality and processability. In general, oats comprise a very large proportion of husk (25–30% of total grain weight) compared to barley (6–15% of total grain weight) *(19)*. Oat husk consists of cellulose and hemicellulose (each around 30–35%), lignin (2–10%), ash (3.5–9%), protein (1.6–5%), oil (1–2.2%), starch (<2%), and water-soluble carbohydrates (<1%). It is a poor quality material and the most significant improvement in oat grain quality could be made by breeding for reduced husk content *(20)*. In addition, oats are unique among the cereals in having high contents of β-glucan, protein, and fat *(21)*, which are undesirable characteristics when used as brewing adjunct. Oat β-glucan can interfere with the brewing process by increasing the viscosity of mashes, worts, and beers, depending on molecular weight and concentration *(22,23)*. Crude protein, the most variable of major components in oats, is negatively correlated with starch *(21,24)*. Oats containing a high percentage of fat are particularly susceptible to the development of bitter off-flavors and rancidity during processing *(25)*. In contrast to oats, sorghum kernels exhibit no husks but high polyphenol concentrations *(26,27)* and a high starch gelatinization temperature *(28,29)*. Sorghum is the fifth most important cereal crop in terms of world production after rice, wheat, maize, and barley. It is uniquely well-

adapted to cultivation in the semi-arid tropics of Asia, Africa, and Latin America *(30,31)*. Nevertheless, its versatility makes sorghum a very promising crop for exploitation in Europe, particularly in areas with inferior soil quality *(32)*. The substitution of barley malt with sorghum adjunct at a commercial scale was born out of necessity: 1) the U.S. brewing industry used considerable amounts of sorghum grain in 1943 when brewing materials were scarce *(33)*; 2) the Federal Government of Nigeria banned barley malt imports in 1988, resulting in the establishment of a unique brewing technology on the basis of sorghum *(34)*. Hence, numerous publications on the use of unmalted sorghum in brewing are available to date *(33,35–52)*. All of these studies are based on sorghum types cultivated in Africa (mainly Nigeria), Latin America, or Asia; within this Ph.D. project, the brewing performance of European grown red sorghum was compared to that of established white Nigerian sorghum. Sorghum cultivars are divided into three types based on their genetics and chemical analyses: Type I (non-tannin sorghum) – non-pigmented testa, no tannins, low levels of phenols; type II (moderate-tannin sorghum) – tannins present in pigmented testa; type III (high-tannin sorghum) – tannins present in pigmented testa and pericarp *(53,54)*. High-tannin sorghum cultivars are not suitable for brewing since condensed tannins (proanthocyanidins) can inhibit enzyme activities (e.g. α-amylase) and cause astringent taste as well as dark beer colors *(33,34,53)*. It has been reported that pericarp (seed) color and its intensity are inadequate indicators of presence or content of tannins in sorghum. White, yellow, red, or brown colored sorghum seeds may or may not contain tannins depending on the presence of a pigmented testa *(53,55)*; however, most sorghum cultivars do not have condensed tannins *(34,53)*. Brewing with sorghum generally necessitates the pregelatinization of starch by cooking due to its considerably higher starch gelatinization temperature compared to barley (malt) *(56)*. Furthermore, unmalted cereals exhibit very low/negligible levels of cytolytic, proteolytic, as well as amylolytic enzyme activities in comparison to barley malt since hydrolytic preexisting enzymes are activated and new enzymes are synthesized during the malting process (limited germination of cereal seeds under controlled conditions) *(57)*. As a consequence of this, endogenous barley malt enzymes become the limiting factor when using up to 40% unmalted oats or sorghum for beer production.

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Chapter 2

Brewing with up to 40% unmalted oats (*Avena sativa*) and sorghum (*Sorghum bicolor*): Application of exogenous enzymes? – A review

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Abstract

Brewing with up to 40% unmalted cereals such as barley, wheat, rice, and maize is allowed as well as practiced in many European countries. The use of oats and sorghum as brewing adjuncts has great potential for creating new beer types/flavors and saving costs. In contrast to oats, sorghum is little known within Europe; however, its versatility makes sorghum a very promising crop for exploitation in these temperate-zone regions. This review will describe the brewing-relevant characteristics of unmalted oat and sorghum grain, investigate the role and properties of endogenous as well as exogenous enzymes during mashing, discuss the processability/quality of mashes, worts, and beers produced with up to 40% oat or sorghum adjunct, and reveal the effectiveness/limitations of endogenous enzymes as well as the benefits of the application of exogenous enzymes.

Introduction

In many European countries (e.g. Belgium, the Netherlands, Luxembourg, and France), brewing with up to 40% unmalted cereals, also referred to as adjuncts, is allowed and realized *(1)*. The substitution of barley malt with adjuncts in beer production has the potential to reduce the cost of raw materials and to create a unique beer flavor/aroma amongst others *(2–5)*. Oat (*Avena sativa* L.) and sorghum [*Sorghum bicolor* (L.) Moench] grain are very interesting but different brewing adjuncts. The former is well-established in Europe *(6)*, whereas the latter is produced on a very limited scale. However, its versatility makes sorghum a very promising crop for exploitation in Europe *(7)*. Oats, an annual grass that has its origin most likely in Asia *(6)*, belong to the subfamily *Pooideae* within the family *Poaceae (8)*. They are more cold- and rain-tolerant than other cereals and mainly grown in the Russian Federation, Canada, Poland, Finland, and Spain *(8,9)*. Oat grain is used for both animal feed and human nutrition; it is a staple food in Germany, Ireland, Scotland, and the Scandinavian countries. In recent years, the interest in oats has increased due to the cholesterol-lowering properties of oat β-glucan reducing the risk of coronary heart disease *(6,10)*. Oats are not labeled as gluten-free but can be tolerated by most celiac disease patients *(11)*. Celiac disease, one of the most common lifelong food intolerances worldwide, is an immune-mediated enteropathy triggered by the ingestion of gluten-containing cereals such as wheat (gliadins), barley (hordeins), and rye (secalins) as well as their products in genetically susceptible individuals *(12–14)*. In the meantime, special oat brands are available ensuring minimal cross-contamination with other cereals by strict controls throughout the whole production chain *(15)*. Oat grain is used in a wide variety of products such as breakfast cereals (porridge, muesli), snacks (biscuits, cereal bars), breads, pancake mixes, ice creams, oat-based drinks (oat milk, oat-berry beverages), and yoghurts suitable for people suffering from celiac disease, milk allergy or lactose intolerance *(15–21)*. Furthermore, oat β-glucans are technologically feasible thickening agents used to modify the texture and appearance of food formulations such as soups or salad dressings *(22–24)*. Oat-containing products have not only a high consumer acceptance but also a high market potential *(15,24)*.

Sorghum has its origin in central Africa from where it spread to Asia as well as India *(25)* and belongs to the subfamily *Andropogonoideae* within the family *Poaceae (8)*. It is closely related to maize in terms of both genomic organization and plant form *(26)*. Sorghum is the fifth most important cereal crop in the world after maize, rice, wheat, as well as barley and largely produced in India, Nigeria, the United States of America, Argentina, and Ethiopia *(9,27)*. It is more drought-tolerant than other cereal crops and therefore an important staple food in many semi-arid regions of the developing world, whereas in Western countries it is primarily used as animal feed *(28)*. Unlike wheat, barley, and rye, sorghum contains no gluten proteins being the causative agent for celiac disease and thus has great potential to be used for the production of gluten-free foods and beverages *(29)*. Food products made from sorghum grain include breads *(30–32)*, cakes *(31)*, cookies *(33,34)*, noodles *(35)*, flat breads *(36)*, tortilla chips *(37)*, and other snacks *(38)*.

This review will deal with the use of unmalted oats and sorghum in beer production with a focus on

- their brewing-relevant characteristics;
- the role and properties of endogenous/exogenous enzymes during mashing;
- the processability/quality of mashes, worts, and beers produced with up to 40% adjunct;
- the effectiveness/limitations of endogenous enzymes and the benefits of the application of exogenous enzymes.

Brewing-relevant characteristics of unmalted oats and sorghum

The use of oats or sorghum as brewing adjunct can be innovative and challenging because of some specific grain characteristics. Oats consist of 25–30% DM (= dry matter) husk (Table 1) (barley 6–15% DM) *(39)* composed of cellulose/hemicellulose (each around 30–35%), lignin (2–10%), ash (3.5–9%), protein (1.6–5%), oil (1–2.2%), starch (<2%), and water-soluble carbohydrates (<1%) *(40)*. Several oat cultivars with lower husk contents or without husks (naked oats) and thus higher energy/nutritive values are already available today *(41–43)*. Furthermore, oats differ from other cereals in having relatively high β-glucan, protein, and fat contents *(44)* being undesirable characteristics when used in brewing. In contrast to oats, sorghum kernels have no husks but high polyphenol concentrations and a high starch gelatinization temperature as described in more detail below.

β-Glucan

Oat β-glucan, primarily located in the endosperm cell walls (β-glucan content 75–78% DM *(45)*), is a linear, unbranched polysaccharide build up from about 70% 4-linked and 30% 3-linked β-D-glucopyranosyl units (molecular weight 1–2 × 10^6 g/mol) *(46)*. Its level in oat kernels (groats), influenced by both genetic (predominant) and environmental factors, varies quite widely *(47)* (Table 1). Schnitzenbaumer and Arendt *(43)* reported that naked oats contain significantly less β-glucan than husked oats. The solubility/extractability of mixed-linkage (1→3)(1→4)-β-D-glucan in aqueous systems depends on particle size, temperature, and pH amongst others *(48,49)*. Oat β-glucan exhibits not only a higher solubility/extractability but also a higher molecular weight compared to barley β-glucan *(46,50,51)*. Its high viscosity, controlled by molecular weight and concentration, can adversely affect the brewing process *(3,52,53)*.

Sorghum has a very low β-glucan content (Table 1) in comparison to oats and malting barley (2.8–5.0% DM *(54,55)*). Its cell walls, water-unextractable solids accounting for around 5% of total grain dry weight, consist of predominantly arabinoxylans and cellulose (non-starch polysaccharides). The major part of these cell wall components is located in the pericarp of the sorghum kernel *(56)*. Arabinoxylans present in sorghum are more complex than those present in barley;

the former are highly substituted and contain considerable amounts of uronic acids as well as acetyl groups (glucuronoarabinoxylans) *(56,57)*. Barley (malt) arabinoxylans were positively correlated with wort/beer viscosity *(58,59)* and negatively correlated with beer filtration efficiency *(59)*, whereas glucuronoarabinoxylans from sorghum seem to have little or no impact on the brewing performance *(60)*.

Protein

Crude protein, the most variable of major components in oats *(44)* (Table 1), is negatively correlated with starch *(61)*. Hence, low-protein oat cultivars exhibiting similar protein contents than malting barley (9.0–11.5% DM *(54,62)*) are preferable for brewing purposes *(43)*. It has been found that naked oats have a higher percentage of protein compared to husked oats *(43,63)*, which is primarily caused by the low protein content of hulls (lemma and palea) *(64,65)*. In general, cereal seed proteins are classified into three groups based on their biological functions: 1) storage proteins; 2) structural and metabolic proteins; 3) protective proteins *(66)*. All cereals contain a high proportion of prolamins (alcohol-soluble protein fraction) except for oats and rice whose major endosperm storage proteins are globulins (sedimentation coefficient 11–12S) contributing 70–80% of total groat proteins *(66–68)*. Oat 12S globulins (salt-soluble protein fraction) are hexameric proteins (native molecular weight approximately 330 kDa) consisting of acidic and basic polypeptides linked by disulfide bonds with molecular weights of approximately 33 kDa and 23 kDa, respectively *(65,69)*. Avenins, oat endosperm storage prolamins, account for around 10% of total groat proteins and possess mainly polypeptides with molecular weights from 22 kDa to 43 kDa *(70,71)*. Cereal seed prolamins exhibit lower levels of essential amino acids such as lysine than 12S (legumin-type) globulins explaining the high nutritional value of oat protein compared to other cereal proteins (e.g. wheat, barley, rye) *(66,68,72)*. Furthermore, Robert *et al. (67)* found only a low percentage of glutelins (acid/alkali-soluble protein fraction) in oat groats; this result indicates that, generally speaking, 12S globulins and avenins are the true storage proteins of oats acting as a store of nitrogen, carbon, and sulfur. In contrast, albumins (water-soluble protein fraction) comprising 9–20% of total groat proteins contain most of the metabolically active proteins (e.g. enzymes, enzyme inhibitors) whose major components have molecular weights of 14–17 kDa, 20–27 kDa, and 36–47 kDa *(10,65)*.

Sorghum proteins can be divided into kafirins (prolamin storage proteins) accounting for approximately 70% of the total grain protein and non-kafirins being involved in cellular functions. Kafirins are subclassified based on their molecular weight, solubility, and structure into α-kafirins (molecular weight 23 kDa, 25 kDa; 66–84% of total kafirins), β-kafirins (molecular weight 16 kDa, 18 kDa, 20 kDa; 7–13% of total kafirins), and γ-kafirins (molecular weight 28 kDa; 10–20% of total kafirins). They are found primarily in spherical protein bodies within the sorghum endosperm *(73–76)*; more precisely, α-kafirins are located mainly in the interior of protein bodies, while β- and γ-kafirins are present on the surface of those *(77)*. Sorghum grain hardness (strength), an important economic and end-use quality trait, is influenced by γ- and possibly β-kafirins due to the formation of cross-links (with themselves, other kafirins, matrix protein) *(77,78)*. Ioerger *et al. (78)* reported that vitreous sorghum endosperm (hard) has a greater level of protein cross-linking and thus a larger molecular weight distribution than floury sorghum endosperm (soft).

Fat

The lipid concentration in oat grain (triacylglycerols, phospholipids, glycolipids, free fatty acids, sterols) also varies considerably among different cultivars *(43,79–81)* (Table 1). Peterson and Wood *(82)* reported that the β-glucan and protein contents of oats increased with increasing oil content, whereas the starch content decreased. Besides, Brown and Craddock *(80)* found a low but statistically significant positive correlation between groat oil content and groat weight (Table 1). In contrast to other cereals, oat lipids are not only concentrated in the aleurone layer and the germ but also in the starchy endosperm *(83–85)*. The latter, surface and internal lipids of starch granules, largely affect the gelatinization/pasting properties of oat starch due to a complex formation between fatty acids and amylose *(84,86–88)*. Once the integrity of the oat kernel is disrupted (e.g. milling), the enzyme systems (lipase, lipoxygenase, peroxygenase) are activated and a rapid buildup of free fatty acids occurs, followed by oxidative breakdown *(83,89)*. Oats with a high fat content are particularly susceptible to the development of bitter off-flavors and rancidity during processing *(83)*.

The lipid content of sorghum (Table 1) is generally lower than that of oats, but higher compared to that of barley (1.8–3.6% DM *(90–92)*). Sorghum and barley lipids are mostly located in the germ and bran (pericarp, testa, aleurone layer) region *(91,93)*. Liu *(91)* found a similar fatty acid composition for sorghum and oats differing from that of barley. The former exhibited considerably higher/lower relative percentages of oleic acid (C 18:1)/linoleic acid (C 18:2). Polyunsaturated fatty acids are most sensitive to oxidation (autoxidation, photo-oxidation, enzymatic oxidation) during the mashing process *(94)*.

Starch

Starch represents the major reserve carbohydrate in the endosperm of cereal seeds (Table 1), stored in the form of water-insoluble, osmotically inactive granules *(95)*. Oat starch granules are composed of two types of α-glucan, amylose (22.1–29.8% *(96–98)*) and amylopectin, accounting for approximately 97–98% of the dry weight (minor non-carbohydrate constituents: protein, lipids, ash, phosphorus *(99)*). Amylose is an essentially linear polysaccharide containing around 99% (1→4)-linked and only very few (1→6)-linked α-D-glucopyranosyl units (molecular weight 1×10^5–1×10^6 g/mol). In contrast, amylopectin is a highly branched polysaccharide build up from about 95% 4-linked and 5% 6-linked α-D-glucopyranosyl units (molecular weight 1×10^7–1×10^9 g/mol) *(100)*. Oat starch is present as large compound granules (20–80 μm) and single granules (2–15 μm) that are smooth and irregular in shape *(99,101,102)*. Morphology and size of starch granules, affecting gelatinization and pasting properties, crystallinity, swelling, solubility, as well as enzyme susceptibility, are genetically controlled. However, starch granule size and size distribution are also influenced by environmental factors *(99,102,103)*. Wang and White *(104)* found a positive correlation between the gelatinization temperature of oat starch and its amylose/lipid contents (amylose-lipid complexes). In general, oat starch has a lower gelatinization temperature (Table 1) than barley starch (59.0–64.6°C) *(105,106)* allowing the use of a standard infusion mashing process when brewing with oats.

Sorghum kernels contain both a vitreous (also called translucent, hard, glassy, horny, corneous) and a floury (also called opaque, soft) endosperm fraction *(28,93,107)*. However, the relative proportions of vitreous and floury endosperm vary highly between different sorghum cultivars *(108,109)*. The outer vitreous endosperm is tightly packed with polygonal starch granules that are surrounded by protein bodies embedded in a continuous protein matrix. In contrast, the inner floury endosperm is loosely packed with spherical starch granules covered with a discontinuous protein matrix comprising fewer protein bodies (starch granule size 10–25 μm) *(93,102,107,109–111)*. As a consequence of this, starch of the vitreous endosperm is more resistant to gelatinization than starch of the floury endosperm *(74)*. Furthermore, Beta *et al. (112)* found a significant negative correlation between amylose content of normal, non-waxy sorghum starch (20.9–30.2% *(112–114)*) and floury endosperm proportion, pericarp thickness, as well as polyphenol content of the grain; they also reported a significant positive correlation between starch amylose content and gelatinization temperature, likely due to amylose-lipid complexes. Brewing with sorghum (high starch gelatinization temperature (Table 1)) necessitates the use of a double infusion mashing procedure in which sorghum starch is pregelatinized by cooking before its enzymatic conversion into fermentable sugars.

Polyphenols

Phenolic compounds such as phenolic acids, flavonoids, and condensed tannins are secondary plant metabolites acting as pigments, reducing agents, as well as hydrogen-donating antioxidants amongst others *(115–117)*. Oat hulls exhibit similar levels of polyphenols than oat groats; however, the latter have a significantly higher antioxidant capacity *(118)*. Oat groats are rich in avenanthramides, phenolic antioxidants that are unique to oats *(116,118)*. In general, oats have a considerably lower polyphenol content (Table 1) compared to barley (0.09–0.24% DM gallic acid equivalents *(119–121)*). Polyphenols can improve the flavor stability of beer but also contribute to color, astringency, and haze *(122)*.

Unlike other cereals, some sorghum cultivars have a pigmented testa containing condensed tannins (proanthocyanidins) that protect the grain against fungi, insects, etc. *(123,124)*. Sorghum tannins can inhibit enzyme activities and adversely affect beer quality *(107,125)*. However, most sorghum cultivars do not contain condensed tannins (non-pigmented testa) *(123,126)*. The pericarp (seed) color and its intensity are not reliable indicators of presence or content of tannins in sorghum; grain colors range from white, yellow, red to brown and are caused by anthocyanins (flavonoids) *(107,126,127)*.

Ash

Oats generally have a high ash content (inorganic compounds (Table 1)) in comparison to other cereals *(39,44)*; in particular, they are rich in potassium, phosphorus, magnesium, calcium (major minerals), iron, zinc, and manganese (minor minerals) *(44)*. However, the high content of phytic acid (anti-nutritional factor) in oats combined with their low phytase activity adversely affect mineral solubility. Phytic acid has a strong binding affinity for multivalent metal ions (especially calcium, iron, zinc) resulting in phytate-mineral complexes (insoluble salts) that may be resistant to hydrolysis by phytase *(128–131)*. It is relatively homogenously distributed in oat groats (bran, endosperm), whereas most of the minerals are located in the outer parts of the oat grain (husk, bran) *(132)*. The latter explains the higher levels of ash in husked oats compared to naked oats *(39,43)*.

Sorghum has not only a considerably lower ash content (Table 1) than oats or barley (1.6–2.4% DM *(133,134)*) but also a lower phytic acid content (less chelation of metal ions) *(135,136)*. The major proportion of minerals and phytic acid is present in the germ region of sorghum kernels *(135–137)*. Kayodé *et al. (138)* reported that the concentration of minor minerals (iron, zinc) in sorghum is predominantly influenced by environmental conditions, while its phytic acid concentration is affected by both environmental and genetic factors. Furthermore, Wu *et al. (139)* found a significant positive/negative correlation between ash content and protein/starch contents of sorghum.

Table 2–1. Characteristics of unmalted oat (*Avena sativa*) and sorghum (*Sorghum bicolor*) grain.

Grain characteristics	References	Unit	Oats	Sorghum
Physical properties				
Husk (lemma and palea)	Grausgruber *et al. (39)*; Welch *et al. (40)*	% DM[a]	25–30	N/A[b]
Kernel/groat weight	Brown and Craddock *(80)*; Wu *et al. (139)*; Li *et al. (140)*	mg DM	20–32	20–42
Chemical composition				
Moisture	Schnitzenbaumer and Arendt *(43)*; Girardet and Webster *(89)*; Schnitzenbaumer *et al. (109)*; Agu and Palmer *(141)*	%	12–14	9–12
β-Glucan	Schnitzenbaumer and Arendt *(43)*; Schnitzenbaumer *et al. (109)*; Miller *et al. (142)*; Niba and Hoffman *(143)*	% DM	1.9–5.0	<0.2
Protein[c]	Schnitzenbaumer and Arendt *(43)*; Peterson *(65)*; Schnitzenbaumer *et al. (109)*; Wu *et al. (139)*; Miller *et al. (142)*	% DM	9.7–16.8	9.0–13.5
Fat	Schnitzenbaumer and Arendt *(43)*; Brown and Craddock *(80)*; Wu *et al. (139)*; Schnitzenbaumer *et al. (144)*	% DM	3.8–9.0	2.8–4.8
Starch	Schnitzenbaumer and Arendt *(43)*; Åman *(61)*; Schnitzenbaumer *et al. (109)*; Wu *et al. (139)*; Paton *(145)*	% DM	46.2–66.3	61.0–74.8
Polyphenols[d]	Emmons and Peterson *(118)*; Schnitzenbaumer *et al. (144)*; Afify *et al. (146)*; Dicko *et al. (147)*	% DM	0.02–0.03	0.11–1.40
Ash	Grausgruber *et al. (39)*; Schnitzenbaumer and Arendt *(43)*; Givens *et al. (63)*; Wu *et al. (139)*; Vannalli *et al. (148)*	% DM	2.1–2.8	1.2–1.8
Other properties				
Starch gelatinization temperature[e]	Tester and Karkalas *(96)*; Schnitzenbaumer and Arendt *(106)*; Beta *et al. (112)*; Beta and Corke *(114)*; Rhymer *et al. (149)*	°C	56.2–61.7	65.8–71.0

[a] DM = dry matter.

[b] N/A = not applicable.

[c] Total nitrogen (% DM) × 6.25.

[d] Data expressed in gallic acid equivalents.

[e] Differential scanning calorimetry (peak gelatinization temperature).

Role and properties of endogenous/exogenous enzymes during the mashing process

Enzymes are a large group of proteins that have evolved into highly active and specific catalysts for virtually all physiological reactions. In general, enzymatic catalysis has two main advantages over nonenzymatic catalysis: 1) very high catalytic rates under relatively mild conditions; 2) high reaction selectivity and in many cases stereospecificity *(150)*. Today, enzymes are classified based on the reactions they catalyze into the following six categories *(151)*:

1. Oxidoreductases – Enzymes catalyzing biological oxidation-reduction reactions;

2. Transferases – Enzymes transferring a chemical group, e.g. a methyl or glycosyl group, from one compound to another compound;

3. Hydrolases – Enzymes catalyzing the hydrolytic cleavage of C-C, C-O, C-N and some other bonds, including phosphoric anhydride bonds;

4. Lyases – Enzymes cleaving C-C, C-O, C-N and other bonds by elimination, leaving double bonds/rings, or conversely adding groups to double bonds;

5. Isomerases – Enzymes catalyzing geometric or structural changes within one molecule;

6. Ligases – Enzymes catalyzing the joining together of two molecules coupled with the hydrolysis of a diphosphate bond in ATP or a similar triphosphate.

Unmalted oats and sorghum exhibit very low/negligible levels of cytolytic, proteolytic, as well as amylolytic enzyme activities in comparison to barley malt *(43,109)* since hydrolytic preexisting enzymes are activated and new enzymes are synthesized during the malting process (limited germination of cereal seeds under controlled conditions) *(152)*. Besides, barley proved to be more suitable for malting/brewing purposes than oats or sorghum due to the development of higher hydrolytic enzyme activities (e.g. β-amylase) during germination amongst others *(107,153–156)*. The synthesis of endosperm-degrading enzymes such as endo-β-glucanases, endopeptidases, and α-amylase in the aleurone layer of germinating barley grains is induced by gibberellins (phytohormones), that are primarily

produced in the embryo *(157,158)*. Those enzymes are secreted into the starchy endosperm, where β-amylase is released and activated by cysteine endopeptidase activity (partial proteolysis) *(157–160)*. As a consequence of modifications during the malting process, barley malt contains considerably less β-glucan (79–98% DM reduction) due to the breakdown of endosperm cell walls by β-glucanases *(134,161,162)*, less fat (13–33% DM reduction) due to the hydrolysis of triacylglycerols and the metabolism of released fatty acids *(92,163,164)*, and less phytate (15–50% DM reduction) due to enhanced phytase activity *(128,165)* than unmalted barley (see above). Protein, starch, ash, and arabinoxylan contents show comparatively little change during malting, whereas the polyphenol content increases (8–66% DM) due to the enzymatic release of bound phenolic compounds (higher extractability) *(119,134,161,166,167)*. Endogenous barley malt enzymes become the limiting factor when brewing with up to 40% unmalted oats or sorghum. During the mashing process (temperature/time-controlled extraction of milled cereal grain with water), primarily hydrolases play a key role in the production of easy-processable, high-quality mashes, worts, and beers as discussed in the following:

Cytolytic or cell wall-hydrolyzing enzymes

It is generally assumed that enzymatic hydrolysis of β-glucan involves the esterolytic activity of acidic carboxypeptidase (solubilase; optimum temperature/pH 62°C/6.35) cleaving peptide linkages that bind β-glucan to the cell wall matrix *(168)*. This assumption is, however, in strong contrast to findings reported by Yin and MacGregor *(169,170)* and Yin *et al. (171)* who came to the conclusion that solubilase activity is most likely associated with an endo-1,4-β-glucanase (cellulase; see below) present in barley husks. Their findings were confirmed by Wilhelmi and Morgan *(172)* who demonstrated that the hydrolysis of barley β-glucan by cellulase of type EC 3.2.1.4 under dilute conditions is identical to that of solubilase. Kanauchi and Bamforth *(173)* found that endo-xylanases, arabinofuranosidase, xyloacetylesterase, and feruloyl esterase also promote the solubilization of β-glucan from barley endosperm cell walls, indicating that arabinoxylans (pentosans) together with their ester-linked ferulic acid and acetyl groups restrict β-glucan extraction. Several endo-β-glucanases were identified in barley malt: Endo-1,3-1,4-β-glucanase (licheninase/lichenase; EC 3.2.1.73), endo-1,3-β-glucanase (glucan endo-1,3-β-D-glucosidase; EC 3.2.1.39), and endo-1,4-β-glucanase (cellulase; EC 3.2.1.4)

(151,174) (Table 2). However, the activity of endo-1,4-β-glucanases, hydrolyzing interior (1→4)-β-D-glucosidic bonds in cereal β-glucans and cellulose, is very low and arises predominantly from the husk (fungal origin) *(151,175)*. Endo-1,3-1,4-β-glucanases have a more distinct function; they exclusively cleave interior (1→4)-β-D-glucosidic bonds in mixed-linkage β-glucan (depolymerization), releasing the characteristic tri- and tetrasaccharides 3-*O*-β-cellobiosyl-D-glucose and 3-*O*-β-cellotriosyl-D-glucose, respectively (major oligomeric products) *(176,177)*. In contrast, endo-1,3-β-glucanases do not act on β-glucan chains in which contiguous (1→3)-β-D-glucosyl residues are absent. These enzymes represent pathogenesis-related proteins; they protect seedlings against potential pathogens through their ability to hydrolyze β-glucans commonly found in fungal cell walls *(176)*. Leah *et al. (178)* characterized a β-glucosidase (EC 3.2.1.21) from barley seeds whose substrates include a number of endo-β-glucanase degradation products, indicating its importance in complete hydrolysis of endosperm cell wall polysaccharides (Table 2). Hrmova *et al. (177,179)* reported a β-glucosidase (isoenzyme βII), having a specificity and action pattern characteristic of both β-glucosidases (EC 3.2.1.21) and exo-1,4-β-glucosidases (EC 3.2.1.74), as well as broad-specificity exo-β-glucosidases in germinated barley. Kotake *et al. (180)* identified an exo-1,3-β-glucosidase (EC 3.2.1.58) in barley seedlings, exhibiting a higher activity than exo-1,4-β-glucosidases (EC 3.2.1.74) *(180)* or β-glucosidases (EC 3.2.1.21) *(177)*. Endogenous endosperm cell wall-hydrolyzing enzymes are very heat-sensitive and extensively destroyed during malting (kilning) *(174)*. When substituting barley malt with unmalted cereals in mashing, the combined application of heat-stable exogenous xylanases (solubilization) and β-glucanases (degradation) has proven most effective in reducing mash consistency/wort viscosity and increasing extract yield *(181)*.

Proteolytic or protein-hydrolyzing enzymes

Barley storage proteins are initially solubilized by endopeptidases (hydrolysis of internal peptide bonds) and then further degraded by exopeptidases during malting or mashing. Most endopeptidases (Table 2) belong to one of four classes based on catalytic mechanisms and active site residues: 1) serine endopeptidases (EC 3.4.21.-); 2) cysteine endopeptidases (EC 3.4.22.-); 3) aspartic endopeptidases (EC 3.4.23.-); 4) metalloendopeptidases (EC 3.4.24.-) *(182)*. Zhang and Jones *(183)* found 42 different endopeptidase activities in germinated barley grains of which 64% were

cysteine endopeptidases (optimum pH 3.8–4.8). The latter play together with metalloendopeptidases (optimum pH 5.3–6.5 *(183)*) a major role in protein solubilization during malting/mashing, whereas aspartic and serine endopeptidases play a minor or no role *(182,184)*. Jones *et al. (185)* demonstrated that the overall endoproteolytic activity is not reduced due to kilning (maximum temperature 85°C). In mashing, however, most endopeptidases are rapidly inactivated/denatured at temperatures of 72°C *(186)*. Besides, some of these enzymes are strongly inhibited by endogenous barley/malt compounds; for example, lipid transfer protein 1 forms tight soluble complexes with cysteine endopeptidases *(187)*. Jones and Budde *(184)* reported that approximately one third of the total soluble protein content of worts is already present in unmalted barley, half of it is released during malting, and the remaining part (around 20%) is solubilized during mashing (pH 6.0). Exopeptidases, catalyzing the liberation of free amino nitrogen (amino acids, small peptides), can be classified based on their site of action into carboxypeptidases (carboxy-terminal cleavage products) and aminopeptidases (amino-terminal cleavage products) *(188)*. Mikola *(189)* and Dal Degan *et al. (190)* identified several serine-type carboxypeptidases (EC 3.4.16.-) with complementary substrate specificities in germinating barley grains (optimum pH 4.8–5.7; acid carboxypeptidases); these play a major role in free amino nitrogen production during malting/mashing *(191,192)* (Table 2). In contrast, Strelec *et al. (193)* reported at least six aminopeptidases (EC 3.4.11.-) with optimum activities at neutral/alkaline pH in germinated barley, which therefore have limited relevance in malting/mashing *(194)* (Table 2). Furthermore, a proline-specific dipeptidyl-peptidase IV (EC 3.4.14.5) *(195)* and dipeptidases (EC 3.4.13.-) *(194,196)* are also present in germinating barley grains (Table 2). The use of unmalted cereal adjuncts in brewing can result in inadequate breakdown of endosperm storage proteins (soluble nitrogen/free amino nitrogen deficiency) adversely affecting fermentation and filtration processes as well as beer quality (haze formation, poor foam stability, off-flavors) *(1,194)*; in order to prevent problems like these, exogenous metalloendopeptidases (EC 3.4.24.-) derived from *Bacillus* species are commonly added to brewery mashes *(197,198)*. Besides, the application of a prolyl oligopeptidase (EC 3.4.21.26; proline-specific endopeptidase) from *Aspergillus niger* during fermentation was found to be highly effective in reducing the haze risk and gluten content in final beers *(199,200)*.

Amylolytic or starch-hydrolyzing enzymes

Four endogenous barley enzymes are involved in the conversion of starch into metabolizable/fermentable sugars during germination/mashing: α-Amylase (EC 3.2.1.1), β-amylase (EC 3.2.1.2), limit dextrinase (pullulanase; EC 3.2.1.41), and α-glucosidase (maltase; EC 3.2.1.20) *(1,151,201)*. Sun and Henson *(202,203)* demonstrated that α-amylases and α-glucosidases play the most important roles in the hydrolysis of native starch granules in germinating barley grains. The latter, releasing glucose units from the non-reducing end of mostly short-chain oligosaccharides and maltose (exoamylases), are very heat-sensitive (pH-dependent) and thus of little importance in brewery mashes *(204–207)* (Table 2). Barley malt α-amylases cleave interior (1→4)-α-D-glucosidic linkages of amylose/amylopectin chains (endoenzymes) and are more heat-tolerant than β-amylases acting on the exterior (1→4)-α-D-glucosidic bonds of amylose/amylopectin (exoenzymes) *(1,204)* (Table 2). The activity of α- and particularly β-amylases is adversely affected in very thin as well as very thick/high-gravity mashes due to a shortage of protective colloids (reduced heat stability) and product inhibition, respectively *(208–210)*. In contrast to α- and β-amylases, the debranching enzyme limit dextrinase exclusively hydrolyzes (1→6)-α-D-glucosidic linkages in amylopectin and its α- and β-limit dextrins *(151)* (Table 2). Stenholm and Home *(211)* found a highly significant positive correlation between free (uninhibited) limit dextrinase activity of malts and the fermentability of corresponding worts. During mashing, the limit dextrinase activity can be considerably increased by lowering the mash pH, most likely as a result of cysteine endopeptidase action (disruption of enzyme-inhibitor complex) *(211–213)*. When brewing with unmalted cereals, the addition of bacterial α-amylases (normal/heat-stable; EC 3.2.1.1) and/or pullulanase (EC 3.2.1.41) to mashes has the potential to considerably increase extract yields (high degree of fermentation) and prevent haze formation/turbidity (complete starch degradation) *(1,198,201)*.

Lipolytic or fat-hydrolyzing and other enzymes

Unmalted oats exhibit not only a relatively high fat content (see above) but also a remarkably high lipase activity (EC 3.1.1.3) compared to barley *(214,215)*; however, no correlation between these characteristics was found *(216)*. During processing, oat lipase rapidly catalyzes the conversion of triacylglycerols (non-polar storage lipids)

into free fatty acids, apparently without accumulation of di- or monoacylglycerols; in contrast, the hydrolysis of oat polar lipids is minimal *(217,218)*. Approximately 80% of total fatty acids in oat grain are either monounsaturated (oleic acid (C 18:1), eicosenoic acid (C 20:1)) or polyunsaturated (linoleic acid (C 18:2), linolenic acid (C 18:3)), and therefore can undergo different oxidation/isomerization reactions *(86)*. However, the lipoxygenase activity in oats is very low compared to that in barley, possibly due to the inhibition by natural antioxidants *(218)* (see above). Lipoxygenases (13*S*-lipoxygenase (EC 1.13.11.12), 9*S*-lipoxygenase (EC 1.13.11.58)) catalyze the peroxidation of free polyunsaturated fatty acids to their corresponding hydroperoxides *(151,219)*. Meesapyodsuk and Qiu *(220)* recently identified the gene *AsLOX2* encoding oat lipoxygenase, which catalyzes the synthesis of 9-hydroperoxydienoic/9-hydroperoxytrienoic acids from linoleic/linolenic acids. Hamberg and Hamberg *(221)* demonstrated that these fatty acid hydroperoxides are reduced to their corresponding alcohols and converted into epoxy-hydroxy acids by the activity of oat peroxygenase; the gene *AsPXG1* encoding this enzyme in oats was also recently determined *(220)*. Oat peroxygenase (optimum temperature/pH 45°C/7) catalyzes the strictly hydroperoxide-dependent epoxidation of unsaturated fatty acids and prefers hydroperoxytrienoic over hydroperoxydienoic acids as oxygen donors to oxidize, for instance, oleic acid (most preferred substrate) *(220)*. The resulting epoxy-hydroxy fatty acids are further transformed by oat epoxide hydrolase activity into trihydroxyoctadecenoic acids *(221)*, which may contribute to bitter taste and aging of beers *(1,222)*. In terms of barley, the lipolytic potential increases markedly during malting, resulting in the hydrolysis of more than 80% of triacylglycerols and polar lipids by different lipases (Table 2) after milling and mixing with water. During mashing, a lipid loss of 12–43% (depending on process conditions) occurs, caused by both complexation of free fatty acids with water-insoluble materials and oxidation *(215)*. Arts *et al. (94)* found that enzymatic oxidation of polyunsaturated fatty acids is more important than non-enzymatic oxidation during the mashing process. Doderer *et al. (219)* purified and characterized two lipoxygenase isoenzymes from germinating barley: lipoxygenase 1 (formation of 9-hydroperoxides) and lipoxygenase 2 (formation of 13-hydroperoxides) (Table 2). Their 9-/13-fatty acid hydroperoxide products are cleaved by hydroperoxide lyases (EC 4.1.2.-) (Table 2) and further converted by 3*Z*:2*E*-enal isomerase to generate volatile aldehydes such as 2(*E*)-nonenal (cardboard flavor)/hexanal as well as non-volatile oxo fatty acids

during mashing *(223)*. Hirota *et al. (224)* reported that the use of a malted lipoxygenase-1 null barley line in brewing resulted in improved flavor and foam stabilities of beer. Nevertheless, oxygen-scavenging enzyme activities such as superoxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.6), and peroxidase (EC 1.11.1.7) in barley malt are likely to provide some protection against oxidative damage caused by oxygen radicals during mashing *(151,225)*. Peroxidases are heat-tolerant and catalyze the oxidation of polyphenols (proanthocyanidins) in the presence of hydrogen peroxide (Table 2), adversely affecting polyphenol content, color, flavor, and colloidal stability (haze formation) of beer *(225,226)*. In contrast, polyphenol oxidase (tyrosinase; EC 1.14.18.1) is extremely heat-sensitive and almost completely destroyed during the malting process *(225)*.

Table 2–2. Specificity and optimum conditions of endogenous barley malt enzymes in mashes.

Enzyme activities	References	Substrate	Product	Temperature	pH
Cytolytic enzymes					
Endo-1,3-1,4-β-glucanase (EC 3.2.1.73)	Hrmova *et al. (177)*; Woodward and Fincher *(227)*	(1→3)(1→4)-β-glucan	Tri-, tetrasaccharides	40–45°C	4.7
Endo-1,3-β-glucanase (EC 3.2.1.39)	Heyse *(167)*; Høj *et al. (228)*	(1→3)-β-glucan	Laminaribiose, laminaritriose	40–45°C	4.7–5.0
Endo-1,4-β-glucanase (EC 3.2.1.4)	Sherief *et al. (229)*; Bauer *et al. (230)*	(1→3)(1→4)-β-glucan, cellulose, arabinoxylan	Short-chain oligosaccharides, cellobiose	40–50°C	5.5
β-Glucosidase (EC 3.2.1.21)	Leah *et al. (178)*; Hrmova *et al. (179)*	Tri-, tetrasaccharides; cellobiose, laminaribiose, laminaritriose	β-Glucose	50°C	5.0
Exo-1,3-β-glucosidase (EC 3.2.1.58)	Kotake *et al. (180)*; Hrmova and Fincher *(231)*	(1→3)-β-glucan, (1→3)(1→4)-β-glucan, Tetrasaccharides	α-Glucose	35–40°C	5.0–5.3
Proteolytic enzymes					
Endopeptidases (EC 3.4.-.-)	Jones *(182)*; Jones and Marinac *(186)*	Proteins	Poly-, oligopeptides (large/intermediate/ small)	40–60°C	4.8/6.0
Serine carboxypeptidases (EC 3.4.16.-)	Heyse *(167)*; Mikola *(189)*	Poly-, oligopeptides	Amino acids	50–60°C	4.8–5.7
Aminopeptidases (EC 3.4.11.-)	Heyse *(167)*; Strelec *et al. (193)*	Poly-, oligopeptides	Amino acids	40–45°C	7.2–8.2
Dipeptidyl-peptidase IV (EC 3.4.14.5)	Davy *et al. (195)*	Small oligopeptides	Dipeptides	–	7.2
Dipeptidases (EC 3.4.13.-)	Heyse *(167)*; Sopanen *(196)*	Dipeptides	Amino acids	40–45°C	8.8
Amylolytic enzymes					
α-Amylase (EC 3.2.1.1)	Back *(1)*; van der Maarel *et al. (204)*	Amylose, amylopectin	Oligosaccharides, α-limit dextrins (branched oligosaccharides)	65–75°C	5.6–5.8
β-Amylase (EC 3.2.1.2)	Back *(1)*; van der Maarel *et al. (204)*	Amylose, amylopectin	Maltose, β-limit dextrins	60–65°C	5.4–5.6
Limit dextrinase (EC 3.2.1.41)	Heyse *(167)*; Stenholm and Home *(211)*	α-, β-limit dextrins	Oligosaccharides (unbranched)	60–63°C	5.0–5.5
α-Glucosidase (EC 3.2.1.20)	Back *(1)*; Muslin *et al. (205)*	Maltose, short-chain oligosaccharides	Glucose	35–40°C	6.0
Lipolytic and other enzymes					
Triacylglycerol lipase (EC 3.1.1.3)	Back *(1)*; Poutanen *(232)*	Triacylglycerols	Free fatty acids	55–65°C	6.8–7.0
Lysophospholipase (EC 3.1.1.5)	Poutanen *(232)*; Fujikura and Baisted *(233)*	Lysophospholipids	Free fatty acids	–	8.0
Lipoxygenases (EC 1.13.11.-)	Back *(1)*; Doderer *et al. (219)*	Free polyunsaturated fatty acids	Fatty acid hydroperoxides	45–55°C	6.5
Hydroperoxide lyases (EC 4.1.2.-)	Kuroda *et al. (223)*	Fatty acid hydroperoxides	Aldehydes, oxo fatty acids	–	6.5
Peroxidase (EC 1.11.1.7)	Clarkson *et al. (225,234)*	Polyphenols	Phenoxyl radicals	55–65°C	4.0–5.0

Processability and quality of mashes, worts, and beers produced with up to 40% unmalted oats or sorghum

Oats were the most widespread brewing grain in the Middle Ages *(235)*; nowadays, they are only used in some specialty beers such as oatmeal stouts (flavor, mouthfeel) *(236)*. Hence, very few brewing-related studies on malted oats *(154,155,236–243)* and even fewer on unmalted oats *(3,43,106,144,244–247)* exist at present. In contrast, numerous publications on the use of unmalted sorghum in brewing are available to date *(4,5,109,125,248–264)*. All of these studies are based on sorghum types cultivated in Africa (mainly Nigeria), Latin America, or Asia except the ones previously published by Schnitzenbaumer *et al. (4,109)* comparing the brewing performance of white Nigerian and red Italian sorghum. The substitution of barley malt with sorghum adjunct at a commercial scale was born out of necessity: for example, the U.S. brewing industry used considerable amounts of sorghum grain in 1943 when brewing materials were scarce *(125)*; the Federal Government of Nigeria banned barley malt imports in 1988, resulting in the establishment of a unique brewing technology on the basis of sorghum *(107)*.

Processability of mashes and worts produced with up to 40% oat or sorghum adjunct

More than 70 years ago, Hopkins *(244)*, Thompson *(245)*, and Moritz *(246)* looked into the use of up to 20% flaked oats (including husks) in brewing to overcome shortages in the supply of barley. They arrived at the conclusion that the substitution of malted or flaked barley with unmalted oats should be limited to 10–15% of the total grist in order to maintain product quality and processability. However, Schnitzenbaumer *et al. (3)* recently demonstrated that brewing with up to 40% hammer-milled oats results in acceptable beers, even without the addition of exogenous enzymes. The use of a hammer mill for grinding unmalted oats improves extract yields and prevents pipeline blockages (high husk volume); though, the reported positive effects of intact oat husks on lautering/filtration performance *(155,237,238,244,246)* do not occur. Furthermore, hammer versus roller milling causes lower final mash β-glucan contents (higher solubility/extractability, higher enzyme susceptibility) *(265)* and lower wort viscosities *(3)*. Nevertheless, Schnitzenbaumer *et al. (3)* reported a 97-fold increase of β-glucan in final mashes when substituting 40% barley malt with hammer-milled oats. The

solubility/extractability of oat β-glucan in aqueous systems increases with decreasing particle size (see above) and increasing temperature or pH *(48,49)*. However, naked oats contain more water-soluble and less water-insoluble β-glucan than hulled oats *(43,266)*. The rheological behavior of solubilized oat β-glucan is primarily controlled by its molecular dimensions (molecular weight, intrinsic viscosity) *(46,53)*; in general, larger molecules contribute more to viscosity than smaller but more numerous molecules *(43,49)*. When using unmalted oats in mashing, a rapid increase of β-glucan between 60°C and 65°C occurs in consequence of starch gelatinization (release of cell wall materials) and solubilase activity (see above); at these temperatures, β-glucan hydrolyzing enzymes are largely inactivated (Table 2). This imbalance between solubilization and degradation of high-molecular-weight β-glucan is reflected in mash consistency/wort viscosity *(3,106)*. High viscosities of mashes, worts, and beers can lower the efficiency of many unit operations involved in the brewing process including mixing, stirring, pumping, lautering, wort boiling/cooling, as well as beer clarification/filtration *(267)*. It has been found that the substitution of 20–40% barley malt with hammer-milled oat grain results in significantly decreased filtration/lautering rates, whereas the use of 10% oats has no effect on processability *(3,43)*.

In terms of brewing with sorghum, Schnitzenbaumer *et al. (144)* successfully demonstrated the use of up to 50% commercial wholegrain flour applying a common infusion mashing process (without cooking). When using sorghum grain, however, it is essential to pregelatinize its starch by cooking in order to enable an effective enzymatic hydrolysis *(251,264)* (see above). Gelatinization is defined as the thermal disordering of crystalline structures in native starch granules *(268)*. Pasting, the phenomenon following gelatinization, involves granular swelling, exudation of molecular components from the granule and, eventually, total disruption of the granule *(268)*. As a consequence of these events, the mash consistency/viscosity increases enormously with increasing sorghum levels during cooking (without heat-stable α-amylase); though, sorghum cultivars rich in floury starch were found to cause significantly lower mash consistencies than those rich in vitreous starch. Nevertheless, a good processability can only be ensured by adding heat-stable α-amylase to sorghum mashes before cooking *(109)*; for this reason, all information given below is based on mashing with heat-stable α-amylase. When substituting 10–

40% barley malt with unmalted sorghum, the wort viscosity decreases with increasing adjunct concentration due to lower β-glucan contents. However, the filterability of mashes produced with 20% or more sorghum (variety dependent) decreases as a result of decreasing husk proportions in the total grist (reduced filter cake permeability) *(4,109,255,257,262,263)*. Hence, it is recommended to apply mash filters when using high amounts of cereal adjunct in brewing in order to reduce mash separation times *(4,254)*.

Quality of worts and beers produced with up to 40% oat or sorghum adjunct

Worts produced with 20% or more husked oat grain have significantly lower extract contents than 100% barley malt worts *(3,43,106)*; the substitution of 10–40% barley malt with naked oat grain leads to constant extract levels *(43)*. However, the use of both husked and naked oats causes a marked reduction of nitrogenous compounds in mashes/worts and thus higher pH values (lower buffering potential) *(3,43,106)*. The fermentability (apparent attenuation limit) of worts drops noticeably with increasing amounts of husked/naked oat grain *(3,43)*. On the other hand, worts containing up to 70% commercial wholegrain oat flour show significantly higher extract contents and similar apparent attenuation limits compared to standard worts *(144)*. Furthermore, significant decreases in wort polyphenol concentration and color have been observed when using 20% or more oat grain *(43)*. Final worts (12% w/w extract) brewed with 40% unmalted oats exhibit considerably less glucose, fructose, sucrose, maltose, and maltotriose (total fermentable sugars) as well as higher total fatty acid contents; all amino acids decreased with increasing adjunct levels except for asparagine, which increased in oat worts. Nevertheless, the values for alcohol, residual extract, degree of fermentation, pH, and color obtained from 40% oat beers were found to be still within the range stated for all-malt beers *(3)*. Besides, a positive effect of oats on yeast growth has been observed, probably as a result of higher zinc and fatty acid contents in worts *(3,237,238)*. Yano *et al. (269)* reported that beers produced with 25% or 40% unmalted barley show a higher foam stability than 100% barley malt beers. However, Schnitzenbaumer *et al. (3)* found significantly reduced beer foam stabilities when using 20% or more oat adjunct, most likely caused by lower amounts of total soluble nitrogen and high-molecular-weight proteins, respectively. Yano *et al. (269)* further reported that the use of 40% unmalted barley in brewing adversely affects beer sensory quality. In contrast, Schnitzenbaumer *et al. (3)* demonstrated that

the sensory quality of oat beers improves with increasing adjunct level; 30% and 40% oat-containing beers are rated higher in terms of aroma and purity of taste than 100% barley malt beers. The former beers exhibit considerably lower concentrations of 2-furfural and γ-nonalactone (heat indicators/staling components) as well as acetaldehyde. Besides, their content of higher alcohols (*n*-propanol, isobutanol) is lower, that of esters (ethyl acetate, isoamyl acetate) higher compared to standard beers. Hanke *et al. (237,238)* and Klose *et al. (155)* who brewed with 100% oat malt determined remarkably lower levels of aging indicators in fresh/forced-aged beers, higher flavor stabilities (high reducing power/antioxidant activity) but poor foam stabilities compared to barley malt beers.

With regard to sorghum grain, Goode *et al. (255)* noted significant decreases in wort extract content when substituting 20% or 40% barley malt with adjunct. This is, however, in strong contrast to the findings of Schnitzenbaumer *et al. (4,109)* who reported an increase in extract with increasing sorghum levels, even using a more time- and energy-efficient mashing procedure. Furthermore, brewing with 10–40% unmalted sorghum results in considerably lower total soluble and free amino nitrogen contents as well as higher wort pH values *(4,109,255,263)*. Nevertheless, it has been found that some sorghum cultivars provide significantly more soluble/assimilable nitrogen than others *(109)*. Besides, Bajomo and Young *(264)* demonstrated that mash pH adjustments have little effect on sorghum wort quality. The polyphenol content and color of worts produced with 10–40% white sorghum are lower compared to those of 100% barley malt worts *(4,109,255,262,263)*; in contrast, the replacement of barley malt with red sorghum causes higher wort polyphenol contents and color values *(4,109)*. In terms of fermentability (apparent attenuation limit), Goode *et al. (255)* reported considerable decreases with increasing amounts of unmalted sorghum, even though heat-stable and fungal α-amylases as well as endoprotease have been used. However, Schnitzenbaumer *et al. (109)* did not observe significant differences in fermentability between 40% sorghum and 100% barley malt worts applying only 50% of the recommended heat-stable α-amylase dose. Final worts (12% w/w extract) brewed with 40% sorghum adjunct were found to contain less glucose, fructose, and sucrose but more maltose and maltotriose than standard worts. Their total fatty acid content and composition are similar to those of 100% barley malt worts *(4)*, whereas the concentration of each amino acid is

decreased *(4,263)*. Nevertheless, sorghum worts (up to 50% adjunct) do not seem to have serious adverse effects on yeast fermentation performance *(4,254,263)*. On the other hand, the foam stability of beers brewed with 25% or more unmalted sorghum is considerably reduced compared with that of all-malt beers (protein deficiency) *(4,252,254,263)*. However, white sorghum has a less adverse impact on beer foam than red sorghum, which may result from its lower polyphenol content (less protein-polyphenol complex formation/precipitation) *(4)*. Furthermore, the sensory quality of lager-type beers containing up to 50% sorghum grain was found to be similar to that of standard beers *(4,252,254)*. Delcour *et al. (249)* demonstrated that beers produced with 50% extruded sorghum (infusion mashing) have a significantly better foam stability but an inferior sensory quality compared to those produced with 50% non-extruded sorghum (cooking before infusion mashing). In general, the use of high levels of unmalted sorghum results in beers revealing less acetaldehyde, esters (e.g. isoamyl acetate), and staling components (γ-nonalactone, 3-methylbutanal, 2-phenylethanal) as well as more higher alcohols (isobutanol, 2- and 3-methylbutanol) *(4,253)*. With regard to flavor stability, Schnitzenbaumer *et al. (4)* reported acceptable test scores for 40% white and red sorghum beers (forced-aged) exhibiting considerably lower concentrations of aging indicators than 100% barley malt beers. Besides, the substitution of 40% barley malt with different sorghum types significantly reduced the gluten content of beers; 40% red sorghum beers were even found to be very low in gluten *(4)*. Table 3 shows the quality parameters of worts and their corresponding beers brewed with 40% oat or sorghum adjunct under similar process conditions (e.g. identical brewing water, barley malt, hops, yeast, milling, fermentation).

Table 2–3. Quality of worts (12% w/w extract) and their corresponding beers produced with 40% unmalted oats or sorghum.

Quality criteria	Method	Unit	40% oats[a]	40% sorghum[b]
Worts (12% w/w extract)				
Viscosity	Falling ball viscometer	mPa·s	1.901	1.787
Total soluble nitrogen	Kjeldahl method	mg/L	817	501
Free amino nitrogen	Ninhydrin method	mg/L	131	98
pH	pH meter		5.73	5.63
Fermentable sugar composition				
Glucose	HPLC	g/L	3.7	5.3
Fructose	HPLC	g/L	0.9	0.6
Sucrose	HPLC	g/L	1.2	2.8
Maltose	HPLC	g/L	56.3	62.6
Maltotriose	HPLC	g/L	8.9	13.8
Amino acid composition				
Class A amino acids	HPLC	mg/100 mL	52.9	42.2
Class B amino acids	HPLC	mg/100 mL	27.4	18.3
Class C amino acids	HPLC	mg/100 mL	21.9	16.8
Fatty acid composition				
Saturated fatty acids	GC	mg/100 mL	1.48	0.30
Single unsaturated fatty acids	GC	mg/100 mL	0.44	0.05
Polyunsaturated fatty acids	GC	mg/100 mL	0.03	0.17
Beers (based on worts 12% w/w extract)				
Alcohol	Anton Paar Alcolyzer	% v/v	4.9	5.0
Apparent extract	Anton Paar Alcolyzer	% w/w	2.1	2.7
Apparent degree of fermentation	Anton Paar Alcolyzer	%	81.3	77.4
pH	pH meter		4.4	4.3
Foam stability	NIBEM-T meter	s	223	241
Aroma compounds				
Acetaldehyde	Headspace GC	mg/L	7.1	8.4
Higher aliphatic alcohols	Headspace GC	mg/L	104.2	119.6
Esters (ethyl acetate, isoamyl acetate)	Headspace GC	mg/L	11.2	8.9
Vicinal diketones (diacetyl, 2,3-pentanedione)	Headspace GC	mg/L	0.4	0.2
Aging indicators				
Heat indicators	GC	µg/L	27.0	46.5
Oxygen indicators	GC	µg/L	26.5	19.5
Staling components	GC	µg/L	72.5	66.0
Sensory quality (5-point scale)				
Aroma	DLG		3.9	3.7
Purity of taste	DLG		3.9	3.7
Fullness of body	DLG		4.4	4.3
Carbonation	DLG		4.5	4.5
Quality of bitterness	DLG		4.1	4.1

[a] Reference: Schnitzenbaumer *et al. (3)*; Brewing with 40% oats 'Lutz' (60% barley malt 'Fr Sebastian').

[b] Reference: Schnitzenbaumer *et al. (4)*; Brewing with 40% commercial red Italian sorghum (60% barley malt 'Fr Sebastian').

Effectiveness/limitations of endogenous enzymes and benefits of the application of exogenous enzymes

While brewing with up to 40% unmalted oats without the addition of industrial enzymes is technically feasible *(3)*, there is considerable room for improvement with respect to processability and quality. The degradation of high-molecular-weight substances such as β-glucan, protein, and starch by endogenous/exogenous enzymes during the mashing process leads to continuous changes in mash consistency/viscosity. Schnitzenbaumer *et al. (247)* developed a highly precise rheological method for monitoring those consistency changes while mashing, suitable for the use of unmalted oats amongst others. The determination of mash consistency is of great importance when brewing with adjuncts, particularly with regard to process/enzyme optimization and quality control. In terms of oats, the mash consistency increases considerably with increasing adjunct concentration *(247)*. It was found that it is impossible to reduce the viscosity of 40% oat-containing mashes to a level comparable to that obtained with 100% barley malt by extending the cytolytic/proteolytic rest (0.5–4.0 h) as shown in Figure 1 (Schnitzenbaumer and Arendt, *unpublished results*). This reduction in mash consistency has, however, been achieved with the addition of exogenous enzymes. Hence, it is recommended to apply commercial β-glucanase to mashes containing 30% or more unmalted oats (rich in β-glucan) in order to prevent lautering/filtration problems *(106)*. With respect to nitrogenous compounds, Schnitzenbaumer *et al. (3,4)* found that free amino nitrogen levels obtained with 40% oat adjunct and without enzyme addition are still sufficient for optimal yeast growth and fermentation. Nevertheless, the application of exogenous endoprotease to mashes may enhance yeast fermentation performance when substituting more than 20% barley malt with oats, particularly with regard to high-gravity brewing. Side effects of extensive protein degradation are lower wort pH values (higher buffering potential) and increased wort colors (excessive formation of Maillard products) *(106)*. Extract levels of worts produced with 20–40% oat adjunct can only be slightly (but statistically significantly) improved by adding α-amylase and pullulanase; this indicates the high effectiveness of endogenous amylolytic enzymes in brewery mashes.

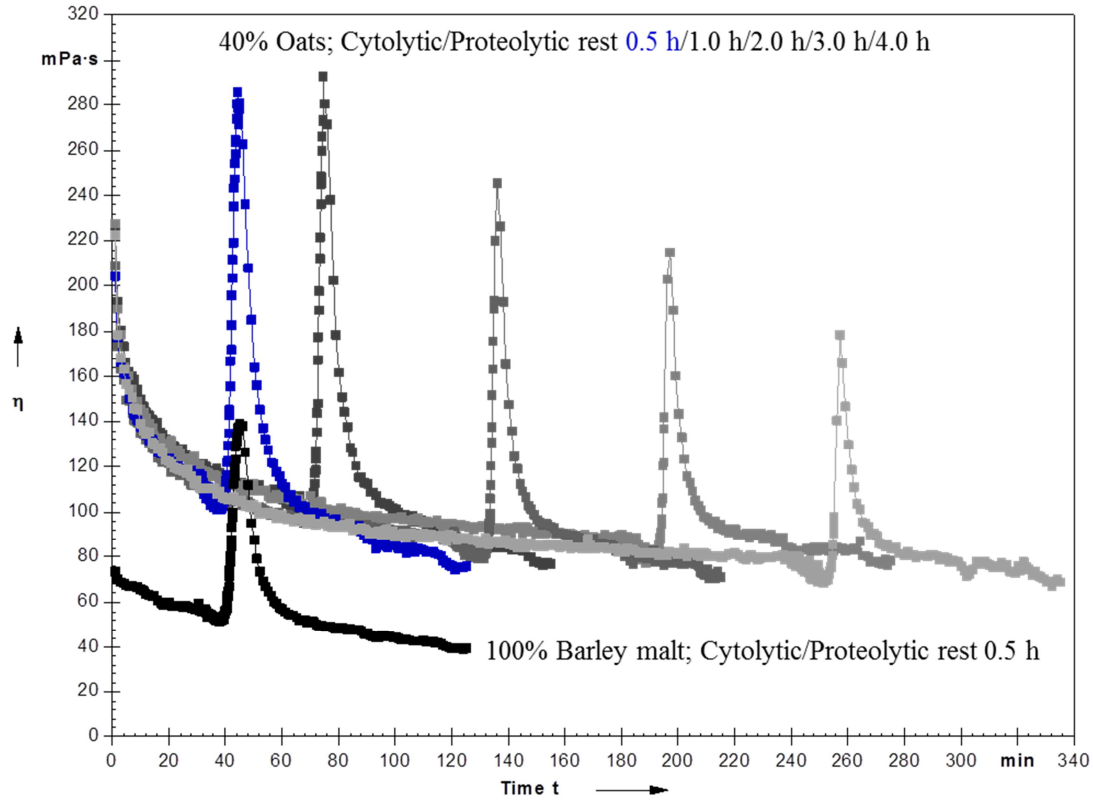


Figure 2–1. Rheological profile of 100% barley malt mash (reference) and 40% oat-containing mashes with varying cytolytic/proteolytic mash rest times at 50°C (Schnitzenbaumer and Arendt, *unpublished results*).

The use of up to 40% unmalted sorghum in brewing necessitates the application of heat-stable α-amylase to sorghum mashes before cooking (high starch gelatinization temperature) in order to reduce the high mash consistency/viscosity caused by pregelatinized sorghum starch and to increase wort extract *(109)*. Some studies about brewing with 100% unmalted sorghum indicate that wort quality and processability increase with increasing enzyme concentrations *(256,259)*; however, Desobgo and Nso *(260)* observed a rise in wort turbidity with increasing dosage of heat-stable α-amylase. Schnitzenbaumer *et al. (109)* recently demonstrated that 50% of the recommended heat-stable α-amylase dose is sufficient for 10–40% sorghum adjunct to ensure a good processability and high extract yields. Besides, the addition of endoprotease (sorghum mash) and β-glucanase (total mash) as recommended had no significant effect on mash consistency/filterability or wort quality (e.g. viscosity, pH, total soluble and free amino nitrogen) *(109,264)*. Goode *et al. (255)* showed that combinations of heat-stable (sorghum mash) and fungal (total mash) α-amylases are

most effective in improving the filtration rates of 20% or 40% sorghum-containing mashes. The combined application of heat-stable α-amylase and endoprotease (sorghum mash) was found to increase total soluble nitrogen levels but also wort color (Maillard reaction); though, the highest free amino nitrogen levels were obtained by adding fungal α-amylase over and above these enzymes. In terms of wort extract, viscosity, and pH, however, the addition of different enzyme combinations (endoprotease, heat-stable and fungal α-amylases) to mashes had no significant effect *(255)*. Discrepancies between reported findings concerning the effectiveness of exogenous endoprotease when brewing with up to 40% unmalted sorghum might be due to large differences in performed mashing procedures (e.g. proteolytic mash rest times) *(109,255)*. A somewhat different approach to maximize extract yields was taken by Omidiji and Okpuzor *(258)* who investigated the enzymatic recovery of extract from cold trub derived from brewing with unmalted sorghum (non-alcoholic beverages); they achieved promising results applying a combination of heat-stable α-amylase and β-glucanase. Nevertheless, the course for successful brewing with up to 40% unmalted cereal grain must be set in the brewhouse by optimizing both mashing parameters and enzyme applications.

Conclusion

The use of oats and sorghum as brewing adjuncts can be innovative but also challenging. Oats generally have relatively high contents of husk, β-glucan, protein, as well as fat, and thus low extract contents. In contrast, sorghum exhibits no husks but usually high polyphenol concentrations and a high starch gelatinization temperature compared to barley malt. However, when substituting up to 40% barley malt (main enzyme source) with unmalted oats or sorghum, endogenous enzyme activities become the limiting factor. In order to take maximum advantage of endogenous enzymes during mashing, it is essential to know their roles and properties. This review provides comprehensive and up-to-date information on endogenous barley malt enzymes as well as commercial enzyme applications in mashing. Brewing with up to 40% unmalted oats and sorghum is not only technically feasible taking into account their specific grain characteristics but has also great potential. The flavor/aroma of oat- or sorghum-containing beers is rated similar or even higher than that of all-malt beers. By means of commercial enzymes, both processability and quality of mashes, worts, and beers produced with high amounts of oat or sorghum adjunct can be considerably improved; for economic reasons, however, the application of enzymes has to be optimized/minimized. Further research is needed to overcome problems such as reduced beer foam stabilities when brewing with up to 40% unmalted oats and sorghum.

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Chapter 3

Objectives

Objectives

The overall objectives of this Ph.D. project were: 1) to get a better understanding of the impact of various types and levels of unmalted oats and sorghum on the quality and processability of mashes, worts, and beers; 2) to provide solutions in terms of the application of industrial enzymes to overcome potential problems involved with their use as brewing adjuncts.

The specific objectives were:

- to develop a highly precise rheological method for monitoring changes in mash consistency during the mashing process; this method represents a tool for the optimization of commercial enzyme additions, mashing parameters, material and product qualities (Chapter 4).
- to compare different oat cultivars in terms of their suitability as brewing adjuncts in order to identify the most promising types (Chapter 5).
- to investigate the limitations of endogenous barley malt enzymes and the benefits of the application of industrial enzymes in high-gravity brewing with oat adjunct (Chapter 6).
- to evaluate the impact of various levels of unmalted oats on the quality and processability of mashes, worts, and beers produced at pilot-plant scale (Chapter 7).
- to determine the advantages and limitations of the use of commercially available oat and sorghum flours for beer production (Chapter 8).
- to compare white Nigerian and red Italian sorghum as brewing adjuncts as well as to optimize the application of commercial enzymes to sorghum mashes (Chapter 9).
- to evaluate and compare the impact of white Nigerian and red Italian sorghum on the quality of worts and beers brewed at pilot-plant scale (Chapter 10).

Chapter 4

Statistical comparison of a new rheological method for defining changes in mash consistency during mashing with the established Rapid Visco Analyser

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Abstract

The determination of mash consistency proved to be difficult but is of great importance particularly with regard to process and quality control. Therefore, the aim of this study was to develop a new rheological method for precisely determining changes in mash consistency occurring during the mashing process. For that purpose, five mashes with various levels of unmalted oats (0–40%) have been analyzed using a Physica MCR rheometer equipped with a paddle-shaped rotor enabling mash particles to be kept in suspension throughout the rheological measurement. For validating this new method, a statistical comparison with the established Rapid Visco Analyser (RVA) has been carried out. For this purpose, the mash consistency curves have been described by regression functions with the aim to determine characteristic curve points mathematically correct. As a result, the start and end point of starch gelatinization/liquefaction have been well-defined. By calculating the coefficients of determination, good to very good linear correlations between respective curve values and adjunct levels have been found for both methods (MCR and RVA). By calculating the repeatability, however, it has been revealed that the precision of the MCR method is significantly better than that of the RVA method.

Introduction

Mashing is a time-consuming and cost-intensive process step in the production of beer. Moreover, it has a substantial effect on both quality and processability of mashes, worts, and beers *(1,2)*. The purpose of mashing is to economically produce easy-processable mashes and worts providing the basis for high-quality beers *(3)*. During mashing, three important enzymatic degradation processes occur:

1. Cytolysis – Degradation of cell wall polysaccharides, particularly β-glucans by β-glucanases;

2. Proteolysis – Degradation of proteins into peptides and free amino acids by proteases;

3. Amylolysis – Degradation of gelatinized starch into fermentable carbohydrates by α- and β-amylases *(4–6)*.

The degradation of high-molecular-weight substances by endogenous malt enzymes leads to continuous changes in mash viscosity *(7,8)* or more precisely mash consistency. At present, mainly standard methods for determining the viscosity of worts and beers (Newtonian fluids) using falling ball viscometers, rotational viscometers or capillary viscometers are known *(9,10)*. However, only a few promising approaches for monitoring the consistency of mashes (non-Newtonian fluids) during the mashing process have been published: Hoog *et al. (11,12)*, Herrmann and Sommer *(13)*, Herrmann *et al. (14)*, Götz *et al. (15,16)* and Goode *et al. (17,18)*. The determination of the consistency of disperse systems like brewery mashes proved to be difficult but is of great importance particularly with regard to process and quality control *(19)*. While the viscosity of worts and beers is constant when the shear rate is increased at a constant temperature, mashes exhibit shear-thinning behavior, meaning that they show lower consistencies at higher shear rates *(20)*. However, two Newtonian ranges regarding the rheological behavior of non-Newtonian fluids have been observed. The 1st Newtonian range is characterized by low shear rates having no significant impact on polymers. During the non-Newtonian range, macromolecules are unwound and orientated as a consequence of increasing shear rates resulting in decreasing consistencies. At very high shear rates, the macromolecules are completely unwound and orientated which is why the

consistency is constant when the shear rate is further increased (2nd Newtonian range) *(16,21).* Götz *et al. (15,16)* demonstrated that the correlation between the Nuclear Magnetic Resonance (NMR) relaxation time T_2 and the dynamic viscosity η is applicable to not only Newtonian fluids (water, wort, beer) but also non-Newtonian fluids like brewery mashes. Suspensions, however, do not exhibit that simple potential dependence between consistency and corresponding relaxation time. Nevertheless, the T_2-η correlation persists for characteristic consistencies at very low and very high shear rates (η_0, η_∞) as noted above and can therefore be employed to determine mash consistency by means of the corresponding T_2-relaxation time (indirect method). With the help of so-called Rheo-NMR probeheads, demixing of mashes can be prevented *(19).* Goode *et al. (17)* developed a rheological method to detect changes in mash consistency during mashing using a Bohlin CS-50 rheometer. The instrument, however, had to be equipped with a specially designed but non-defined six-paddle rotor for keeping mash particles in suspension throughout the rheological measurement. Thus, it was neither possible to exactly define the applied shear rate nor to give the mash consistency data in standard units (absolute consistency) instead of arbitrary units (relative consistency). Moreover, Goode *et al. (18)* successfully used a Rapid Visco Analyser (RVA) to characterize the effect of different levels of barley adjunct on mash consistency. They concluded that the fully designed and calibrated RVA, giving results in standard viscosity units (mPa·s), was easy to operate and therefore more user-friendly than the previously developed method using the Bohlin CS-50 rheometer.

The objective of this study was to develop a highly precise rheological method for defining changes in mash consistency during mashing using a Physica MCR rheometer. For this reason, mash samples with various levels of unmalted oats, also referred to as adjunct, have been used in the trials. Oats are well-known for their high content of β-glucan *(22)* benefitting the rheological analyses by increasing the mash consistency. For validating this new rheological method, it has been compared with the established Rapid Visco Analyser by means of statistical tools.

Materials and methods

Mashing materials

Malted barley (*Hordeum vulgare* L. 'Fr Sebastian') of 4.8% moisture and 9.4% protein (dry weight), harvested in 2008 and obtained from Greencore Group plc (Dublin, Ireland) and unmalted oats (*Avena sativa* L. 'Lutz') of 12.7% moisture and 10.5% protein (dry weight), harvested in 2009 in Ravensburg, Germany were used in the mashing trials.

Milling

Malted barley was milled with a laboratory disk mill (Bühler GmbH, Braunschweig, Germany) set at a 0.2 mm-disk distance. Unmalted oats were milled using a hammer mill equipped with a 1.5-mm sieve (A.M.A. S.p.A., San Martino in Rio, Italy). Milling of mashing materials was carried out directly before mashing-in.

Mashing

For mashing, a commonly used infusion process has been chosen, taking the three important enzymatic degradation processes cytolysis, proteolysis, and amylolysis into consideration as follows: 30 min at 50°C, 40 min at 65°C, 20 min at 72°C, and 5 min at 78°C (mashing-off) with a heating rate of 1°C per min. In all mashing trials, a sample mass of 7.740 g (dry weight) was mixed with distilled water to give a total mash mass of 27.000 g at a constant moisture basis of 14%. Five mashes with increasing levels of unmalted oats (0%, 10%, 20%, 30%, 40% of sample mass) with a constant liquor-to-grist ratio of 2.488:1 (dry weight) were prepared. Mashing-in was performed by putting the homogenized grist into the 50°C preheated distilled water in the respective mash cup and stirring it properly. Then, the mash cup was applied to the temperature controlled heating block of the respective rheological measuring instrument.

Rheological measuring instruments

Physica MCR rheometer

The controlled stress rheometer Physica MCR 301 (Anton Paar Germany GmbH, Ostfildern, Germany) has been equipped with a paddle-shaped rotor enabling mash

particles to be kept in suspension throughout the rheological measurement. This rotor is based on the principle of a counter current stirrer where the stirring blades are arranged at right angles to each other. Furthermore, the mash cup and rotor system have been covered with a specially designed aluminum lid (School of Food and Nutritional Sciences, University College Cork, Ireland) for preventing evaporation during mashing. Before starting the infusion mashing process, the mash was homogenized for 60 s at a constant shear rate of 200 rpm. During the mashing process, a constant shear rate of 100 rpm was applied to the mash sample.

Rapid Visco Analyser

The RVA-Super3 with Thermocline for Windows software (Newport Scientific Pty. Ltd., Warriewood/NSW, Australia) is used with one-way aluminum sample canisters and plastic stirrers. Before starting the mashing regime, the mash was homogenized for 10 s at a constant shear rate of 960 rpm as described in the manufacturers' manual. During the rheological measurement, a constant shear rate of 160 rpm was applied to the mash as recommended by the manufacturer.

Mash sample description

In the following, the mash samples are described by means of numerical codes. The first code number refers to the rheological method used for determining mash consistency: 1 refers to MCR method and 2 refers to RVA method. The second code number indicates the adjunct concentration of the mash: 0 indicates 0% oats, 1 indicates 10% oats, 2 indicates 20% oats, 3 indicates 30% oats, and 4 indicates 40% oats.

Statistical evaluation of mash consistency data

All rheological mashing trials were performed in triplicate. On the basis of the individual mash consistency curves, the mean value curves have been calculated. The mash consistency represents the shear viscosity and is given in mPa·s. All calculations with respect to regression functions, as shown below on the basis of the mean value curve of mash sample 1.0, have been accomplished by applying the software program TableCurve 2D (Systat Software Inc., Chicago, U.S.A.). Further calculations have been done by using the manufacturers' software of the Physica

MCR rheometer. For the characterization and comparison of rheological mash curves, the following statistical tools have been used:

Confidence interval

For determining the statistical significance, the Student's *t*-test has been applied *(23)*. The confidence interval with a probability of $P = 95\%$ was calculated for each mean value (arithmetic mean). In general, the smaller the confidence intervals are, the better the repeatability.

Coefficient of determination

For the evaluation of results, one parameter is compared to another. With the help of a regression line, it can be tested whether a correlation exists. Basically, the coefficient of correlation *R* compares the statistical spread of the values to the regression line with the total spread of the method *(24)*. The coefficient of correlation *R* is an index number indicating whether a pair of variables *x* and *y* are connected to each other. The square of the coefficient of correlation *R* is called the coefficient of determination R^2. It has always a positive value and this index value is more precise than *R*.

Standard deviation of repeatability and repeatability

The standard deviation of repeatability s_r and the repeatability *r* have been determined according to DIN ISO 5725 *(25)*.

Outlier test

An outlier test according to Grubbs *(26)* as well as Grubbs and Beck *(27)* has been performed. This test is recommended by DIN 53804-13 *(28)* for a data volume *n* higher than 30. On the basis of the authors' experience, the Grubbs' test for outliers has also been found adequate for data volumes below 30.

Results and discussion

Mathematical characterization of mash consistency curves

The rheological behavior of different mashes during the mashing process has been described by means of recorded consistency curves. Each analysis was done in triplicate to determine the quality of the consistency measurement, resulting in three consistency curves for each mash type. A mean value curve has been calculated from 3 × 210 individual consistency values, which have been detected in defined time intervals of 1 min except for the heating-up period from 50°C to 65°C, where the consistency has been detected every 0.15 min. Hence, the mean value curve is based on 630 individual measuring points and 210 mean values, respectively. For each mean value of the calculated mash consistency curve, the confidence interval has been determined by applying the *t*-distribution with 2 degrees of freedom and a confidence level of 95%. By calculating the mean value curve, it was possible to compensate for initial uncertainties in the interpretation of individual data points. For the exact determination of specific curve characteristics such as slope or integral, it is necessary to know the functional equation of the mean value curve. Therefore, the measuring points of the mean value curve were approximated using a mathematical regression function $f(x)$ by assigning mash consistency data y to $f(x)$; the variable x represents the time of measurement. This was achieved by applying a software program as mentioned above. The approach to approximation describes an adaption by means of mathematical functional definitions for each respective curve progression, aiming at the highest correlation between the variance of mean value curve data and regression function data $f(x)$. By determining the coefficient of determination R^2, the quality of the approximation of the mean value curve by $f(x)$ can be expressed. The aim of using a regression or approximation function $f(x)$ is to determine characteristic points like extreme values or to calculate integrals in a mathematically correct way. A function can have a relative extremum only at the points where its first derivative is equal to zero or does not exist *(29)*. For determining maximum or minimum points, it is common practice to set the first and second derivative of a function equal to zero, respectively. This approach is successful in most cases. Nonetheless, a first derivative being equal to zero does not necessarily have to be a criterion for an extreme value. However, a sufficient condition for the existence of an extremum is given by the sign change check of the

first derivative *(29)*. The domain for the variable x has been predetermined by the mashing process and specifies for which x- and y-values the regression function has to be defined. It can be formulated for all x-values as follows:

$$\mathbb{D} = \{x \in \mathbb{R} \,|1\ \text{min} \leq x \leq 125\ \text{min}\} \quad \text{(Eq. 1).}$$

Due to the complex progression of the mean value curve, it was not possible to find one regression function $f(x)$ for the total domain showing a satisfactory approximation ($R^2 \approx 1$). For this reason, the mean value curve had to be described by a composite function consisting of several sub-functions. The total domain for x has been split into adequate sub-intervals for which the determined sub-functions showed the best possible approximation to the mean value curve ($R^2 \approx 1$). In principle, regression functions in the form of composite functions could be found for all mean value curves. These functions generally consist of three sub-functions. By means of the mean value curve of mash sample 1.0, the approach for determining a regression function $f(x)$ will be described and the associated advantages discussed in the following. The regression function $f(x)$ used for the mean value curve of mash sample 1.0 consists of three sub-functions $f_1(x)$, $f_2(x)$, and $f_3(x)$:

$$f(x) = \begin{cases} a_1 + b_1 \cdot x + c_1 \cdot x^2 + d_1 \cdot x^3 + e_1 \cdot x^4 + f_1 \cdot x^5 + g_1 \cdot x^6 + h_1 \cdot x^7 + i_1 \cdot x^8, & x \leq x_1 = 35.5\ min \\ \dfrac{(a_2 + c_2 \cdot x + e_2 \cdot x^2 + g_2 \cdot x^3 + i_2 \cdot x^4 + k_2 \cdot x^5 + m_2 \cdot x^6)}{(1 + b_2 \cdot x + d_2 \cdot x^2 + f_2 \cdot x^3 + h_2 \cdot x^4 + j_2 \cdot x^5 + l_2 \cdot x^6 + n_2 \cdot x^7)}, & x_1 \leq x \leq x_2 \\ a_3 + b_3 \cdot x + \dfrac{c_3}{x} + d_3 \cdot x^2 + \dfrac{e_3}{x^2} + f_3 \cdot x^3 + \dfrac{g_3}{x^3} + h_3 \cdot x^4 + \dfrac{i_3}{x^4} + j_3 \cdot x^5, & x \geq x_2\ = 55.2\ min \end{cases} \quad \text{(Eq. 2).}$$

The sub-function $f_1(x)$ is a standard polynomial (8th degree), $f_2(x)$ is a Chebyshev converted rational function (6th/7th degree), and $f_3(x)$ is a balanced order polynomial (5th degree). The function parameters $a_1, \ldots, j_3$ are listed in the supplementary material (Table S1). For the sub-functions $f_1(x)$ and $f_2(x)$ as well as $f_2(x)$ and $f_3(x)$ the following is valid: $f_1(x_1) = f_2(x_1)$ and $f_2(x_2) = f_3(x_2)$. This means that the function $f(x)$ is continuous at the points x_1 and x_2 if a limit $\lim f(x)$ exists, being equal to the function value $f(x_1)$ and $f(x_2)$, respectively, as follows: $\lim_{x \to x_1} f(x) = f(x_1)$ or $\lim_{x \to x_2} f(x) = f(x_2)$. With regard to the regression function, this means that insignificant changes in time result in insignificant changes in consistency. The continuity of a function implies that no jumps appear in the function values. Hence, the continuity condition for $f(x)$ is fulfilled over the entire domain. Since continuous functions do not necessarily have to be differentiable, both internal points of the sub-

intervals were proved in terms of their differentiability. The term differentiability describes the characteristic of a function to be approximated locally around a point in a linear manner. The function f is differentiable at the points x_1 and x_2 if the limits $\lim_{x \to x_1} \frac{f(x) - f(x_1)}{x - x_1}$ and $\lim_{x \to x_2} \frac{f(x) - f(x_1)}{x - x_2}$ exist. These limits are defined as the derivatives of f at the points x_1 and x_2. This condition is fulfilled when the function has exactly one limit at the points x_1 and x_2, respectively, for which the following is valid: $f_1'(x_1) = f_2'(x_1)$ and $f_2'(x_2) = f_3'(x_2)$. The mean value curve of mash sample 1.0 could be approximated using a composite function consisting of three sub-functions, which are characterized by the coefficient of determination: $f_1(x)$ R^2 = 0.9837, $f_2(x)$ R^2 = 0.9981, and $f_3(x)$ R^2 = 0.9964. In Figure 1, three sub-functions and the composite function $f(x)$ are shown. For interval points, the following function values have been determined: $f_1(x_1 = 35.5 \text{ min}) = f_2(x_1) = 68.88$ mPa·s as well as $f_2(x_2 = 55.2 \text{ min}) = f_3(x_2) = 80.26$ mPa·s.

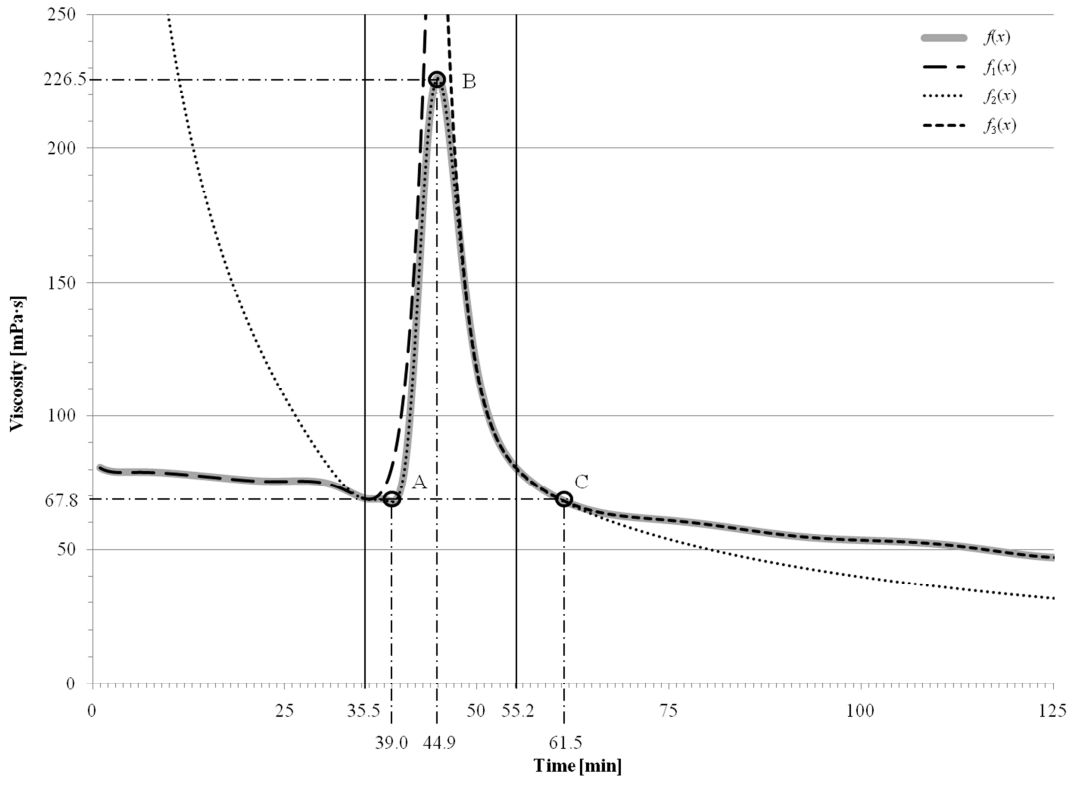


Figure 4–1. Graphical representation of the sub-functions f_1, f_2, and f_3 of the composite function f within the domain (Eq. 1). The following characteristic points have been marked: Interval points x_1 = 35.5 min, x_2 = 55.2 min; local minimum $f(x$ = 39.0 min) = 67.8 mPa·s (A), local maximum $f(x$ = 44.9 min) = 226.5 mPa·s (B); limits of integration x = 39.0 min (A), x = 61.5 min (C).

In Figure 2, the derivatives $f'(x)$, $f_1'(x)$, $f_2'(x)$, and $f_3'(x)$ are shown. It can be seen that the derivative values of the respective sub-functions are equal at the interval points: $f_1'(x_1 = 35.5 \text{ min}) = f_2'(x_1) = -0.62$ mPa·s/min and $f_2'(x_2 = 55.2 \text{ min}) = f_3'(x_2) = -3.12$ mPa·s/min. Thus, it has been proven that the regression or approximation function $f(x)$ is differentiable at any point within the domain.

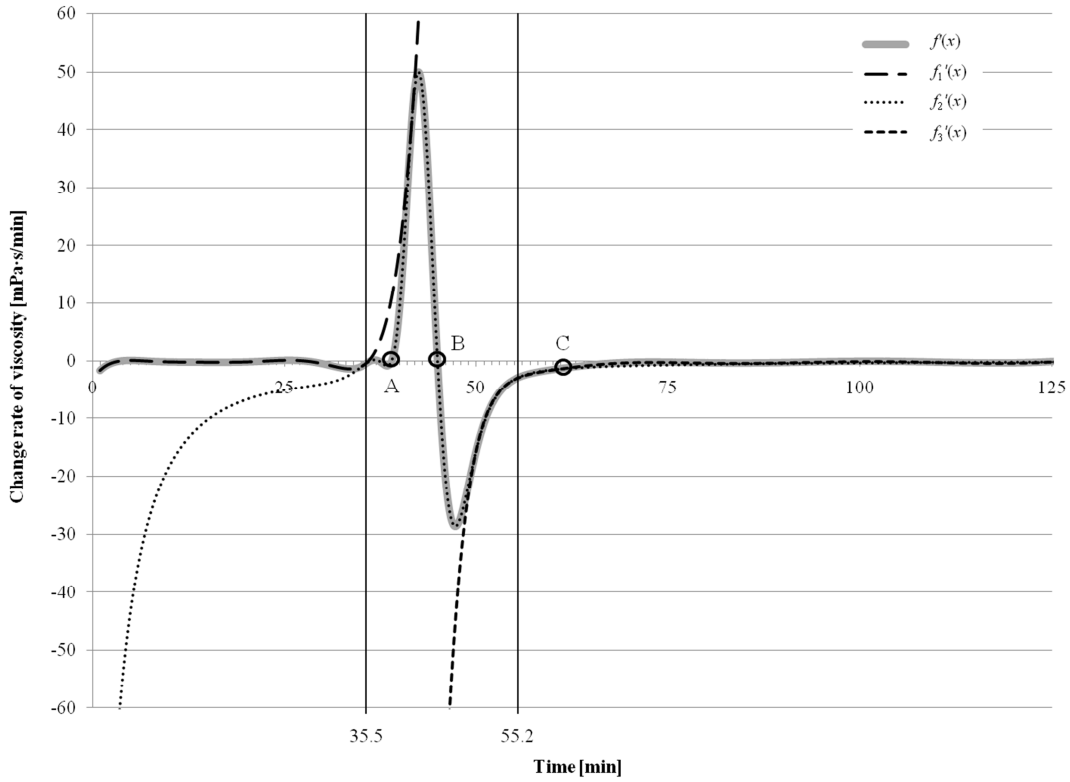


Figure 4–2. Graphical representation of the derivatives f_1', f_2', and f_3' of the sub-functions f_1, f_2, and f_3 as well as the derivative f' of the composite function f within the domain (Eq. 1). The following characteristic points have been marked: Interval points $x_1 = 35.5$ min, $x_2 = 55.2$ min; zero points $f'(x = 39.0 \text{ min}) = 0$ mPa·s/min (A), $f'(x = 44.9 \text{ min}) = 0$ mPa·s/min (B).

For determining the quality of approximation, it has been proved in which way the divergences from the mean values are distributed to the regression function values. For this purpose, the difference between each measuring point of the mean value curve and the corresponding function value ($n = 210$) has been calculated. An outlier test according to Grubbs *(26)* as well as Grubbs and Beck *(27)* was performed and a total of 8 outliers detected. The frequency distribution of the measuring value differences after subtracting the outliers ($n = 202$) is shown in the supplementary

material (Figure S1). According to the test of David *et al. (30)*, the distribution of measuring value differences can be assumed to be normal distributed for five confidence levels (90.0%, 95.0%, 97.5%, 99.0%, and 99.5%). The 95% confidence level for the true mean value μ of all differences is given by -0.221 mPa·s $\leq \mu \leq$ 0.032 mPa·s. This confidence interval shows that zero is included. Hence, it can be assumed with a significance level of 5% that the regression function does not systematically deviate from the mean value curve.

With the help of a regression function $f(x)$, the characteristic points of a consistency curve can be determined. The local minimum at x = 39.0 min can be defined as the start of gelatinization since the consistency increases continuously after that point. In general, the gelatinization temperature is defined as the temperature at which the starch granules swell tangentially and at the same time lose their crystalline properties *(10)*. According to a RVA method described by MEBAK *(10)*, the gelatinization of starch starts when the viscosity increases by 24 mPa·s within 1 sec. However, the automatic determination of the gelatinization temperature by a Rapid Visco Analyser and Thermocline for Windows software can result in inaccuracies with a coefficient of variation (CV) = 16.4%. Noisy baselines can lead to misinterpretations, which is why MEBAK *(10)* recommends the manual interpretation of data. Such problems can be eliminated using regression functions as described in this study.

After reaching a peak of 226.5 mPa·s at x = 44.9 min (Figure 1, B), the mash consistency (mash sample 1.0) decreases continuously due to the activity of liquefying malt enzymes. The integral within the time interval of starch gelatinization/liquefaction may be of importance for many analyses. However, the end point of liquefaction has not been well-defined yet and therefore selected arbitrarily. It is reasonable to define the end point of liquefaction as that curve point at which the consistency after gelatinization is equal to the minimum consistency before gelatinization (Figure 1, C). The start point A and end point C (Figure 1) represent the relevant integration limits. On the basis of the mash consistency function $f(x)$, the primitive function $F(x)$ can be determined. The area under the curve between the points A and C can be calculated as follows:

$$F(x = 61.5\ \text{min}) - F(x = 39.0\ \text{min}) = \int_{A}^{C} f(x)dx \qquad \text{(Eq. 3).}$$

The most significant slope occurs within the interval [A,B] during the gelatinization of starch as shown in Figure 3.

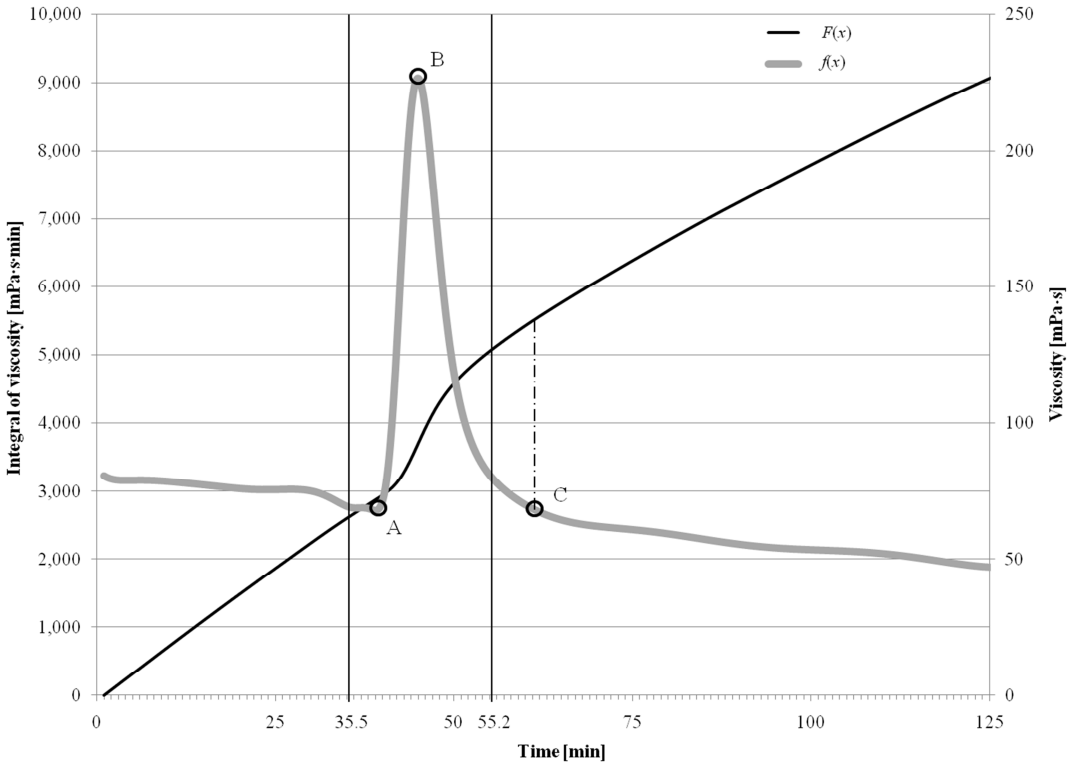


Figure 4–3. Graphical representation of the primitive function $F(x)$ of the regression function $f(x)$ within the domain (Eq. 1). The following characteristic points have been marked: Interval points x_1 = 35.5 min, x_2 = 55.2 min.

Statistical comparison of the developed rheological method with the established RVA

In Table 1, the mean values of start and end mash consistencies of starch gelatinization/liquefaction, peak consistency, peak area within the interval [A,C], and the viscosity breakdown rate during liquefaction of all mash samples being analyzed by the MCR rheometer and RVA, respectively, are summarized.

Table 4–1. Start and end mash consistencies of gelatinization/liquefaction (A,C), peak consistency (B), peak area (A-C), and rate of viscosity breakdown during liquefaction (B-C), calculated by using a regression function $f(x)$, are shown. The coefficients of determination R^2 show the relation between A, B, C, peak area, or rate of viscosity breakdown and the adjunct concentration.

Sample no.	A		B		C		Peak area (A-C)	Rate of viscosity breakdown (B-C)
	[min]	[mPa·s]	[min]	[mPa·s]	[min]	[mPa·s]	[mPa·s·min]	[mPa·s/min]
MCR method								
1.0	39.0	67.8	44.9	226.5	61.5	67.8	2651	9.5602
1.1	38.4	77.1	45.0	249.7	63.7	77.1	3256	9.2300
1.2	37.7	88.0	44.5	282.3	63.4	88.0	3732	10.2804
1.3	37.8	100.7	44.3	324.6	64.5	100.7	4376	11.0842
1.4	37.6	119.9	44.3	378.5	65.2	119.9	5076	12.3732
R^2		**0.9784**		**0.9754**		**0.9784**	**0.9958**	**0.8750**
RVA method								
2.0	37.4	86.4	43.0	200.6	69.4	86.4	3548	4.3258
2.1	38.3	98.0	43.5	217.0	66.3	98.0	3576	5.2193
2.2	37.1	126.3	43.4	251.1	67.6	126.3	4783	5.1570
2.3	36.4	138.0	43.6	280.1	65.2	138.0	5012	6.5787
2.4	37.6	158.5	43.4	323.3	65.2	158.5	5544	7.5596
R^2		**0.9839**		**0.9800**		**0.9839**	**0.9230**	**0.9252**

As shown in Table 1, characteristic points of all mash consistency curves could be calculated with the help of a regression function. By determining the coefficient of determination R^2, correlations between the respective curve values and the level of adjunct could be revealed. For example, a linear correlation between peak area and

adjunct level (MCR method) with a coefficient of determination $R^2 = 0.9958$ has been found, revealing that the peak area increases with an increasing level of adjunct as shown in Figure 4.

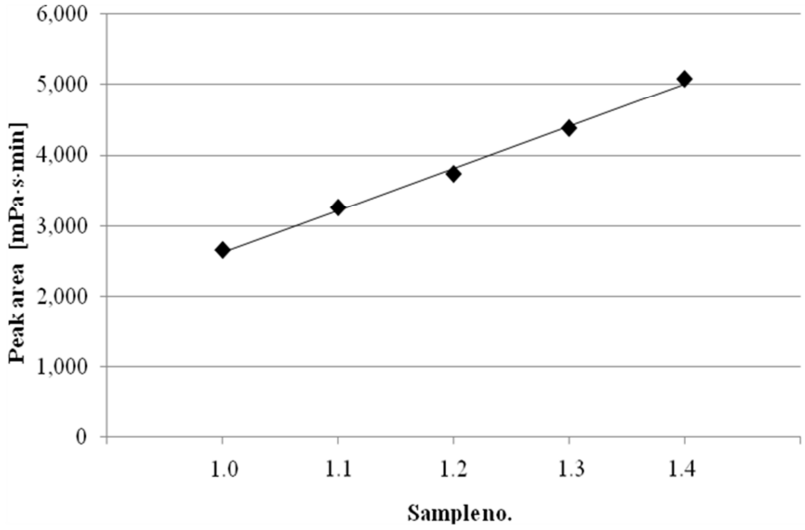


Figure 4–4. Graphical representation of the linear relation between peak area (A-C) and adjunct concentration based on the MCR method ($R^2 = 0.9958$).

Besides the characteristic points of the mean value curves shown in Table 1, other mash consistency values may be of importance. By using a regression function, the start and end consistency values of each mash rest could be determined. In Table 2, the mean values of start and end mash consistencies of the rests at 50°C, 65°C, 72°C, and 78°C (mashing-off) are given. Tables 1 and 2 demonstrate that both rheological methods (MCR and RVA) are able to detect changes in mash consistency during mashing. This has been shown by calculating the coefficients of determination R^2. For both methods, good to very good linear correlations have been found between respective curve values and adjunct levels. However, these statistical numbers need to be interpreted carefully. Coefficients of determination R^2 express only the quality of the linear approximation, but not whether the model has been correctly specified. Models which have been estimated by the method of least squares will therefore receive the highest R^2. Another disadvantage is the sensitivity with respect to trends. Provided that an exogenous variable develops in parallel with an explaining one, high R^2 are shown regardless of the real explanatory power of the model. Thus, a high R^2 in Tables 1 and 2 indicates that the estimated regression line shows a good approximation to the data.

Table 4–2. Start and end mash consistencies of the rests at 50°C, 65°C, 72°C, and 78°C (mashing-off), calculated by using a regression function $f(x)$, are shown. The coefficients of determination R^2 show the relation between the respective start or end consistency and the adjunct concentration.

Sample no.	**50°C**		**65°C**		**72°C**		**78°C**	
	Start	End	Start	End	Start	End	Start	End
	[mPa·s]	[mPa·s]	[mPa·s]	[mPa·s]	[mPa·s]	[mPa·s]	[mPa·s]	[mPa·s]
MCR method								
1.0	80.5	74.7	214.7	57.7	53.4	51.5	47.4	47.2
1.1	98.9	84.6	237.0	68.6	63.5	58.7	54.7	54.6
1.2	119.3	97.7	263.3	76.1	69.6	65.2	59.9	60.2
1.3	145.7	111.0	290.7	89.2	80.1	75.1	69.2	69.9
1.4	190.3	133.0	334.3	104.6	92.2	88.2	81.0	79.8
R^2	**0.9632**	**0.9773**	**0.9822**	**0.9845**	**0.9889**	**0.9778**	**0.9777**	**0.9883**
RVA method								
2.0	86.7	94.3	168.3	83.7	77.7	73.3	70.0	68.0
2.1	103.7	100.7	181.7	91.7	86.0	85.7	82.7	77.3
2.2	145.3	131.7	213.7	120.3	113.7	109.7	106.7	108.0
2.3	175.3	145.0	237.0	126.7	120.7	114.3	102.7	107.7
2.4	206.7	166.3	269.7	150.7	140.0	137.0	125.0	127.7
R^2	**0.9888**	**0.9715**	**0.9850**	**0.9645**	**0.9689**	**0.9715**	**0.9169**	**0.9333**

By means of the graphical representation of mean value curves, differences between the rheological methods could be revealed as shown in Figure 5. The curve progression of the mean value curves based on the RVA method is not as symmetric as that of the mean value curves based on the MCR method. More information about the reliability of mean values could be gained by the graphical representation of the minimum and maximum consistency curves together with the 95% confidence intervals for each mean value as shown in Figure 6. By using the RVA, almost no significant differences between the minimum and maximum consistency curve (0% and 40% oats) could be detected at $t > 80$ min due to overlapping confidence intervals.

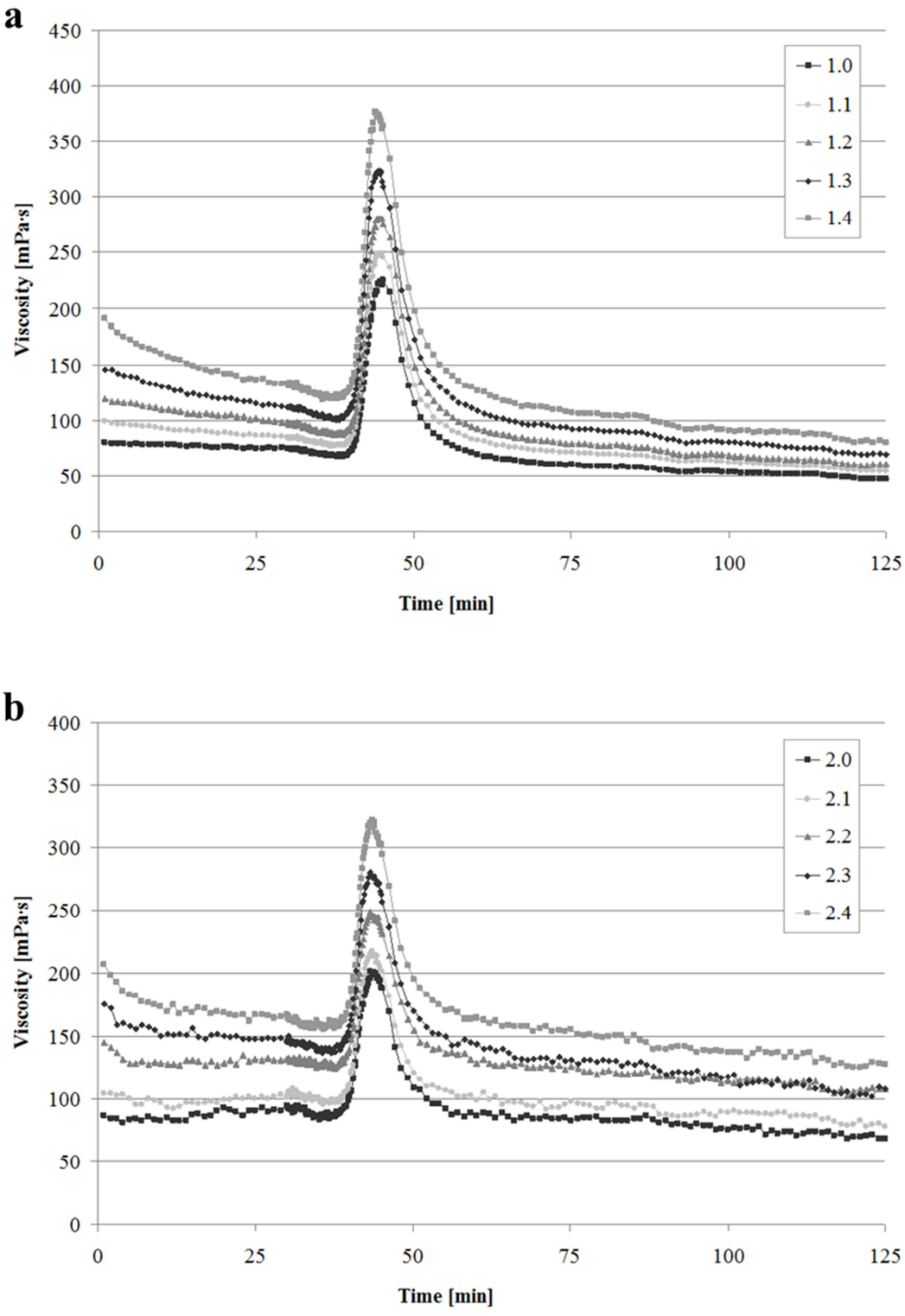


Figure 4–5. Graphical representation of the mean value curves of five different mash samples (0–40% oats) based on (a) the MCR method and (b) the RVA method.

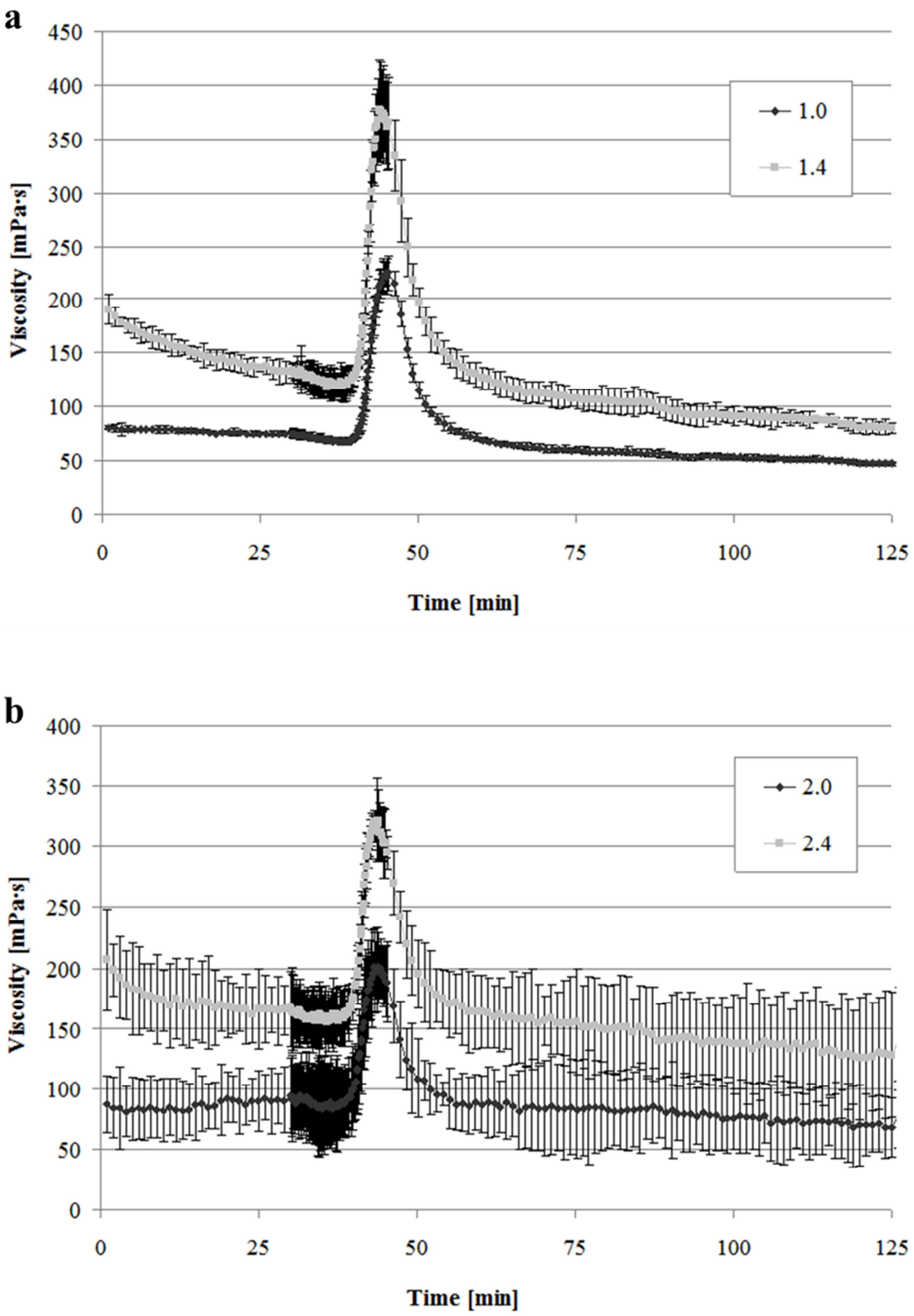


Figure 4–6. Graphical representation of the mean value curves of 0% and 40% oat mashes with confidence intervals (P = 95%) based on (a) the MCR method and (b) the RVA method.

In order to finally assess the quality of the MCR and RVA measuring system, tests on repeatability have been carried out. For this purpose, the precision measure repeatability *r* according to DIN ISO 5725 *(25)* has been used. Under the terms of DIN 55350-13 *(31)*, precision is the qualitative description for the closeness of agreement between results which have been achieved by reapplying a defined measuring method. In general, the standard deviation of repeated measurements is used as a measure of precision. With the help of the standard deviation of repeatability σ_r, the repeatability *r* can be determined as "critical difference". That is, the absolute value of the difference between two test results which can be expected with a given probability (mostly 95%). The repeatability *r* according to DIN ISO 5725 *(25)* is calculated as follows:

$$r = k \cdot \sqrt{2} \cdot s_r \qquad \text{(Eq. 4).}$$

The factor $\sqrt{2}$ arises from *r* referring to the difference between two test results of repeated measurements. Furthermore, the factor *k* is dependent on both the number of measuring values which are used for estimating the standard deviation of repeatability s_r and the shape of their distribution. If the distribution is approximately normally distributed (unimodal) as shown in Figure S1 (see supplementary material) and the total number of measuring results adequate for testing, then the factor *k* will differ only slightly from the value 2. In addition, the use of $k = 2$ is recommended in practice because the determined repeatability *r* can be easily compared to another. Since the real value of the standard deviation of repeatability σ_r is generally unknown, the estimate s_r is used in Eq. 4. The triple analysis of mashes containing various levels of oats resulted in three consistency values for each measuring point. Thus, the total number of individual measurements is $n_j = 3$. The number of different measuring points is $m = 210$ and therefore the total number of measuring points $N = 630$ (3 × 210). Furthermore, the mean value of the individual measurement is $\bar{x}_j$. According to DIN ISO 5725 *(25)*, the standard deviation of repeatability can be calculated from individual standard deviations as follows:

$$s_r = \sqrt{\frac{\sum_{j=1}^{m} \sum_{i=1}^{n_j} (x_{ij} - \bar{x}_j)^2}{N - m}} \qquad \text{(Eq. 5).}$$

In accordance with Eq. 4, the repeatability results from

$$r = 2.83 \cdot s_r \quad \text{(Eq. 6).}$$

As expected, the distribution of the individual repeatabilities followed a chi-square distribution. The results are given as mean value of repeatability r_m in Table 3. In addition, the coefficient of variation (CV) has been calculated for all mash samples. With regard to the RVA method described by MEBAK *(10),* it arises that low coefficients of variation are absolutely necessary in order to avoid misinterpretations. Furthermore, it has been shown that the repeatability depends on the level of oat adjunct. Therefore, the individual repeatabilities could be given as a linear function r_A with $R^2 \approx 1$, depending on the concentration of adjunct c. In addition, the dependence of r on c could be interpreted indirectly as a dependence on the consistency level, since by standardizing the standard deviation, the coefficient of variation is also depending on the mean level of the measured consistencies. Using the RVA method, only the mash samples 2.2, 2.3, and 2.4 showed a linear relation, which is why the corresponding equation r_A is, strictly speaking, only valid for adjunct concentrations $c \geq 20\%$.

By calculating the repeatability, it has been shown that the precision of the MCR method is significantly better than that of the RVA method. Due to its considerably better measuring performance, the MCR method is superior to the RVA method. Finally, it should be noted that not every trial leads to the same values in terms of precision measure, since measuring results are generally random results. Thus, the determined values for repeatability given in Table 3 represent estimates, about whose inaccuracies no statement is made here. Besides, the repeatability allows for no statement about the accuracy of a measurement method.

Table 4–3. Results of repeatability tests for the MCR method and the RVA method.

MCR method						
Sample no.		1.0	1.1	1.2	1.3	1.4
Mean value of repeatability	r_m [mPa·s]	6.17	10.02	11.52	13.80	16.99
Coefficient of variation	CV [%]	1.68	3.65	4.02	4.07	3.28
Mean value of repeatability dependent on the percentage of adjunct	r_A [mPa·s]			$25.42 \cdot c + 6.62$		
	R^2			0.9816		
RVA method						
Sample no.		2.0	2.1	2.2	2.3	2.4
Mean value of repeatability	r_m [mPa·s]	33.79	34.18	15.80	25.75	33.23
Coefficient of variation	CV [%]	12.69	11.37	3.32	6.06	6.93
Mean value of repeatability dependent on the percentage of adjunct	r_A [mPa·s]			$88.07 \cdot c - 1.23$ for $c \geq 20\%$		
	R^2			0.9933		

Conclusion

In summary, this study presents a new rheological method for precisely defining changes in mash consistency during mashing using a Physica MCR rheometer. With the help of statistical tools, it has been proven that the new developed method is superior to the established RVA method. By using regression functions for describing mash consistency curves, characteristic curve points have been mathematically correctly determined. Moreover, it has been possible to well-define the start and end point of starch gelatinization/liquefaction.

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Abbreviations

a_1, ..., j_3	Function parameters;
c	Concentration;
CV	Coefficient of variation;
f	Regression function;
F	Primitive function of f;
f_i	Sub-function;
k	Factor;
m	Number of different measuring points;
n	Number of samples;
N	Total number of measuring points;
n_j	Number of individual measurements;
r	Repeatability;
R	Coefficient of correlation;
R^2	Coefficient of determination;
r_A	Mean value of repeatability dependent on the percentage of adjunct;
r_m	Mean value of repeatability;
s_r	Standard deviation of repeatability;
x	Variable used for time of measurement;
x, y	Variables;
$\bar{x}$	Arithmetic mean;
y	Variable used for mash consistency;
η	Dynamic viscosity;
μ	True arithmetic mean;
σ_r	True standard deviation of repeatability.

Supplementary material

Table 4–S1. Function parameters of equation 2 (values are rounded and given without a unit).

$f_1(x)$		$f_2(x)$		$f_3(x)$	
a_1	8.3121E+01	a_2	8.5590E+02	a_3	1.0145E+07
b_1	-3.4096E+00	b_2	9.5380E-02	b_3	-1.1853E+05
c_1	1.0801E+00	c_2	-1.2182E+02	c_3	-5.6957E+08
d_1	-1.7344E-01	d_2	-2.4785E-02	d_3	9.0807E+02
e_1	1.5692E-02	e_2	7.2290E+00	e_3	2.0235E+10
f_1	-8.4502E-04	f_2	1.6707E-03	f_3	-4.3984E+00
g_1	2.6734E-05	g_2	-2.2891E-01	g_3	-4.1307E+11
h_1	-4.5587E-07	h_2	-5.5596E-05	h_3	1.2221E-02
i_1	3.2147E-09	i_2	4.0790E-03	i_3	3.6943E+12
		j_2	1.0135E-06	j_3	-1.4841E-05
		k_2	-3.8781E-05		
		m_2	-9.7394E-09		
		n_2	1.5370E-07		

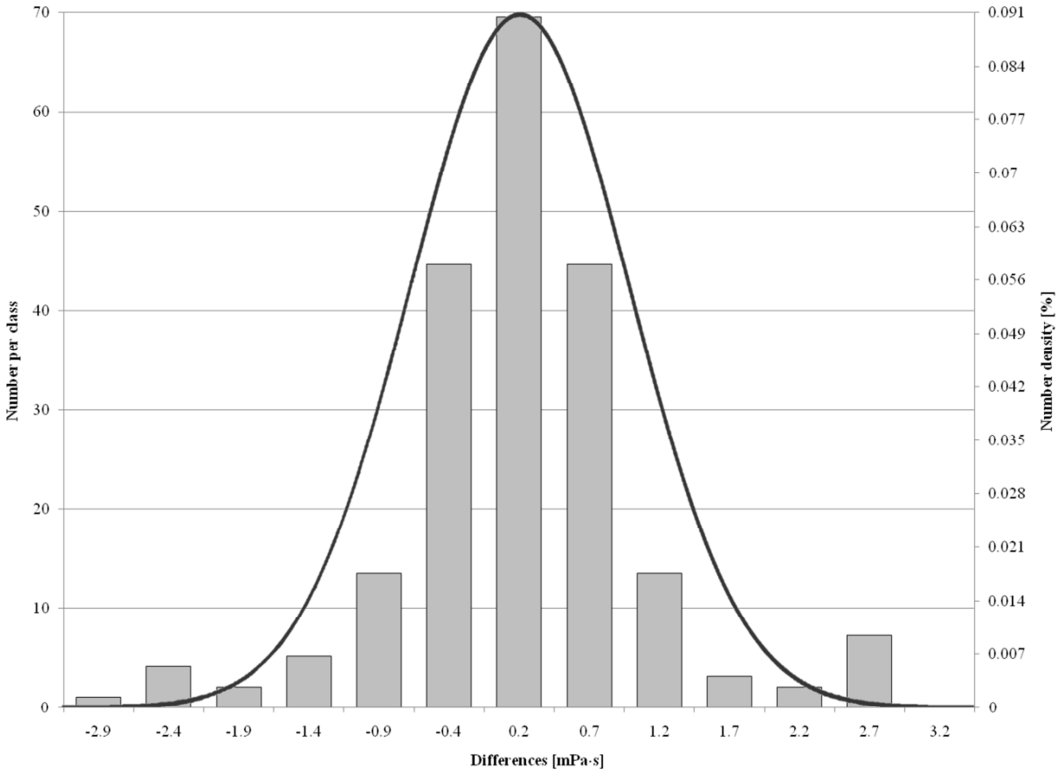


Figure 4–S1. Graphical representation of the frequency distribution of measurement errors between mean value curve and regression function, given as number per class (bars) and number density (curve) function. The number density function is defined by a normal distribution with a mean value $\bar{x}$ = -0.095 mPa·s and a standard deviation s = 0.915 mPa·s at a number of measurement differences n = 202.

Chapter 5

A comparative study of oat (*Avena sativa*) cultivars as brewing adjuncts

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Abstract

Brewing with high levels of unmalted oats (*Avena sativa*) has proven to be successful despite their high contents of β-glucan, protein, and fat. However, little is known about the effect of different oat cultivars on the quality and processability of mashes and worts. Therefore, the aim of this study was to compare the mashing performance of eight oat cultivars, selected because of their low contents of β-glucan, protein, fat, and/or high starch content, when substituting 20% or 40% barley malt. For this purpose, seven husked (*A. sativa* L. 'Lutz', 'Buggy', 'Galaxy', 'Scorpion', 'Typhon', 'Ivory', 'Curly') and one naked oat cultivar (*A. sativa* var. *nuda* 'NORD 07/711') were fully characterized using standard methods, Lab-on-a-Chip capillary electrophoresis, and scanning electron microscopy. The rheological behavior of mashes containing up to 40% of each oat cultivar was measured during mashing by applying a Physica MCR rheometer. In addition, the quality of worts obtained from laboratory-scale mashing trials was analyzed particularly with regard to their cytolytic, proteolytic, and amylolytic properties. The substitution of up to 40% barley malt with husked or naked oats resulted in significantly higher pH values, β-glucan contents, and viscosities as well as significantly lower soluble nitrogen and polyphenol contents, color values, filtration rates, and apparent attenuation limits. Naked oats contained significantly less β-glucan as well as more protein and starch than the seven husked oat cultivars. The replacement of barley malt with naked oats resulted in a constant extract yield, whereas the use of husked oats caused significant extract losses.

Introduction

Oats (*Avena sativa*) are unique among the cereals in having high contents of β-glucan, protein, and fat *(1)*, which are undesirable characteristics when used as brewing adjunct. Oat β-glucan is a linear, unbranched polysaccharide composed of approximately 70% 4-linked and 30% 3-linked β-D-glucopyranosyl units. It is located in the endosperm cell walls, the aleurone cell walls, and the germ of oats, however, there is no β-glucan in the hull *(2)*. The amount of β-glucan in the oat aleurone layer is small compared to that in the starchy endosperm, but its impact on the water-binding properties of the bran is considerable. As a result, oat bran has a much higher water hydration capacity than barley bran *(3,4)*. The solubility (extractability) of oat β-glucan in aqueous systems is affected by several factors such as particle size, temperature, and pH *(5,6)*. Under mild extraction conditions (e.g. water at 45°C or 65°C), it is not possible to extract all the β-glucan present in oats *(2,5)*. The common use of hot water (90–100°C) for extracting β-glucan from cereals results in solutions containing 60% to 75% of the oat β-glucan *(2,7)*. Most of the water-insoluble β-glucan in oat kernels is located in the bran *(8)*. The viscosity of oat β-glucan, depending on molecular weight and concentration, interferes with the brewing process *(9,10)*. It has been reported that the molecular weight of extractable oat β-glucan (around 1–2 million g/mol) is significantly higher than that of extractable barley β-glucan *(7,11)*. In addition, oat β-glucan has a higher extractability and solubility (controlled by structure and molecular weight) than barley β-glucan *(2,7)*. Ajithkumar *et al. (11)* assumed that the extractable β-glucan content of oats is a heritable trait, whereas the molecular weight depends more on environmental factors. At concentrations below 0.2–0.3% (w/v), solutions of high-molecular-weight oat β-glucan (molecular weight ≈ 1 million g/mol; intrinsic viscosity ≈ 950 mL/g) show Newtonian behavior, that is, the viscosity is constant with increasing shear rates. At concentrations above 0.2–0.3% (w/v) (“critical” concentration), solutions of high-molecular-weight oat β-glucan develop non-Newtonian shear-thinning or pseudoplastic behavior (entanglement of individual polymer chains), that is, the viscosity decreases with increasing shear rates above a minimum shear rate (zero-shear viscosity η_0). A doubling of the β-glucan concentration above the “critical” concentration could lead to a 16-fold increase in zero-shear viscosity. The rheological behavior is primarily controlled by the

molecular dimensions (molecular weight, intrinsic viscosity) of the β-glucan (non-hydrolyzed) *(2,10)*. In contrast, partially hydrolyzed oat or barley β-glucan tends to show a more gel-like behavior by forming aggregates, as observed in beer *(10,12)*.

Furthermore, oats containing a high percentage of fat are particularly susceptible to the development of bitter off-flavors (associated with long-chain hydroxy fatty acids) and rancidity (associated with volatile aldehydes, ketones, and alcohols) during processing. Two distinct reactions may detrimentally modify oat lipids: 1) hydrolysis – conversion of triacylglycerols or phospholipids to free fatty acids; 2) oxidation – conversion of polyunsaturated fatty acids to hydroperoxides and further to secondary oxidation products *(4)*. In oats, not only the aleurone layer and the germ are rich in lipids but also the starchy endosperm in contrast to other cereals *(4,13)*. Lipids present in native cereal starches can be divided into surface lipids being attached to the surface of starch granules and internal lipids being inside the starch granules (formation of amylose-lipid inclusion complexes) *(13–15)*. Both surface and internal lipids have a considerable influence on the gelatinization and pasting properties of starch largely due to a complex formation between fatty acids and amylose *(13,16)*. It has been reported that the removal of surface or internal lipids from oat starch had no significant effect on swelling power, whereas the solubility increased enormously *(17)*.

In spite of high β-glucan, protein, and fat contents, brewing with up to 40% unmalted oats was recently found to be successful *(18)*. However, no comparative study of different oat cultivars in terms of their suitability as brewing adjuncts is available to date. Therefore, the objective of this study was to compare the effect of eight oat cultivars on the quality and processability of mashes and worts. For this purpose, husked and naked oats exhibiting low contents of β-glucan, protein, fat, and/or high starch contents were chosen.

Materials and methods

Mashing materials

Malted barley (*Hordeum vulgare* L. 'Fr Sebastian'), harvested in 2008 and obtained from Greencore Group plc (Dublin, Ireland), unmalted oats (*A. sativa* L. 'Lutz'), harvested in 2009 in Ravensburg, Germany, and seven other oat cultivars (*A. sativa* L. 'Buggy', 'Galaxy', 'Ivory', 'Curly', 'Scorpion', 'Typhon'; *A. sativa* var. *nuda* 'NORD 07/711'), harvested in 2009 and provided by Nordsaat Saatzucht GmbH (Granskevitz, Germany) were used in the mashing trials. The oat cultivars were selected because of their low contents of husk, fat, β-glucan, protein, or high starch content.

Characterization of oat cultivars

Standard analysis

Unmalted oats were analyzed applying the methods described by Mitteleuropäische Brautechnische Analysenkommission (MEBAK) – Raw materials *(19)*. Moisture contents were measured according to method 1.5.1.1. Total nitrogen contents were determined using a Tecator™ Digestor combined with a Kjeltec™ 2100 Distillation unit (Foss, Hillerød, Denmark) following method 1.5.2.1. Fat contents were analyzed using the method 2.5. Ash contents were measured according to method 3.1.4.20.1. In addition, β-glucan contents were determined following the McCleary method (Megazyme International Ireland Ltd., Bray, Ireland). Total starch contents were analyzed using the Megazyme Amyloglucosidase/α-Amylase method. All standard analyses were performed in triplicate.

Enzyme activities

β-Glucanase activities were measured according to the Megazyme Azo-Barley Glucan method. One unit of activity equals one micromole of glucose reducing sugar equivalent released per minute at 30°C and pH 4.6. α-Amylase activities were determined using the Megazyme Ceralpha method. One unit of activity corresponds to the amount of enzyme required to release one micromole of *p*-nitrophenol from non-reducing-end blocked *p*-nitrophenyl maltoheptaoside in one minute under the defined assay conditions. β-Amylase activities were analyzed following the

Megazyme Betamyl-3 method. One unit of activity corresponds to the amount of enzyme required to release one micromole of *p*-nitrophenol from *p*-nitrophenyl-β-D-maltotrioside in one minute under the defined assay conditions. Proteolytic enzyme activities were measured applying the slightly modified method of Brijs *et al. (20)*. For the analysis, 5 g of flour was extracted with 50 mL of 0.05 M sodium acetate buffer (pH 5.0) containing 2.0 mM L-cysteine by mechanical shaking for 30 min at 4°C. After the extraction of proteolytic enzymes, the suspension was centrifuged at 5,000 rpm for 15 min at 4°C and the supernatant used for further analysis. The proteolytic activity in this enzyme extract was measured using hemoglobin as substrate. For this purpose, 1.0% (w/v) hemoglobin was solubilized in 0.2 M sodium acetate buffer (pH 4.0). A mixture of 0.25 mL of hemoglobin solution, 0.20 mL of 0.2 M sodium acetate buffer (pH 4.0), and 0.05 mL of enzyme extract was incubated for 150 min at 40°C. After incubation, the reaction was stopped by adding 0.4 mL of cold 10.0% (w/v) trichloroacetic acid, and precipitated proteins were removed by centrifugation at 10,000×*g* for 10 min. Then, the free α-amino nitrogen level of the supernatant was determined with trinitrobenzene-sulfonic acid reagent (0.3% (v/v) TNBS in 0.2 M sodium phosphate buffer, pH 8.0) using L-leucine as standard. For this purpose, 0.025 mL of supernatant and 0.225 mL of TNBS reagent were incubated for 20 min at 50°C. Subsequently, the reaction was stopped by adding 0.75 mL of 0.2 M HCl. Finally, the absorbance of the solution at 340 nm was measured. One unit of activity corresponds to the enzyme activity releasing 1 mg of L-leucine/h·g under the assay conditions. For the determination of enzyme activities, three independent sample extractions were performed and each enzyme extract was analyzed in duplicate.

Lab-on-a-Chip capillary electrophoresis

Total protein profiles were determined following the method described by Klose *et al. (21)*. For the analysis, 40 mg of flour was extracted with 400 µL of a reagent containing 2 M urea, 15% glycerol, 0.1 M Tris-HCl (pH 8.8), and 0.1 M dithiothreitol in the ultrasonic water bath for 15 min at room temperature. After centrifugation at 10,000×*g* for 15 min, 4 µL of supernatant was denatured by heating at 95°C for 5 min with 2 µL of Agilent denaturing solution. Afterward, the denatured sample was diluted with 84 µL of deionized water and 6 µL of this mixture was applied to the Protein 80^{+} LabChip for analysis in the Agilent 2100 Bioanalyzer

according to the manufacturer's instructions (Agilent Technologies, Santa Clara, California). Three independent sample extractions were performed and each total protein extract was analyzed in duplicate.

Scanning electron microscopy

Grain ultrastructures were analyzed according to the method of Oliveira *et al. (22)*. For the analysis, grain cross sections were mounted onto aluminum stubs using double-sided adhesive carbon tape. Then, the samples were coated with a 7-nm gold layer in a Gold Sputter Coater (Bio-Rad Polaron Division, Hemel Hempstead, United Kingdom) and observed under a constant accelerating voltage of 5 kV applying a JEOL scanning electron microscope type 5510 (JEOL Ltd., Tokyo, Japan). Each oat cultivar was analyzed in duplicate.

Mashing performance of oat cultivars

Milling

Malted barley was milled with a laboratory disk mill (Bühler GmbH, Braunschweig, Germany) set at a 0.2-mm disk distance. Unmalted oat cultivars were milled using a hammer mill equipped with a 1.5-mm sieve (A.M.A. S.p.A., San Martino in Rio, Italy). Milling of mashing materials was carried out directly before mashing-in.

Infusion mashing process

For mashing, a commonly used infusion process was chosen as follows: 30 min at 50°C, 40 min at 65°C, 20 min at 72°C, and 5 min at 78°C (mashing-off) applying a heating rate of 1°C per min. Mashes with various levels of each oat cultivar (0%, 20%, and 40% of grist mass) were prepared using a constant liquor-to-grist ratio of 3.55:1. Mashing-in was performed by mixing the homogenized grist into preheated distilled water (50°C) in the respective mash cup, which was then attached to the temperature-controlled heating system of the respective mashing instrument. In all mashing trials, a constant stirring speed of 100 rpm was applied.

Rheological mash profile

The rheological profile of mashes containing different oat cultivars was detected by applying the previously published method of Schnitzenbaumer *et al. (23)*. For this

purpose, a controlled stress rheometer Physica MCR 301 (Anton Paar Germany GmbH, Ostfildern, Germany) equipped with a paddle-shaped rotor, enabling mash particles to be kept in suspension throughout the measurement, was used. In all rheological mashing trials, a total grist mass of 5.097 g dry matter (DM) was mixed with distilled water to give a total mash mass of 27.000 g at a constant moisture basis of 14%. All rheological tests were performed in triplicate.

Laboratory-scale mashing

Mashing with malted barley and unmalted oat cultivars was carried out in a LB 8 – Electronic mashing device (Lochner Labor + Technik GmbH, Berching, Germany). In all laboratory-scale mashing trials, a total grist mass of 96.75 g (DM) was mixed with distilled water to give a total mash mass of 512.50 g at a constant moisture basis of 14%. The saccharification rate was measured 10 min after the mash reached 72°C and repeated every 5 min until the iodine test was negative. After mashing-off at 78°C, the loss of water due to evaporation during the mashing process was determined gravimetrically and replaced. Then, the filtration rate of the hot mash was checked by measuring the filtered wort volume every 2 min. All laboratory-scale mashing trials were performed in triplicate.

Wort analysis

Worts were analyzed applying the standard methods specified by MEBAK, European Brewery Convention (EBC), or American Society of Brewing Chemists (ASBC). pH and color of wort samples were measured according to MEBAK – Raw materials *(19)* methods 3.1.4.2.7 and 3.1.4.2.8.2. Total polyphenols in wort were determined following MEBAK – Band II *(24)* method 2.17.1. Wort viscosities were analyzed using a HAAKE falling ball viscometer (Thermo Scientific, Karlsruhe, Germany). Total soluble nitrogen (TSN) contents of wort samples (10 mL) were measured applying a Tecator™ Digestor combined with a Kjeltec™ 2100 Distillation unit (Foss, Hillerød, Denmark). Free amino nitrogen (FAN) in worts was determined according to MEBAK – Raw materials *(19)* method 3.1.4.5.5.1. The limit of attenuation of worts was analyzed following MEBAK – Raw materials *(19)* method 3.1.4.10.1.2 using dry lager yeast (Saflager S-23; Fermentis, Marcq-en-Baroeul cedex, France). Wort extract, apparent extract, apparent degree of fermentation, and alcohol were measured using an Alcolyzer Beer ME Analyzing System (Anton Paar

GmbH, Graz, Austria). In addition, wort β-glucan contents were determined applying the Megazyme mixed-linkage β-glucan assay procedure (Megazyme International Ireland Ltd., Bray, Ireland). The protein profile of worts was detected using Lab-on-a-Chip capillary electrophoresis. For the analysis, 40 mg of freeze-dried and homogenized sample was extracted and analyzed as described above. All wort analyses were performed in duplicate.

Statistical analysis

For determining the statistical significance, the two-tailed Student's *t*-value for *n*-1 degrees of freedom was calculated *(25)*. The confidence interval with a probability level of 95% ($\alpha = 0.05$) was determined for each mean value (arithmetic mean).

Results and discussion

Characterization of oat cultivars

In this study, mostly husked oat cultivars (Lutz, Buggy, Galaxy, Scorpion, Typhon, Ivory, Curly), where each kernel is wrapped in a hull, but also one huskless or naked oat cultivar (NORD 07/711), where the hull naturally falls off the kernel at harvest, were analyzed. Oat husk consists of cellulose and hemicellulose (each around 30–35%), lignin (2–10%), ash (3.5–9%), protein (1.6–5%), oil (1–2.2%), starch (<2%), and water-soluble carbohydrates (<1%). It is a poor quality feedstuff and the most significant improvement in grain quality could be made by breeding for reduced husk content *(26)*. In general, oats comprise a very large proportion of husk (25–30% of total grain weight) compared to barley (6–15% of total grain weight) *(27)*. At present, the oat cultivar 'Ivory' features the lowest husk content of all German husked oat cultivars according to the breeder (Nordsaat Saatzucht GmbH, Granskevitz, Germany). As shown in Table 1, the fat content varied significantly among the eight oat cultivars from 3.8% to 6.1% (DM) being within the range given in the literature *(14,28)*. Intact hulled or hulless oats stored at typical moisture contents of 12–14% (Table 1) are stable and show little change in free fatty acid content during storage *(29)*. However, once the integrity of the kernel is disrupted, the enzymes lipase (hydrolytic catalyst), lipoxygenase (oxidative catalyst), and peroxidase are activated and a rapid buildup of free fatty acids occurs, followed by oxidative breakdown *(4,29)*. At present, the oat cultivar 'Typhon' features the lowest fat content of all German oat cultivars according to the breeder (Nordsaat Saatzucht GmbH, Granskevitz, Germany). Also the β-glucan content varied significantly among the eight oat cultivars from 1.94% to 3.62% (DM) (Table 1). These findings correspond with data published in the literature *(30)*. The naked oat cultivar 'NORD 07/711' contained significantly lower levels of β-glucan than the seven husked oat cultivars such as 'Lutz' exhibiting the highest β-glucan content. Mixed-linkage (1→3)(1→4)-β-D-glucan is a water-soluble and highly viscous polysaccharide representing the main component of soluble dietary fiber in oats *(2)*. Its solubility (extractability) in aqueous systems generally increases with decreasing particle size and increasing temperature or pH *(5,6)*. In terms of mash consistency/wort viscosity, it is the amount of β-glucan solubilized in the brewery mash that is important, rather than the total β-glucan content *(10,23)*.

The total nitrogen content of all oat cultivars analyzed in this study was significantly higher than that of barley malt (1.41% DM) ranging from 1.55% to 2.19% (DM) (Table 1). It is common practice to multiply the nitrogen content by 6.25 (conversion factor) resulting in crude protein contents of 9.7–13.7% (DM). Naked oats 'NORD 07/711' contained significantly more protein (13.7% DM) than husked oats (9.7–11.2% DM) representing low-protein cultivars. These findings correspond with data published in the literature *(1,31,32)*. The oat cultivars 'Buggy' and 'Galaxy' featured the lowest protein contents among husked oats being in agreement with the breeder (Nordsaat Saatzucht GmbH, Granskevitz, Germany). Furthermore, the total starch content differed significantly among the eight oat cultivars between 58.50% and 66.32% (DM) (Table 1). In comparison with barley malt (65.68% DM), the oat cultivars 'Galaxy', 'Lutz', 'Curly', and 'Buggy' contained significantly less starch, whereas 'NORD 07/711', 'Typhon', 'Scorpion', and 'Ivory' contained similar levels of starch (statistically non-significant different). Naked oats 'NORD 07/711' exhibited the highest starch content of all samples analyzed in this study. The ash content of husked and naked oats was significantly higher than that of barley malt (1.60% DM) ranging from 2.08% to 2.71% (DM) (Table 1). Only the oat cultivar 'Buggy' revealed similar ash levels than 'NORD 07/711' being significantly lower compared to those of 'Lutz'. It has been reported that hulled oats or barley exhibited higher contents of crude fiber and ash as well as lower contents of starch than hulless oats or barley *(27,31,32)* as confirmed in this study.

Table 5–1. Standard analysis of oat cultivars.

Oat cultivar	Moisture	Fat	β-Glucan	Nitrogen	Starch	Ash
	%	% DM	% DM	% DM	% DM	% DM
Lutz	12.7 ± 0.3	6.1 ± 0.9	3.62 ± 0.20	1.76 ± 0.08	58.61 ± 3.55	2.71 ± 0.27
Buggy	12.9 ± 0.2	5.5 ± 0.6	3.39 ± 0.28	1.55 ± 0.05	59.98 ± 1.52	2.08 ± 0.18
Galaxy	12.3 ± 0.1	5.7 ± 0.4	3.18 ± 0.20	1.55 ± 0.12	58.50 ± 2.21	2.39 ± 0.13
Scorpion	12.7 ± 0.8	4.3 ± 0.4	3.04 ± 0.35	1.64 ± 0.04	65.01 ± 4.91	2.30 ± 0.16
Typhon	12.4 ± 0.7	3.8 ± 0.4	2.56 ± 0.21	1.74 ± 0.05	65.60 ± 6.59	2.49 ± 0.20
Ivory	12.2 ± 0.1	4.6 ± 0.4	3.03 ± 0.24	1.75 ± 0.12	65.01 ± 3.83	2.48 ± 0.41
Curly	12.5 ± 0.1	4.3 ± 0.3	2.84 ± 0.17	1.79 ± 0.10	59.67 ± 4.01	2.35 ± 0.11
NORD 07/711	13.3 ± 0.7	5.3 ± 0.5	1.94 ± 0.25	2.19 ± 0.04	66.32 ± 2.74	2.11 ± 0.12

Cytolytic, proteolytic, and amylolytic enzyme activities in all eight oat cultivars (Table 2) were significantly lower than those in barley malt (β-glucanase 359.14 U/kg; proteolytic activity 11.30 mg L-leucine/h·g; α-amylase 165.50 U/g; β-amylase 19.27 U/g), since hydrolytic preexisting enzymes are activated and new enzymes are synthesized during germination *(33)*. Interestingly, naked oats 'NORD 07/711' exhibited a 2.5–3.6-fold higher α-amylase activity than husked oats (statistically significant). However, their enzyme level (0.62 U/g) was still negligible in comparison to that of malted barley.

Table 5–2. Enzyme activities in oat cultivars.

Oat cultivar	β-Glucanase	Proteolytic activity	α-Amylase	β-Amylase
	U/kg	mg L-leucine/h·g	U/g	U/g
Lutz	28.43 ± 8.68	4.23 ± 0.95	0.20 ± 0.01	1.56 ± 0.27
Buggy	15.77 ± 4.31	3.63 ± 1.17	0.17 ± 0.01	1.23 ± 0.27
Galaxy	24.54 ± 4.84	4.05 ± 1.95	0.20 ± 0.01	1.33 ± 0.25
Scorpion	23.93 ± 1.64	4.18 ± 1.65	0.25 ± 0.02	1.37 ± 0.20
Typhon	24.25 ± 0.94	4.76 ± 2.26	0.23 ± 0.01	1.05 ± 0.17
Ivory	25.25 ± 3.61	4.22 ± 1.09	0.24 ± 0.01	1.11 ± 0.10
Curly	21.41 ± 3.13	4.60 ± 1.13	0.19 ± 0.01	1.19 ± 0.06
NORD 07/711	18.31 ± 6.59	4.57 ± 1.00	0.62 ± 0.02	0.89 ± 0.11

The protein profile of barley malt differed significantly from that of unmalted oats such as 'Lutz' as shown in Figure 1a. However, high similarities between the protein profiles of the eight oat cultivars were revealed (Figure 1b). Barley malt proteins ranged from 9.9 kDa to 84.7 kDa, whereas oat proteins ranged from 6.3 kDa to 75.2 kDa. The total peak area in the electropherograms of different oat cultivars was 4.4–5.6-fold higher than that in the electropherogram of barley malt. Hence, high amounts of unmodified high-molecular-weight proteins are brought into solution by replacing barley malt with oats increasing the consistency of mashes.

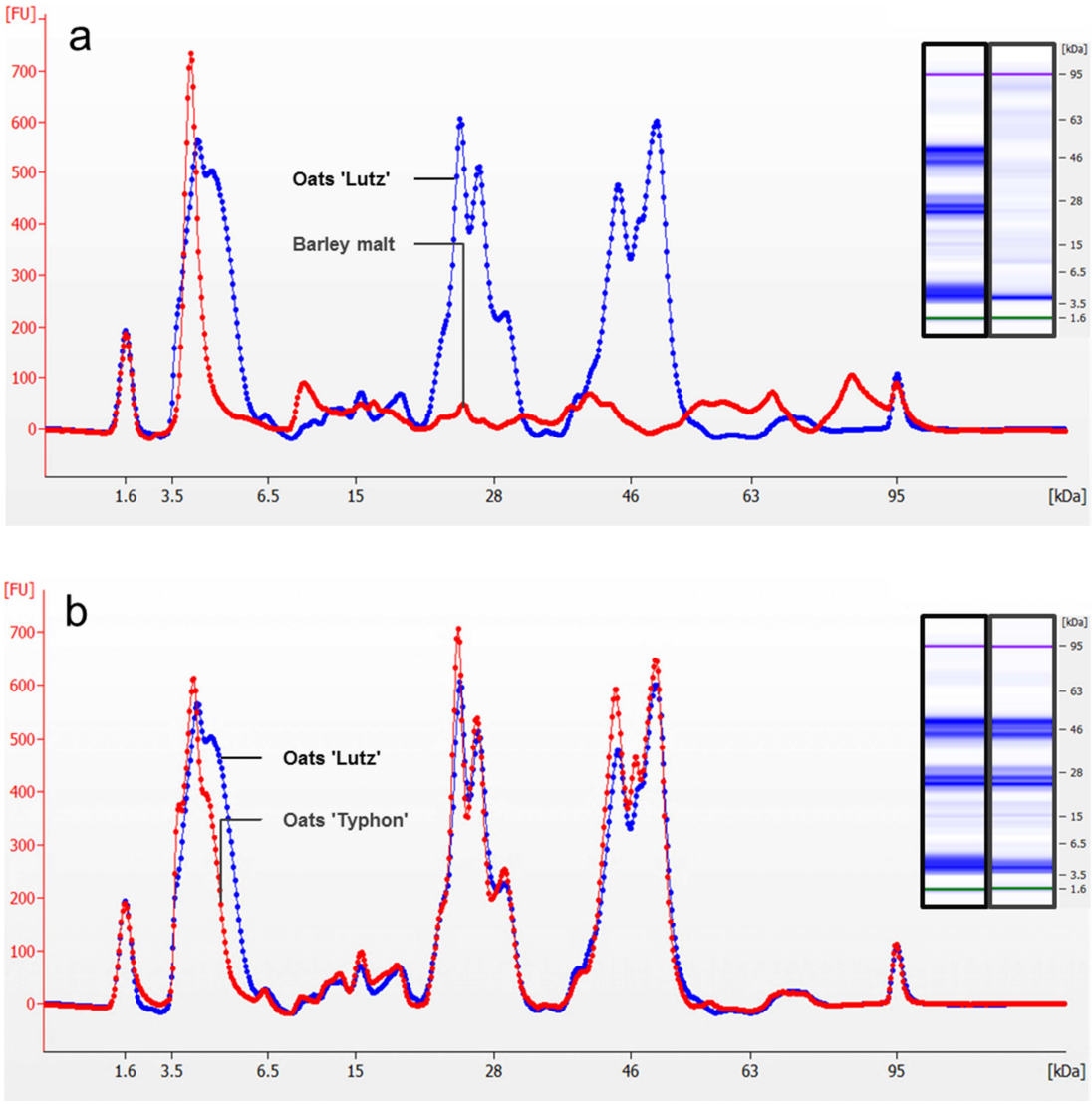


Figure 5–1. Electropherograms/gel-like images of (a) barley malt, oats 'Lutz' and (b) different oat cultivars.

A look inside husked and naked oats using scanning electron microscopy (SEM) illustrated their main difference, the outer layers as shown in Figures 2a and 2b. The oat kernel, also named caryopsis or groat, is the residual part after removal of lemma and palea, commonly termed the hull. That usually remains on the groat after threshing (husked oats) and accounts for around 25–30% of total dry grain weight in most oat cultivars *(3,34)* as mentioned above. Naked oats, when harvested, are groats without their protective hulls having a considerably higher nutritional value than husked oats *(31)*. A closer look into the endosperm of barley malt (Figure 2c) revealed that β-glucans and proteins were degraded to a large extent by cytolytic and proteolytic enzymes during malting, even starch granules showed bite marks from

amylolytic enzymes. Barley starch comprises both large lenticular and small spherical granules *(35)*, whereas oat starch was found to be present in large compound granules (composed of several individual granules) and single granules being smooth and irregular in shape (Figure 2d). Its small granule size, high lipid content (as mentioned above), high relative crystallinity, and small amylose chain length are unique features affecting gelatinization properties *(3,36)*.

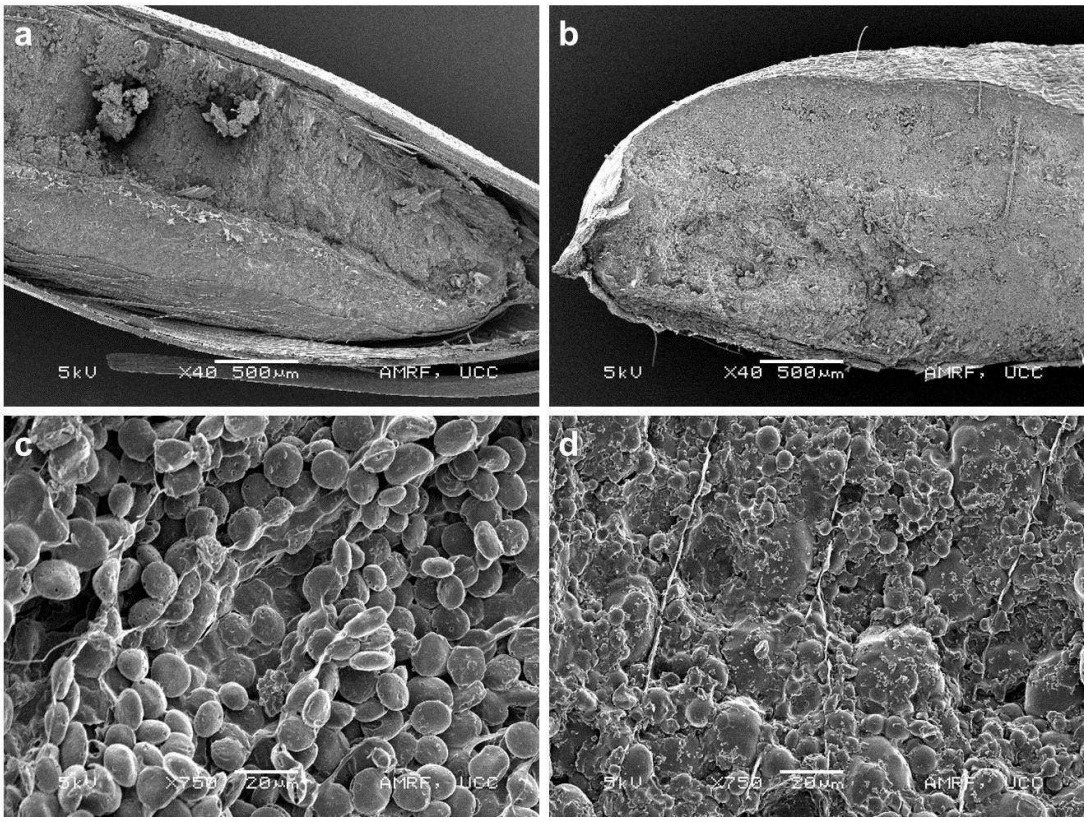


Figure 5–2. SEM images of (a) husked oats 'Lutz', (b) naked oats 'NORD 07/711', (c) barley malt endosperm, and (d) oat endosperm (NORD 07/711).

Mashing performance of oat cultivars

Rheological mash profile

In the present study, the rheological profile of a brewery mash containing 40% naked oats 'NORD 07/711' was similar to that of the reference mash containing 100% barley malt as shown in Figure 3. At the beginning of the cytolytic and proteolytic mash rest, its consistency was slightly higher than that of the reference mash due to significantly higher contents of β-glucan and protein in naked oats (NORD 07/711). Furthermore, the gelatinization of naked oat starch caused a significantly higher peak consistency compared to that of barley malt starch as a consequence of previously mentioned ultrastructural differences (gelatinization temperature 56.5–57.9°C). However, the substitution of 40% barley malt with oats 'NORD 07/711' resulted in a final mash consistency similar to that of the reference. In contrast, husked oats such as 'Lutz' caused significantly higher mash consistencies than naked oats before and after starch gelatinization, most likely because of their significantly higher β-glucan contents and coarser grists (high husk fraction). During starch gelatinization, however, the peak consistencies of mashes containing 40% of different oat cultivars were non-significantly different. The use of the husked oat cultivar 'Typhon' led to the highest final mash consistency (statistically significant).

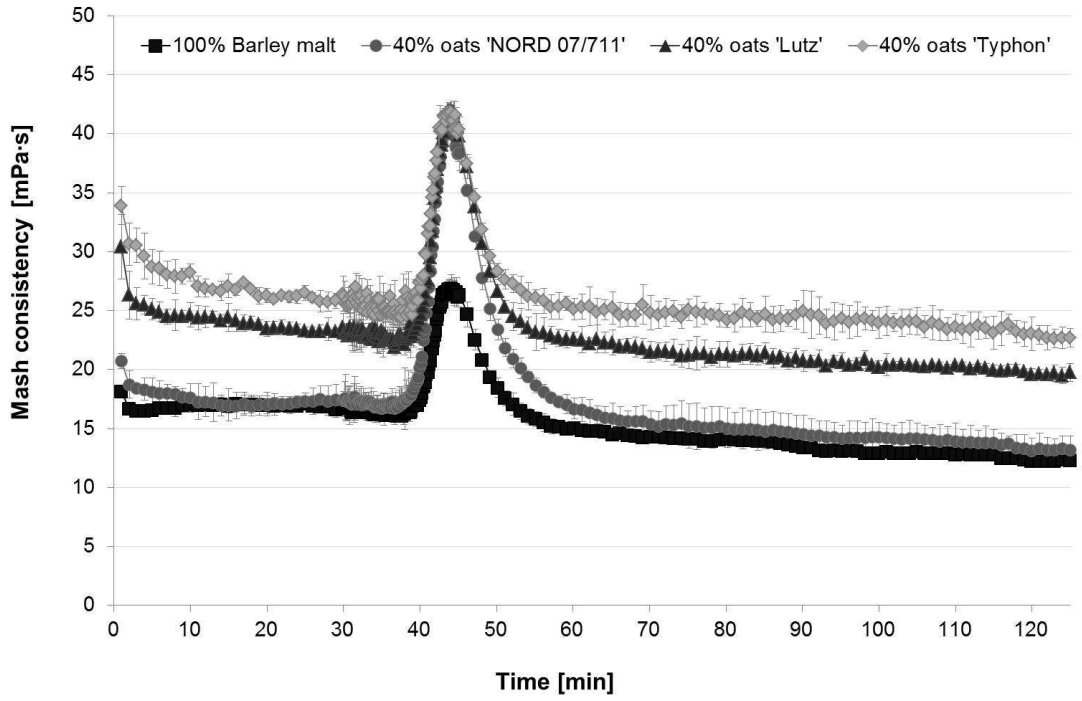


Figure 5–3. Effect of 40% of different oat cultivars on the rheological mash profile.

Laboratory-scale mashing

In the present study, the wort pH increased significantly when replacing 20% (except for naked oats) or 40% malted barley with different oat cultivars (100% barley malt wort pH 5.75; 40% oat worts pH 5.79–5.85). These findings correspond to experimental results published previously *(18)*. Worts produced with naked oats 'NORD 07/711' showed a lower pH than those produced with husked oats (statistically non-significant). The β-glucan content of worts increased significantly from 60 mg/L (100% barley malt reference) to 209–434 mg/L when using 20% of the studied oat cultivars. Doubling the adjunct concentration from 20% to 40% oats led to a 1.7–3.2-fold increase in wort β-glucan to 497–984 mg/L (statistically significant) as shown in Table 3. Worts produced with 20% or 40% oats 'Lutz' exhibited the highest levels of β-glucan among all samples (statistically significant except for 20% oats 'NORD 07/711'). These findings can be explained by the fact that 'Lutz' had the highest β-glucan content of all oat cultivars analyzed in this study (see Table 1). However, worts containing 20% oats 'NORD 07/711', showing the lowest β-glucan content (see Table 1), revealed a significantly higher β-glucan concentration than those containing 20% of the oat cultivars 'Buggy', 'Galaxy', 'Scorpion', 'Typhon', 'Ivory', or 'Curly'. Substituting 40% barley malt with naked oats (NORD 07/711) resulted in significantly higher β-glucan contents compared to those of worts produced with the husked oat cultivars 'Curly' or 'Galaxy'. Gajdošová *et al. (8)* found that naked oats contain more water-soluble and less water-insoluble β-glucan than hulled oats. The surprisingly high β-glucan concentration in worts produced with naked oats (NORD 07/711) might also result from an increased solubility due to finer husk-free grist (higher flour fraction). The TSN content of worts decreased significantly from 1,129 mg/L (100% barley malt reference) to 821–961 mg/L using 20% oats and to 786–943 mg/L using 40% oats (Table 3). Worts produced with 20% oats 'Buggy' or 40% oats 'Galaxy' (low in protein) exhibited the lowest TSN levels, whereas those produced with 'NORD 07/711' (high in protein) or 'Lutz' exhibited constant high TSN levels.

Table 5–3. Effect of 20%/40% of different oat cultivars on wort β-glucan and TSN.

Oat cultivar	β-Glucan [mg/L]		TSN [mg/L]	
	20% oats	40% oats	20% oats	40% oats
Lutz	434 ± 104	984 ± 78	910 ± 57	943 ± 70
Buggy	209 ± 9	677 ± 84	821 ± 102	852 ± 57
Galaxy	239 ± 19	546 ± 27	903 ± 62	786 ± 80
Scorpion	236 ± 12	579 ± 57	961 ± 40	831 ± 81
Typhon	248 ± 26	746 ± 51	947 ± 57	847 ± 71
Ivory	250 ± 17	556 ± 50	866 ± 44	863 ± 72
Curly	218 ± 3	497 ± 43	835 ± 95	842 ± 78
NORD 07/711	369 ± 28	639 ± 55	945 ± 52	912 ± 36

The substitution of 20% barley malt with different oat cultivars also caused a significant decrease in FAN content of worts from 220 mg/L (100% barley malt) to 182–204 mg/L. Doubling the adjunct concentration from 20% to 40% oats resulted in a further significant decrease in wort FAN to 152–172 mg/L as shown in Figure 4. The oat cultivars 'Lutz' and 'Scorpion' provided the highest FAN levels in worts, whereas 'Ivory' and 'Galaxy' provided around 20 mg/L less FAN (lowest levels). However, it has been found that FAN contents of 160 mg/L and lower are adequate for optimal yeast growth and an efficient fermentation *(18,37)*.

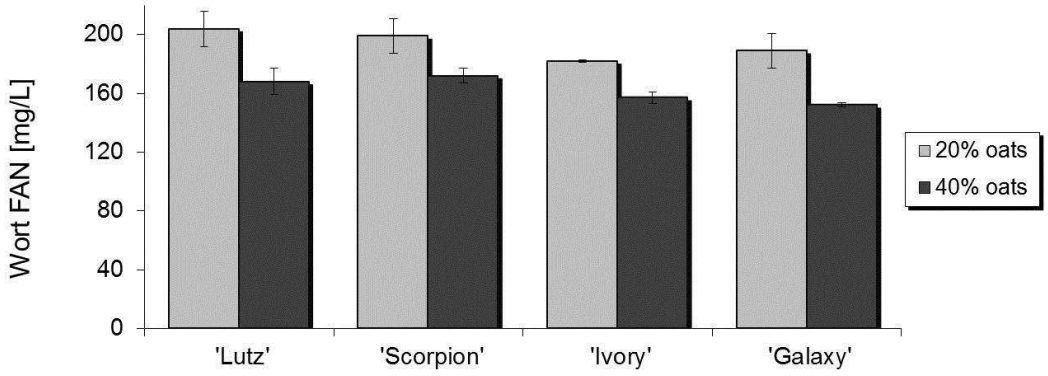


Figure 5–4. Effect of 20%/40% of different oat cultivars on wort FAN content.

In addition, worts containing 40% naked (NORD 07/711) or husked oats exhibited similar protein profiles than the reference wort (100% barley malt) as shown in Figure 5. Thus, the high levels of unmodified high-molecular-weight proteins, brought into solution by replacing barley malt with oats, were extensively degraded by malt proteases or precipitated during the mashing process. However, the protein peaks or bands between 6.3 kDa and 17.4 kDa (foam-positive low-molecular-weight

fraction) as well as around 41.5 kDa and 50.3 kDa (foam-positive high-molecular-weight fraction) were more distinct when using naked instead of husked oats. The first fraction contains lipid transfer protein 1 and the latter mainly protein Z being tolerant to high temperatures and resistant to proteolysis *(38)*. These findings might indicate a better foam stability in beers produced with naked oats compared to that in beers produced with husked oats *(18)*.

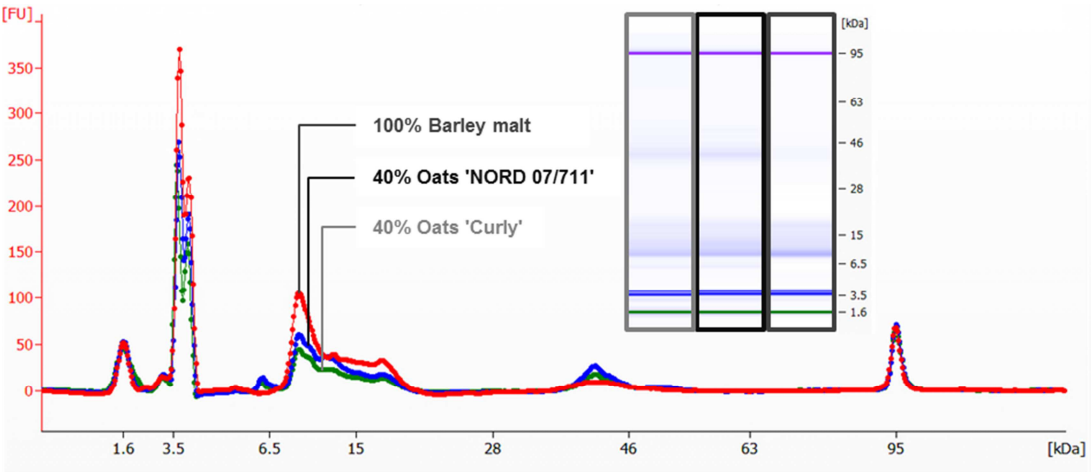


Figure 5–5. Effect of 40% of different oat cultivars on wort protein profile.

Worts containing 20% or 40% of the naked oat cultivar 'NORD 07/711' showed the highest extract contents of all samples. Besides, the replacement of up to 40% barley malt with naked oats resulted in a constant extract yield (100% barley malt wort extract 15.68% w/w), whereas the use of husked oats led to significant extract losses as shown in Figure 6. The highest wort extract contents when using 20%/40% husked oats were achieved with the oat cultivar 'Buggy' (15.40% w/w/14.93% w/w); in contrast, the use of the oat cultivar 'Lutz' resulted in the lowest extract levels.

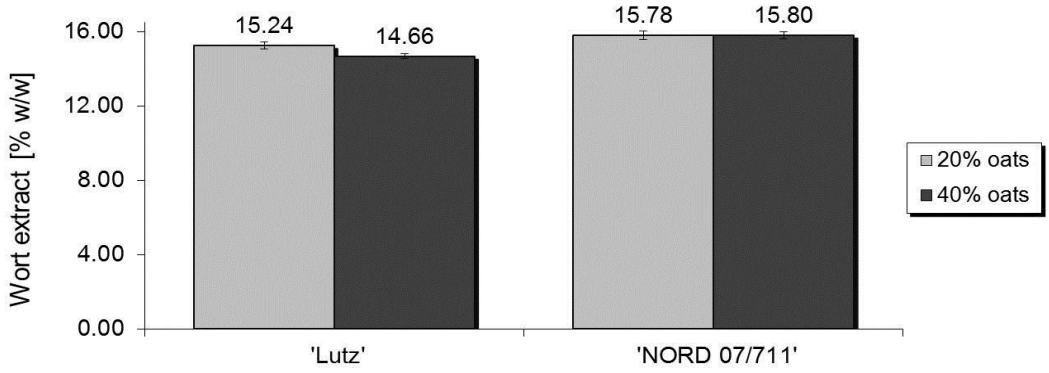


Figure 5–6. Effect of 20%/40% of different oat cultivars on wort extract content.

These findings were partially reflected in the fermentability or apparent attenuation limit of those worts decreasing significantly from 79.0% w/w (100% barley malt reference) to 75.3–76.4% w/w by substituting 40% barley malt with different oat cultivars. The highest apparent attenuation limit was achieved in worts containing 40% oats 'Buggy', being significantly higher than that determined in 40% oats 'Lutz' containing worts (75.3% w/w). However, the higher extract content of worts produced with naked oats (NORD 07/711) was not reflected in a higher fermentability. It should be mentioned here that the apparent attenuation limit of worts was analyzed using the dry yeast Saflager S-23, which was found to attenuate significantly less than other yeast strains *(39)*.

The viscosity of worts based on 12% (w/w) extract increased significantly from 1.674 mPa·s (reference) to 1.709–1.834 mPa·s when replacing 20% malted barley with different oat cultivars. Doubling the adjunct concentration from 20% to 40% oats caused a further significant increase in wort viscosity to 1.777–2.250 mPa·s as shown in Figure 7. Worts produced with the oat cultivar 'Lutz' (high in β-glucan) exhibited the lowest viscosity (statistically significant), despite the fact that their β-glucan concentration was found to be the highest of all worts. In contrast, worts containing up to 40% of the naked oat cultivar 'NORD 07/711' (low in β-glucan) showed the highest viscosity among all samples (statistically significant). Bhatty *(6)* found that viscosity is a poor indicator of total β-glucan content in oats. A high level of extracted β-glucan does not necessarily involve a high viscosity (and vice versa) since different extraction conditions (e.g. particle size) can result in different fractions of β-glucan (larger fragments contribute more to viscosity than smaller but more numerous fragments). In addition, Autio *et al. (40)* compared the viscosities and molecular weight distributions of β-glucan preparations isolated from ten Finnish oat cultivars. It has been found that the most significant differences in viscosity between those β-glucan solutions were caused by differences in their mean molecular weight.

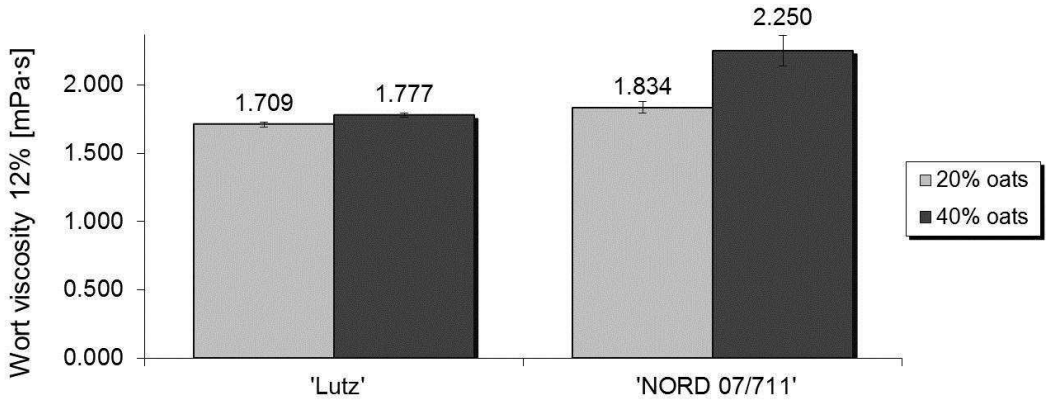


Figure 5–7. Effect of 20%/40% of different oat cultivars on wort viscosity.

In accordance with these results, the filtration rate of the 78°C hot mashes was significantly reduced by using 20% of each hammer-milled oat cultivar and decreased significantly from 17.4 mL wort/min (100% barley malt) to 13.7–15.3 mL wort/min by using 40% oats. These findings also correspond to experimental results published previously *(18)*. The filtration of mashes containing 40% oats 'Lutz' (15.3 mL wort/min) was significantly faster compared with that of mashes containing 40% oats 'NORD 07/711' (13.7 mL wort/min).

The substitution of 20% barley malt with different oat cultivars significantly reduced the polyphenol concentration in wort from 148 mg/L (reference) to 104–121 mg/L. Doubling the adjunct concentration from 20% to 40% oats led to a further significant decrease of total polyphenols in wort to 82–94 mg/L as shown in Figure 8. Worts produced with the oat cultivars 'Lutz' or 'Buggy' tended to have the highest polyphenol contents, whereas those produced with 'Ivory' or 'Curly' showed the lowest levels (statistically non-significant).

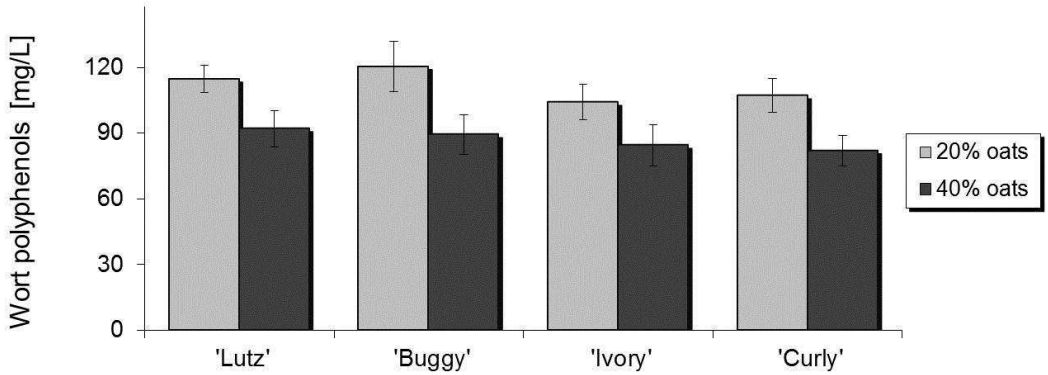


Figure 5–8. Effect of 20%/40% of different oat cultivars on wort polyphenol content.

In accordance with these findings, the wort color decreased significantly from 17.30 EBC units (100% barley malt reference) to 9.25–14.98 EBC units when using up to 40% oat adjunct. Worts containing 20% or 40% of the oat cultivars 'Lutz' or 'Buggy' tended to be darker than those containing 'Ivory' or 'Curly'. The lowest color values were, however, achieved with the naked oat cultivar 'NORD 07/711'. Polyphenols may not only contribute to wort color but also to astringency (harsh taste), haze, and an increased flavor stability of beer *(41)*.

Conclusion

In this study, the effect of eight oat cultivars (low in fat, β-glucan, protein, and/or high in starch) on the quality and processability of mashes and worts was compared. The substitution of up to 40% barley malt with unmalted husked or naked oats resulted in significantly higher pH values, β-glucan contents, and viscosities as well as significantly lower TSN, FAN, and polyphenol contents, color values, filtration rates, and apparent attenuation limits. Naked oats (NORD 07/711) contained significantly less β-glucan as well as more protein and starch than the seven husked oat cultivars (Lutz, Buggy, Galaxy, Scorpion, Typhon, Ivory, Curly). Their use as a substitute for barley malt caused significantly lower mash consistencies before and after starch gelatinization compared to the use of husked oats. However, worts produced with 20% or 40% of the naked oat cultivar 'NORD 07/711' exhibited the highest viscosities of all samples, whereas those produced with 'Lutz' revealed the lowest viscosities. Hence, a high β-glucan content does not necessarily involve a high wort viscosity (and vice versa). The replacement of 40% barley malt with naked oats (NORD 07/711) resulted in a constant extract yield, whereas the use of husked oats led to significant extract losses. However, the oat cultivars 'Lutz' and 'Scorpion' provided more FAN than all other cultivars analyzed in this study. In view of all these results, the oat cultivars 'NORD 07/711' (naked oats) and 'Lutz' (husked oats) have proven to be especially interesting and promising as brewing adjuncts.

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Chapter 6

Effect of unmalted oats (*Avena sativa* L.) on the quality of high-gravity mashes and worts without or with exogenous enzyme addition

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Abstract

Barley malt is the preferred brewing material these days because of its high extract content and high enzyme activities. When substituting malted barley with unmalted oats in order to create a unique beer flavor/aroma amongst others, endogenous malt enzymes become the limiting factor. Therefore, the objectives of this study were to evaluate the effect of 10–40% unmalted oats on the quality of high-gravity mashes/worts and to investigate the limitations of endogenous malt enzymes as well as the benefits of the application of industrial enzymes. The enzyme mix Ondea® Pro was found to be particularly suitable for mashing with unmalted oats and was therefore used in the present rheological tests and laboratory-scale mashing trials. In order to gain detailed information about the biochemical processes occurring during mashing, the quality of mashes was comprehensively analyzed after each mash rest using standard methods described by Mitteleuropäische Brautechnische Analysenkommission (MEBAK) and Lab-on-a-Chip capillary electrophoresis. Mashing with up to 40% oats resulted in increased mash consistencies, color/pH (20°C) values, β-glucan concentrations, wort viscosities 12.0%, and filtration times as well as decreased free amino nitrogen and extract contents. The application of Ondea® Pro enormously increased the color of worts despite lower pH values but considerably improved the quality and processability of 30% or 40% oat-containing mashes/worts. However, the substitution of up to 20% barley malt with unmalted oats can easily be realized without the addition of exogenous enzymes.

Introduction

Malted oats (*Avena sativa* L.) have been used by European brewers for many centuries *(1)*. Today, barley malt is the dominant brewing material; preferred because of its lower husk proportion and therefore higher extract content as well as higher enzyme activities amongst others *(2–6)*. Malting is the initial step in the traditional beer production process and strongly defines type and quality of the final beer. Its main purposes are to produce enzymes and to breakdown cell walls surrounding starch granules *(7)*. The malting process, a limited modification of cereal seeds under controlled conditions, is split into three unit operations: steeping, germination, and kilning. In steeping, seeds absorb moisture to a controlled extent by immersing them in water. Thus, the seeds swell and soften while the living tissues resume their metabolism *(8)*. The germination of cereal seeds comprises three biochemical processes: 1) the initiation or "wake up" period; 2) the period of intensive biosynthesis of proteins; 3) the degradation of storage proteins as well as other macromolecules in the endosperm *(9)*. During germination, hydrolytic preexisting enzymes are activated and new enzymes are synthesized in the aleurone layer *(8)*. Furthermore, the proteinaceous matrix surrounding starch granules within the endosperm cells is degraded into soluble peptides and amino acids providing substrates for the synthesis of proteins in the growing embryo *(7)*. Since enzymes are generally sensitive to heat at high moisture contents, the kilning process starts with a gentle drying of germinating seeds to halt growth without damaging the enzyme activity *(10)*. Then, further heat is applied to produce the required color, flavor, and aroma *(8)*. While malting, a 10% to 20% loss of weight occurs caused by the growth of rootlets being removed during the malt cleaning process *(10)*. In addition, malting is a very time-/energy-consuming and therefore cost-intensive process causing raw material prices to double and triple according to figures provided by Private Brauereien Bayern e.V. The substitution of barley malt with oats has not only the potential to reduce costs but also to create a unique beer flavor and aroma *(11)*. However, unmalted oats contain high levels of unmodified β-glucan, protein, and fat as well as negligibly low enzyme activities; hence, barley malt enzymes become the limiting factor when brewing with oat adjunct.

The aims of this study were: 1) to determine the effect of up to 40% unmalted oats on the quality and processability of high-gravity mashes/worts; 2) to investigate the limitations of barley malt enzymes and the benefits of the application of exogenous enzymes in high-gravity brewing. For these purposes, both rheological tests and laboratory-scale mashing trials were performed.

Materials and methods

Mashing materials

Unmalted oats (*Avena sativa* L. 'Lutz'), harvested in 2009 in Ravensburg, Germany as well as malted barley (*Hordeum vulgare* L. 'Fr Sebastian'), harvested in 2008 and obtained from Greencore Group plc (Dublin, Ireland) were used in the mashing trials. In a previous study *(12)*, the oat cultivar 'Lutz' has proven to be especially interesting and promising as brewing adjunct. The lipolytic, cytolytic, proteolytic, as well as amylolytic characteristics of both well-modified barley malt and unmalted oats are given in Table 1.

Mashing enzymes

The enzyme cocktail applied to mashes was Ondea® Pro (2.0 g/kg oats) containing the following enzyme activities: Pullulanase (declared enzyme 637 U/g; optimum pH/temperature 3.5–6.0/60–65°C) and α-amylase (optimum pH/temperature 5.2–5.5/70–90°C) to increase the degree of fermentation as a consequence of high maltose yields; endoprotease (optimum pH/temperature 6.0/40–50°C) to maintain a good yeast fermentation performance due to higher levels of fast absorbable amino acids; β-glucanase (optimum pH/temperature 4.0–5.8/65°C), xylanase (optimum pH/temperature 5.0/65°C), as well as lipase (optimum pH/temperature 5.0–9.0/45–65°C) to improve the lautering/filtration efficiency by reducing wort viscosity and to ensure wort clarity (Novozymes A/S, Bagsværd, Denmark). This enzyme mix was found to be particularly suitable for mashing with unmalted oats.

Table 6–1. Characteristics of malted barley and unmalted oats.

Analysis	Method	Unit	Barley malt	Oats
Standard analysis				
Moisture	MEBAK *(13)* method 3.1.4.1/1.5.1.1	%	4.8 ± 0.0	12.7 ± 0.3
Total protein[a]	MEBAK *(13)* method 3.1.4.5.1/1.5.2.1	% DM	9.4 ± 0.1	10.5 ± 0.3
β-Glucan	McCleary method[b]	% DM	0.3 ± 0.0	3.6 ± 0.2
Total starch	Amyloglucosidase/α-Amylase method[b]	% DM	65.7 ± 1.9	58.6 ± 3.6
Fat	MEBAK *(13)* method 2.5	% DM	1.8 ± 0.3	6.1 ± 0.9
Ash	MEBAK *(13)* method 3.1.4.20.1	% DM	1.6 ± 0.2	2.7 ± 0.3
Friability	MEBAK *(13)* method 3.1.3.6.1	%	94.8 ± 1.4	N/A
Glassy kernels	MEBAK *(13)* method 3.1.3.5.1	%	0.6 ± 0.1	N/A
Homogeneity	MEBAK *(13)* method 3.1.3.8	%	98.7 ± 1.2	N/A
Gelatinization temperature	Differential scanning calorimetry	°C	64.6 ± 0.6	61.7 ± 1.3
Enzyme activities				
Proteolytic activity	Method of Brijs *et al. (14)*	mg L-leucine/h·g	11.30 ± 2.88	4.23 ± 0.95
β-Glucanase	Azo-Barley Glucan method[b]	U/kg	359.14 ± 15.88	28.43 ± 8.68
α-Amylase	Ceralpha method[b]	U/g	165.50 ± 4.81	0.20 ± 0.01
β-Amylase	Betamyl-3 method[b]	U/g	19.27 ± 0.28	1.56 ± 0.27
Congress mashing				
Extract	MEBAK *(13)* method 3.1.4.2.2	% w/w (DM)	82.9 ± 0.4	N/A
Saccharification time	MEBAK *(13)* method 3.1.4.2.4	min	<10	N/A
pH	MEBAK *(13)* method 3.1.4.2.7		5.93 ± 0.06	N/A
Wort color	MEBAK *(13)* method 3.1.4.2.8.2	EBC	3.2 ± 0.1	N/A
Boiled wort color	MEBAK *(13)* method 3.1.4.2.9	EBC	5.2 ± 0.0	N/A
Viscosity 8.6%	MEBAK *(13)* method 3.1.4.4.2	mPa·s	1.506 ± 0.016	N/A
Soluble nitrogen	MEBAK *(13)* method 3.1.4.5.2.1	mg/100 g (DM)	581 ± 4	N/A
KOLBACH index	MEBAK *(13)* method 3.1.4.5.3	%	38.8 ± 0.3	N/A
Free amino nitrogen	MEBAK *(13)* method 3.1.4.5.5.1	mg/100 g (DM)	127 ± 2	N/A
β-Glucan	MEBAK *(13)* method 3.1.4.9.2	mg/L	78 ± 16	N/A
Apparent attenuation limit	MEBAK *(13)* method 3.1.4.10.1.1	%	77.7 ± 0.6	N/A
65°C Isothermal mashing				
Viscosity 8.6%	MEBAK *(13)* method 3.1.4.4.2	mPa·s	1.517 ± 0.004	N/A
β-Glucan	MEBAK *(13)* method 3.1.4.9.2	mg/L	139 ± 5	N/A

[a] Total nitrogen (% DM) × 6.25.

[b] Megazyme kits (Megazyme International Ireland Ltd., Bray, Ireland).

N/A = not applicable; $n = 3$.

Milling

Unmalted oats were milled using a hammer mill equipped with a 1.5-mm sieve (A.M.A. S.p.A., San Martino in Rio, Italy). Malted barley was milled with a laboratory disk mill (Bühler GmbH, Braunschweig, Germany) set at a 0.2-mm disk distance. Milling of mashing materials was carried out directly before mashing-in. The particle size distribution of barley malt and oat grist, analyzed according to MEBAK *(15)* method 1.1.1 (n = 3), is shown in Figure 1.

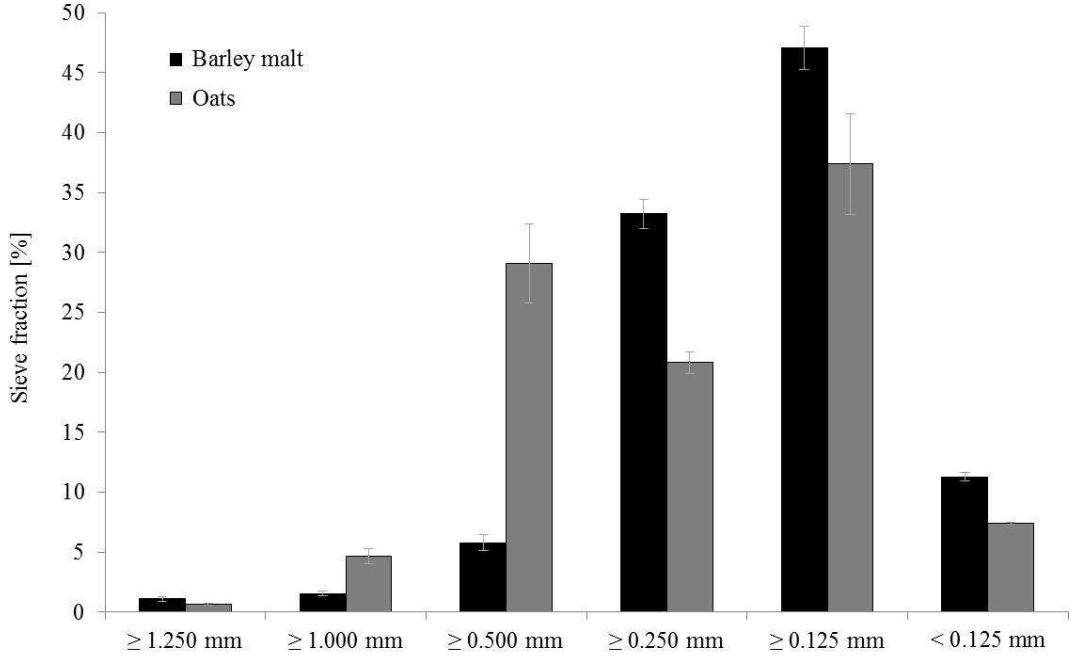


Figure 6–1. Particle size distribution of barley malt and oat grist.

Mashing

For mashing, the following well-proven infusion procedure considering the three important degradation processes cytolysis, proteolysis, and amylolysis was chosen: 30 min at 50°C, 40 min at 65°C, 20 min at 72°C, 5 min at 78°C (mashing-off). Mashes with increasing levels of unmalted oats (0%, 10%, 20%, 30%, and 40% of total grist mass) were prepared using a constant liquor-to-grist ratio of 2.488:1 dry matter (DM). Mashing-in was performed by mixing the homogenized grist into preheated distilled water (50°C) in the respective mash cup being then attached to the temperature-controlled heating system of the respective mashing instrument. In all mashing trials, a stirring speed of 100 rpm and a heating rate of 1°C per min were applied (n = 3).

Rheological mashing trials

The rheological profile of mashes containing various levels of oats was determined according to the previously published method of Schnitzenbaumer *et al. (16)* using a controlled stress rheometer Physica MCR 301 (Anton Paar Germany GmbH, Ostfildern, Germany). In all rheological mashing trials, a total grist mass of 7.740 g (DM) was mixed with distilled water to give a total mash mass of 27.000 g at a constant moisture basis of 14%. Rheological tests were carried out without exogenous enzymes and with recommended enzyme addition (2.0 g Ondea® Pro/kg oats).

Laboratory-scale mashing trials

Worts produced with 0–40% oats using a LB 8 – Electronic mashing device (Lochner Labor + Technik GmbH, Berching, Germany) were comprehensively analyzed after each mash rest at 50°C (End 50°C/total mashing time 30 min), 65°C (End 65°C/total mashing time 85 min), and 72°C (End 72°C/total mashing time 112 min) as well as after mashing-off at 78°C (End 78°C/total mashing time 123 min). The complete infusion mashing process was performed without exogenous enzymes and with recommended enzyme addition (2.0 g Ondea® Pro/kg oats). Saccharification rates were checked 10 min after the mash reached 72°C and the measurement repeated every 5 min until the iodine test was negative. In all laboratory-scale mashing trials, a total grist mass of 154.80 g (DM) was mixed with distilled water to give a total mash mass of 540.00 g at a constant moisture basis of 14%. Water losses due to evaporation during the mashing process were determined gravimetrically and replaced. The filtration rate of hot mashes (78°C) was measured by recording the filtered wort volume every 5 min using folded filter paper (grade 597 ½; Whatman, Dassel, Germany). After filtration, the worts were cooled down to 20°C and then analyzed.

Wort analysis

Worts (20°C) were analyzed according to standard methods specified in MEBAK *(15)*. Free amino nitrogen (FAN) in worts was determined following method 2.6.4.1.1. Wort extract was measured using an Alcolyzer Beer ME Analyzing System (Anton Paar GmbH, Graz, Austria). Color and pH of wort samples were analyzed

according to method 2.12.2 and 2.13, respectively. Wort viscosity was determined applying a HAAKE falling ball viscometer (Thermo Scientific, Karlsruhe, Germany). In addition, wort β-glucan contents were measured following the Megazyme mixed-linkage β-glucan assay procedure (Megazyme International Ireland Ltd., Bray, Ireland). The protein profile of worts was detected using Lab-on-a-Chip capillary electrophoresis as described by Klose *et al. (17)*. For the analysis, 40 mg of freeze-dried and homogenized sample was extracted with 400 µL of a reagent containing 2 M urea, 15% glycerol, 0.1 M Tris-HCl (pH 8.8), and 0.1 M dithiothreitol in the ultrasonic water bath for 15 min at room temperature. After centrifugation at 10,000×*g* for 15 min, 4 µL of supernatant was denatured by heating at 95°C for 5 min with 2 µL of Agilent denaturing solution. Afterward, the denatured sample was diluted with 84 µL of deionized water and 6 µL of this mixture was applied to the Protein 80^{+} LabChip for analysis in the Agilent 2100 Bioanalyzer according to the manufacturer's instructions (Agilent Technologies, Santa Clara, California). All wort analyses were carried out in duplicate ($n = 6$).

Statistical analysis

Results are given as arithmetic means with 95% confidence intervals (two-tailed Student's *t*-values for *n*-1 degrees of freedom). Analysis of variance tests were performed to compare sample means (Bonferroni *t*-test; $\alpha = 0.05$) using SigmaPlot software (Systat Software Inc., San Jose, California).

Results and discussion

In the present study, the effect of up to 40% oats used as brewing adjunct on the quality of high-gravity mashes and worts without or with enzyme addition was evaluated. For these purposes, both rheological tests and laboratory-scale mashing trials were performed.

Rheological mashing trials

The replacement of 10–40% barley malt with oat adjunct adversely affected mash consistency throughout the mashing process as shown in Table 2. Very high correlations between mash consistency and oat concentration were determined. Unmalted oats contain large amounts of unmodified high-molecular-weight β-glucan and protein (see Table 1) increasing the initial mash consistency from 60 mPa·s (0% oats/100% barley malt; reference) to 131 mPa·s (40% oats). During mashing, endogenous malt enzymes (limiting factor) break down viscosity-altering macromolecules such as β-glucan, protein, and starch *(18)* resulting in final mash consistencies from 30 mPa·s (0% oats) to 44 mPa·s (40% oats).

Table 6–2. Effect of 10–40% oats on mash consistency during the mashing process: Start/End 50°C = Start/End of cytolytic/proteolytic mash rest at 50°C; Start/End 65°C = Start/End of first amylolytic mash rest at 65°C; Start/End 72°C = Start/End of second amylolytic mash rest at 72°C; Start/End 78°C = Mashing-off at 78°C.

Adjunct concentration	**Mash consistency**							
	50°C		**65°C**		**72°C**		**78°C**	
	Start	End	Start	End	Start	End	Start	End
	mPa·s	mPa·s	mPa·s	mPa·s	mPa·s	mPa·s	mPa·s	mPa·s
0% Oats	60 ± 2	44 ± 1	90 ± 2	37 ± 0	35 ± 0	33 ± 1	30 ± 0	30 ± 0
10% Oats	66 ± 5	49 ± 0	95 ± 0	42 ± 0	39 ± 1	37 ± 0	34 ± 0	34 ± 1
20% Oats	82 ± 1	54 ± 3	107 ± 3	47 ± 4	43 ± 3	40 ± 3	37 ± 3	38 ± 4
30% Oats	99 ± 6	60 ± 2	121 ± 6	50 ± 1	46 ± 0	43 ± 0	39 ± 2	39 ± 0
40% Oats	131 ± 6	71 ± 6	148 ± 6	55 ± 4	50 ± 2	48 ± 3	43 ± 2	44 ± 1
R^2	**0.9356**	**0.9664**	**0.9272**	**0.9938**	**0.9978**	**0.9908**	**0.9887**	**0.9723**

In preliminary rheological tests was found that it is impossible to reduce the mash consistency of 40% oat-containing mashes to a similar level as that obtained with 100% barley malt by extending the cytolytic/proteolytic mash rest (up to 5 hours). However, this reduction in mash consistency could be achieved by the addition of Ondea® Pro (2.0 g/kg oats) to 40% oat-containing mashes as shown in Figure 2, causing an extensive degradation of high-molecular-weight β-glucans and proteins within 30 min at 50°C. As a consequence of this, the peak consistency decreased from 157 mPa·s (without enzyme addition) to 91 mPa·s (recommended enzyme addition) improving the processability of mashes. The application of Ondea® Pro had significant positive effects on mash consistency when substituting 20% or more barley malt with oats. However, it had relatively little effect on 10% oat-containing mashes.

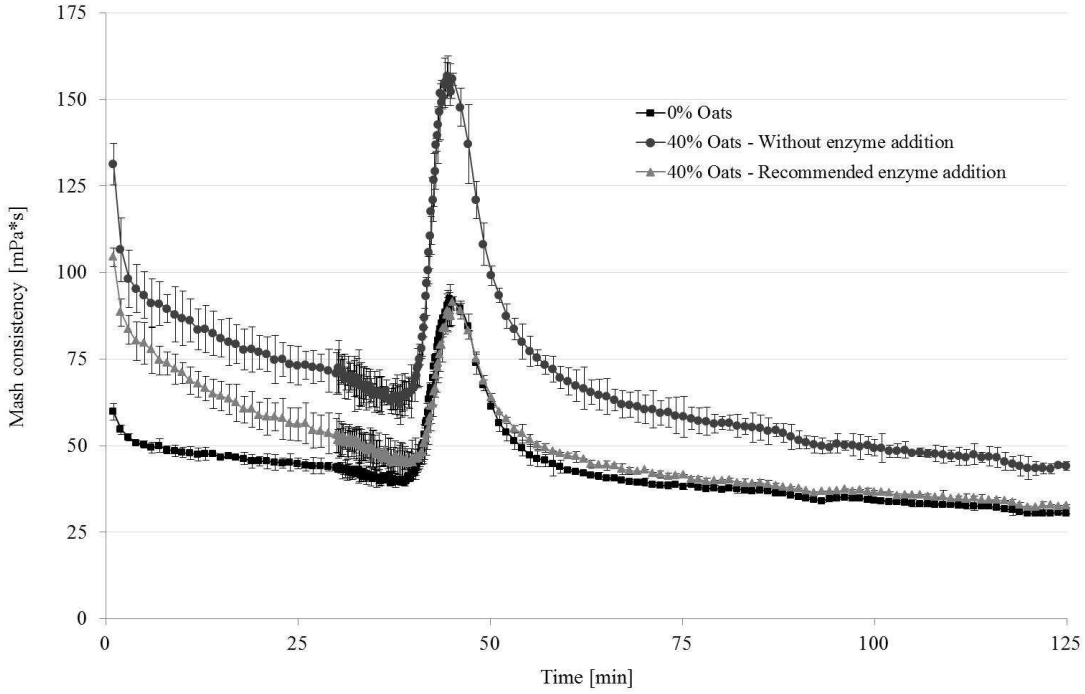


Figure 6–2. Rheological profile of mashes (0%, 40% oats) without and with enzyme addition.

Laboratory-scale mashing trials

In order to closer investigate the performance of endogenous malt enzymes during mashing when using up to 40% unmalted oats, the mashing process was stopped after each mash rest (herein after referred to as End 50°C/End 65°C/End 72°C) and the quality of the respective mashes/worts analyzed. The complete infusion mashing process was performed without exogenous enzymes (End 78°C = final wort) and with recommended enzyme addition (2.0 g Ondea® Pro/kg oats). It has been found that the β-glucan content of final worts increased significantly with increasing oat adjunct level ($P < 0.001$) from 129 mg/L (0% oats/100% barley malt; reference) to 1,773 mg/L (40% oats) as shown in Figure 3. After the cytolytic/proteolytic mash rest, only negligibly low concentrations of β-glucan were determined. During heating up to 65°C and the subsequent mash rest at this temperature, however, enormous amounts of high-molecular-weight oat β-glucan were released into solution ($P < 0.001$) as a result of starch gelatinization (see Table 1) and/or endogenous β-glucan solubilase activity (optimum pH/temperature 6.8/62–65°C) *(18,19)*.

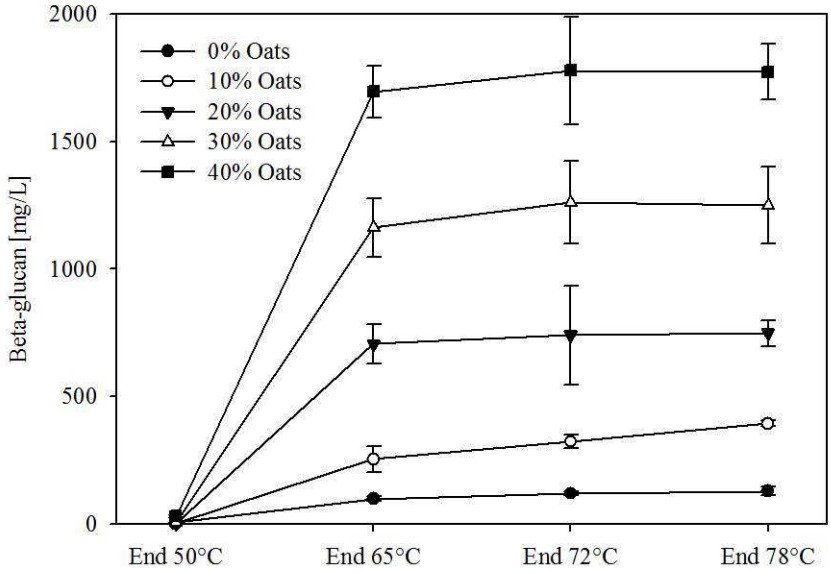


Figure 6–3. Effect of 10–40% oats on mash/wort β-glucan concentration during the mashing process: End 50°C = End of cytolytic/proteolytic mash rest at 50°C; End 65°C = End of first amylolytic mash rest at 65°C; End 72°C = End of second amylolytic mash rest at 72°C; End 78°C = Mashing-off at 78°C.

Malt endo-β-glucanases, degrading solubilized β-glucan to low-molecular-weight molecules, cellobiose, and laminaribiose, are already inactivated at temperatures around 60°C in contrast to β-glucan solubilase (inactivation temperature 73°C) *(19,20)*. This also explains the further increase in β-glucan during the heating up period to 72°C and the following saccharification rest ($P > 0.05$). The addition of Ondea® Pro (2.0 g/kg oats) to mashes significantly reduced the β-glucan concentration in final worts by 55.9–99.6% ($P < 0.001$) (Figure 4). Interestingly, the enzyme activity and degradation rate, respectively, increased with increasing oat adjunct level. Therefore, the application of exogenous β-glucanase in order to prevent lautering and filtration problems seems to be especially reasonable when replacing higher amounts of barley malt with unmalted oats.

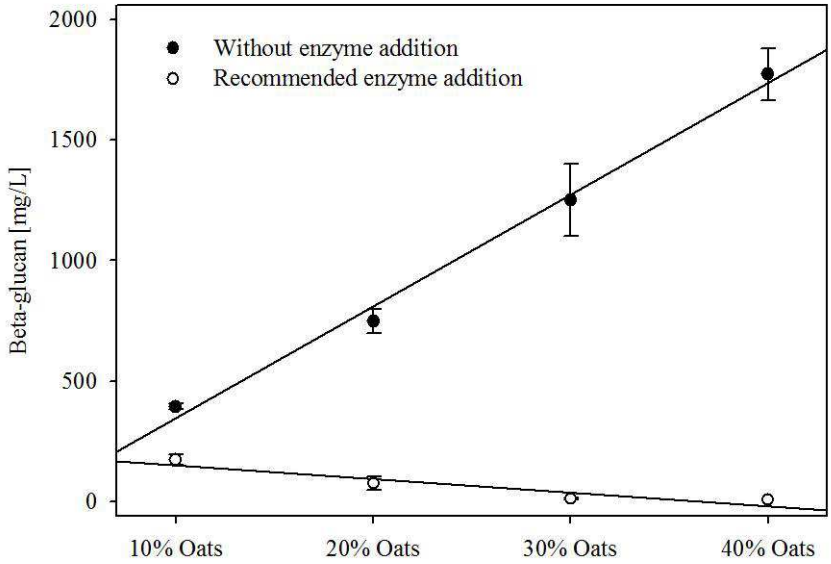


Figure 6–4. Final wort β-glucan concentration (10–40% oats) without and with enzyme addition.

The viscosity of final worts calculated to 12.0% w/w extract increased significantly when using 20% or more oats ($P < 0.001$) from 1.784 mPa·s (100% barley malt) to 1.852 mPa·s (40% oats) as shown in Figure 5. After the mash rest at 50°C, very high viscosities between 2.013 mPa·s (100% barley malt) and 2.130 mPa·s (40% oats) were measured despite negligible β-glucan contents (see above). It has been reported that β-glucan in a concentration below 800 mg/L is not the predominant viscosity-altering component in wort/beer compared to protein and starch *(21)*. During heating up to 65°C and the maltose formation rest, a significant drop in viscosity occurred

($P < 0.001$) being contrary to the immense oat β-glucan concentration determined in those filtrated mashes. However, it has been found that high levels of extracted β-glucan do not necessarily involve a high viscosity and vice versa since larger molecules contribute more to viscosity than smaller but more numerous molecules *(12,22)*. This might also explain the further decline in viscosity during the heating up period to 72°C and the mash rest of 20 min ($P > 0.05$) in spite of increasing β-glucan contents. Besides, the significantly higher viscosity of mashes/worts after mashing-off at 78°C ($P < 0.01$) was most likely caused by their higher pH values *(21)* (see below).

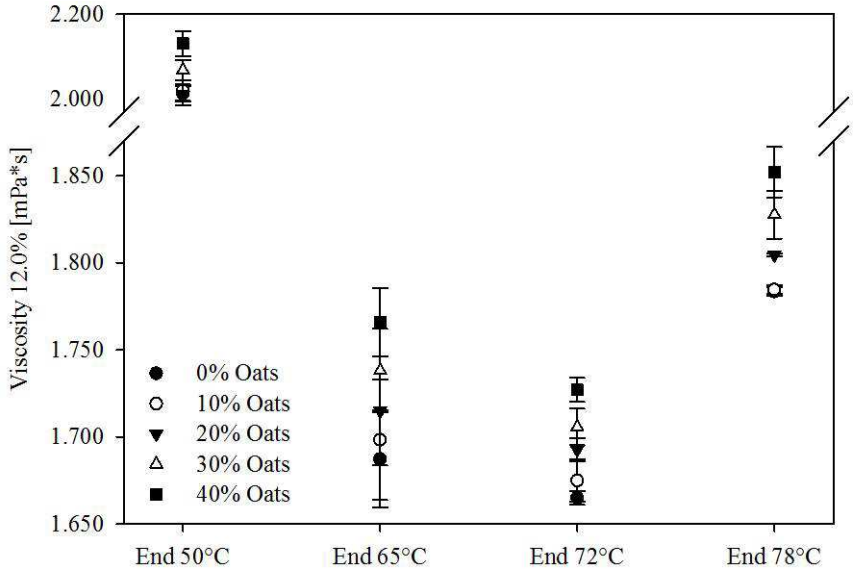


Figure 6–5. Effect of 10–40% oats on mash/wort viscosity 12.0% during the mashing process: End 50°C = End of cytolytic/proteolytic mash rest at 50°C; End 65°C = End of first amylolytic mash rest at 65°C; End 72°C = End of second amylolytic mash rest at 72°C; End 78°C = Mashing-off at 78°C.

The use of Ondea® Pro as recommended significantly lowered the final wort viscosity 12.0% from 1.785 mPa·s to 1.701 mPa·s (10% oats) and from 1.852 mPa·s to 1.668 mPa·s (40% oats), respectively ($P < 0.001$) (Figure 6). These results are in accordance with the β-glucan reductions achieved by adding this enzyme cocktail (see above). However, the substitution of only 10% barley malt with unmalted oats does not require the application of viscosity-reducing enzymes.

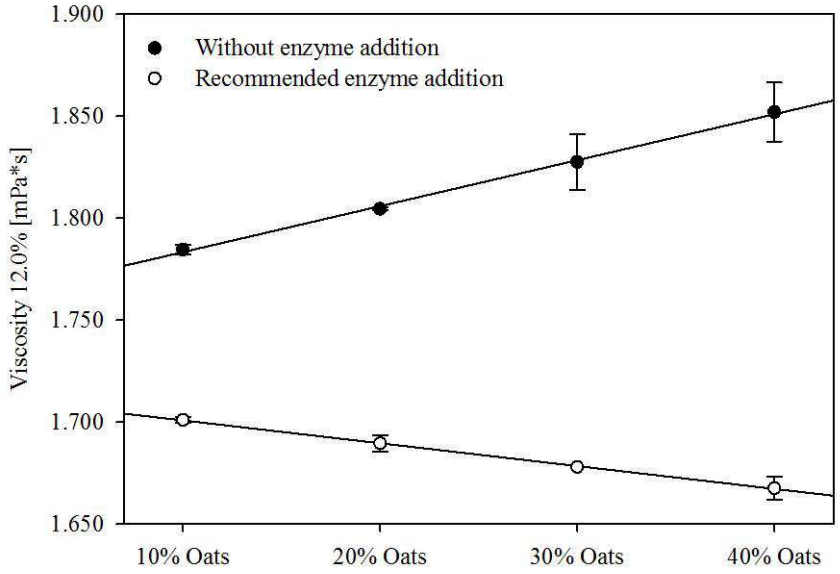


Figure 6–6. Final wort viscosity 12.0% (10–40% oats) without and with enzyme addition.

The findings concerning viscosity were reflected in the filtration rates of the respective hot mashes. After mashing-off at 78°C, mash filtration rates decreased from 3.1 mL wort/min (100% barley malt) to 2.6 mL wort/min (40% oats). Using up to 20% oat adjunct had no or little effect on the filtration performance of mashes/worts, whereas the use of 30% and 40% oats considerably increased filtration times. However, the addition of exogenous enzymes to mashes containing 30/40% unmalted oats clearly improved the filterability of final worts by 34/41%. In practice, mash filters are highly suitable for separating high-gravity worts from spent grains *(23)*.

The replacement of 40% barley malt with oats also had an effect on the protein profile of final worts as shown in Figure 7. Distinct protein peaks at approximately 6.3 kDa and 42.5/51.8 kDa, respectively, have been identified in the electropherogram or gel-like image of worts produced with 40% oats. On the other hand, the concentration of proteins with molecular weights of 5.2–5.6 kDa and 9.5–18.0 kDa, respectively, was considerably lower compared to that in 100% barley malt worts, which could indicate a poorer beer foam quality (less foam-positive lipid transfer protein 1) *(19)*.

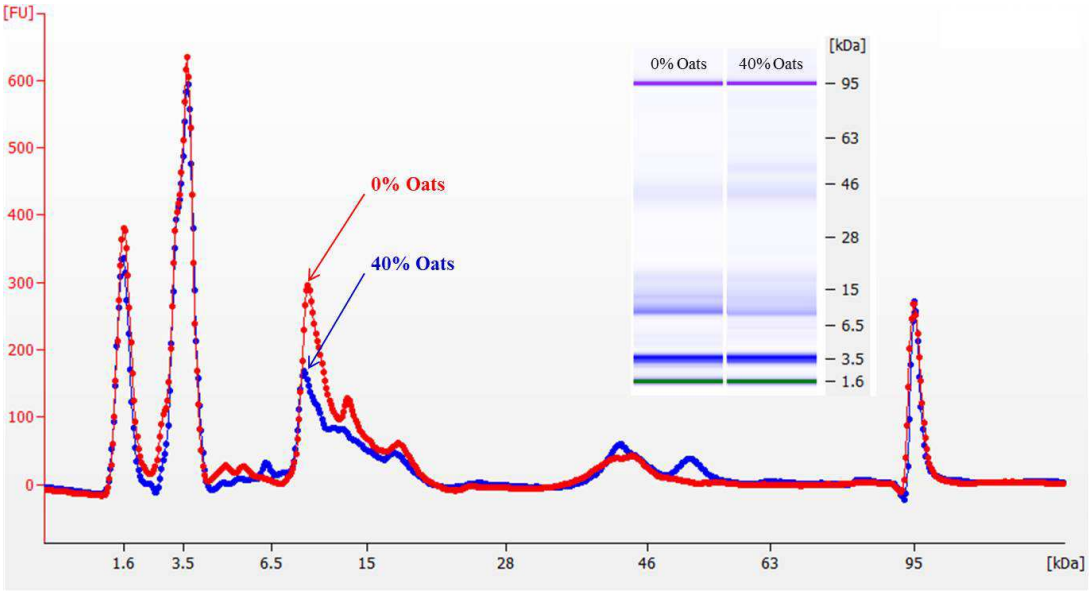


Figure 6–7. Electropherogram/gel-like image of final worts (0%, 40% oats).

The FAN content of final worts decreased significantly with increasing amounts of oats ($P < 0.05$) from 328 mg/L (100% barley malt) to 222 mg/L (40% oats) (Figure 8). At the end of the cytolytic/proteolytic mash rest, the FAN levels in filtrated mashes were 7.9–31.5% higher than those in the respective final worts. Interestingly, the overall relative losses of FAN during mashing increased with increasing oat concentration. The greatest FAN losses (20–65 mg/L), being significant when using 20% or more oats ($P < 0.05$), occurred during heating up to 65°C and the subsequent mash rest of 40 min. These reductions in FAN were probably caused by the high fat content of oats (see Table 1) due to interactions between nitrogenous compounds and lipids *(24,25)* as well as the formation of melanoidins (Maillard reactions between amino acids and reducing sugars) resulting in increased mash/wort color values (see below) *(26)*. The application of Ondea® Pro (2.0 g/kg oats), containing endoprotease and lipase amongst others, significantly increased the FAN concentration in final worts by 21.2–55.9% when substituting 20% or more barley malt with oats ($P < 0.001$) (Figure 9). According to the literature *(27,28)*, recommended values for FAN in high-gravity worts (18–24% w/w) are 250–280 mg/L. Those concentrations have still been reached in final worts produced with up to 20% oats without the addition of exogenous enzymes.

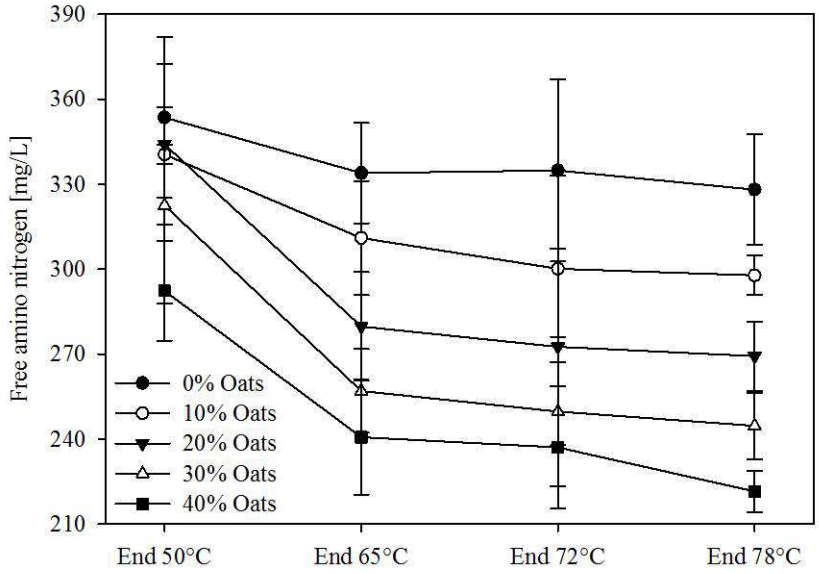


Figure 6–8. Effect of 10–40% oats on mash/wort FAN concentration during the mashing process: End 50°C = End of cytolytic/proteolytic mash rest at 50°C; End 65°C = End of first amylolytic mash rest at 65°C; End 72°C = End of second amylolytic mash rest at 72°C; End 78°C = Mashing-off at 78°C.

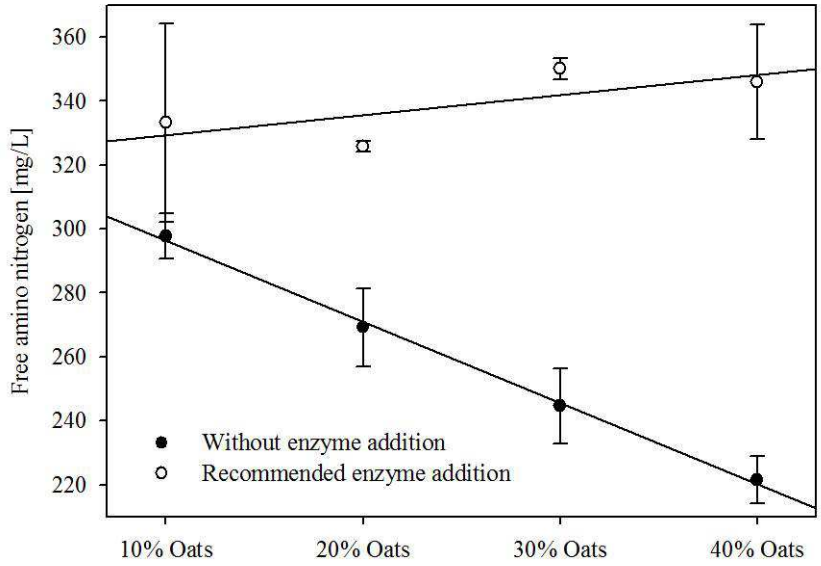


Figure 6–9. Final wort FAN concentration (10–40% oats) without and with enzyme addition.

The final wort extract content decreased significantly when using 20% or more oat adjunct ($P < 0.001$) from 24.5% w/w (100% barley malt) to 23.3% w/w (40% oats) as shown in Figure 10. Oats used in this study exhibited a husk proportion of 26% of

the total grain weight, explaining the lower starch content (see Table 1) and extract yield in comparison to barley malt. After the mash rest at 50°C, extract levels between 8.2% w/w (40% oats) and 10.2% w/w (100% barley malt) were determined, accounting for 35.3–41.4% of the final wort extract. These results also indicate a higher solubility/extractability of barley malt grist compared with oat grist due to a higher flour fraction (see Figure 1). Most of the extract (46.3–52.8% of the final content) was released into solution during the heating up period to 65°C and the maltose formation rest ($P < 0.001$) as a consequence of starch gelatinization (see Table 1) as well as liquefaction by endogenous α- and β-amylases. This time, the amount of released extract increased with increasing oat concentration, indicating the high amylolytic enzyme activity in barley malt (see Table 1). A further significant rise in mash/wort extract ($P < 0.001$) occurred during heating up to 72°C and the saccharification rest due to the activity of malt α-amylase.

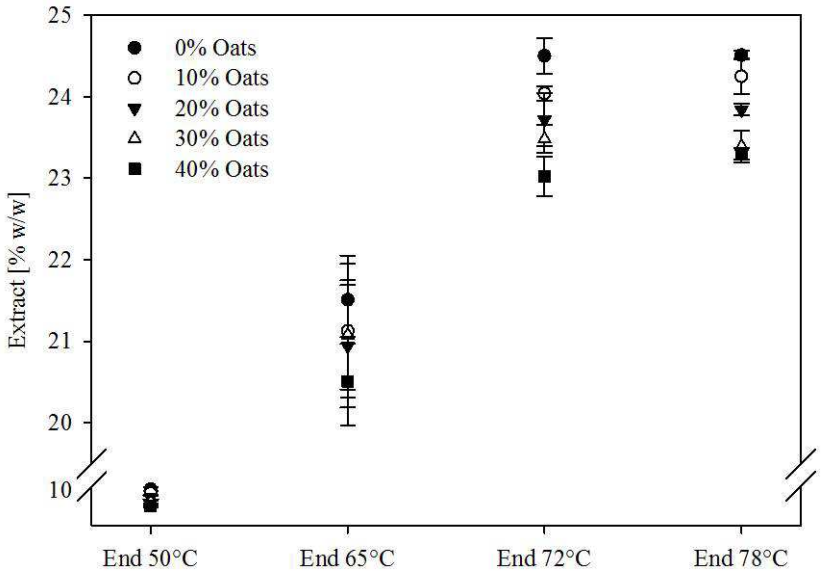


Figure 6–10. Effect of 10–40% oats on mash/wort extract content during the mashing process: End 50°C = End of cytolytic/proteolytic mash rest at 50°C; End 65°C = End of first amylolytic mash rest at 65°C; End 72°C = End of second amylolytic mash rest at 72°C; End 78°C = Mashing-off at 78°C.

The use of Ondea® Pro as recommended also significantly enhanced the extract content of final worts produced with 20% or more oats by up to 0.6% w/w ($P < 0.001$) (Figure 11). All mashes (10–40% oats) were completely saccharified (negative iodine test) after 10 min at 72°C without or with enzyme addition.

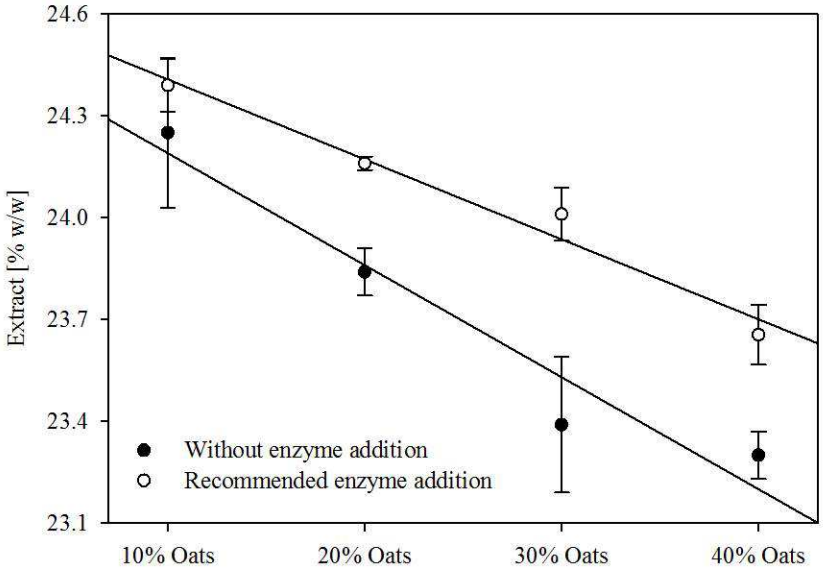


Figure 6–11. Final wort extract content (10–40% oats) without and with enzyme addition.

Furthermore, the pH of final worts (20°C) ranging from 5.77 (100% barley malt) to 5.89 (40% oats) was significantly higher when substituting 30% or 40% barley malt with unmalted oats ($P < 0.05$) (Figure 12). This increase in pH might be explained by a reduced buffering potential of oat-containing mashes/worts due to lower concentrations of buffer substances such as FAN *(29)*. At the end of the cytolytic/proteolytic mash rest, pH values of 5.87–5.99 (0–40% oats) were measured in the filtrated mashes (20°C), decreasing significantly to 5.75–5.86 during heating up to 65°C and the subsequent mash rest of 40 min ($P < 0.05$). A further decline in mash pH (20°C) was observed after the heating up period to 72°C and the saccharification rest ($P > 0.05$) followed by a slight rise at the end of mashing ($P > 0.05$). These pH changes, however, were quite small in comparison to those obtained by applying Ondea® Pro (2.0 g/kg oats), significantly reducing the final wort pH (20°C) from 5.80 to 5.52 (10% oats) and from 5.89 to 5.63 (40% oats), respectively ($P < 0.001$) as shown in Figure 13. According to the literature *(30)*, a mash pH of 5.5–5.6 benefits protein degradation, viscosity, lautering and filtration rates, wort color, and attenuation limit.

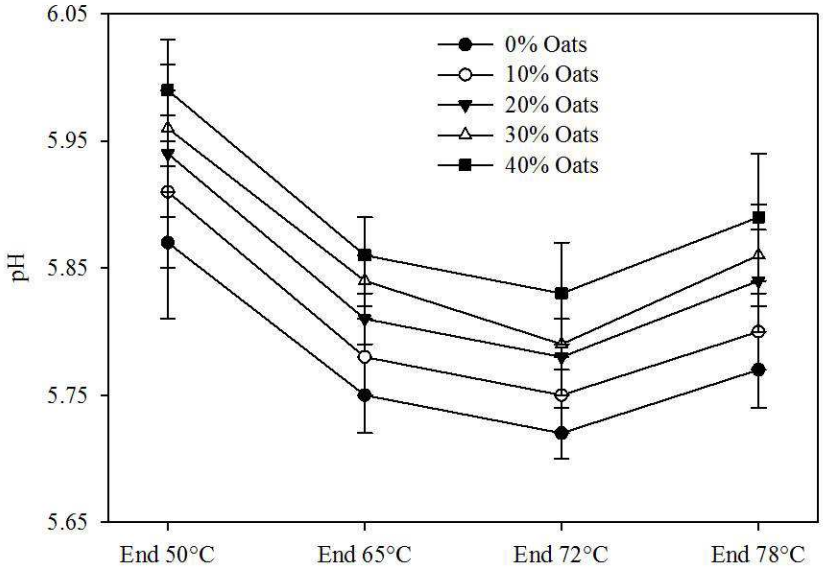


Figure 6–12. Effect of 10–40% oats on mash/wort pH (20°C) during the mashing process: End 50°C = End of cytolytic/proteolytic mash rest at 50°C; End 65°C = End of first amylolytic mash rest at 65°C; End 72°C = End of second amylolytic mash rest at 72°C; End 78°C = Mashing-off at 78°C.

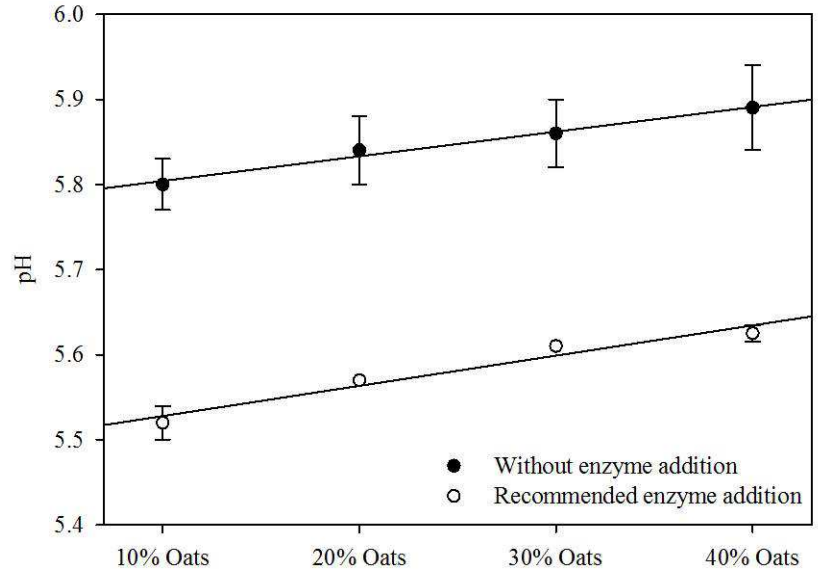


Figure 6–13. Final wort pH (10–40% oats) without and with enzyme addition.

Final worts produced with 10–40% oats tended to higher color values (9.51–10.09 EBC units) compared to 100% barley malt worts (9.49 EBC units; $P > 0.05$) (Figure 14). After the cytolytic/proteolytic mash rest, color values between 9.05 EBC units (100% barley malt) and 9.58 EBC units (40% oats) were determined, increasing to

9.83–10.35 EBC units during heating up to 65°C and the maltose formation rest ($P > 0.05$). This rise in mash/wort color was most likely due to the formation of melanoidins as a result of Maillard reactions *(26,31)* being in accordance with FAN reductions occurring in this period (see above). In general, the extent of those non-enzymatic browning reactions and the extraction of coloring substances from mashing materials are greater at higher pH values *(30–32)* explaining the increase in color with increasing oat concentration. Interestingly, however, mash/wort color and pH behaved contrary to each other during mashing; after the saccharification rest at 72°C, for instance, filtrated mashes exhibited the highest color but lowest pH values.

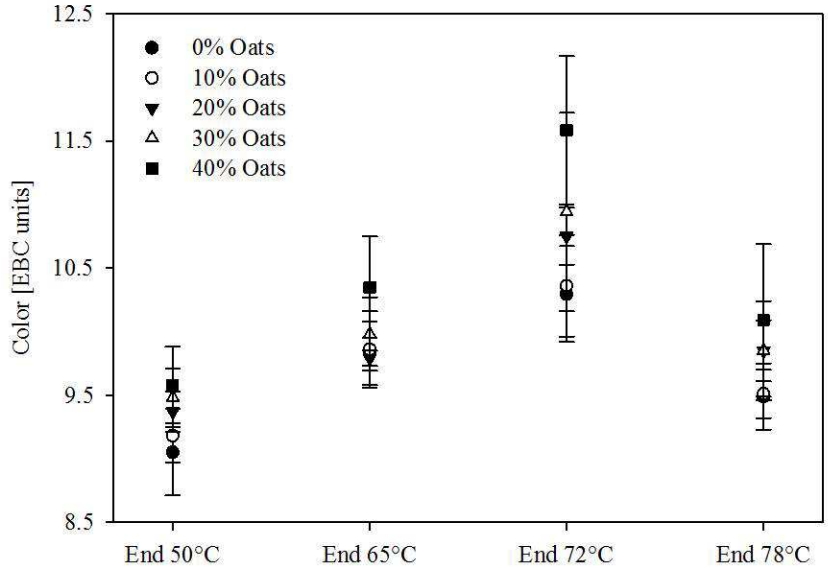


Figure 6–14. Effect of 10–40% oats on mash/wort color during the mashing process: End 50°C = End of cytolytic/proteolytic mash rest at 50°C; End 65°C = End of first amylolytic mash rest at 65°C; End 72°C = End of second amylolytic mash rest at 72°C; End 78°C = Mashing-off at 78°C.

Even more surprising is that the addition of Ondea® Pro to mashes as recommended significantly increased the final wort color ($P < 0.001$) from 9.51 EBC units to 11.15 EBC units (10% oats) and from 10.09 EBC units to 12.49 EBC units (Figure 15) despite significantly reduced pH values. These findings indicate an excessive formation of Maillard products as a consequence of the extensive protein degradation *(31)* being undesirable since wort color further increases during wort boiling (see Table 1).

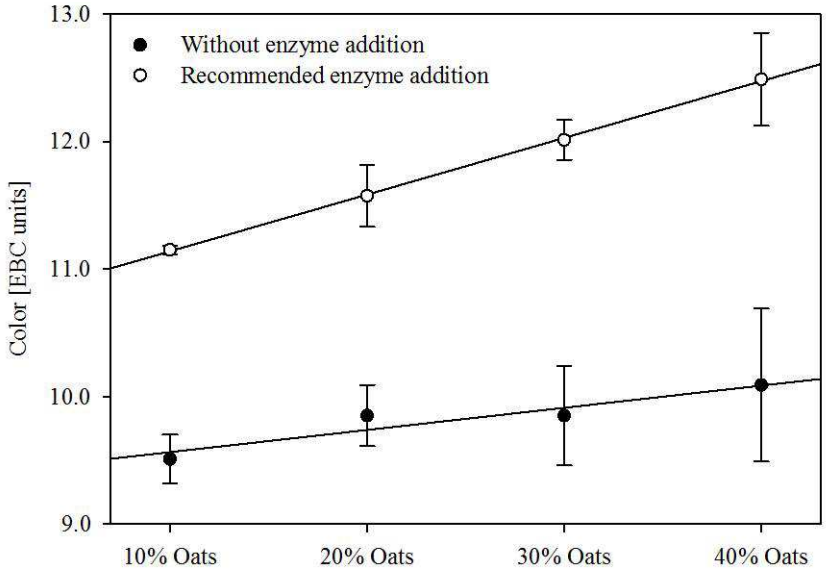


Figure 6–15. Final wort color (10–40% oats) without and with enzyme addition.

Conclusion

In the present study, the effect of up to 40% unmalted oats on the quality of high-gravity mashes and worts without or with enzyme addition was investigated. The enzyme mix Ondea® Pro, which was found to be particularly suitable for brewing with oats, was used in the rheological and laboratory-scale mashing trials (2.0 g/kg oats). High-gravity mashes or worts produced with 10–40% oat adjunct revealed increased mash consistencies, color/pH (20°C) values, β-glucan concentrations, wort viscosities 12.0%, and filtration times as well as decreased FAN and extract contents. However, the substitution of up to 20% barley malt with unmalted oats had no or only little effect on mash/wort quality. The application of Ondea® Pro as recommended enormously increased the color of worts despite lower pH values but considerably improved the quality and processability of 30% or 40% oat-containing mashes/worts.

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Chapter 7

Impact of various levels of unmalted oats (*Avena sativa* L.) on the quality and processability of mashes, worts, and beers

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Abstract

The brewing industry is facing an ever increasing challenge to become more cost-effective, while at the same time maintaining or improving product quality. Brewing with unmalted oats (*Avena sativa* L.) has the potential to reduce the costs of raw materials. However, the replacement of malted barley with unmalted oats can also adversely affect the quality and processability of mashes, worts, and beers. In this study, brewing with unmalted oats (0–40%) and malted barley was carried out in a 60-L pilot plant. The impact of various levels of oats on mashing, lautering, and fermentation performance was monitored in detail and the quality of the final beers was evaluated using Lab-on-a-Chip capillary electrophoresis as well as standard methods specified by Mitteleuropäische Brautechnische Analysenkommission, European Brewery Convention, or American Society of Brewing Chemists. It has been found that the β-glucan content and viscosity of mashes/worts increased significantly with increasing amounts of oats. In addition, the use of 20% or more oat adjunct resulted in a clearly increased lautering time. The replacement of barley malt with unmalted oats also had adverse effects on total soluble nitrogen, free amino nitrogen, and extract levels in worts. The foam stability of the final beers decreased significantly using 20% oats or more. However, their sensory quality improved with increasing levels of oat adjunct.

Introduction

In recent years, the interest in oats (*Avena sativa* L.) for the production of foods and beverages has significantly increased due to their excellent health benefits *(1)*. Oats represented the predominant brewing cereal during the Middle Ages and were used for beer production long afterward. Today, however, they have lost their significance in brewing *(2)* since barley (*Hordeum vulgare* L.) proved to be more suitable for malting and brewing purposes. Only a few specialty beers are still produced with unmalted and/or malted oats (lagers, ales, and stouts) as flavoring ingredients *(3,4)*. As a result, very little brewing-related publications on oats are available at present *(4–11)*. It has been reported that oats as brewing adjunct can benefit flavor properties of the final product *(12)*. Beers produced with up to 10% oats exhibited a distinct toasted, biscuit-like flavor and aroma combined with a relatively intense, creamy mouthfeel *(4)*. Hanke *et al. (5,6)* determined an oat-typical flavor and good reduction properties in beer brewed with 100% oat malt. Oat kernels comprise a higher proportion of husks (approximately 30% of grain weight) compared to barley kernels (approximately 10% of grain weight), resulting in significantly shorter lautering times. However, the extract content of malted oats amounts to only 70–75% of that of malted barley *(5,6,13)*. In addition, oat malt is deficient in α- and β-amylase activities, causing insufficient extract recovery *(14)*. Oats are unique with regard to the distribution of protein fractions. Common cereals such as barley, wheat, and rye contain mainly prolamins (approximately 80% of total protein) and only a small proportion of globulins (approximately 10% of total protein). In contrast, globulins represent the predominant protein fraction in oats (approximately 80% of total protein), whereas prolamins comprise a minor proportion of oat proteins (approximately 10% of total protein) *(15)*. Oats are also known for their high contents of β-glucan, protein, and fat. High β-glucan contents can adversely affect the processability of mashes and worts due to an increased viscosity *(2,14)*.

However, most of these findings are based on malted oats. Therefore, the objective of the present study was to evaluate the impact of various levels of unmalted oats (10%, 20%, 30%, and 40% of grist mass) on the quality and processability of mashes, worts, and beers produced at pilot-plant scale (60 L). For this purpose, their brewing performance, particularly during mashing, lautering, and fermentation, was monitored in detail and the quality of the final beers was comprehensively

determined applying advanced protein analysis (Lab-on-a-Chip capillary electrophoresis) as well as standard methods described by Mitteleuropäische Brautechnische Analysenkommission (MEBAK), European Brewery Convention (EBC), or American Society of Brewing Chemists (ASBC).

Materials and methods

Brewing materials

Malted barley (*Hordeum vulgare* L. 'Fr Sebastian'), harvested in 2008 and obtained from Greencore Group plc (Dublin, Ireland), and unmalted oats (*Avena sativa* L. 'Lutz'), harvested in 2009 in Ravensburg, Germany, were used in the brewing trials. Oats are characterized by high contents of β-glucan (3.6% DM) and protein (10.5% DM) as well as a low starch content (58.6% DM) in comparison to barley malt (0.3% β-glucan (DM), 9.4% protein (DM), and 65.7% starch (DM)).

Milling

Malted barley was milled with a two-roller mill (Engl Maschinen-Großhandels GmbH, Schwebheim, Germany) set at a 0.7-mm roller distance. Unmalted oats were milled using a hammer mill equipped with a 1.5-mm sieve (A.M.A. S.p.A., San Martino in Rio, Italy). Milling of brewing materials was carried out directly before mashing-in.

Brewing

Brewing with unmalted oats and malted barley was carried out in a 60-L pilot plant. For mashing, a commonly used infusion process has been chosen as follows: 30 min at 50°C, 40 min at 65°C, 20 min at 72°C, and 5 min at 78°C (mashing-off). In all brewing trials, mashing-in was performed by mixing 9 kg of grist into 32 L of brewing water at 50°C. Five mashes with increasing levels of unmalted oats (0%, 10%, 20%, 30%, 40% of grist mass) with a constant liquor-to-grist ratio of 3.55:1 were prepared. During mashing, changes in pH were monitored and mash samples were taken for further analysis at the start and end of each mash rest and before mashing-off. After reaching 72°C, saccharification was checked every 5 min until discoloration of iodine disappeared. Wort separation was performed in a lauter tun. After a lauter rest of 20 min and turbid wort pumping for 10 min, 20 kg of first wort was collected. Three sparging steps using tempered brewing water (78°C) were then carried out to reach a preboil wort volume of 55 L (volume measurement at 95°C). The lautering rate of first and sparged worts was determined gravimetrically. Wort turbidity was detected at the start and end of each wort collecting step as well as in the preboil wort. Hop pellets (Hallertau Magnum; Hopsteiner, Mainburg, Germany)

were added at the start of wort boiling, aiming for 18 EBC bitterness units in the final beer. After wort boiling for 60 ± 10 min and a whirlpool rest of 20 min, the worts were cooled and aerated. During brewing, samples of first wort, preboil wort, boiled wort, and cold wort were taken for further analysis. Wort fermentation was implemented by adding 100 g dry lager yeast (Saflager S-23; Fermentis, Marcq-en-Baroeul cedex, France) with prior rehydration according to the manufacturer's recommendation. Fermentation was performed at 10°C until the apparent extract no longer changed significantly (approximately 10 days). During fermentation, beer samples were taken every day from the middle of the fermentation tank for further analysis. After fermentation, the young or 'green' beer was filled into 50-L stainless steel kegs and a maturation period of 4 weeks at 4°C was performed. Filtration of the final beers was carried out using a plate filter with standard depth filter sheets (K 900; Pall SeitzSchenk Filtersystems GmbH, Bad Kreuznach, Germany). The filtered beers were bottled using a manual bottling unit (Esau & Hueber GmbH, Schrobenhausen, Germany). The bottled beers were stored in the dark at 4°C prior to analysis. All brewing trials were performed in duplicate.

Standard analysis

Mashes, worts, and beers were analyzed according to standard methods specified by MEBAK, EBC, or ASBC. Color and pH of mash, wort, and beer samples were determined according to MEBAK II *(16)* methods 2.13.2 and 2.14. Wort viscosities were measured using a HAAKE falling ball viscometer (Thermo Scientific, Karlsruhe, Germany). Total soluble nitrogen (TSN) contents of wort and beer samples (10 mL) were analyzed using a Tecator™ Digestor combined with a Kjeltec™ 2100 Distillation unit (Foss, Hillerød, Denmark). Free amino nitrogen (FAN) in worts and beers was determined according to MEBAK II *(16)* method 2.8.4.1.1. Wort and beer amino acid profiles were measured by reversed-phase high-performance liquid chromatography (RP-HPLC) according to MEBAK III *(17)* method 3.3. Wort extract, apparent extract, apparent degree of fermentation, and alcohol of beer were analyzed using a SCABA™ 5610 Automatic Beer Analyzer (Foss Tecator, Höganäs, Sweden). Yeast cell counts were carried out using a haemocytometer (Thoma chamber, 0.100 mm cell depth) and methyl red as an indicator for yeast viability. Beer foam stability was determined using the foam stability tester NIBEM-T (Haffmans BV, Venlo, The Netherlands) according to

MEBAK II *(16)* method 2.19.2. Sensory analysis of the final beers was performed according to the Deutsche Landwirtschafts-Gesellschaft e.V. (DLG) scheme. Volatile by-products of fermentation and vicinal diketones were measured according to MEBAK III *(17)* methods 1.1.1 and 1.2.1. In addition, aging factors (heat indicators, oxygen indicators, staling components) were analyzed by pervaporation followed by gas chromatography (PV-GC). Wort and beer sugar profiles were determined by HPLC. Fatty acids in worts and beers were measured by GC using trimethylsulfonium hydroxide as derivatization reagent according to Deutsche Gesellschaft für Fettwissenschaft e.V. method C-VI 11e. Mash and wort β-glucan contents were analyzed applying the Megazyme mixed-linkage beta-glucan assay procedure (Megazyme International Ireland Ltd., Bray, Ireland). All mash, wort, and beer standard analyses were performed in duplicate.

Lab-on-a-Chip analysis

The protein profile of mashes, worts, and beers was analyzed using Lab-on-a-Chip capillary electrophoresis. The principles of these electrophoretic assays are based on traditional gel electrophoresis principles that have been transferred to a chip format as described by Klose *et al. (9)*. For the analysis, 40 mg of freeze-dried and homogenized sample was extracted with 400 µL of a reagent containing 2 M urea, 15% glycerol, 0.1 M Tris-HCl (pH 8.8), and 0.1 M dithiothreitol in an ultra-sonic water bath for 5 min at room temperature. After centrifugation at 18,890×*g* for 15 min, 4 µL of each supernatant was denatured by heating at 95°C for 5 min with 2 µL of Agilent denaturing solution. Afterward, the denatured samples were diluted with 84 µL of deionized water and 6 µL of this mixture was applied to the Protein 80^+ LabChip for analysis in the Agilent 2100 Bioanalyzer according to the manufacturer's instructions (Agilent Technologies, Santa Clara, California). A Protein 80^+ LabChip includes a ladder comprising reference proteins of 3.5, 6.5, 15, 28, 46, and 63 kDa plus a lower marker of 1.6 kDa and an upper marker of 95 kDa. Each sample contained an internal standard comprising the lower and upper marker of 1.6 and 95 kDa. According to the manufacturer's manual, any peak detected below 5 kDa is termed a system peak and is not included in the analysis. All samples were analyzed in triplicate.

Statistical analysis

Graphical representations of data were generated using SigmaPlot software (Systat Software Inc., San Jose, California). The Student's *t*-test was applied for determining the statistical significance. Results are given as arithmetic means with confidence intervals ($P = 95\%$). Correlations are indicated by the coefficient of determination R^2.

Results and discussion

Impact of unmalted oats on the processability of mashes, worts, and beers

Mash pH is an important quality control parameter affecting not only cytolytic, proteolytic, and amylolytic enzyme activities as well as wort composition, but also flavor, foam, colloidal, and microbiological stabilities of the final beer. The main factors influencing the pH of mashes and worts are grist and brewing water composition as well as temperature *(18,19)*. In the present study, the mash pH increased considerably with increasing levels of oat adjunct as shown in Figure 1.

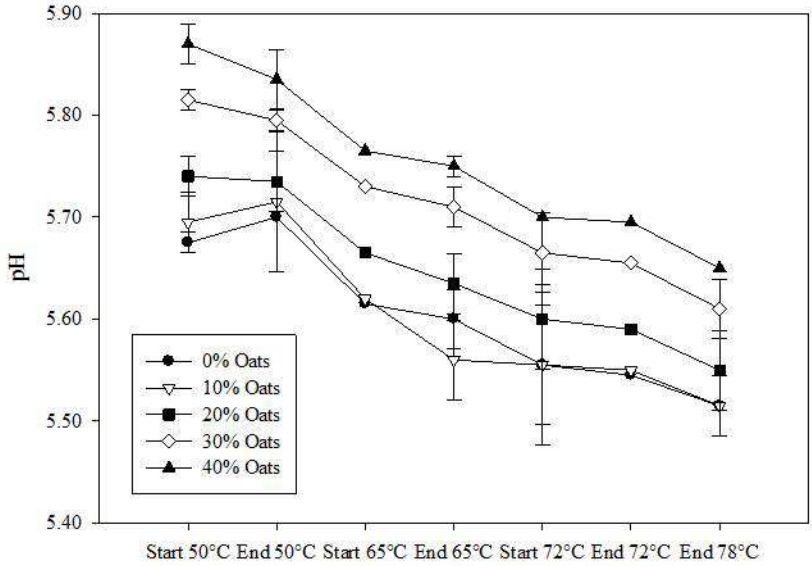


Figure 7–1. Impact of various levels of unmalted oats (10–40%) on mash pH during mashing (Start/End 50°C = Start/End of cytolytic/proteolytic mash rest at 50°C; Start/End 65°C = Start/End of amylolytic mash rest at 65°C; Start/End 72°C = Start/End of amylolytic mash rest at 72°C; End 78°C = Mashing-off at 78°C).

At the start of the cytolytic/proteolytic mash rest at 50°C, the mash pH of the reference (100% barley malt) was 5.68 and increased to 5.87 by replacing 40% barley malt with unmalted oats. This pH increase is likely the result of a lower concentration of buffer substances such as peptides and polypeptides with aspartate and glutamate residues in the mash *(18)*. During mashing, the mash pH decreased continuously to 5.52 using 0% oats (R^2 = 0.9176) and 5.65 using 40% oats (R^2 = 0.9660), except for 0% and 10% oats, where the pH increased non-significantly during the cytolytic/proteolytic mash rest. The pH decrease during the mashing

process is mainly caused by increasing temperatures leading to an increased dissociation of acidic materials *(18)*. The replacement of 10% barley malt with unmalted oats had no significant impact on the mash pH.

The lautering rate is also affected by the mash pH. It has been reported that a low mash pH benefits the filter cake permeability *(19)*. In addition, the particle size distribution of grist influences the lautering performance. Excessive milling leads to a reduced permeability of the filter cake resulting in longer lautering times *(20)*. In the present study, unmalted oats were milled using a hammer mill in order to increase the extract yield and prevent a pipeline blockage due to the high husk volume. As a result, the lautering rate decreased from 0.65 kg wort/min to 0.37 kg wort/min by replacing 40% barley malt with unmalted oats. The use of 10% oat adjunct had no significant impact on the lautering rate. These findings correspond with experimental results published in the literature *(19,21)*.

Another reason for poor lautering performance when using high levels of oat adjunct is the higher β-glucan content of mashes as shown in Figure 2. Already the use of 10% oats considerably increased the final mash β-glucan content by 393 mg/L (final mash β-glucan content of reference (0% oats) 20 mg/L). As mentioned above, no significant difference between the lautering rates of reference mash and 10% oat mash was determined. However, using 20% oats, the final mash β-glucan content increased to 858 mg/L, resulting in a clearly increased lautering time. The replacement of 40% barley malt with unmalted oats caused a 97-fold increase of β-glucan in the final mash from 20 mg/L to 1,949 mg/L. Most of the oat β-glucan was released into solution during the heating up period to 65°C (20%, 30%, and 40% oats) or within the 65°C mash rest (10% oats). The rapid increase of β-glucan in mashes between 60°C and 65°C could be due to the release of cell wall materials as a consequence of starch gelatinization. On the other hand, solubilization of high-molecular-weight β-glucan from cell walls could be caused by β-glucan solubilase activity. This heat-stable enzyme has its temperature optimum in mash at 62–65°C and can still be active at 73°C. The released β-glucan is broken down by endo-1,3-β-glucanase and endo-1,4-β-glucanase to low-molecular-weight β-glucan, cellobiose, and laminaribiose. However, β-glucanases are heat-labile and rapidly inactivated at temperatures of 60–65°C *(22–24)*.

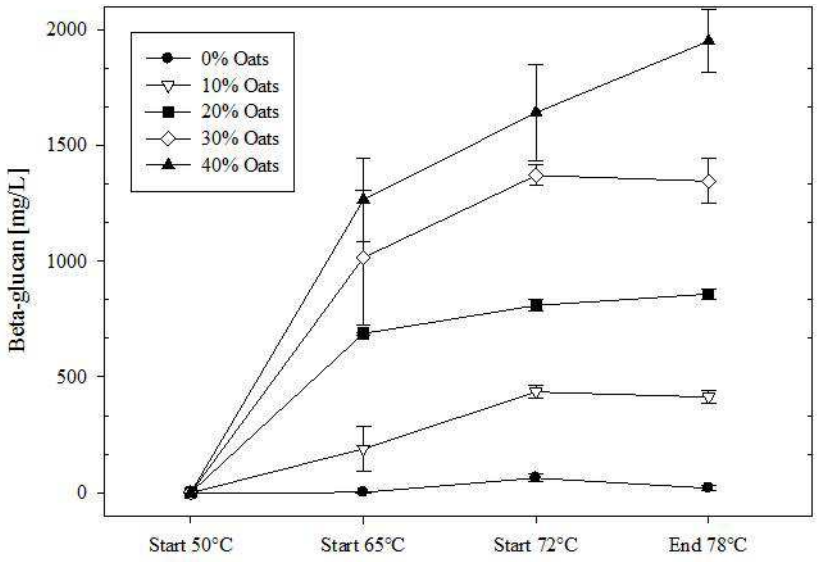


Figure 7–2. Impact of various levels of unmalted oats (10–40%) on mash β-glucan during mashing (Start 50°C = Start of cytolytic/proteolytic mash rest at 50°C; Start 65°C = Start of amylolytic mash rest at 65°C; Start 72°C = Start of amylolytic mash rest at 72°C; End 78°C = Mashing-off at 78°C).

This imbalance between solubilization and degradation of high-molecular-weight β-glucan is reflected in the viscosity of mashes and worts. In the present study, the viscosity of the preboil wort increased considerably with increasing levels of oat adjunct as shown in Figure 3.

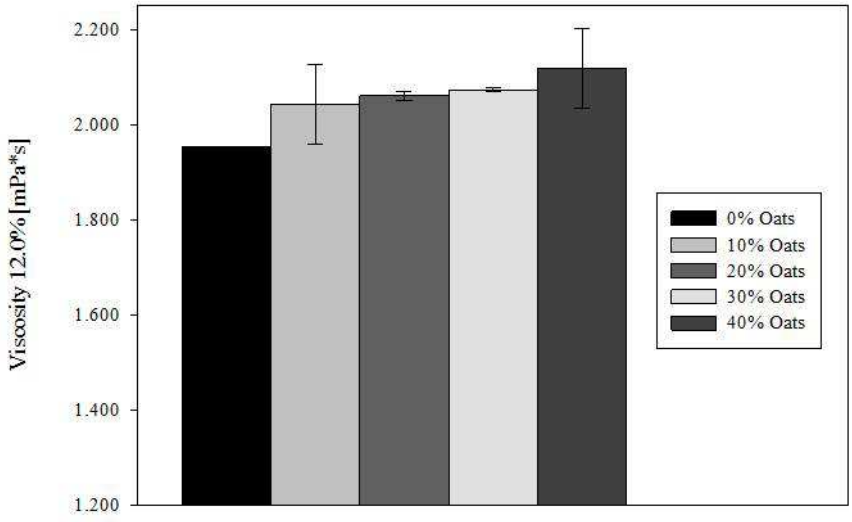


Figure 7–3. Impact of various levels of unmalted oats (10–40%) on preboil wort viscosity.

β-Glucan macromolecules increase the viscosity of mashes, worts, and beers when solubilized from cell walls. It has been reported that viscosity increases linearly with concentration and molecular weight of β-glucans. However, β-glucan in a concentration lower than 800 mg/L is not the predominant viscosity-altering substance in wort and beer. Also proteins (nitrogen compounds) and starch (sugars) contribute to their viscosities *(23,25)*. Oats used in the present study contain high levels of protein (10.5% dry weight) and mixed-linked β-glucan polymers (3.6% dry weight). The latter are mainly found in the endosperm cell walls and consist of β-D-glucopyranose units linked together by 1,3-glycosidic (30%) and 1,4-glycosidic (70%) bonds *(1,26)*. During malting of oats, β-glucans are almost completely degraded by β-glucanases *(27,28)*. When brewing with unmalted oats, however, unmodified high-molecular-weight β-glucans and proteins are released into the mash, increasing its viscosity *(25,29,30)*. In the present study, a high correlation between the β-glucan content of the final mash and the viscosity of the preboil wort was determined (R^2 = 0.9455) when using 10%, 20%, 30%, and 40% oats as shown in Figure 4.

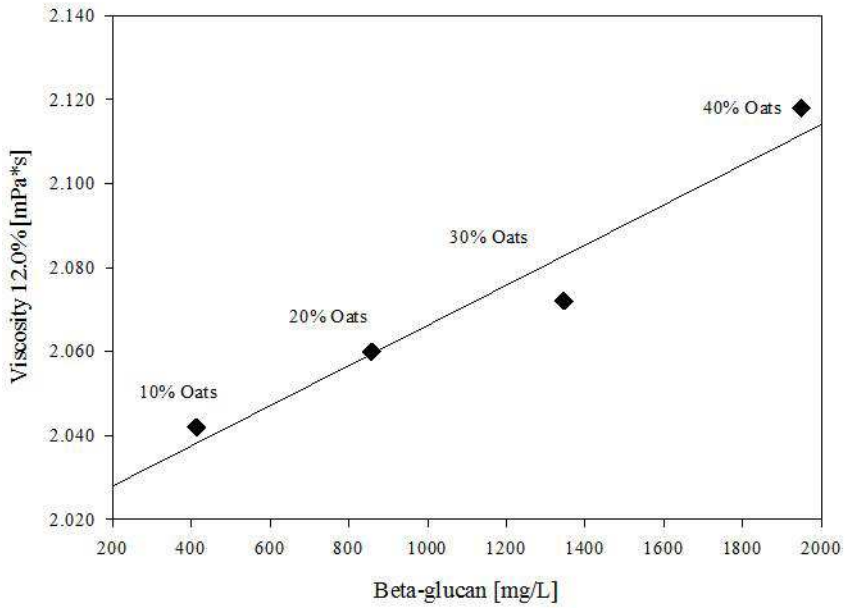


Figure 7–4. Correlation between final mash β-glucan content and preboil wort viscosity using 10–40% unmalted oats (R^2 = 0.9455).

Schneider *(31)* reported that barley malt grist fineness has almost no influence on the β-glucan content of mashes when using a liquor-to-grist ratio of 3.5–4:1. However, at higher mash concentrations (liquor-to-grist ratio 2.5–3:1), finer malt grists result in lower β-glucan contents and wort viscosities. Kühbeck *et al. (24)* determined the effect of roller- and hammer-milled grist on the β-glucan content of mashes during mashing using poorly modified malt. It has been found that the final mash β-glucan concentration is lower when using a hammer mill. The mechanical decomposition leads to an extensive degradation of high-molecular-weight β-glucans at low mashing temperatures, resulting in a reduced β-glucan solubilization after the inactivation of endo-β-glucanases. These findings correspond with results obtained from preliminary brewing trials concerning the present study using roller-milled oats (0.5-mm roller distance). The viscosity (12.0%) of the preboil wort containing 40% oats decreased from 2.419 mPa·s to 2.118 mPa·s by using hammer-milled oats. High viscosities of mashes, worts, and beers can adversely affect many unit operations of the brewing process such as mixing, stirring, pumping, lautering, wort boiling, wort cooling, beer clarification, and beer filtration. For these reasons, exogenous enzymes are applied when brewing with high amounts of adjuncts since barley malt enzymes become the limiting factor *(21,24,25)*. However, the viscosity of mashes, worts, and beers affects not only their processability but also the final beer quality. A high viscosity of beer benefits its body and improves foam stability (head retention) by reducing the liquid drainage rate *(25,32)*.

Impact of unmalted oats on the quality of mashes, worts, and beers

Numerous substances have an impact on the quality of beer, however, first and foremost proteinaceous compounds and alcohol. It has been reported that stability and organoleptic properties of beer are affected by interactions between proteins, amino acids, and polyphenols. The levels of those substances in mashes, worts, and beers depend on brewing materials and technology *(22,24,33)*. In the present study, the protein profile of filtered mashes before and after the proteolytic mash rest changed considerably by replacing 40% barley malt with unmalted oats as shown in Figure 5. When brewing with oats, distinct protein peaks at around 8 kDa and between 32 kDa and 48 kDa were detected using Lab-on-a-Chip capillary electrophoresis, representing oat albumins and globulins. However, the protein profiles of final beers revealed no significant differences. These findings correspond with experimental results published in the literature *(9,10)*.

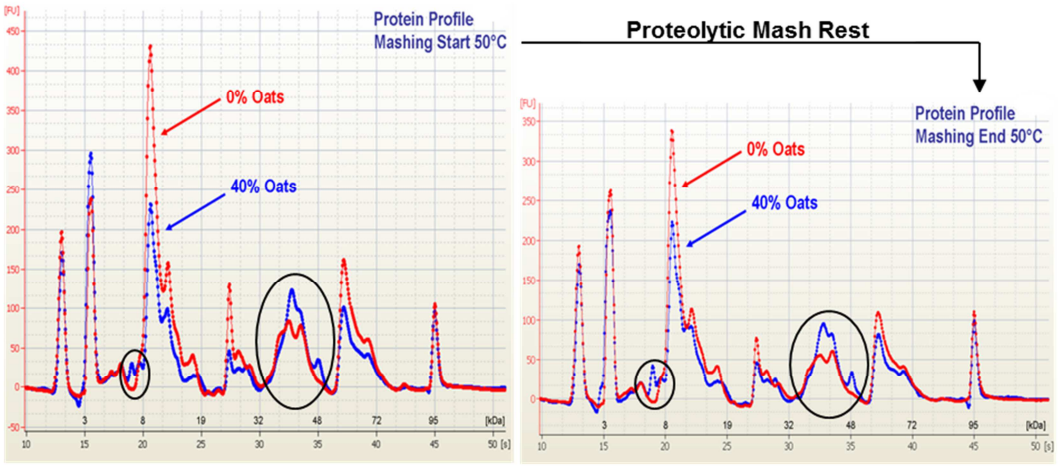


Figure 7–5. Impact of 40% unmalted oats on the protein profile of filtered mashes before and after the proteolytic mash rest at 50°C.

In cold worts, the total soluble nitrogen (TSN) contents decreased significantly from 940 mg/L to 817 mg/L by replacing 40% barley malt with oats. As a result, the free amino nitrogen (FAN) contents dropped significantly from 177 mg/L using 0% oats to 131 mg/L using 40% oats (R^2 = 0.9630) as shown in Figure 6. According to the literature *(34)*, recommended values for TSN are 900–1,200 mg/L and for FAN 200–240 mg/L based on all-malt worts (12% w/w). Those values have not been reached in oat-containing worts; however, it has recently been found that FAN contents of 160 mg/L and lower might be adequate *(22)*. In the final beers, the FAN content

significantly decreased with increasing amounts of oats, ranging from 86.50 mg/L using 0% oats (51% reduction) to 30.00 mg/L using 40% oats (77% reduction) (R^2 = 0.9394).

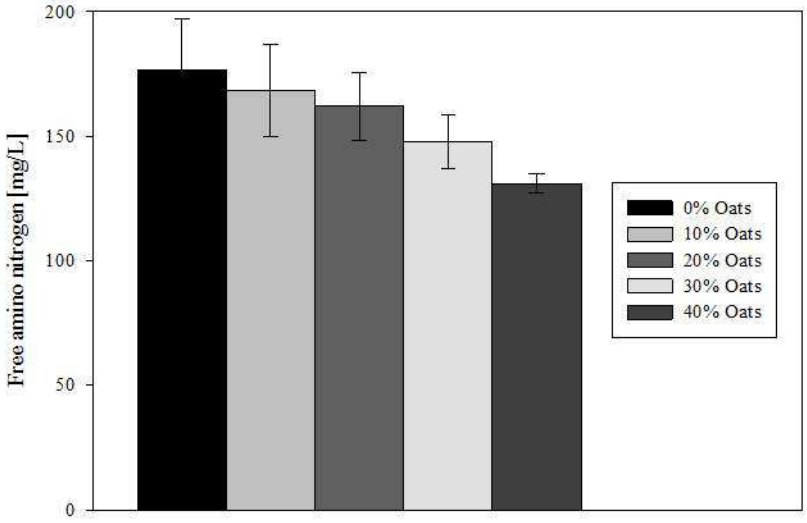


Figure 7–6. Impact of various levels of unmalted oats (10–40%) on cold wort FAN contents (R^2 = 0.9630).

In accordance with FAN values, the total amino acid content of cold worts continuously decreased with increasing adjunct levels from 143.51 mg/100 mL (0% oats) to 106.56 mg/100 mL (40% oats) by approximately 26% (R^2 = 0.9649) as shown in Table 1. Aspartic and glutamic acids slightly increased by replacing 10% or 20% barley malt with oats (statistically non-significant). Asparagine significantly increased when using 20% oats or more, whereas all other amino acids decreased with increasing levels of adjunct. During the fermentation process, 73.23 mg/100 mL (51% of total amino acids) of the reference wort and 82.59 mg/100 mL (78% of total amino acids) of the 40% oat-containing wort were metabolized. The concentration of each amino acid in final beers was lower compared to that in the respective cold worts except for γ-aminobutanoic acid, whose concentration approximately doubled from 6.77 mg/100 mL to 13.68 mg/100 mL (0% oats) and from 4.36 mg/100 mL to 7.31 mg/100 mL (40% oats). It has been reported that yeast cells excrete amino acids such as γ-aminobutanoic acid during growth because of a change in membrane permeability *(35)*. In the final beers, the total amino acid content significantly decreased with increasing adjunct levels from 70.28 mg/100 mL using 0% oats to 23.97 mg/100 mL using 40% oats (R^2 = 0.9352).

Table 7–1. Impact of various levels of unmalted oats (10–40%) on cold wort amino acid composition [mg/100 mL].

Amino acid	0% Oats	10% Oats	20% Oats	30% Oats	40% Oats
Aspartic acid	6.71 ± 0.27	7.10 ± 0.73	6.86 ± 0.32	6.38 ± 0.25	5.86 ± 0.15
Glutamic acid	8.28 ± 1.20	9.05 ± 1.21	8.92 ± 0.29	7.92 ± 0.04	7.37 ± 0.02
Asparagine	8.81 ± 0.47	9.31 ± 0.90	9.95 ± 0.46	10.31 ± 0.48	9.94 ± 0.98
Serine	6.25 ± 0.65	5.90 ± 0.56	5.63 ± 0.67	5.05 ± 0.53	4.39 ± 0.16
Glutamine	2.28 ± 0.65	2.15 ± 0.65	1.77 ± 0.10	1.72 ± 0.08	1.34 ± 0.76
Histidine	5.07 ± 0.71	4.30 ± 0.18	3.83 ± 0.45	3.54 ± 0.29	3.40 ± 0.01
Glycine	3.45 ± 0.49	3.14 ± 0.28	3.15 ± 0.09	2.95 ± 0.20	2.60 ± 0.24
Threonine	5.27 ± 1.40	4.93 ± 1.65	5.06 ± 1.92	4.56 ± 1.74	4.00 ± 1.21
Alanine	6.19 ± 1.24	6.01 ± 0.44	5.84 ± 0.47	5.31 ± 0.67	4.70 ± 1.17
Arginine	20.11 ± 4.05	18.32 ± 2.09	17.58 ± 2.10	15.71 ± 1.62	13.66 ± 0.33
γ-Aminobutanoic acid	6.77 ± 1.76	5.08 ± 0.37	4.93 ± 1.11	4.89 ± 1.12	4.36 ± 0.12
Tyrosine	7.81 ± 0.76	7.36 ± 0.83	6.99 ± 0.59	6.23 ± 0.46	5.46 ± 0.07
Valine	9.30 ± 1.33	8.62 ± 0.95	8.26 ± 0.55	7.38 ± 0.46	6.49 ± 0.05
Methionine	2.07 ± 0.19	1.96 ± 0.28	1.83 ± 0.22	1.61 ± 0.16	1.44 ± 0.06
Tryptophan	4.47 ± 0.33	4.26 ± 0.43	3.86 ± 0.40	3.51 ± 0.33	3.04 ± 0.08
Isoleucine	6.34 ± 0.82	5.97 ± 0.66	5.77 ± 0.61	5.19 ± 0.53	4.50 ± 0.04
Phenylalanine	8.79 ± 0.97	8.49 ± 0.88	7.99 ± 0.58	7.12 ± 0.43	6.12 ± 0.39
Leucine	16.70 ± 1.25	16.06 ± 2.08	15.00 ± 1.89	13.18 ± 1.40	11.56 ± 0.31
Lysine	8.85 ± 0.59	8.81 ± 1.05	8.25 ± 0.86	7.28 ± 0.70	6.32 ± 0.17
Total amino acids	143.51 ± 16.51	136.82 ± 14.76	131.47 ± 11.63	119.84 ± 9.30	106.56 ± 3.11

Furthermore, the extract content of preboil worts (55 L) decreased from 10.4% (w/w) using 0% oats to 9.6% (w/w) using 40% oats. The replacement of 20% barley malt with unmalted oats resulted in a significantly lower extract value. Reasons for decreasing extract levels when brewing with increasing amounts of oats are their relatively low starch and extract content due to the high proportion of husks *(11,13,36)*. Besides, endogenous barley malt enzymes were limited in terms of compensating for α- and β-amylase deficiencies in unmalted oats since no exogenous enzymes were applied *(5,6,14)*. As a result, the total fermentable sugar content of cold worts (12% w/w) decreased from 85.1 g/L to 71.0 g/L by replacing 40% barley malt with oats. Fructose and glucose dropped by approximately 50% and all other sugars by 12–18%. However, the achieved total fermentable sugar value in 40% oat-containing worts still lies within the recommended range of 69.0–98.0 g/L *(22)*. During the fermentation process, 78.4 g/L (approximately 92% of total sugars) of the reference wort and 70.2 g/L (approximately 99% of total sugars) of the 40% oat-

containing wort were metabolized. In the final beers, the remaining sugar content continuously decreased with increasing adjunct levels from 6.7 g/L (0% oats) to 0.8 g/L (40% oats) by approximately 87%.

The color of cold worts increased non-significantly from 21.45 to 23.15 EBC units by replacing 40% barley malt with unmalted oats. After 10 days of fermentation, however, statistically significant increases in beer color with increasing adjunct levels from 8.10 EBC units using 0% oats to 14.14 EBC units using 40% oats (R^2 = 0.9258) were observed. Furthermore, the alcohol content decreased from 5.32% (v/v) to 4.91% (v/v) by replacing 40% barley malt with unmalted oats (R^2 = 0.8788) in accordance with the total fermentable sugar content of cold worts. As a result, the apparent extract content increased from 1.97% (w/w) in the reference beer to 2.14% (w/w) in the 40% oat-containing beer. The apparent degree of fermentation (ASBC) decreased significantly with increasing adjunct levels from 83.6% (0% oats) to 81.3% (40% oats). The beer pH was not significantly affected by the use of oats (reference beer pH 4.43; 40% oat-containing beer pH 4.41). According to the literature *(22,32,37),* general values for lager-type beer based on 12% (w/w) original extract are as follows: Color 8–15 EBC units, alcohol 4.7–5.2% (v/v), apparent residual extract 1.7–3.0% (w/w), apparent degree of fermentation 80–85%, and pH 4.3–4.6. Hence, the replacement of 40% barley malt with unmalted oats still resulted in acceptable values without the addition of exogenous enzymes. Furthermore, a positive effect of oats on the lag phase of yeast cells was observed; the yeast growth in oat-containing brews was up to 93% higher compared to the reference brew after the first and second day of fermentation. The reduced lag time resulting in accelerated yeast growth might have been stimulated by higher zinc and lipid contents in oat-containing worts *(2,5,6,38)*. These findings correspond with the total fatty acid content of cold worts increasing from 1.47 mg/100 mL to 1.95 mg/100 mL by approximately 33% (statistically non-significant) because of the replacement of 40% barley malt with oats. During the fermentation process, 0.29 mg/100 mL (approximately 20% of total fatty acids) of the reference wort and 1.47 mg/100 mL (approximately 76% of total fatty acids) of the 40% oat-containing wort were metabolized.

The foam stability of the final beers decreased significantly from 295 s to 223 s by substituting 40% barley malt with unmalted oats as shown in Figure 7. The use of 10% oats had almost no impact on beer foam; however, 20% and higher adjunct levels clearly affected beer foam quality adversely.

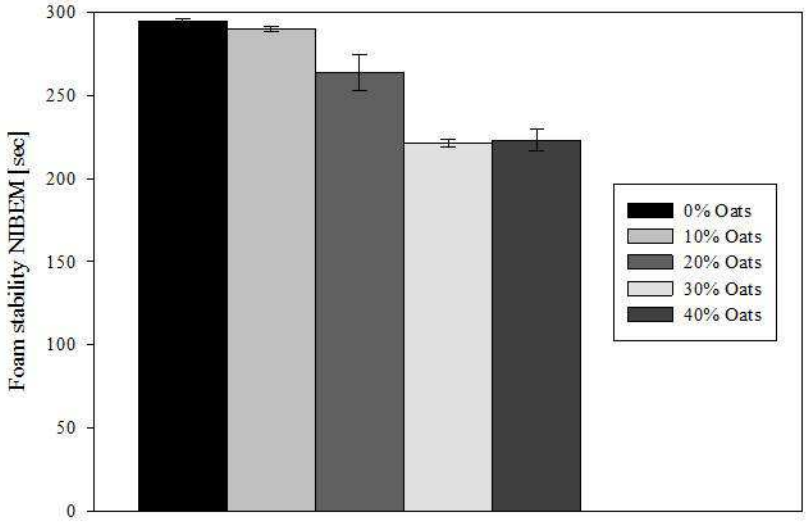


Figure 7–7. Impact of various levels of unmalted oats (10–40%) on beer foam stability.

Beers brewed with high levels of oats (30% and 40%) might have poor foam stabilities because of insufficient amounts of TSN and high-molecular-weight proteins, respectively. Taylor *et al. (36)* reported that the reduced foam stability of oat malt beer is almost certainly related to the lower TSN content and probably not related to the high fat content of oats. These findings also correspond to the fatty acid content of the final beers surprisingly decreasing from 1.18 mg/100 mL to 0.48 mg/100 mL (statistically significant) by replacing 40% barley malt with unmalted oats as shown in Table 2. It can be seen that the composition of fatty acids in the reference beer (relative weight of saturated fatty acids 80%, single unsaturated fatty acids 19%, polyunsaturated fatty acids 1%) differs from that in the 40% oat-containing beer (relative weight of saturated fatty acids 92%, single unsaturated fatty acids 8%, polyunsaturated fatty acids 0%).

Table 7–2. Impact of various levels of unmalted oats (10–40%) on final beer fatty acid composition [mg/100 mL].

Fatty acid	0% Oats	10% Oats	20% Oats	30% Oats	40% Oats
Caproic acid (C 6:0)	0.05 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.00
Caprylic acid (C 8:0)	0.11 ± 0.03	0.10 ± 0.01	0.07 ± 0.00	0.10 ± 0.01	0.09 ± 0.01
Capric acid (C 10:0)	0.04 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Lauric acid (C 12:0)	0.09 ± 0.06	0.06 ± 0.02	0.05 ± 0.03	0.09 ± 0.08	0.09 ± 0.08
Myristic acid (C 14:0)	0.12 ± 0.07	0.06 ± 0.05	<0.05 ± 0.03	0.06 ± 0.01	0.03 ± 0.01
Palmitic acid (C 16:0)	0.37 ± 0.19	0.20 ± 0.14	0.13 ± 0.10	0.15 ± 0.03	0.10 ± 0.02
Stearic acid (C 18:0)	0.20 ± 0.14	0.15 ± 0.14	0.10 ± 0.09	0.13 ± 0.05	0.09 ± 0.03
Oleic acid (C 18:1)	0.22 ± 0.06	<0.09 ± 0.07	<0.06 ± 0.05	0.08 ± 0.02	0.04 ± 0.01
Linoleic acid (C 18:2)	<0.03 ± 0.01	<0.02 ± 0.00	<0.02 ± 0.00	<0.02 ± 0.00	<0.02 ± 0.00
Linolenic acid (C 18:3)	<0.02 ± 0.00	<0.02 ± 0.00	<0.02 ± 0.00	<0.02 ± 0.00	<0.02 ± 0.00
Saturated fatty acids	0.95 ± 0.31	0.63 ± 0.32	0.44 ± 0.22	0.57 ± 0.01	0.44 ± 0.10
Single unsaturated fatty acids	0.22 ± 0.06	0.08 ± 0.08	0.05 ± 0.05	0.08 ± 0.02	0.04 ± 0.01
Polyunsaturated fatty acids	<0.03 ± 0.01	<0.01 ± 0.01	<0.01 ± 0.01	<0.02 ± 0.00	<0.02 ± 0.00
Total fatty acids	1.18 ± 0.35	0.70 ± 0.41	0.49 ± 0.27	0.65 ± 0.03	0.48 ± 0.11

The sensory quality of the final beers determined according to the DLG scheme showed a positive trend with increasing levels of unmalted oats as shown in Table 3. In particular, 30% and 40% oat-containing beers revealed an acceptable aroma and purity of taste, receiving an overall score of 4.1 and 4.2, respectively, out of 5. These results may be explained by their lower contents of acetaldehyde, decreasing from 11.05 mg/L to 7.10 mg/L (36% reduction) by replacing 40% barley malt with unmalted oats *(22,39,40)*. Furthermore, the higher alcohol content (*n*-propanol, isobutanol) decreased by 7%, whereas the ester content (ethyl acetate, isoamyl acetate) increased by 14% when brewing with an adjunct level of 40% *(22,39)*.

Table 7–3. Impact of various levels of unmalted oats (10–40%) on beer sensory quality [5-point scale].

DLG criteria	0% Oats	10% Oats	20% Oats	30% Oats	40% Oats
Aroma	3.7 ± 0.7	3.6 ± 1.1	3.5 ± 0.8	3.8 ± 0.3	3.9 ± 0.4
Purity of taste	3.7 ± 0.7	3.6 ± 1.0	3.5 ± 0.8	3.8 ± 0.3	3.9 ± 0.4
Fullness of body	4.4 ± 0.2	4.4 ± 0.2	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.2
Carbonation	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1
Quality of bitterness	3.9 ± 0.7	4.0 ± 0.5	3.9 ± 0.2	4.0 ± 0.5	4.1 ± 0.2
Overall score	4.0 ± 0.5	4.0 ± 0.6	3.9 ± 0.4	4.1 ± 0.3	4.2 ± 0.3

In contrast, it has been reported that the use of 40% unmalted barley clearly affected the sensory quality of beer adversely *(21)*. The positive DLG test results for beers brewed with high levels of oat adjunct may also be caused by a significant decrease of aging indicators in the final beers (30% and 40% oats) as shown in Figure 8. By replacing 40% barley malt with unmalted oats, the heat indicators and staling components 2-furfural and γ-nonalactone decreased significantly from 89.5 µg/L to 27.0 µg/L by approximately 70%. The first was reduced by 45 µg/L (approximately 83%) and the latter by 17 µg/L (approximately 49%) using 40% oat adjunct. However, no significant differences between the reference beer and oat-containing beers in terms of other aging indicators were determined. According to the literature *(41)*, reference values for heat indicators are 10–50 µg/L and for staling components 50–100 µg/L in fresh beer. Those values have only been achieved in beers brewed with 30% and 40% oats, which is probably the reason for their better sensory qualities *(22)*. However, it has to be mentioned that no specific aging indicators for oats used in brewing have been defined at present.

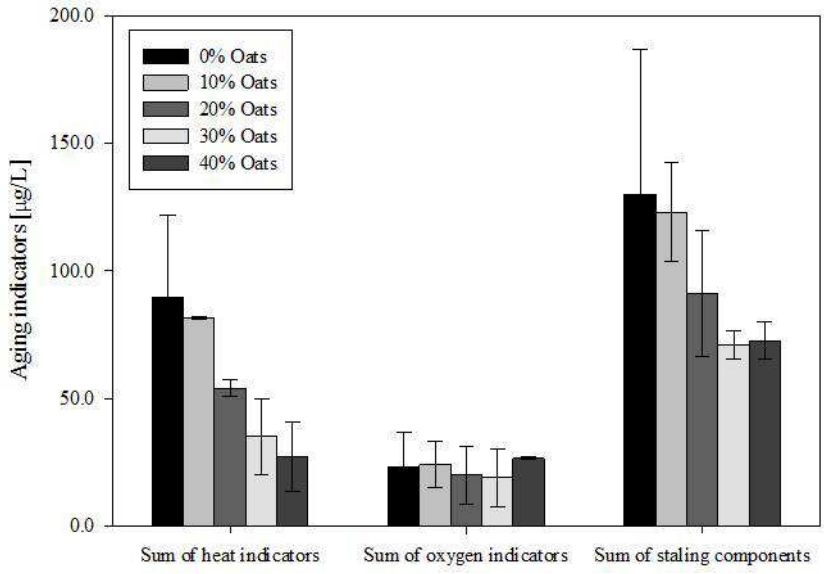


Figure 7–8. Impact of various levels of unmalted oats (10–40%) on beer aging indicators.

Conclusion

In this study, the impact of unmalted oats (10–40%) on the quality and processability of mashes, worts, and beers was evaluated. The use of 10% oats had no significant effect on mash/wort pH and lautering performance, whereas using 20% oats or more clearly adversely affected those parameters. The β-glucan content and viscosity of mashes/worts increased significantly with increasing amounts of oats. Besides, a very high correlation between the β-glucan contents of final mashes and the viscosity of preboil worts was found. Furthermore, the substitution of barley malt with unmalted oats caused lower levels of TSN, FAN, and extract in worts. Nevertheless, brewing with 40% oat adjunct resulted in acceptable values for lager-type beer as regards alcohol, apparent residual extract, apparent degree of fermentation, pH, and color, even without the addition of exogenous enzymes. However, the foam stability of final beers decreased significantly when using 20% oats or more. In contrast, the sensory quality of oat beers improved with increasing adjunct levels. In particular, 30% and 40% oat-containing beers revealed an acceptable aroma and purity of taste, which may be the result of their significantly lower contents of aging indicators.

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Chapter 8

Implementation of commercial oat and sorghum flours in brewing

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Abstract

Brewing with commercial flours has the potential to reduce mashing times and improve brewhouse efficiency. At present, however, no studies are available assessing the application of commercial oat and sorghum flours as brewing adjuncts. Therefore, the objectives of this study were to evaluate the quality and processability of mashes/worts produced with 10–90% oat or sorghum flour as well as to reveal the advantages and limitations of their use as a substitute for barley malt. For these purposes, both flour types were fully analyzed in terms of brewing-relevant characteristics using standard methods, Lab-on-a-Chip capillary electrophoresis, and scanning electron microscopy. Laboratory-scale mashing trials were performed to assess the effect of up to 90% flour adjunct on mash/wort quality. Equivalent factors were introduced to determine the performance efficiency of different oat/sorghum flour concentrations. Commercial oat flour sourced in Ireland exhibited significantly more protein, β-glucan, and fat, less starch, ash, and polyphenols, as well as a lower starch gelatinization temperature than commercial sorghum flour obtained from the United States. Worts produced with 10–90% oat or sorghum flour had lighter colors, higher pH values, and lower concentrations of foam-positive proteins as well as free amino nitrogen compared to 100% barley malt worts. In terms of extract yields, the use of up to 70% oat flour and 50% sorghum flour, respectively, has proven economically beneficial. Worts containing up to 70% oat flour showed a very good or good fermentability, those containing 30–50% sorghum flour resulted, however, in a lower alcohol production.

Introduction

The use of commercial oat (*Avena sativa* L.) and sorghum [*Sorghum bicolor* (L.) Moench] flours in brewing can reduce mashing times due to the high solubility (extractability) of very finely milled cereals, and thus lower energy consumption and costs *(1,2)*. On the other hand, the substitution of barley malt with unmalted oats (rich in fat and β-glucan) or sorghum (rich in polyphenols; high starch gelatinization temperature) may adversely affect product quality and processability *(3,4)*. Oat grains mainly consist of hulls (25–30% of total grain dry weight), cell walls (bran), and endosperm fractions *(3,5)*. When used in food production, their hulls are removed and endogenous lipid-modifying enzymes (lipase, lipoxygenase, lipoperoxidase) are inactivated. The dehulled and heat-treated oat groats can be processed into various products such as rolled oats, steel-cut oats, (whole) oat flour, and oat bran differing in appearance, composition, taste, and technological functionality. In general, the handling and further processing of fine oat flakes (produced from steel-cut oats) are easier compared with those of oat flour which tends to form lumps *(5,6)*. Milling of sorghum (hulless grain) is more challenging as it still lacks advanced technology in order to reduce milling losses and improve flour quality *(4,7,8)*. It has been reported that dry roller milling is not appropriate for sorghum resulting in products with undesirable characteristics *(9)*, whereas semi-wet roller milling (moderate pre-conditioning to 20% moisture) was found to be applicable. However, semi-wet milled products are more susceptible to microbiological growth and usually not suitable for long-term storage *(7,8)*. An alternative to the use of roller mills is abrasive decortication or attrition milling in which the outer layers (bran) of sorghum grains are removed *(10)* reducing tannin/phytic acid contents and improving product color *(8,11)*. After decortication, the endosperm is reduced by using a hammer mill *(10)*. Successful milling processes also consider the differences in physical hardness between floury (soft) and vitreous (hard) endosperm. In the first milling step (coarse grinding), high-quality flour (low starch damage) and high amounts of coarse grits are produced which can be separated and used for different purposes. The latter can be re-milled to fine flour which involves, however, high starch damage affecting its functionality *(12)*. At present, no studies are available evaluating the use of commercial oat and sorghum flours in brewing, despite their remarkable potential.

Therefore, the aims of this study were: 1) to fully characterize oat flour sourced in Ireland and sorghum flour obtained from the United States; 2) to determine the quality of worts produced with up to 90% of these commercial flours; 3) to reveal the advantages as well as limitations of their use as brewing adjuncts.

Materials and methods

Mashing materials

Barley malt (*Hordeum vulgare* L. 'Fr Sebastian') obtained from Greencore Group plc (Dublin, Ireland) in 2009, commercial wholegrain oat flour (E. Flahavan & Sons Limited, Kilmacthomas, Ireland), as well as commercial wholegrain sorghum flour (Twin Valley Mills LLC, Ruskin, Nebraska) were used in the mashing trials. Whole oat flour was produced as described above; whole sorghum flour was stone ground.

Characterization of commercial oat and sorghum flours

Standard analysis

Moisture and fat contents of oat/sorghum flours were determined according to AACC International *(13)* methods 44-15.02 and 30-10.01, respectively. Total nitrogen contents were analyzed using a Tecator™ Digestor combined with a Kjeltec™ 2100 Distillation unit (Foss, Hillerød, Denmark) following AACC International *(13)* method 46-12.01. Ash contents were measured applying the method described by Matissek and Steiner *(14)*. Polyphenols were quantified carrying out the method of Alvarez-Jubete *et al. (15)*. β-Glucan contents were determined using the McCleary method (Megazyme International Ireland Ltd., Bray, Ireland). Total starch contents were analyzed according to the Megazyme Amyloglucosidase/α-Amylase method. α-Amylase activities in oat and sorghum flours were measured following the Megazyme Ceralpha method. β-Amylase activities were determined applying the Megazyme Betamyl-3 method. The gelatinization temperature of oat/sorghum flour starch was detected by differential scanning calorimetry using a Mettler-Toledo DSC821e (Mettler-Toledo GmbH, Gießen, Germany). All standard analyses were performed in triplicate (n = 3).

Lab-on-a-Chip capillary electrophoresis

The protein profile of commercial oat and sorghum flours was detected according to the method described by Klose *et al. (16)*. For the analysis, 40 mg of freeze-dried and homogenized sample was extracted with 400 µL of a reagent containing 2 M urea, 15% glycerol, 0.1 M Tris-HCl (pH 8.8), and 0.1 M dithiothreitol in the ultrasonic water bath for 15 min at room temperature. After centrifugation at

10,000×*g* for 15 min, 4 µL of supernatant was denatured by heating at 95°C for 5 min with 2 µL of Agilent denaturing solution. Afterward, the denatured sample was diluted with 84 µL of deionized water and 6 µL of this mixture was applied to the Protein 80^{+} and Protein 230^{+} LabChip, respectively, for analysis in the Agilent 2100 Bioanalyzer according to the manufacturer's instructions (Agilent Technologies, Santa Clara, California). Each flour protein profile was analyzed in triplicate.

Scanning electron microscopy

Oven-dried (103°C; 1 hour) oat and sorghum flour samples were mounted onto aluminum specimen stubs using double-sided adhesive carbon tape. After this, the samples were coated with a 25-nm gold layer in a Gold Sputter Coater (Bio-Rad Polaron Division, Hemel Hempstead, United Kingdom) and observed under a constant accelerating voltage of 5 kV applying a JEOL scanning electron microscope type 5510 (JEOL Ltd., Tokyo, Japan). Each flour ultrastructure was analyzed in triplicate.

Mashing performance of commercial oat and sorghum flours

Milling

Barley malt was milled using a laboratory disk mill (Bühler GmbH, Braunschweig, Germany) set at a 0.2-mm disk distance. The milling process was performed directly before mashing-in.

Mashing

Mashing with malted barley and commercial flours from unmalted oat/sorghum grain was carried out in a LB 8 – Electronic mashing device (Lochner Labor + Technik GmbH, Berching, Germany). A commonly used infusion mashing procedure taking the three important enzymatic degradation processes cytolysis (cell wall hydrolysis), proteolysis (protein hydrolysis), and amylolysis (starch hydrolysis) into consideration was chosen: 30 min at 50°C, 40 min at 65°C, 20 min at 72°C, 5 min at 78°C (mashing-off). In all laboratory-scale mashing trials, a total grist mass of 96.75 g dry matter (DM) was mixed with distilled water to give a total mash mass of 512.50 g at a constant moisture basis of 14%. Mashes with increasing levels of commercial oat or sorghum flour (0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% of total

grist mass) were prepared using a constant liquor-to-grist ratio of 4.3:1 (DM). Mashing-in was performed by putting the homogenized grist into 50°C preheated distilled water in the mash cup and stirring the mixture properly. Afterward, the mash cup was attached to the temperature-controlled heating system of the mashing device. In all mashing trials, a stirring speed of 100 rpm and a heating rate of 1°C per min were applied. The saccharification rate was checked 10 min after the mash reached 72°C and then every 5 min until the iodine test was negative. After mashing-off at 78°C, the loss of water due to evaporation during the mashing process was determined gravimetrically and replaced. Finally, the hot mash was filtered and the filtrate (wort) used for further analysis. All laboratory-scale mashing trials were carried out in triplicate.

Wort analysis

Worts were analyzed applying the standard methods specified by Mitteleuropäische Brautechnische Analysenkommission (MEBAK) – Raw materials *(17)*. pH and color of wort samples were determined following methods 3.1.4.2.7 and 3.1.4.2.8.2. Wort viscosities were measured using a HAAKE falling ball viscometer (Thermo Scientific, Karlsruhe, Germany). Total soluble nitrogen (TSN) contents of wort samples (10 mL) were analyzed applying a Tecator™ Digestor combined with a Kjeltec™ 2100 Distillation unit (Foss, Hillerød, Denmark). Free amino nitrogen (FAN) in worts was determined according to method 3.1.4.5.5.1. The apparent attenuation limit (AAL) of worts was measured following method 3.1.4.10.1.2 using dry lager yeast (Saflager W-34/70; Fermentis, Marcq-en-Baroeul cedex, France). Wort extract, apparent extract, apparent degree of fermentation, and alcohol were determined applying an Alcolyzer Beer ME Analyzing System (Anton Paar GmbH, Graz, Austria). In addition, the wort protein profile was detected using the Lab-on-a-Chip method described above. All wort analyses were performed in duplicate ($n = 6$).

Statistical analysis

Results are given as arithmetic means with 95% confidence intervals (two-tailed Student's *t*-values for *n*-1 degrees of freedom). Analysis of variance tests were performed to compare sample means (Holm-Sidak method; $\alpha = 0.05$) using SigmaPlot software (Systat Software Inc., San Jose, California). Correlations are indicated by the coefficient of determination R^2.

Results and discussion

Characterization of commercial oat and sorghum flours

In the present study, commercially available oat and sorghum flours were used as adjuncts in brewing. Their composition and characteristics are given in Table 1. Oat flour contained significantly more protein, β-glucan, and fat as well as less starch, ash, and polyphenols than sorghum flour. These findings are largely in agreement with the literature *(18,19)*. In comparison to barley malt (Table 1), both commercial flours showed lower levels of protein as well as higher levels of starch and fat. The gelatinization temperature of sorghum flour starch was considerably higher than that of oat flour starch as reported by Delcour and Hoseney *(10)*. Furthermore, both commercial flours exhibited negligibly low enzyme activities compared to malted barley, representing the main source of endogenous enzymes such as α-amylase and β-amylase (Table 1).

Table 8–1. Standard analysis of barley malt and commercial oat/sorghum flours.

Analysis	Unit	Barley malt	Oat flour	Sorghum flour
Moisture	%	4.82 ± 0.03	10.36 ± 0.20	11.08 ± 0.18
Total protein[a]	% DM	9.37 ± 0.06	7.71 ± 0.08	5.26 ± 0.04
β-Glucan	% DM	0.28 ± 0.02	1.10 ± 0.06	0.09 ± 0.01
Total starch	% DM	65.68 ± 1.86	77.43 ± 1.66	82.34 ± 1.52
Fat	% DM	1.82 ± 0.26	7.52 ± 0.80	3.94 ± 0.31
Ash	% DM	1.60 ± 0.16	0.92 ± 0.01	1.09 ± 0.01
Polyphenols	% DM	NA[b]	0.02 ± 0.00	0.12 ± 0.01
Gelatinization temperature	°C	64.62 ± 0.61	56.43 ± 0.30	68.64 ± 0.05
α-Amylase	U/g	165.50 ± 4.81	ND[c]	0.13 ± 0.01
β-Amylase	U/g	19.27 ± 0.28	0.02 ± 0.02	0.10 ± 0.02

[a] Total nitrogen (% DM) × 6.25.

[b] NA = not analyzed.

[c] ND = not detectable.

The protein profile of commercial oat flour differed considerably from that of commercial sorghum flour (Figure 1). The electropherogram/gel-like image of oat flour revealed highly distinct protein peaks/bands at 6.4–18.4 kDa (11.4% of total peak area), 24.5–33.7 kDa (44.8% of total peak area), 38.4–48.7 kDa (42.5% of total peak area), and 68.4–74.3 kDa (1.3% of total peak area). These results are in accordance with those of a previous study conducted using oat grain *(20)*. On the

other hand, sorghum flour exhibited proteins with a molecular weight of 5.6–15.0 kDa (2.8% of total peak area), 18.7–26.4 kDa (92.3% of total peak area), 39.0–45.5 kDa (2.7% of total peak area), 50.0–64.2 kDa (1.7% of total peak area), and 73.4–83.1 kDa (0.5% of total peak area). When applying a Protein 230^{+} instead of a Protein 80^{+} LabChip, additional protein peaks/bands at 99.0–103.4 kDa were detected in commercial sorghum flour. These findings correspond largely to those obtained in previous work based on sorghum grain *(21)*. The substitution of high levels of barley malt with commercial oat flour is likely to result in insufficient protein degradation without the addition of exogenous enzymes.

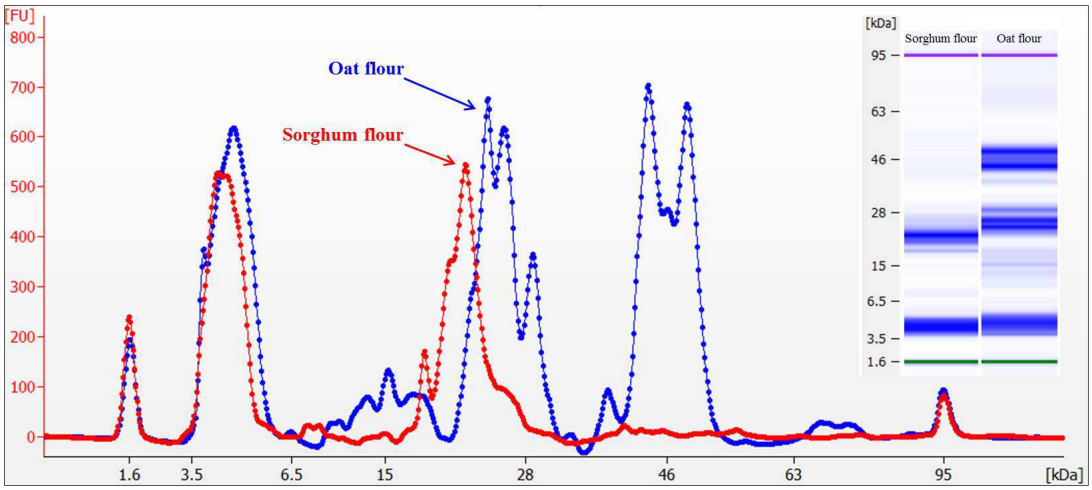


Figure 8–1. Electropherogram/gel-like image of commercial oat and sorghum flours.

With the help of scanning electron microscopy (SEM), considerable ultrastructural differences between barley malt and commercial oat/sorghum flour starch granules were revealed (Figure 2). Barley malt starch consists of both large lenticular granules (20–32 µm) and small spherical granules (2–6 µm). In contrast, oat flour starch is present as large compound granules that are composed of many small polygonal granules (2–15 µm). Sorghum flour starch comprises spherical starch granules (inner floury endosperm) as well as polygonal starch granules (outer vitreous endosperm) (10–25 µm) *(10,22,23)*. The biosynthesis of starch occurs in amyloplasts and/or chloroplasts (plastids), whose membranous structures and physical characteristics not only impart a particular shape or morphology to starch granules but also affect the arrangement and association of amylose/amylopectin molecules within granules *(22)*.

Starch morphology and granule size are genetically controlled; however, starch granule size and size distribution are also affected by environmental conditions such as temperature *(22,23)*. The granule size has an impact on gelatinization and pasting properties, enzyme susceptibility, crystallinity, swelling, as well as solubility *(22)*.

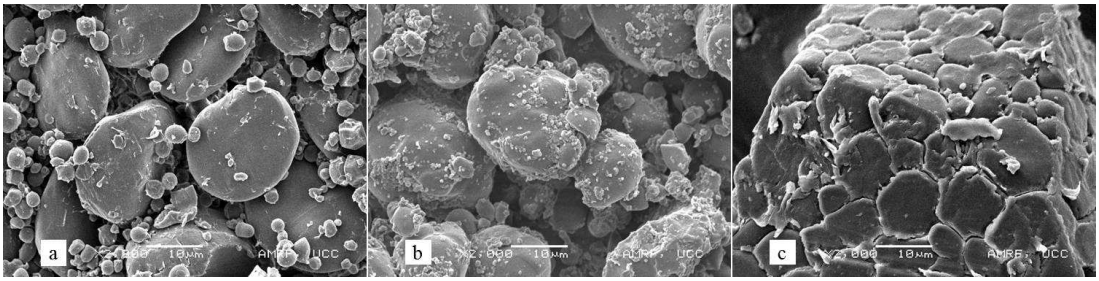


Figure 8–2. SEM images of starch granules in (a) barley malt, (b) oat flour, and (c) sorghum flour.

Mashing performance of commercial oat and sorghum flours

The extract content of worts produced with 10–90% commercial oat flour (15.73–16.24% w/w) was significantly higher ($P < 0.001$) compared to that of 100% barley malt wort (15.63% w/w) (Figure 3). A high correlation between extract level and oat flour concentration was determined ($R^2 = 0.9598$). Oat flour used in this study had a considerably higher starch content than barley malt. Furthermore, its particle size was very small (high surface area) due to the fine grinding process at industrial scale causing an increased solubility (extractability) of oat substances in mashes and a higher degradation rate of high-molecular-weight compounds *(1,24,25)*. The substitution of barley malt with sorghum flour also significantly enhanced ($P < 0.001$) extract yields from 15.63% w/w (100% barley malt) to a maximum of 15.93% w/w (50% sorghum flour; $R^2 = 0.9316$) (Figure 3). At adjunct concentrations beyond 50%, however, the extract content of worts rapidly decreased to 13.89% w/w (90% sorghum flour), whereas their viscosity (12.0% w/w) clearly increased (poor mash filterability) (see below). These findings indicate the presence of high-molecular-weight starch/dextrins in worts produced with 60% or more sorghum flour as confirmed by positive iodine tests after the saccharification rest at 72°C (20 min). Hence, the replacement of barley malt with commercial sorghum flour is limited to 50% when using a common infusion mashing process (no pregelatinization of sorghum starch) without the addition of exogenous enzymes. It has been reported that the use of up to 50% very finely ground rice (gelatinization temperature 68–78°C *(10)*) applying a standard infusion mashing process (120 min) resulted in similar extract levels as the use of up to 50% coarser ground rice (lauter tun grist) applying a complex double infusion process (170 min) including rice cooking with heat-stable α-amylase addition. Besides, mashing with up to 50% very finely ground rice using a standard infusion procedure led to significantly better saccharification rates *(1)*. In the present study, all mashes containing up to 50% sorghum flour and up to 90% oat flour, respectively, exhibited iodine normality after 10–15 min at 72°C as well as a good filterability resulting in worts with a clear appearance. The gelatinization temperature of oat flour starch is lower than that of barley malt starch (see Table 1) explaining the unproblematic processability of high oat flour concentrations in terms of starch degradation.

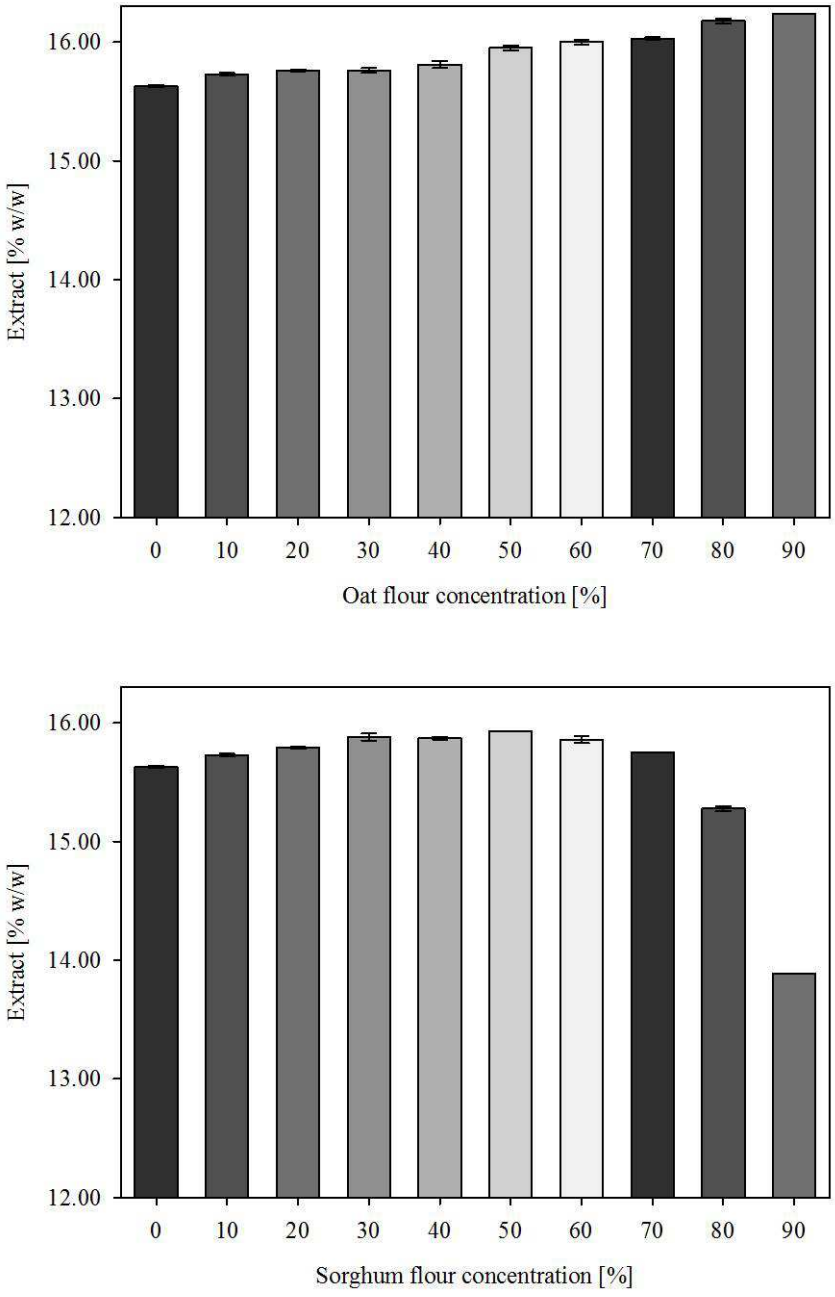


Figure 8–3. Effect of different oat/sorghum flour concentrations (0–90%) on wort extract content.

The viscosity of worts based on 12.0% w/w extract increased significantly ($P < 0.001$) when replacing 60% or more barley malt with oat flour from 1.791 mPa·s (100% barley malt) to 2.208 mPa·s (90% oat flour; $R^2 = 0.9145$) (Figure 4). Unmalted oats contain high amounts of high-molecular-weight β-glucan which contributes to mash consistency, wort/beer viscosity, and thus wort separation as well as beer filtration problems *(26–29)*. In the present study, the β-glucan content of oat flour was 3.9-fold higher than that of barley malt (see Table 1). The findings indicate that the level of endogenous malt β-glucanases (359.14 U/kg) was sufficient for degrading the β-glucan of up to 50% commercial oat flour. However, higher oat flour concentrations combined with lower enzyme levels are not practical with regard to processability of mashes, worts, and beers. In terms of commercial sorghum flour, the wort viscosity (12.0% w/w) was significantly reduced ($P < 0.001$) by substituting up to 80% barley malt from 1.791 mPa·s (100% barley malt) to a minimum of 1.737 mPa·s (50% sorghum flour; $R^2 = 0.7237$) (Figure 4). These findings can be explained by the very low β-glucan content of sorghum flour being 3.1-fold lower than that of barley malt. However, using 90% sorghum flour caused a significant increase ($P < 0.001$) in wort viscosity to 1.853 mPa·s, most likely as a consequence of insufficient starch degradation *(29–31)*. Reasons for this might be the high gelatinization temperature of sorghum flour starch (see Table 1) and the very limited levels of endogenous malt α- and β-amylase activities *(21)*. Mashes containing 90% sorghum flour (10% barley malt) resulted in incomplete saccharification (positive iodine test), poor filterability, and worts with an opalescent appearance.

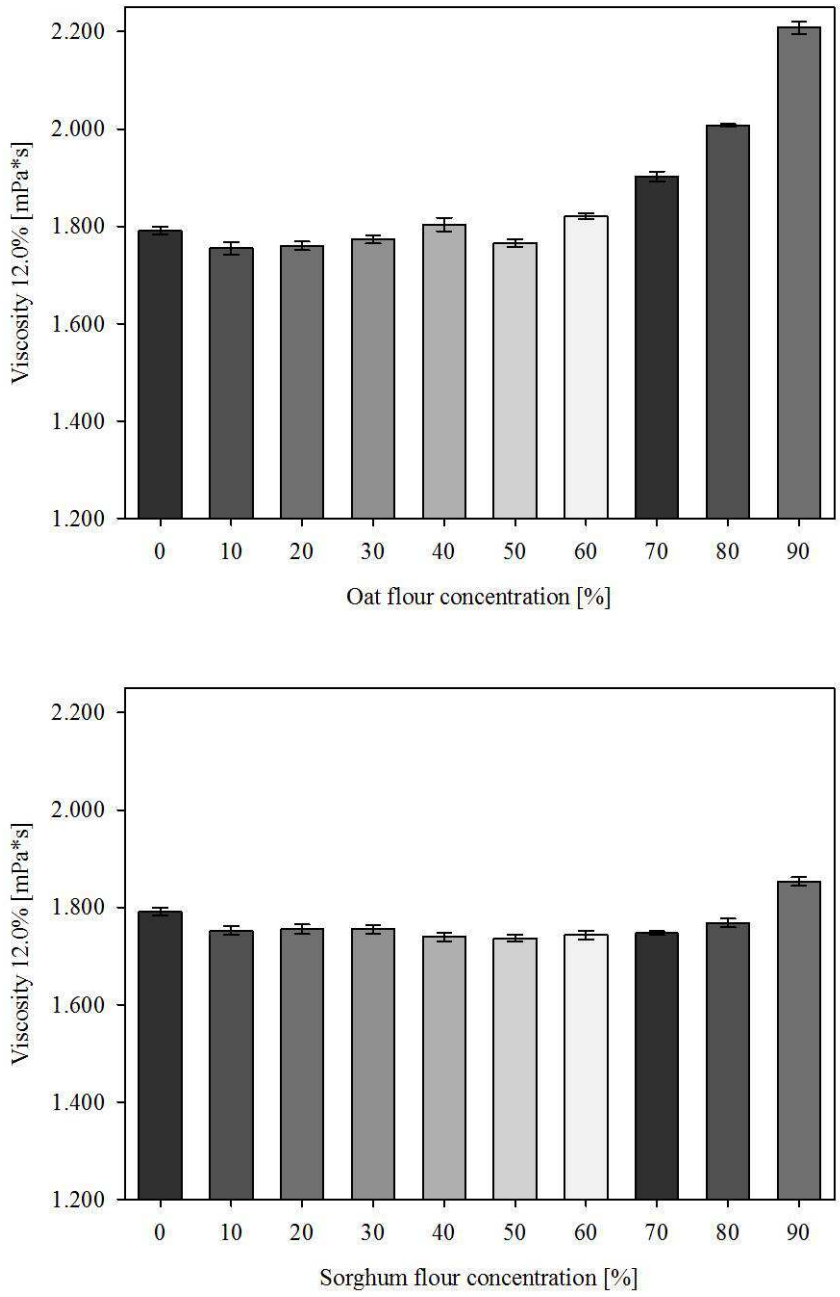


Figure 8–4. Effect of different oat/sorghum flour concentrations (0–90%) on wort viscosity.

Furthermore, the protein profiles of worts produced with different levels of commercial oat or sorghum flour were very similar (Figure 5). The large amounts of unmodified high-molecular-weight proteins brought into solution by substituting barley malt with flour adjuncts were extensively degraded by endogenous malt proteases or precipitated during the mashing process. All worts containing up to 90% oat/sorghum flour revealed predominantly foam-positive low-molecular-weight proteins of 5.3–17.7 kDa/5.0–17.5 kDa but also very low levels of foam-positive

high-molecular-weight proteins of 36.8–52.6 kDa/36.0–52.4 kDa (Lab-on-a-Chip capillary electrophoresis). However, the concentrations of those proteins clearly decreased with increasing levels of oat and sorghum flours, indicating poorer beer foam qualities *(28,32)*.

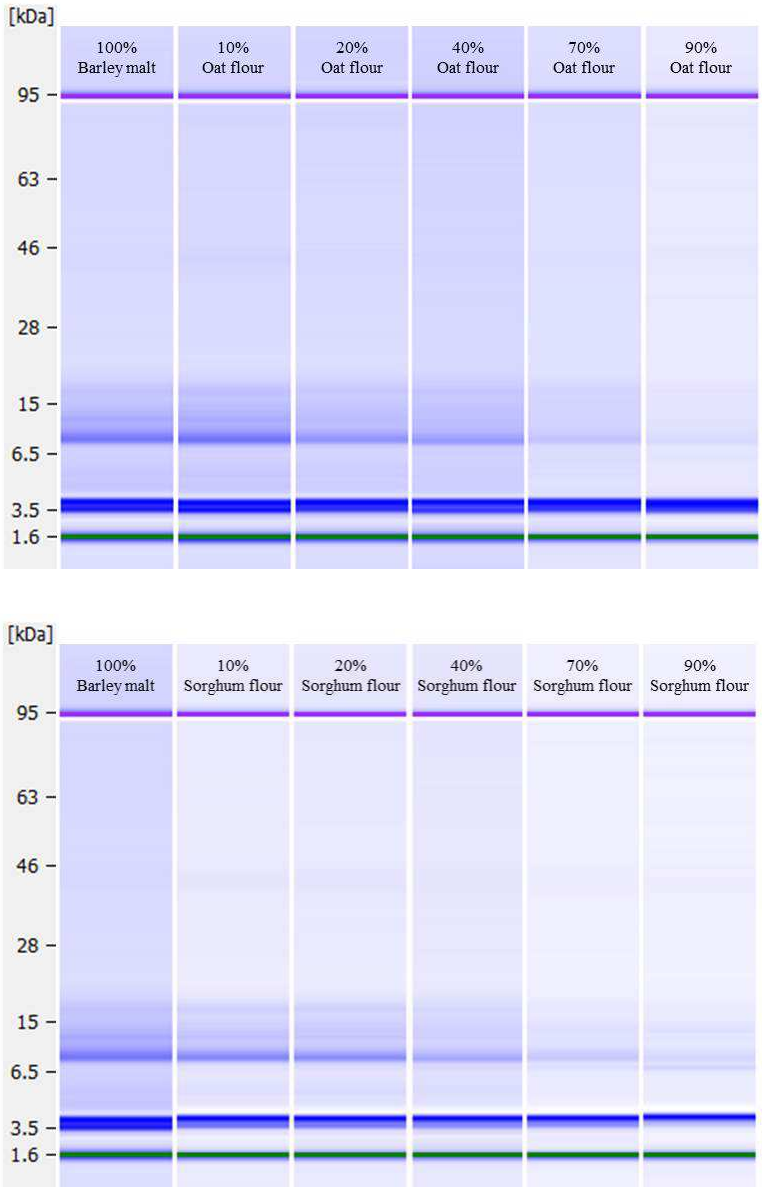


Figure 8–5. Effect of different oat/sorghum flour concentrations (0–90%) on wort protein profile.

In accordance with these findings, the TSN content of worts decreased significantly ($P < 0.001$) with increasing levels of flour adjunct from 1,236 mg/L (100% barley malt) to 335 mg/L (90% oat flour; $R^2 = 0.9972$) and 368 mg/L (90% sorghum flour; $R^2 = 0.9921$), respectively (Figure 6).

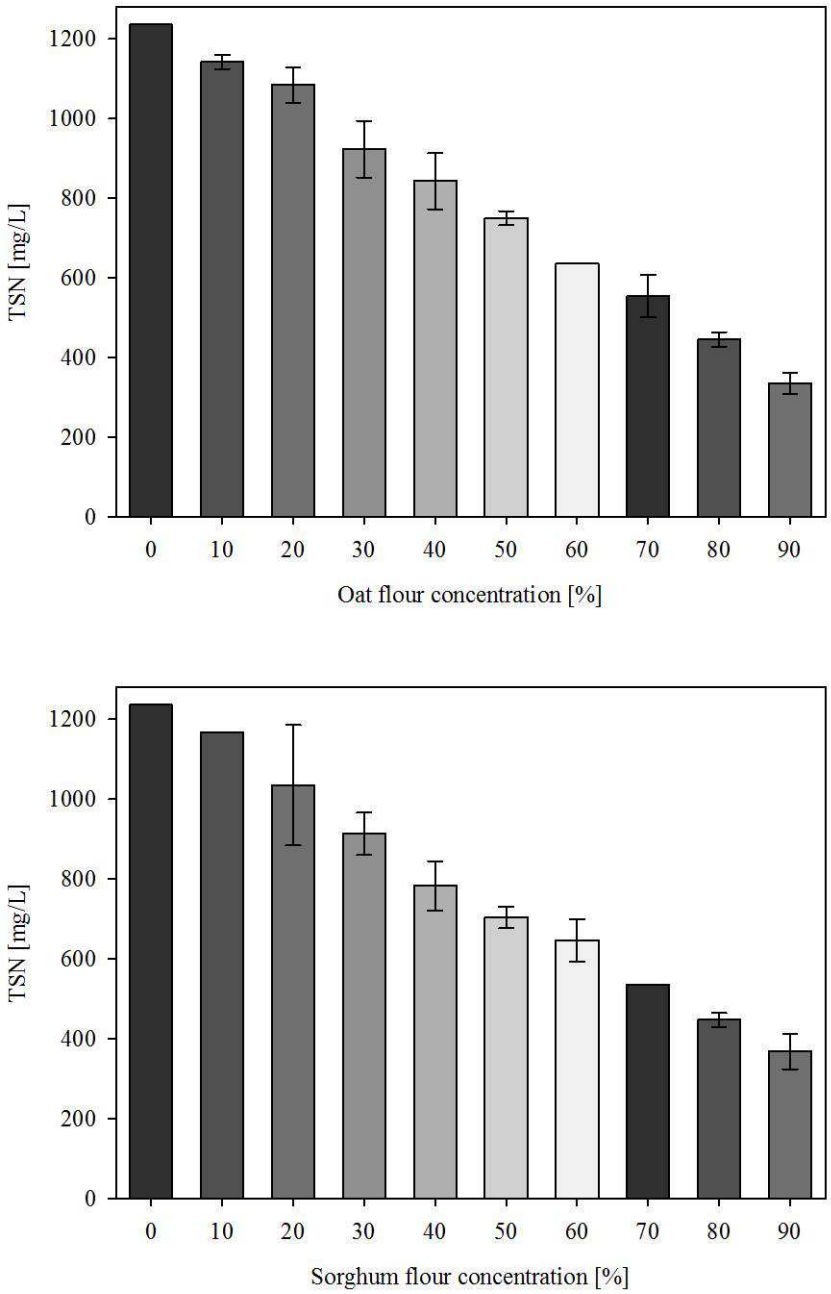


Figure 8–6. Effect of different oat/sorghum flour concentrations (0–90%) on wort TSN content.

As a consequence of this, the FAN content of worts was significantly reduced ($P < 0.001$) by substituting 20% or more barley malt with commercial flours from 249 mg/L (100% barley malt) to 36 mg/L (90% oat flour; $R^2 = 0.9808$) and 66 mg/L (90% sorghum flour; $R^2 = 0.9791$), respectively (Figure 7).

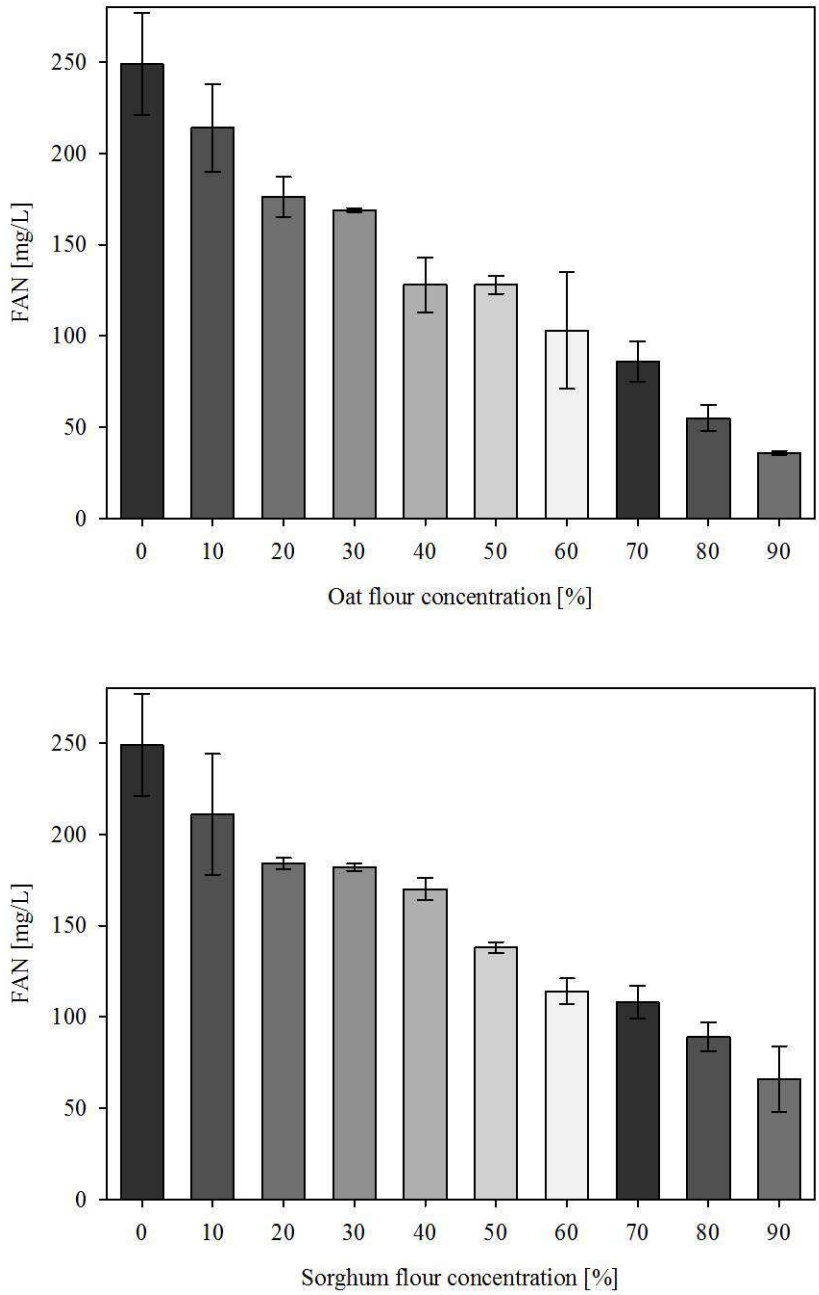


Figure 8–7. Effect of different oat/sorghum flour concentrations (0–90%) on wort FAN content.

The rapid reduction of nitrogenous compounds in worts produced with up to 90% oat and sorghum flours was due to the considerably lower protein contents of those in comparison to barley malt. According to the literature *(33,34)*, adequate amounts for TSN and FAN in all-malt worts (12.0% w/w) are 900–1,200 mg/L and 140–240 mg/L, respectively, depending on the yeast strain (that is, some yeast strains need more assimilable nitrogen than others). Nitrogen sources (amino acids, low-molecular-weight peptides) are essential for cellular biosyntheses, enzyme and nucleic acid functions in yeast cells allowing for yeast growth and a sufficient fermentation performance *(33)*. A lack of FAN in worts might cause inefficient fermentation processes, adversely affecting aroma profile (formation of undesirable by-products) and foam stability (secretion of proteinase A due to yeast autolysis) of beers *(32,35)*. However, it has been found that much lower wort FAN concentrations (85–130 mg/L) resulted in optimal yeast growth and fermentation *(28,36)*. Hence, the substitution of up to 50% barley malt with commercial oat/sorghum flours seems to be appropriate for beer production taking into account the losses of nitrogenous compounds during wort boiling and clarification.

The use of 10% oat flour had no statistically significant effect ($P > 0.05$) on wort FAN, whereas using 10% sorghum flour had a statistically significant effect ($P < 0.05$) performing analysis of variance tests. In both cases, however, the 95% confidence intervals (two-tailed Student's *t*-values) are clearly overlapping. In order to prevent confusion, it is worth noting that non-overlapping 95% confidence intervals demonstrate a statistically significant difference between group means, whereas overlapping 95% confidence intervals do not necessarily demonstrate a statistically non-significant difference between group means in terms of formal statistical tests producing *P*-values ($\alpha = 0.05$) *(37,38)*. Confidence intervals convey more information than *P*-values by indicating something about the magnitude of the difference, the precision of estimates, or the power of a procedure *(39,40)*.

The pH of worts produced with 10–90% commercial oat flour (5.78–6.09) was significantly higher ($P < 0.001$) compared to that of 100% barley malt wort (5.75) (Figure 8). However, it increased only slightly with increasing oat flour concentration up to 70% (5.75–5.86; $R^2 = 0.9593$). The substitution of more than 70% barley malt with oat flour resulted in a strong increase in wort pH (5.86–6.09; $R^2 = 0.9994$). Worts containing 10–90% commercial sorghum flour also had a

significantly higher pH (5.78–6.11; $P < 0.001$) than the reference wort (5.75) (Figure 8). The pH rise was, however, more rapid and consistent ($R^2 = 0.9775$) in comparison to that observed by replacing up to 90% barley malt with oat flour.

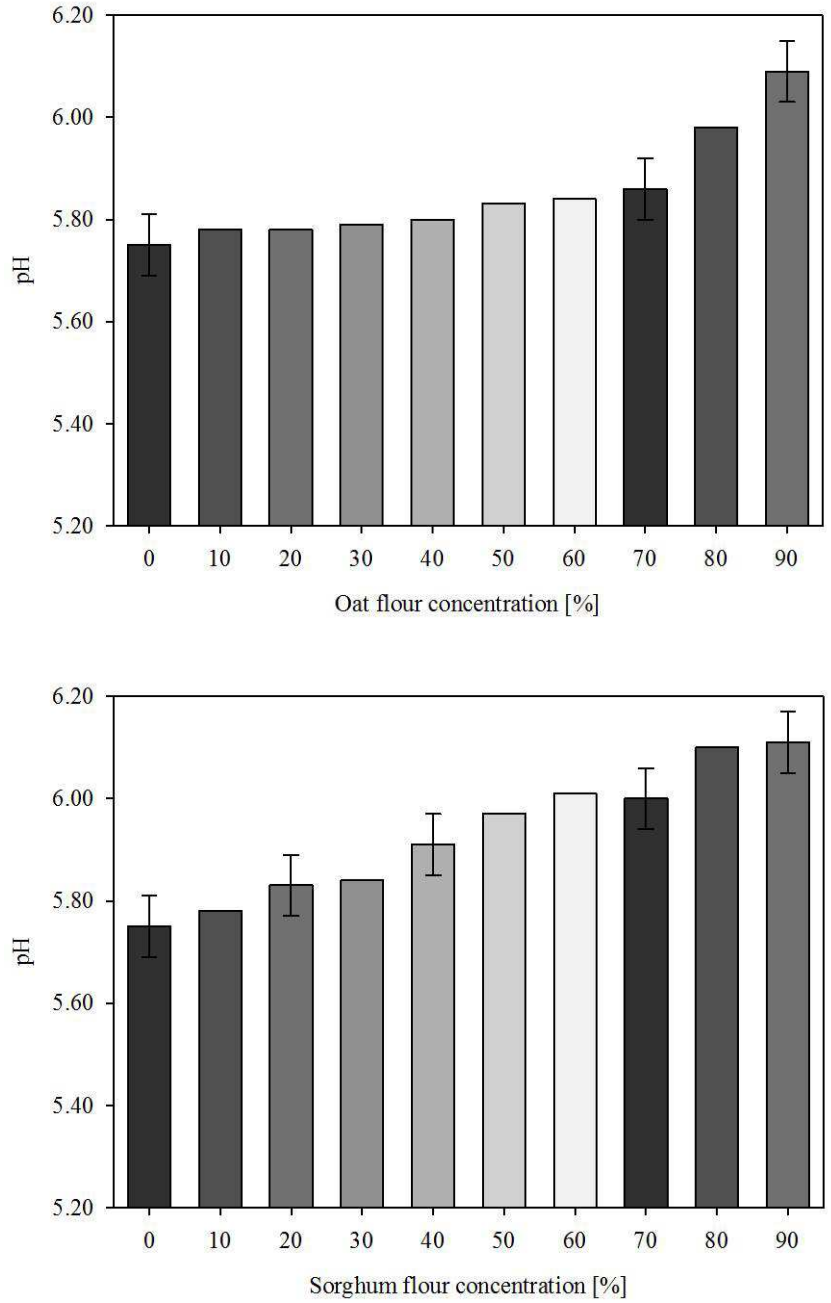


Figure 8–8. Effect of different oat/sorghum flour concentrations (0–90%) on wort pH.

In general, extracts of malted grain have a lower pH compared to those of unmalted grain due to modification processes during malting *(41,42)*; this explains the increase in wort pH when using commercial oat and sorghum flours. Furthermore, higher nitrogen contents of mashing materials usually involve higher buffering capacities of worts (e.g. peptides/polypeptides with aspartate and glutamate residues act as buffer substances) *(42)*, which is probably why the use of sorghum flour (5.26% DM protein) resulted in higher wort pH values than the use of oat flour (7.71% DM protein).

The color of worts filtered through 0.45-µm membrane filters did not change when substituting 10% barley malt with commercial oat flour (reference value 13.0 EBC units) (Figure 9). However, the use of 20% or more adjunct caused a significant decrease in wort color ($P < 0.001$) from 12.8 EBC units (20% oat flour) to 8.0 EBC units (90% oat flour). In addition, a very high correlation between wort color and oat flour concentration was found ($R^2 = 0.9729$). Worts produced with 10–90% commercial sorghum flour exhibited considerably lower color values (12.5–6.2 EBC units) (Figure 9) than those produced with oat flour; already the use of 10% sorghum flour resulted in a significant decrease in wort color ($P < 0.001$). During kilning of green malt, non-enzymatic browning or Maillard reactions between amino acids and reducing sugars take place imparting color and flavor to malt *(42)*, which explains the loss of color (melanoidins) when replacing barley malt with commercial flours. The color differences between worts produced with commercial oat and sorghum flours might be due to their different fat contents (7.52% DM and 3.94% DM, respectively) since Maillard-type reactions also occur between amino compounds and substances produced during the oxidation of lipids (free carbonyl group) *(42)*.

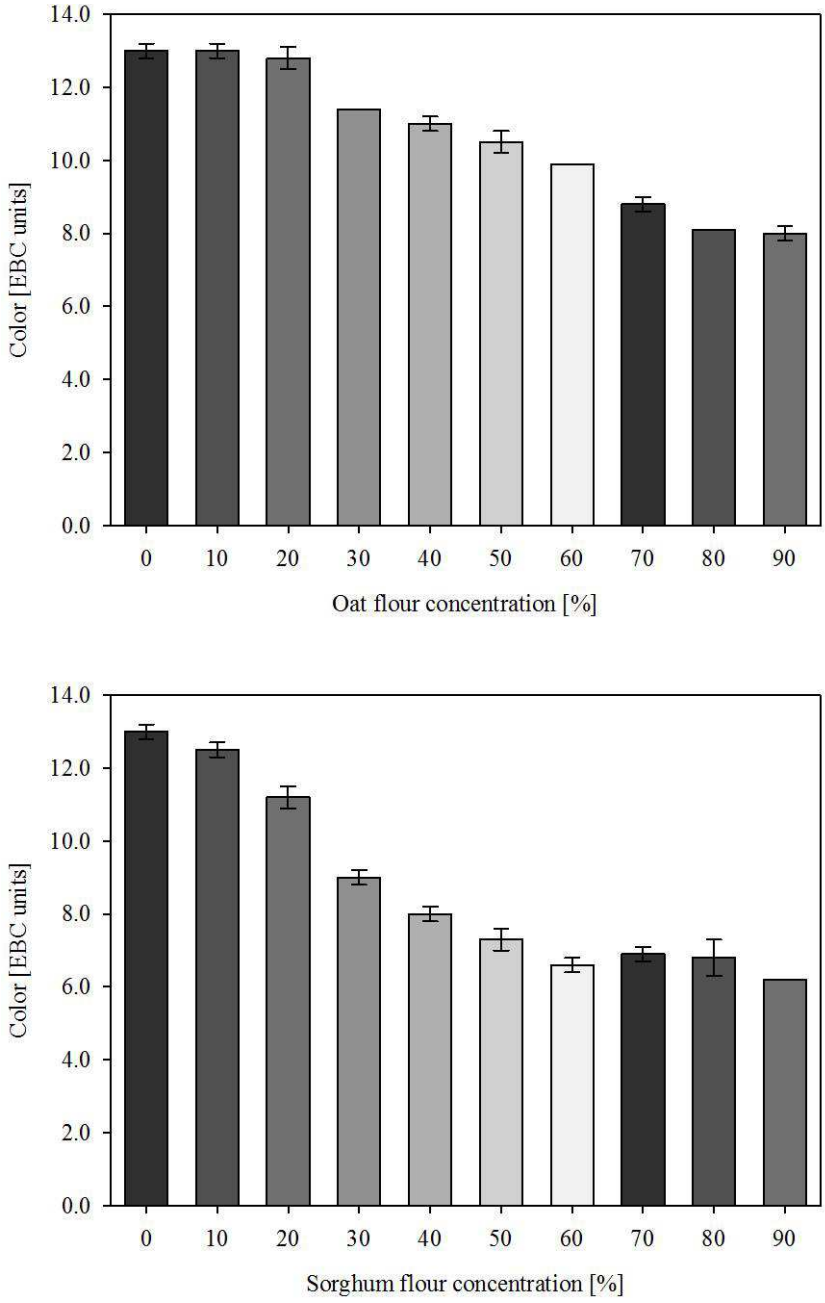


Figure 8–9. Effect of different oat/sorghum flour concentrations (0–90%) on wort color.

The AAL of worts increased significantly when replacing 10/20% barley malt with commercial oat flour from 82.0% w/w (100% barley malt) to 83.5/83.2% w/w ($P < 0.001$); the use of 30/40% oat flour resulted in similar AALs (82.1/81.9% w/w) (Figure 10). Higher adjunct concentrations, however, caused significantly lower ($P < 0.001$) AALs compared to the reference ranging from 80.5–74.2% w/w (50–90% oat flour; $R^2 = 0.8576$). According to the literature *(32)*, recommended values concerning the AAL of worts (11–14% w/w extract) are 80–85%. AALs in this range were still achieved in worts containing 70% commercial oat flour (79.8% w/w) without the addition of exogenous enzymes. The increasing extract content of worts produced with up to 50% commercial sorghum flour was not reflected in a higher AAL; the latter decreased significantly ($P < 0.05$) with rising adjunct concentration from 82.0% w/w (100% barley malt) to 50.2% w/w (90% sorghum flour) (Figure 10). Besides, a very high correlation between AAL of worts and sorghum flour concentration was determined ($R^2 = 0.9831$). These findings indicate that most of the extract obtained from sorghum flour was not fermentable by the yeast strain used in this study (Saflager W-34/70; *Saccharomyces cerevisiae*) such as dextrins *(43,44)*. As a consequence of this, the use of 30–50% commercial sorghum flour without pregelatinization and enzyme addition is only suitable for the production of beers with lower alcohol content *(45,46)*.

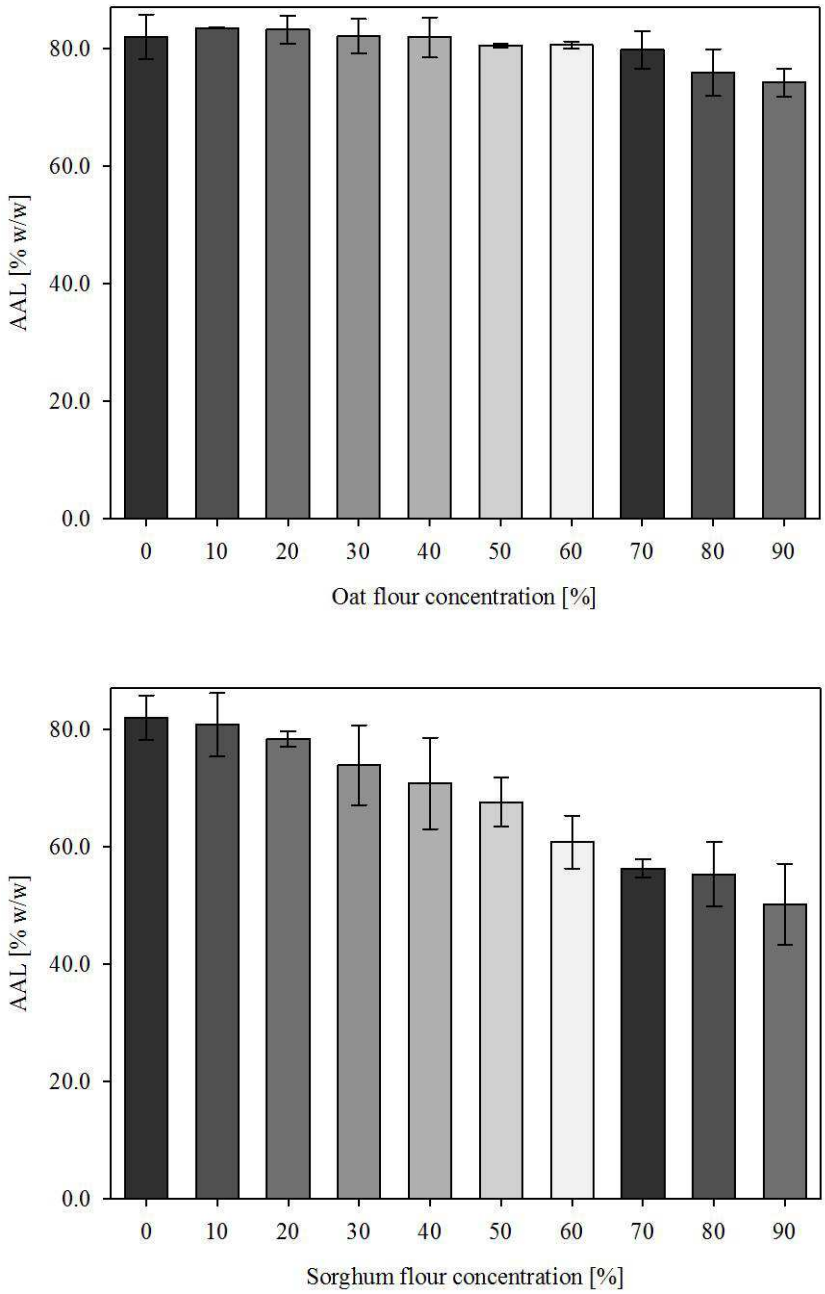


Figure 8–10. Effect of different oat/sorghum flour concentrations (0–90%) on wort fermentability.

Introduction of equivalent factors into brewing

Equivalent factors are a measure of the amount of adjunct (that is, oat or sorghum flour) required to substitute a defined amount of barley malt without causing relevant changes in wort extract. On the basis of extract yields, equivalent curves for the substitution of up to 90% barley malt with commercial oat or sorghum flour were generated, aiming at a constant wort extract content of 15.8 ± 0.2% w/w (Figure 11). By means of equivalent curves, the performance efficiency of different oat/sorghum flour concentrations could be determined. Each equivalent factor was calculated to a baseline value of 1.00 representing the extract yield obtained with 100% barley malt. As shown in Figure 11, at adjunct concentrations of approximately 50% (sorghum flour; equivalent factor 1.00) and 70% (oat flour; equivalent factor 1.00), respectively, 100 kg barley malt (random amount) has to be replaced by 100 kg commercial flour in order to produce worts with constant extract levels. When substituting 10% barley malt, for example, only 87 kg oat flour (equivalent factor 1.15) and 86 kg sorghum flour (equivalent factor 1.16), respectively, is needed to compensate for the wort extract content achieved with 100 kg barley malt (positive substitution). However, the use of 60% sorghum flour (equivalent factor 0.98) and 80% oat flour (equivalent factor 0.99), respectively, involves a high raw material charge with poor extract recovery (negative substitution, that is, 102 kg sorghum flour/101 kg oat flour is necessary to replace 100 kg barley malt). Hence, equivalent factors clearly indicate the limitations of substituting barley malt with commercial flours from an economic point of view.

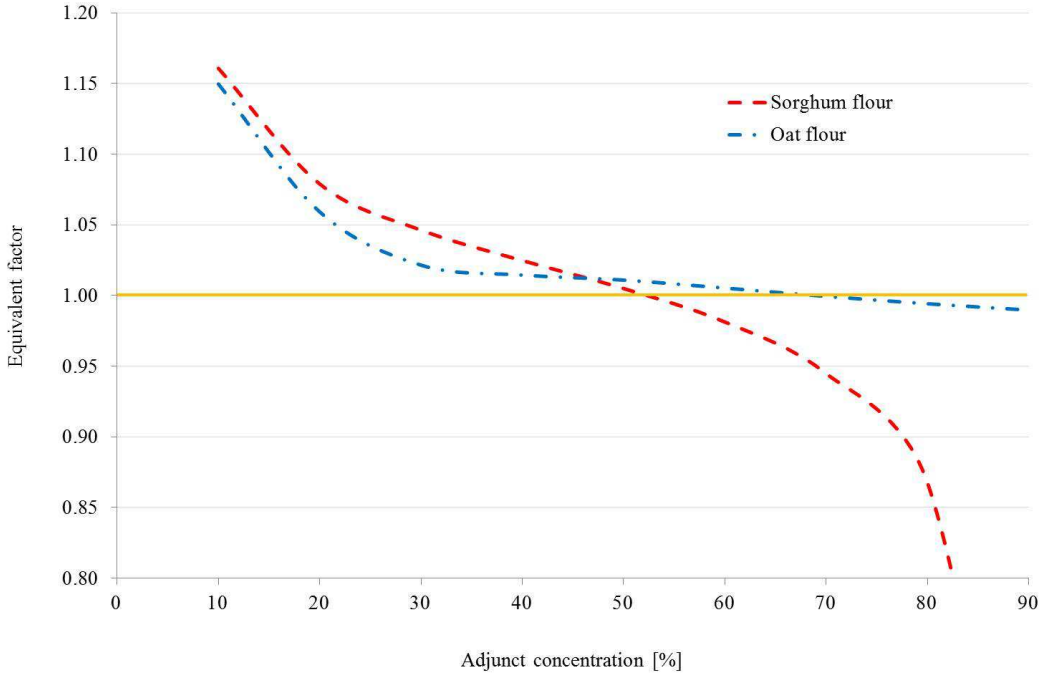


Figure 8–11. Equivalent curves for the substitution of barley malt with oat/sorghum flour (0–90%); the baseline value of 1.00 represents the extract yield obtained with 100% barley malt; equivalent factor = 1.00: e.g. 100 kg barley malt has to be replaced by 100 kg commercial flour in order to obtain a constant wort extract; equivalent factor > 1.00: positive substitution; equivalent factor < 1.00: negative substitution.

Conclusion

In the present study, the use of 10–90% commercial oat and sorghum flours as a substitute for barley malt in brewing was evaluated. Oat flour contained significantly more protein, β-glucan, and fat as well as less starch, ash, and polyphenols than sorghum flour. The gelatinization temperature of sorghum flour starch was considerably higher compared to that of oat flour starch. Mashing with up to 50% sorghum flour or up to 90% oat flour resulted in complete saccharified and easily filterable mashes. Worts containing 10–90% oat or sorghum flour exhibited lower concentrations of foam-positive proteins, TSN, and FAN, higher pH values as well as lighter colors in comparison to 100% barley malt worts. With regard to wort viscosity (12.0% w/w), it has been found that the level of endogenous malt β-glucanases was sufficient for the use of up to 50% oat flour (rich in β-glucan). The extract content of worts steadily increased with increasing oat flour concentration; however, the application of more than 50% sorghum flour caused a rapid decrease in wort extract combined with a rising wort viscosity (12.0% w/w). Hence, the replacement of barley malt with sorghum flour is limited to 50% when using an infusion mashing process (no pregelatinization of sorghum starch) without the addition of exogenous enzymes. With the help of equivalent factors, the performance efficiency of different oat and sorghum flour concentrations could be determined. The use of more than 70% oat flour did not prove economically advantageous because of endogenous malt α- and β-amylase deficiencies. Worts produced with up to 70% oat flour showed a very good or good fermentability, whereas those containing 30–50% sorghum flour resulted in a lower alcohol production.

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Chapter 9

A comparison of white Nigerian and red Italian sorghum (*Sorghum bicolor*) as brewing adjuncts based on optimized enzyme additions

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Abstract

Sorghum has been used for thousands of years in human food products. In Western countries, however, it is primarily used as bird and animal feed, although there is considerable interest in its use as gluten-free alternative to wheat, barley, and rye. The aim of this study was to compare the mashing performance of white Nigerian and red Italian sorghum based on optimized enzyme additions. For this purpose, both sorghum types were fully characterized using standard methods, Lab-on-a-Chip capillary electrophoresis, and scanning electron microscopy. The application of exogenous enzymes was optimized by monitoring changes in mash consistency during mashing using a Physica MCR rheometer. Furthermore, laboratory-scale mashing trials were carried out to compare the quality of worts produced with up to 40% white or red sorghum and optimized enzyme levels. Both sorghum types are characterized by higher starch and lower protein/β-glucan contents in comparison to barley malt. The addition of protease/β-glucanase as recommended had no significant effect on mash consistency and wort quality. Besides, 50% of the recommended heat-stable α-amylase dose was sufficient for 40% sorghum adjunct. Worts produced with 40% white or red sorghum had significantly lower TSN/FAN contents and viscosities than the reference wort (100% barley malt). However, white sorghum provided significantly more TSN/FAN compared to red sorghum. Its use as a substitute for barley malt also resulted in significantly higher extract contents.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop in terms of world production after rice, wheat, maize, and barley. It is uniquely well-adapted to cultivation in the semi-arid tropics of Asia, Africa, and Latin America *(1,2)*. Thus, sorghum is a staple food in many developing countries, whereas in Western countries it is primarily used as animal feed. However, sorghum has considerable potential as gluten-free alternative to wheat, barley, and rye *(3)*. In addition, the use of unmalted sorghum as brewing adjunct can be cost-saving and highly successful. In Mexico, rice and maize grits have been replaced by sorghum grits (higher protein and lower fat content) for many years *(4,5)*. Brewing with sorghum adjunct is generally unproblematic, despite the fact that sorghum starch is more difficult to gelatinize than maize starch due to the presence of protein bodies organized around the starch granule *(6,7)*. Besides, the gelatinization temperature of sorghum starch ranging from 68°C to 78°C is approximately 5°C higher compared to that of maize starch *(8)*. Sorghum is unique among major cereals because some cultivars contain condensed tannins conferring resistance to deterioration of the grain (molds, insects, weather, etc.). Condensed tannins, also known as proanthocyanidins, are high-molecular-weight polyphenols consisting of polymerized flavan-3-ol units (catechin, epicatechin). For this reason, sorghum cultivars are divided into three types based on their genetics and chemical analyses: Type I (non-tannin sorghum) – non-pigmented testa, no tannins, low levels of phenols; type II (moderate-tannin sorghum) – tannins present in pigmented testa; type III (high-tannin sorghum) – tannins present in pigmented testa and pericarp *(9,10)*. In general, type II and type III sorghum cultivars contain tannin levels of 0.5–1.5 mg/100 mg and 1.0–6.0 mg/100 mg catechin equivalents, respectively. It has been reported that pericarp (seed) color and its intensity are inadequate indicators of presence or content of tannins in sorghum. White, yellow, red, or brown colored sorghum seeds may or may not contain tannins depending on the presence of a pigmented testa *(9,11)*. High-tannin sorghum cultivars are not suitable for brewing since condensed tannins can inhibit enzyme activities (e.g. α-amylase) and cause astringent taste as well as dark beer colors *(4,7,9)*. However, most sorghum cultivars do not contain condensed tannins (anti-nutritional substances). Many sorghum cultivars contain flavonoids such as

anthocyanins contributing the red, purple, and blue colors in plants but all contain phenolic acids (e.g. ferulic acid), both being not anti-nutritional factors *(7,9)*.

When brewing with unmalted sorghum, exogenous enzymes such as heat-stable α-amylase are required. High levels of industrial enzymes usually improve both extract content and processability of sorghum worts. However, a balance between product quality and production costs has to be established. Therefore, the objectives of this study were: 1) to optimize the addition of industrial enzymes to mashes using a Physica MCR rheometer; 2) to compare the impact of white Nigerian and red Italian sorghum on the quality and processability of mashes and worts produced with optimized enzyme levels.

Materials and methods

Mashing materials

Malted barley (*Hordeum vulgare* L. 'Fr Sebastian'), harvested in 2008 and obtained from Greencore Group plc (Dublin, Ireland), unmalted white Nigerian sorghum [*Sorghum bicolor* (L.) Moench 'Short Kaura 5912'], harvested in 2011, and commercially available red sorghum, harvested in 2011 in Ancona, Italy were used in the mashing trials.

Mashing enzymes

The exogenous enzymes applied to mashes were Bioprotease N120MG (0.13 g/kg sorghum; optimum pH/temperature 6.0/55°C) to increase the free amino nitrogen (FAN) content of worts, Hitempase 2XP (1.0 g/kg sorghum; optimum pH/temperature 6.0/90°C) to hydrolyze sorghum starch to dextrins (heat-stable α-amylase), and Bioglucanase TX (0.25 g/kg malt; optimum pH/temperature 5.5/60°C) to improve mash filtration (Kerry Ingredients & Flavours, Carrigaline, Ireland).

Characterization of unmalted sorghum types

Standard analysis

White and red sorghum grains were analyzed according to the methods of MEBAK – Raw materials *(12)*. Moisture contents were determined using method 1.5.1.1. Total nitrogen contents were measured applying a Tecator™ Digestor combined with a Kjeltec™ 2100 Distillation unit (Foss, Hillerød, Denmark) following method 1.5.2.1. In addition, β-glucan contents were analyzed using the McCleary method (Megazyme International Ireland Ltd., Bray, Ireland). Total starch contents were determined following the Megazyme Amyloglucosidase/α-Amylase method. β-Glucanase activities were measured according to the Megazyme Azo-Barley Glucan method. One unit of activity equals one micromole of glucose reducing sugar equivalent released per minute at 30°C and pH 4.6. α-Amylase activities were analyzed following the Megazyme Ceralpha method. One unit of activity corresponds to the amount of enzyme required to release one micromole of *p*-nitrophenol from non-reducing-end blocked *p*-nitrophenyl maltoheptaoside in one minute under the defined assay conditions. β-Amylase activities were determined

using the Megazyme Betamyl-3 method. One unit of activity corresponds to the amount of enzyme required to release one micromole of *p*-nitrophenol from *p*-nitrophenyl-β-D-maltotrioside in one minute under the defined assay conditions. Furthermore, the particle size distribution of hammer-milled sorghum grist was evaluated according to MEBAK – Würze, Bier, Biermischgetränke *(13)* method 1.1.1. All standard analyses were carried out in triplicate.

Scanning electron microscopy

Grain ultrastructures were determined following the method of Oliveira *et al. (14)*. For the analysis, grain cross sections were mounted onto aluminium stubs using double-sided adhesive carbon tape. Then, the samples were coated with a 7-nm gold layer in a Gold Sputter Coater (Bio-Rad Polaron Division, Hemel Hempstead, United Kingdom) and observed under a constant accelerating voltage of 5 kV applying a JEOL scanning electron microscope type 5510 (JEOL Ltd., Tokyo, Japan). All scanning electron microscopy (SEM) analyses were carried out in duplicate.

Lab-on-a-Chip capillary electrophoresis

Total protein profiles were detected using the method described by Klose *et al. (15)*. For the analysis, 40 mg of flour was extracted with 400 µL of a reagent containing 2 M urea, 15% glycerol, 0.1 M Tris-HCl (pH 8.8), and 0.1 M dithiothreitol in an ultrasonic water bath for 15 min at room temperature. After centrifugation at 18,890×*g* for 15 min, 4 µL of supernatant was denatured by heating at 95°C for 5 min with 2 µL of Agilent denaturing solution. Afterward, the denatured sample was diluted with 84 µL of deionized water and 6 µL of this mixture was applied to the Protein 80^{+} and Protein 230^{+} LabChip, respectively, for analysis in the Agilent 2100 Bioanalyzer according to the manufacturer's instructions (Agilent Technologies, Santa Clara, California). All Lab-on-a-Chip analyses were performed in triplicate.

Mashing performance of unmalted sorghum types

Milling

Malted barley was milled with a laboratory disk mill (Bühler GmbH, Braunschweig, Germany) set at a 0.2-mm disk distance. White and red sorghum grains were milled

using a hammer mill equipped with a 1.5-mm sieve (A.M.A. S.p.A., San Martino in Rio, Italy). Milling of mashing materials was carried out directly before mashing-in.

Optimization of enzyme addition

The addition of exogenous enzymes to mashes was optimized by monitoring changes in mash consistency (viscosity) during mashing. For this purpose, the previously published method of Schnitzenbaumer *et al. (16)* using a controlled stress rheometer Physica MCR 301 (Anton Paar Germany GmbH, Ostfildern, Germany) was applied. The rheometer was equipped with a paddle-shaped rotor enabling mash particles to be kept in suspension throughout the measurement. In all rheological mashing trials, a total grist mass of 6.020 g dry matter (DM) was mixed with distilled water to give a total mash mass of 33.000 g at a constant moisture basis of 14%. For the optimization process, rheological trials without enzyme addition, with recommended enzyme addition, and with different levels (0%, 25%, 50%, 75%, 100%, 200% of recommended enzyme dose) of Bioprotease N120MG, Hitempase 2XP, and Bioglucanase TX were carried out. All rheological tests were performed in triplicate using a double infusion mashing process (see below).

Laboratory-scale mashing

The quality of worts produced with optimized levels of exogenous enzymes was verified by comparing their analysis data with those of worts produced with recommended enzyme levels and without enzyme addition, respectively. Mashing with malted barley and unmalted sorghum types was carried out in a LB 8 – Electronic mashing device (Lochner Labor + Technik GmbH, Berching, Germany). In all laboratory-scale mashing trials, a total grist mass of 84.28 g (DM) was mixed with distilled water to give a total mash mass of 462.00 g at a constant moisture basis of 14%. The saccharification rate was analyzed 10 min after the total mash reached 70°C (see double infusion mashing process below) and the measurement repeated every 5 min until the iodine test was negative. After mashing-off at 78°C, the loss of water due to evaporation during the mashing process was determined gravimetrically and replaced. Then, the filtration rate of the hot mash was evaluated by measuring the filtered wort volume every 2 min. All laboratory-scale mashing trials were performed in triplicate.

Double infusion mashing process

For all rheological and laboratory-scale mashing trials, a slightly modified double infusion process used in the brewing industry (Kerry Ingredients & Flavours, Carrigaline, Ireland) was chosen (Figure 1). First, sorghum mash was cooked at 90°C for 30 min to gelatinize starch and then mixed with barley malt mash to enzymatically convert gelatinized starch into fermentable sugars. It is recommended to add protease and heat-stable α-amylase to the sorghum mash and β-glucanase to the total mash (Kerry Ingredients & Flavours, Carrigaline, Ireland). Mashing-in was performed by mixing homogenized sorghum grist into preheated distilled water (55°C) in the respective mash cup, which was then attached to the temperature-controlled heating system of the respective mashing instrument. Total mashes containing various levels of each sorghum type (0%, 10%, 20%, 30%, 40% of total grist mass) were prepared using a constant liquor-to-grist ratio of 4.5:1 (DM). In all mashing trials, a stirring speed of 100 rpm and a heating rate of 1°C per min were applied.

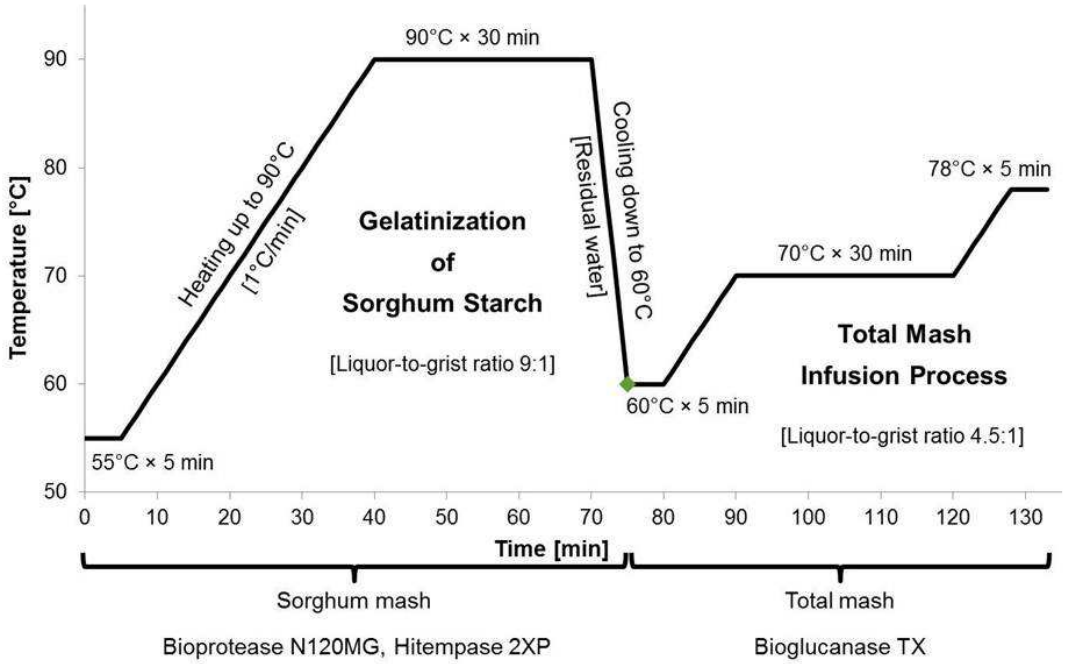


Figure 9–1. Graphical representation of the double infusion mashing process used in this study.

Wort analysis

Worts were analyzed applying the standard methods specified by Mitteleuropäische Brautechnische Analysenkommission (MEBAK). pH and color of wort samples were determined according to MEBAK – Raw materials *(12)* methods 3.1.4.2.7 and 3.1.4.2.8.2. Total polyphenols in wort were measured following MEBAK – Würze, Bier, Biermischgetränke *(13)* method 2.16.1. Wort viscosities were analyzed using a HAAKE falling ball viscometer (Thermo Scientific, Karlsruhe, Germany). Total soluble nitrogen (TSN) contents of wort samples (10 mL) were determined applying a Tecator™ Digestor combined with a Kjeltec™ 2100 Distillation unit (Foss, Hillerød, Denmark). Free amino nitrogen (FAN) in worts was measured according to MEBAK – Raw materials *(12)* method 3.1.4.5.5.1. The limit of attenuation of worts was analyzed following MEBAK – Raw materials *(12)* method 3.1.4.10.1.2 using dry lager yeast (Saflager S-23; Fermentis, Marcq-en-Baroeul cedex, France). Wort extract, apparent extract, apparent degree of fermentation, and alcohol were determined using an Alcolyzer Beer ME Analyzing System (Anton Paar GmbH, Graz, Austria). In addition, the protein profile of worts was detected by Lab-on-a-Chip capillary electrophoresis. For this purpose, 40 mg of freeze-dried and homogenized sample was extracted and analyzed as described above.

Statistical analysis

For determining the statistical significance, the two-tailed Student's *t*-value for *n*-1 degrees of freedom was calculated. The confidence interval with a probability level of 95% ($\alpha = 0.05$) was determined for each mean value (arithmetic mean). Correlations are indicated by the coefficient of determination R^2.

Results and discussion

Characterization of unmalted sorghum types

In the present study, white Nigerian and red Italian sorghum were compared as brewing adjuncts. Both unmalted sorghum types exhibited similar levels of β-glucan, protein, starch, and enzyme activity as shown in Table 1. In comparison to barley malt (0.3% DM β-glucan, 9.4% DM protein, 65.7% DM starch), they both contained less β-glucan and protein as well as more starch. Furthermore, their enzyme activities were negligible compared to those in malted barley (β-glucanase 359.1 U/kg, α-amylase 165.5 U/g, β-amylase 19.3 U/g). These findings are in accordance with data published in the literature *(10,17–21)*.

Table 9–1. Standard analysis of sorghum types.

Sorghum type	Moisture	β-Glucan	Protein[a]	Starch	β-Glucanase	α-Amylase	β-Amylase
	%	% DM	% DM	% DM	U/kg	U/g	U/g
White sorghum	11.6 ± 0.5	0.1 ± 0.0	9.0 ± 0.1	67.8 ± 2.6	37.6 ± 10.4	0.2 ± 0.0	0.5 ± 0.3
Red sorghum	11.9 ± 0.1	0.0 ± 0.0	9.1 ± 0.1	68.1 ± 3.9	22.6 ± 05.5	0.0 ± 0.0	0.2 ± 0.1

[a] Total nitrogen (% DM) × 6.25.

A sieve analysis of hammer-milled white and red sorghum used in this study revealed significant differences in their grist particle size distributions as shown in Figure 2. Hence, milling of white sorghum led to a higher flour fraction in the total grist. These results can be explained by examining white and red sorghum grains using SEM. The sorghum kernel consists of the pericarp region (pericarp, testa, aleurone), the germ, and the endosperm. Unlike other cereals, sorghum may contain starch granules in the pericarp. Under the aleurone layer is the outer vitreous (also called translucent, hard, glassy, horny, corneous) endosperm fraction surrounding an inner floury (also called opaque, soft) core *(7,8,22)*. The relative proportion of vitreous to floury endosperm can vary widely among sorghum cultivars *(23)*. In the present study, white sorghum contained more floury and less vitreous endosperm than red sorghum as shown in Figures 3A and B. With regard to milling properties, the floury part pulverizes more easily than the vitreous part. The latter gives coarse grits which are commonly used in brewing *(24)*.

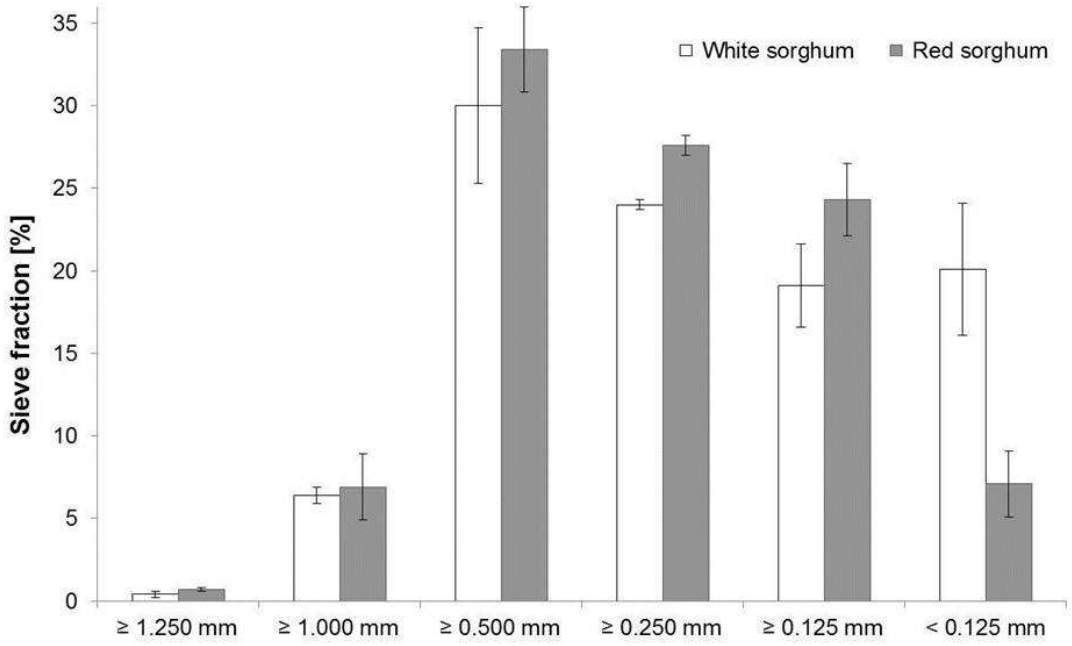


Figure 9–2. Particle size distribution of hammer-milled white and red sorghum grist.

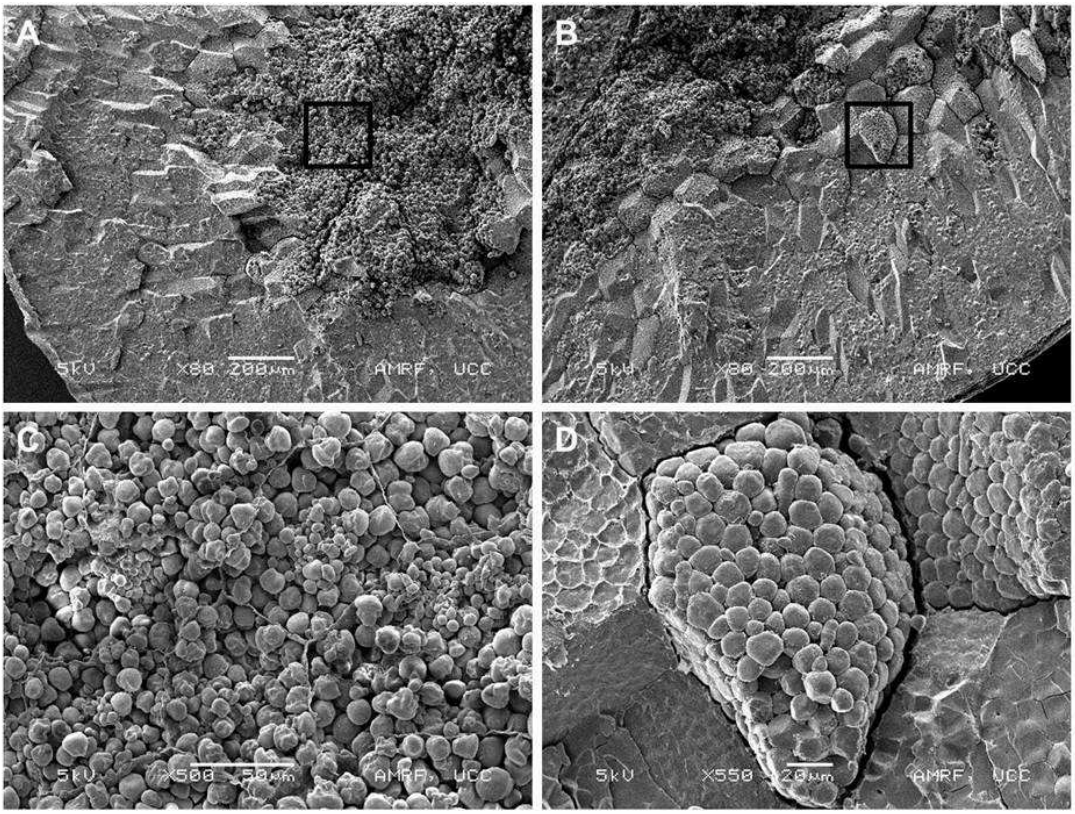


Figure 9–3. SEM images of white and red sorghum grains. White sorghum: (A) vitreous (outer part) and floury (inner part) endosperm (magnification ×80); (C) floury endosperm (magnification ×500). Red sorghum: (B) vitreous and floury endosperm (magnification ×80); (D) vitreous endosperm (magnification ×550).

A closer look (Figures 3C and D) revealed that the inner floury endosperm consists of round, loosely packed starch granules covered with a thin, weakly adhering protein layer. In contrast, the outer vitreous endosperm is tightly packed with polygonal starch granules held together by a protein network consisting of protein bodies embedded in a matrix protein (strong protein-starch adhesion) *(7,25,26)*. The presence of protein bodies around sorghum starch granules has a limiting effect on starch gelatinization *(6)*. Starch of the vitreous endosperm has not only a higher gelatinization temperature but also a higher intrinsic viscosity and a lower iodine binding capacity than starch of the floury endosperm *(27)*.

With the help of Lab-on-a-Chip capillary electrophoresis separating proteins based on their molecular weight, a distinct protein peak/band at around 28 kDa was determined in the electropherogram/gel-like image of white sorghum (Figure 4). Sorghum proteins are classified into kafirins (prolamins) and non-kafirins. The first are storage proteins comprising around 70% of the total grain protein, the latter are involved in cellular functions. Kafirins are found in protein bodies within the sorghum endosperm (more protein bodies in vitreous than in floury endosperm) and subclassified as α-, β-, and γ-kafirins based on similarities in molecular weight, solubility, and structure. Their approximate molecular weights using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) are as follows: α-kafirins (66–71%/80–84% of total kafirins in floury/vitreous endosperm) 23 kDa and 25 kDa; β-kafirins (7–13% of total kafirins) 16 kDa, 18 kDa, and 20 kDa; γ-kafirins (10–20% of total kafirins) 28 kDa *(6,28–30)*. Hence, the distinct protein peak/band at around 28 kDa in the electropherogram/gel-like image of white sorghum represents γ-kafirins. Using a Protein 230^+ instead of a Protein 80^+ LabChip revealed that red sorghum contains proteins with a molecular weight of around 103 kDa. Those might represent heat shock proteins which are synthesized in response to high temperatures *(31,32)*.

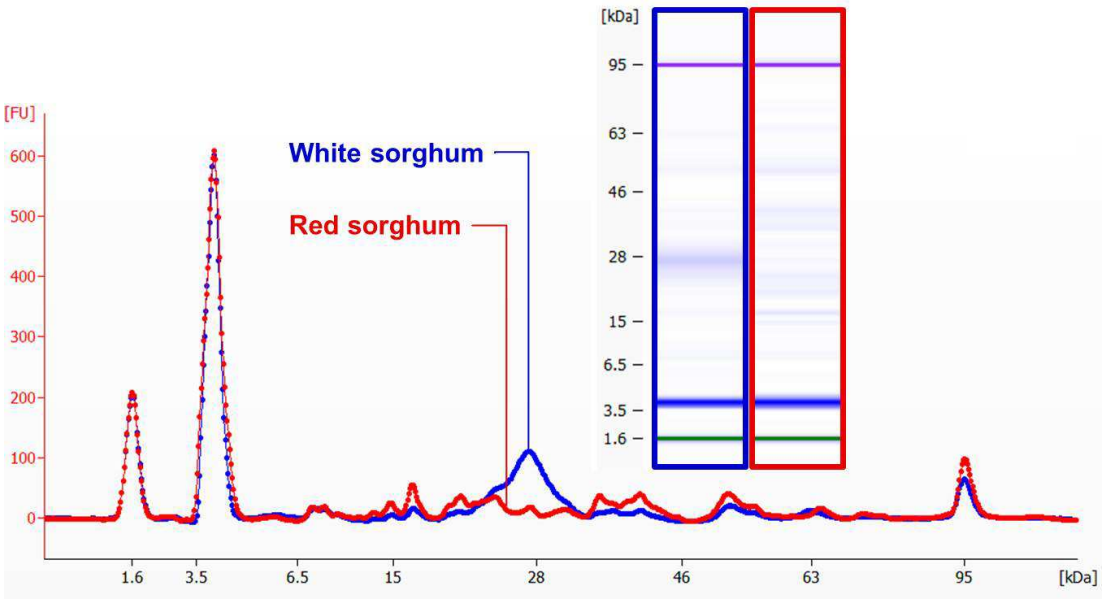


Figure 9–4. Electropherogram/gel-like image of the protein profile of white and red sorghum.

Mashing performance of unmalted sorghum types

Optimization of enzyme addition

In the present study, the application of industrial enzymes was optimized by monitoring changes in mash consistency during mashing using a Physica MCR 301 rheometer. As shown in Figure 5A, the mash consistency increased significantly with increasing levels of white sorghum. The addition of heat-stable α-amylase to sorghum mashes as recommended caused a decrease in mash consistency to a large extent (Figure 5B). A closer look revealed that the mash consistency was still significantly increased with increasing amounts of white sorghum. Doubling the recommended α-amylase dose from 100% to 200% had no impact on mash consistency. In contrast, it was found that 50% of the recommended heat-stable α-amylase dose was sufficient for the use of 40% white sorghum adjunct (non-significant changes in mash consistency). A reduction of the recommended α-amylase dose by 75% resulted, however, in significantly higher mash consistencies. The addition of protease to sorghum mashes as recommended had no significant effect on mash consistency.

After mixing of sorghum and barley malt mash, the mash consistency of the total mash was also significantly increased with increasing levels of white sorghum as shown in Figure 6A. The addition of heat-stable α-amylase to sorghum mashes as recommended resulted in significantly lower total mash consistencies when using 20% or more white sorghum adjunct (Figure 6B). It had, however, no significant impact on the final mash consistency of 10% sorghum-containing mashes. Furthermore, the addition of β-glucanase to total mashes as recommended had no significant effect on mash consistency. The addition of different levels of Bioprotease N120MG, Hitempase 2XP, and Bioglucanase TX to mashes produced with up to 40% red sorghum adjunct led to similar results as discussed above on the basis of white sorghum.

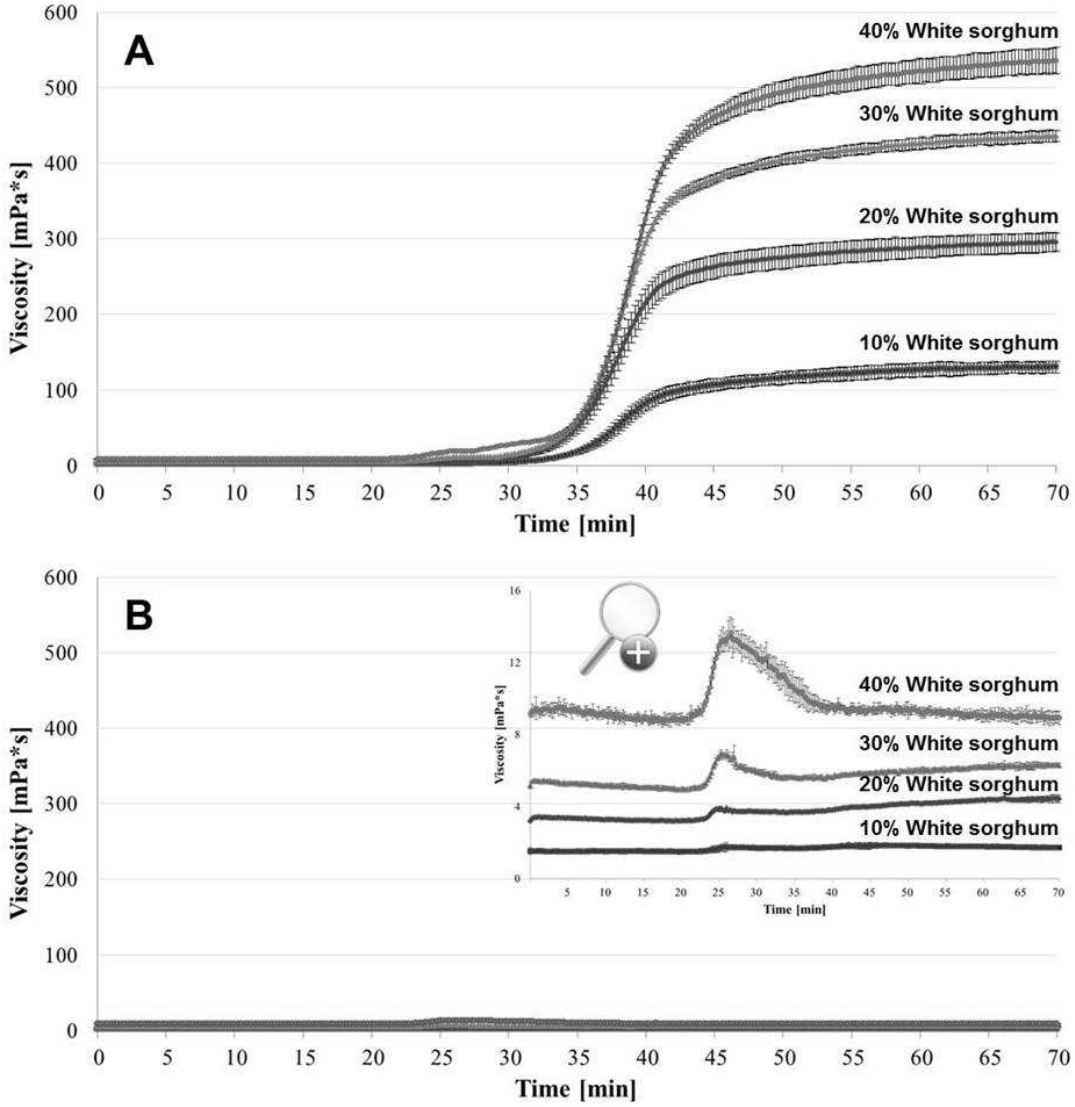


Figure 9–5. Rheological profiles of white sorghum mashes (10–40%) during the gelatinization process. (A) Without enzyme addition; (B) with recommended enzyme addition.

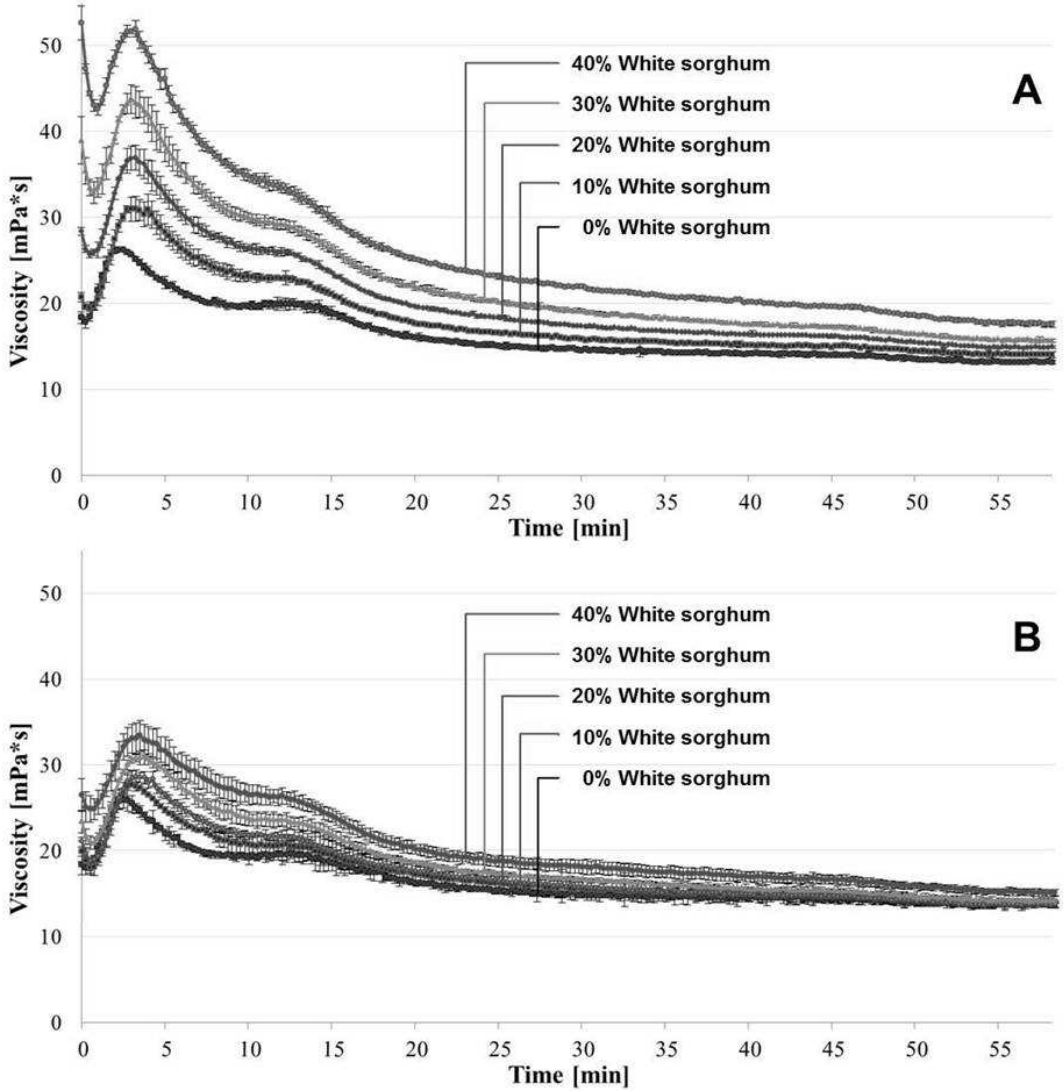


Figure 9–6. Rheological profiles of white sorghum mashes (0–40%) during the infusion process (total mashes). (A) Without enzyme addition; (B) with recommended enzyme addition.

Comparison of rheological profiles of white and red sorghum mashes

When comparing the rheological behavior of white and red sorghum used in this study, it has been found that red sorghum caused a significantly higher mash consistency than white sorghum as shown in Figure 7A. In addition, a time-delayed gelatinization of red sorghum starch compared to that of white sorghum starch has been observed. Both can be explained by the higher proportion of vitreous starch in red sorghum having a higher intrinsic viscosity as well as a higher gelatinization temperature. After mixing of sorghum and barley malt mash, the start consistency of the total mash was around 4 mPa·s to 19 mPa·s higher when using up to 40% red sorghum (without enzyme addition). However, within 5 min at 60°C the mash consistency has been reduced to a similar level caused by white sorghum. The addition of heat-stable α-amylase to red sorghum mashes as recommended resulted in extensive reductions of mash consistency (Figure 7B) as already shown on the basis of white sorghum. A closer look revealed, however, that the consistency of red sorghum mashes was still significantly higher than that of white sorghum mashes. As a consequence of this, the use of 20% or more red sorghum caused significantly higher total mash consistencies in comparison to that of white sorghum (with recommended enzyme addition).

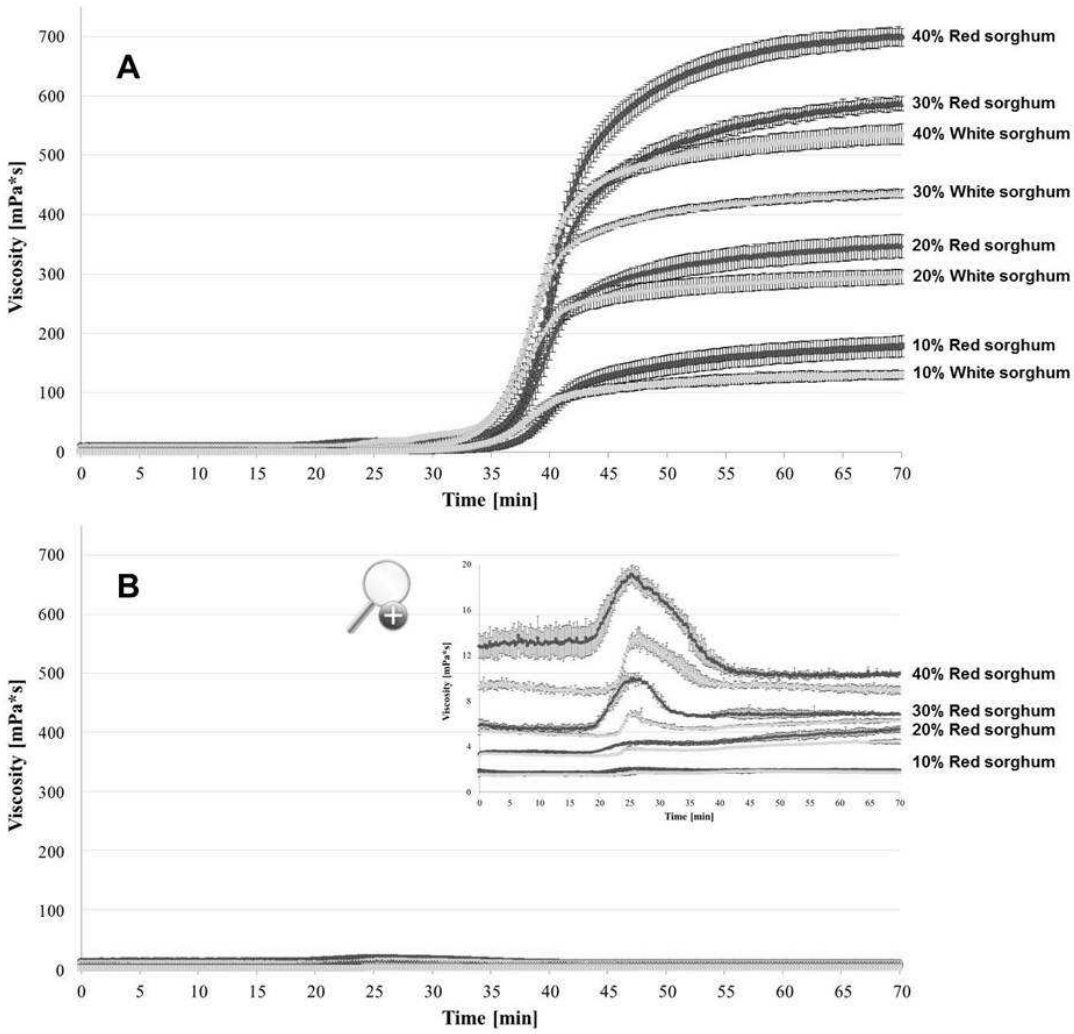


Figure 9–7. Comparison of rheological profiles of white and red sorghum mashes (10–40%) during the gelatinization process. (A) Without enzyme addition; (B) with recommended enzyme addition.

Laboratory-scale mashing

The quality of white and red sorghum worts (0%, 10%, 20%, 30%, 40% adjunct) produced with optimized levels of industrial enzymes (no protease, 50% of recommended heat-stable α-amylase dose, no β-glucanase) was assessed by comparing their analysis data with those of worts produced with recommended enzyme levels (100% protease, 100% heat-stable α-amylase, 100% β-glucanase) or without exogenous enzymes. It has been found that the wort pH increased significantly from 5.62 to 5.80 by replacing 40% barley malt with white (R^2 = 0.9918) or red (R^2 = 0.9963) sorghum, already the use of 10% adjunct caused a significantly higher wort pH (optimized enzyme addition). The application of different levels of Bioprotease N120MG, Hitempase 2XP, and Bioglucanase TX had no significant effect on wort pH. These findings are in agreement with experimental results published in the literature *(33,34)*. As shown in Tables 2 and 3, the wort viscosity (based on 12% w/w extract) decreased significantly from 1.750 mPa·s (100% barley malt) to 1.637 mPa·s/1.636 mPa·s when using 40% white/red sorghum which is in agreement with the literature *(33)*. Already the use of 10% red and 20% white sorghum caused a significant drop in viscosity with or without exogenous enzymes. These findings are most likely the result of a reduction in β-glucan content by substituting barley malt (0.3% DM β-glucan) with red/white sorghum (0.0%/0.1% DM β-glucan) *(20)*. It should be mentioned here that worts produced with recommended β-glucanase levels tended to lower viscosities than those produced without β-glucanase addition. Nonetheless, the filtration rate of 78°C hot mashes showed a negative trend with increasing levels (0–40%) of white (15.2–11.6 mL wort/min; R^2 = 0.8300) or red (15.2–13.8 mL wort/min; R^2 = 0.8599) sorghum. The use of 20% or more white and 40% red sorghum caused significantly lower filtration rates resulting in longer filtration times (optimized enzyme addition). The application of different levels of industrial mashing enzymes, in particular β-glucanase, had no significant impact on the filterability of mashes. These results can be explained by decreasing husk proportions in the total grist with increasing amounts of white or red sorghum (huskless grains) *(35,36)*. The permeability of the filter cake is further reduced by high flour proportions in the total grist when using hammer-milled white sorghum as mentioned above.

A very high linear correlation was found between TSN content of worts and white (R^2 = 0.9993) or red (R^2 = 0.9986) sorghum concentration (Tables 2 and 3). Worts produced with 40% white or red sorghum had significantly lower TSN contents (746 mg/L and 688 mg/L, respectively) than the reference wort (1,101 mg/L) which is in agreement with the literature *(33)*. Already the use of 10% sorghum adjunct led to significant reductions in TSN with or without industrial enzymes. White sorghum provided, however, significantly higher levels of TSN compared to red sorghum. The reason for this might be its finer grist (higher flour fraction) causing a higher solubility/extractability of proteins in aqueous systems *(37,38)*. In accordance with the TSN results, the FAN content of worts decreased significantly from 203 mg/L to 133 mg/L and 106 mg/L, respectively, by replacing 40% barley malt with unmalted white or red sorghum (Tables 2 and 3). However, it has been reported that FAN levels of 85–130 mg/L are sufficient for optimal yeast growth and fermentation *(39,40)*. Already the use of 10% red or 20% white sorghum resulted in significant FAN losses with or without enzyme application. Bajomo and Young *(41)* found that the addition of protease to sorghum mashes before starch gelatinization had no significant effect on the FAN content of worts as confirmed in this study. Besides, white sorghum also provided significantly higher FAN levels in comparison to red sorghum.

The protein profile of worts produced with 40% sorghum adjunct was similar to that of 100% barley malt worts. In all samples, proteins with a molecular weight of 7.4–17.5 kDa (foam-positive low-molecular-weight fraction) and approximately 41.0 kDa (foam-positive high-molecular-weight fraction) were detected *(42)*. However, the protein peaks/bands in the electropherogram/gel-like image of white/red sorghum worts were less/much less pronounced compared to those in the electropherogram/gel-like image of barley malt worts. Therefore, it is likely that beers containing 40% adjunct, particularly red sorghum, have a lower foam stability than 100% barley malt beers.

As shown in Table 2, worts produced with 40% white sorghum had a significantly higher extract content (15.43% w/w) than the reference wort (15.16% w/w). The use of 40% red sorghum (Table 3) resulted, however, in a constant extract yield. These findings can also be explained by the higher flour proportion in white sorghum grist, making starch granules more easily accessible to amylolytic enzymes *(43)*. A

reduction of the recommended heat-stable α-amylase dose by 50% had no significant impact on wort extract. In terms of fermentability or apparent attenuation limit (AAL), no significant differences between 40% white (75.7% w/w) or red (77.1% w/w) sorghum worts and the reference wort (77.4% w/w) were determined (optimized enzyme addition). The higher extract content of 40% white sorghum worts was not reflected in a higher fermentability. Interestingly, red sorghum worts tended to higher AALs compared to white sorghum worts. It should be mentioned here that the AAL of worts was analyzed using the dry yeast Saflager S-23, which was found to attenuate significantly less than other yeast strains *(44)*.

The polyphenol content of 40% red sorghum worts (191 mg/L) was, as expected, significantly higher compared to that of 100% barley malt worts (166 mg/L). In contrast, the use of 40% white sorghum resulted in a significantly lower polyphenol content (134 mg/L) (Tables 2 and 3). The substitution of up to 20% barley malt with sorghum adjunct had no significant effect on wort polyphenol content. When using 30% or more adjunct, however, red sorghum caused significantly higher polyphenol levels in worts than white sorghum. The addition of different amounts of industrial enzymes had no significant impact on the polyphenol content of worts. As a result, worts produced with 10–40% red sorghum had significantly higher color values (14.4–11.3 EBC units; $R^2 = 0.9744$) compared to those produced with 10–40% white sorghum (10.3–8.8 EBC units; $R^2 = 0.9963$). Already the use of 10% white sorghum caused a significant decrease in wort color from 11.7 EBC units (100% barley malt) to 10.3 EBC units (optimized enzyme addition). The replacement of barley malt with red sorghum resulted in significantly higher (10–30% adjunct) or constant (40% adjunct) color values.

Table 9–2. Effect of white sorghum on wort quality based on optimized enzyme additions.

Adjunct concentration	**Viscosity 12%** [mPa·s]	**TSN** [mg/L]	**FAN** [mg/L]	**Extract** [% w/w]	**Polyphenols** [mg/L]
0% Sorghum	1.750 ± 0.013	1,101 ± 08	203 ± 04	15.16 ± 0.03	166 ± 12
10% Sorghum	1.726 ± 0.021	1,017 ± 11	185 ± 16	15.21 ± 0.01	153 ± 22
20% Sorghum	1.699 ± 0.015	0,926 ± 09	173 ± 22	15.30 ± 0.02	147 ± 16
30% Sorghum	1.669 ± 0.019	0,828 ± 09	148 ± 14	15.35 ± 0.02	138 ± 04
40% Sorghum	1.637 ± 0.014	0,746 ± 03	133 ± 08	15.43 ± 0.02	134 ± 10
R^2	0.9963	0.9993	0.9909	0.9942	0.9663

Table 9–3. Effect of red sorghum on wort quality based on optimized enzyme additions.

Adjunct concentration	**Viscosity 12%** [mPa·s]	**TSN** [mg/L]	**FAN** [mg/L]	**Extract** [% w/w]	**Polyphenols** [mg/L]
0% Sorghum	1.750 ± 0.013	1,101 ± 8	203 ± 04	15.16 ± 0.03	166 ± 12
10% Sorghum	1.703 ± 0.020	0,986 ± 6	158 ± 08	15.19 ± 0.07	173 ± 06
20% Sorghum	1.682 ± 0.036	0,887 ± 8	119 ± 09	15.21 ± 0.08	177 ± 20
30% Sorghum	1.646 ± 0.026	0,779 ± 8	110 ± 05	15.24 ± 0.09	188 ± 04
40% Sorghum	1.636 ± 0.038	0,688 ± 6	106 ± 10	15.26 ± 0.11	191 ± 03
R^2	0.9609	0.9986	0.8606	0.9926	0.9727

Conclusion

This comparative study of white Nigerian and red Italian sorghum as brewing adjuncts revealed high similarities in cytolytic, proteolytic, and amylolytic parameters. Both types are characterized by higher starch and lower protein/β-glucan contents as well as negligible enzyme levels in comparison with barley malt. A sieve analysis of hammer-milled white and red sorghum showed, however, significant differences in their grist particle size distribution. Besides, high similarities were found in protein profile and ultrastructure. However, white sorghum exhibited more floury and less vitreous starch granules than red sorghum. As a result, white sorghum caused a significantly lower mash consistency compared to red sorghum. It has been found that the addition of protease and β-glucanase as recommended had no significant effect on mash consistency. Furthermore, 50% of the recommended heat-stable α-amylase dose was sufficient for 40% sorghum adjunct. Worts produced with white sorghum had a significantly higher extract content than the reference wort using 100% barley malt, whereas the use of red sorghum resulted in a constant extract yield. A reduction of the recommended heat-stable α-amylase dose by 50% had no adverse impact on the extract level of worts. Besides, white sorghum provided significantly more TSN and FAN than red sorghum. However, the substitution of up to 40% barley malt with sorghum adjunct led to significant nitrogen losses. The addition of protease as recommended had no positive effect on wort quality. Furthermore, the wort viscosity decreased significantly with increasing amounts of white or red sorghum with or without the addition of β-glucanase. The polyphenol content of worts produced with 30% or more red sorghum was significantly higher than that of the reference wort. In contrast, the use of 30% or more white sorghum resulted in significantly lower polyphenol contents. It must be mentioned here that these findings are based on two sorghum types which is why no general statements can be made. However, it can be concluded that the application of a Physica MCR rheometer for optimizing the addition of industrial enzymes to mashes is highly successful.

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Chapter 10

Impact of unmalted white Nigerian and red Italian sorghum (*Sorghum bicolor*) on the quality of worts and beers applying optimized enzyme levels

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Abstract

Brewing with sorghum adjunct is well established in many countries such as Nigeria. However, no brewing-related publications based on the use of unmalted sorghum grown in Europe are available to date. Therefore, the objectives of this study were to determine and compare the impact of 40% white Nigerian and red Italian sorghum on wort and beer quality adding optimized levels of exogenous enzymes. Brewing with sorghum adjunct was carried out in a 60-L pilot plant applying a double infusion mashing process. Worts and beers were analyzed with regard to processability, flavor and sensory characteristics, foam stability, shelf life, and gluten content (competitive ELISA). The substitution of 40% barley malt with white or red sorghum caused significant increases in wort pH as well as significant decreases in wort viscosity (calculated to 12.0% w/w extract), TSN, FAN, and total amino acids. Worts produced with 40% red sorghum contained higher levels of total polyphenols and total fermentable sugars than those produced with 40% white sorghum. Beers brewed with 40% unmalted sorghum exhibited significantly lower foam stabilities compared to 100% barley malt beers. However, white sorghum had a considerably less adverse impact on beer foam than red sorghum. The sensory analysis performed according to the Deutsche Landwirtschafts-Gesellschaft e.V. (DLG) scheme revealed no significant differences between 40% sorghum and 100% barley malt beers. Furthermore, the gluten content of beers was significantly reduced by replacing 40% barley malt with sorghum adjuncts. According to the Codex Alimentarius, red sorghum beer could even be labeled as ‘very low gluten’ beverage.

Introduction

Unmalted sorghum [*Sorghum bicolor* (L.) Moench] has been successfully used as a brewing adjunct in different parts of the world *(1)*. Beers produced with sorghum adjunct generally have a paler color and milder flavor than all-malt beers *(2)*. Another positive attribute of sorghum-containing beers is their reduced gluten content. In contrast to barley malt, sorghum has no gluten-like proteins which are the causative agent for celiac disease, one of the most common lifelong disorders worldwide. Celiac disease is an immune-mediated enteropathy triggered by the ingestion of gluten-containing grains/products in genetically susceptible individuals *(3)*. However, sorghum lacks in breeding programs aiming for new cultivars particularly suitable for beer production (high extract content, high enzyme activities). In general, sorghum cultivars used in brewing are large seeded (high in starch) and have either white or yellow endosperm (low in polyphenols) *(4)*. Sorghum grains have no husk which is why mash filters are usually applied to separate sorghum mashes *(5–7)*. When substituting high levels of barley malt with unmalted sorghum, commercial enzymes (hemicellulases, proteases, α-amylases) are needed to compensate for malt enzymes. Furthermore, yeast nutrients (FAN, vitamins, minerals) must be supplied for an efficient fermentation process in order to achieve the desired beer flavor and aroma *(4,7–9)*. Lager beers produced with sorghum adjunct and commercial enzymes exhibited higher pH values, lighter colors, poorer foam stabilities, and lower concentrations of volatile compounds such as ethyl acetate, isoamyl acetate, and diacetyl than all-malt lager beers *(4,9,10)*. In contrast, beers brewed with sorghum malt often have a distinctive diacetyl off-flavor *(11)*. Diacetyl is an aromatic volatile by-product of the yeast amino acid metabolism *(12)*. Generally, the production of high-quality sorghum malt beers still involves the addition of exogenous enzymes since sorghum malt lacks in endosperm cell wall-degrading enzymes and β-amylase. On the other hand, brewing with primarily unmalted sorghum and commercial enzymes usually results in an excellent beer quality. Hence, the use of sorghum adjunct and industrial enzymes can be more efficient than the use of sorghum malt *(1,4)*. Several publications dealing with the application of sorghum as adjunct in brewing exist *(5,10,13–19)*. In all of these studies, only sorghum types cultivated in Africa (mainly Nigeria), Latin America, or

Asia were used. However, no brewing-related publications based on the use of unmalted sorghum grown in Europe are available to date.

The aim of this study was to determine and compare the impact of 40% white Nigerian and red Italian sorghum on the quality and processability of worts and beers produced at pilot-plant scale (60 L). A previous study demonstrated by means of a new rheological method applying a controlled stress rheometer (Physica MCR 301) that 50% of the recommended heat-stable α-amylase dose was sufficient for using 40% of those sorghum types (optimized enzyme addition); the addition of protease and β-glucanase to sorghum-containing mashes as recommended had no significant effect on mash consistency and wort quality *(20)*. The quality of beers brewed with 40% white Nigerian and red Italian sorghum was evaluated with regard to flavor and sensory attributes (fresh/forced-aged), foam stability, and gluten content amongst others.

Materials and methods

Brewing materials

Malted barley (*Hordeum vulgare* L. 'Fr Sebastian'), harvested in 2008 and obtained from Greencore Group plc (Dublin, Ireland), unmalted white Nigerian sorghum [*Sorghum bicolor* (L.) Moench 'Short Kaura 5912'], harvested in 2011, and commercially available red sorghum, harvested in 2011 in Ancona, Italy were used in the brewing trials. Both sorghum types (white/red) are characterized by lower β-glucan (0.1% DM/0.0% DM) and protein (9.0% DM/9.1% DM) as well as higher starch (67.8% DM/68.1% DM) contents in comparison with barley malt (0.3% DM β-glucan, 9.4% DM protein, 65.7% DM starch).

Mashing enzyme

The exogenous enzyme applied to sorghum mashes was Hitempase 2XP (recommended dose 1.0 g/kg sorghum; optimum pH/temperature 6.0/90°C) to hydrolyze sorghum starch to dextrins (Kerry Ingredients & Flavours, Carrigaline, Ireland). Schnitzenbaumer *et al. (20)* recently found that 50% of the recommended heat-stable α-amylase dose (0.5 g/kg sorghum) is sufficient for the use of 40% sorghum adjunct (optimized enzyme addition).

Milling

Barley malt was milled with a two-roller mill (Engl Maschinen-Großhandels GmbH, Schwebheim, Germany) set at a 0.7-mm roller distance. White and red sorghum were milled using a hammer mill equipped with a 1.5-mm sieve (A.M.A. S.p.A., San Martino in Rio, Italy). Milling of brewing materials was carried out directly before mashing-in.

Brewing

Brewing with white or red sorghum (0%, 40%) and barley malt (100%, 60%) was performed in a 60-L pilot plant (Fooding. Nahrungsmitteltechnik GmbH, Stuttgart, Germany). For mashing, a slightly modified double infusion process used in the brewing industry (Kerry Ingredients & Flavours, Carrigaline, Ireland) was chosen. First, the sorghum mash was cooked to gelatinize starch (5 min at 55°C, 30 min at 90°C, cooling down to 60°C) and then mixed with barley malt (total mash) to

enzymatically convert gelatinized starch into fermentable sugars (5 min at 60°C, 30 min at 70°C, mashing-off at 78°C). In all brewing trials, a total grist mass of 7.3 kg (dry weight) was mixed with brewing water to give a total mash mass of 40.0 kg at a constant moisture basis of 14%. Mashing-in was carried out by mixing 3.2 kg (wet weight) of white/red sorghum grist (40% of total grist mass) and 1.6 g of Hitempase 2XP (50% of recommended heat-stable α-amylase dose) into 22.0 L of preheated brewing water (55°C). After cooking, the sorghum mash was cooled down by adding the residual brewing water (10.0 L) and mixed with 4.8 kg (wet weight) of barley malt grist (60% of total grist mass) at a temperature of 60°C. The reference brew was performed by mixing 7.7 kg (wet weight) of barley malt grist (100% of total grist mass) into 32.3 L of preheated brewing water (60°C) applying the second step of the double infusion mashing process. In all brewing trials, a constant liquor-to-grist ratio of 4.5:1 (dry weight) was used (total mash). During mashing, the pH was monitored (sorghum/total mash) and the saccharification rate checked every 10 min after reaching 70°C until the iodine test was negative (total mash). The wort was separated from the spent grains using a lauter tun. After a lauter rest of 20 min and turbid wort pumping for 10 min, 20 kg of first wort was collected. Then, defined sparging steps with tempered brewing water (78°C) were carried out to reach a constant preboil wort extract of 10% w/w. The lautering rate of first and sparged worts was measured gravimetrically. Hop pellets (Hallertau Magnum; Hopsteiner, Mainburg, Germany) were added at the start of wort boiling, aiming for 18 EBC bitterness units in the final beer. After wort boiling for exactly 60 min and a whirlpool rest of 20 min, the wort (12% w/w) was cooled and aerated. During brewing, samples of first wort, preboil wort, boiled wort, and cold wort were taken and filtered before further analysis. The final wort volume was split into two 20-L stainless steel Cornelius kegs (each with 15 kg of cold wort). Wort fermentation was implemented by adding 30 g of dry lager yeast (Saflager S-23; Fermentis, Marcq-en-Baroeul cedex, France) with prior rehydration according to the manufacturer's recommendation to each Cornelius keg. Fermentation was performed in a temperature-controlled water bath at 10°C for 10 days. During fermentation, beer samples were taken every day from the middle of the Cornelius kegs for further analysis. After fermentation, the young or 'green' beer was transferred into another 20-L Cornelius keg (excluding the settled yeast at the bottom) and a maturation period of 4 weeks at 4°C was carried out. Filtration of the final beers was performed using a plate filter with standard depth filter sheets

(K 200; Pall SeitzSchenk Filtersystems GmbH, Bad Kreuznach, Germany). The filtered beers were bottled applying a manual bottling unit (Esau & Hueber GmbH, Schrobenhausen, Germany). Finally, the bottled beers were stored in the dark at 4°C prior to analysis. All brewing trials were carried out in duplicate.

Standard wort and beer analysis

Worts and beers were analyzed according to standard methods described in Mitteleuropäische Brautechnische Analysenkommission (MEBAK) – Würze, Bier, Biermischgetränke *(21)*. Total soluble nitrogen (TSN) contents of wort and beer samples (10 mL) were determined using a Tecator™ Digestor combined with a Kjeltec™ 2100 Distillation unit (Foss, Hillerød, Denmark). Free amino nitrogen (FAN) in worts and beers was measured following method 2.6.4.1.1. Wort and beer amino acid profiles were analyzed by high-performance liquid chromatography (HPLC) according to method 2.6.4.1.2. Color and pH of mash/wort and beer samples were determined as described in methods 2.12.2 and 2.13. Wort viscosities were measured using a HAAKE falling ball viscometer (Thermo Scientific, Karlsruhe, Germany) and calculated to 12.0% w/w extract. Total polyphenol contents in worts and beers were determined by spectrophotometry following method 2.16.1. Wort and beer sugar profiles were analyzed by HPLC. EBC bitterness units in beer were determined according to method 2.17.1. Beer foam stability was measured using the foam stability tester NIBEM-T (Haffmans BV, Venlo, The Netherlands) as described in method 2.18.2. Volatile fermentation by-products in beers were analyzed following method 2.21.1. Vicinal diketones in beers were determined according to method 2.21.5.1 (diacetyl, 2,3-pentanedione) and 2.21.5.4 (acetoin), respectively. Organic acids in beers were measured using method 2.21.7.2. Chloride and sulfate in beers were analyzed as described in method 2.22.2. Free dimethyl sulfide (DMS) in beers was determined following method 2.23.1.1. Fatty acids and fatty acid esters in beers were measured according to method 2.21.4 and 2.23.6, respectively. 2-Phenylethanol in beers was analyzed using method 2.23.6. Potassium, magnesium, and zinc in beers were determined as described in method 2.24.12. Sensory analysis of fresh and forced-aged beers was performed by a panel of 10 professional tasters on the basis of the Deutsche Landwirtschafts-Gesellschaft e.V. (DLG) scheme (5-point scale; 1 = dislike extremely, 5 = like extremely). Aging indicators in fresh and forced aged beers were analyzed by pervaporation followed by gas chromatography

(PV-GC). Wort extract, apparent extract, apparent degree of fermentation (ADF), and alcohol of beer were measured by an Alcolyzer Beer ME Analyzing System (Anton Paar GmbH, Graz, Austria). Yeast cell counts were carried out using a haemocytometer (Thoma chamber, 0.100 mm cell depth) and methylene blue as an indicator for yeast viability. In addition, wort fatty acid profiles were analyzed by GC using trimethylsulfonium hydroxide as derivatization reagent according to Deutsche Gesellschaft für Fettwissenschaft e.V. method C-VI 11e. All wort and beer standard analyses were performed in duplicate.

Lab-on-a-Chip analysis

The protein profile of worts and beers was determined using Lab-on-a-Chip capillary electrophoresis as described by Klose *et al. (22)*. For the analysis, 40 mg of freeze-dried and homogenized sample was extracted with 400 µL of reagent (2 M urea, 15% glycerol, 0.1 M Tris-HCl (pH 8.8), 0.1 M dithiothreitol) in the ultra-sonic water bath for 15 min at room temperature. After centrifugation at 18,890×*g* for 15 min, 4 µL of supernatant was denatured by heating at 95°C for 5 min with 2 µL of Agilent denaturing solution. The denatured sample was then diluted with 84 µL of deionized water and 6 µL of this mixture was applied to the Protein 80^{+} LabChip for analysis in the Agilent 2100 Bioanalyzer according to the manufacturer's instructions (Agilent Technologies, Santa Clara, California). Any peak detected below 5 kDa is termed a system peak and is not included in the analysis. All wort and beer protein profiles were determined in duplicate.

ELISA analysis

Peptide fragments of prolamins in beers were quantitatively determined using a R5 gliadin competitive enzyme-linked immunosorbent assay (R-Biopharm AG, Darmstadt, Germany) representing the official standard method for gluten determination according to the Codex Alimentarius. All ELISA tests were carried out in triplicate.

Statistical analysis

Results are given as arithmetic means with 95% confidence intervals (two-tailed Student's *t*-values for *n*-1 degrees of freedom). Analysis of variance tests were performed to compare sample means (Bonferroni *t*-test; $\alpha = 0.05$) using SigmaPlot software (Systat Software Inc., San Jose, California).

Results and discussion

Impact of unmalted white Nigerian and red Italian sorghum on wort quality

In the present study, the quality of mashes/worts and beers brewed with 40% white or red sorghum adjunct and optimized levels of industrial enzymes (no protease, 50% of recommended heat-stable α-amylase dose, no β-glucanase) *(20)* was determined and compared. It was observed that red sorghum tended to lump formation during mashing and caused a significantly higher ($P = 0.01$) mash pH than white sorghum at the end of cooking at 90°C (pH 6.17 and pH 6.07, respectively). However, the use of both sorghum types (white/red) resulted in a significantly increased total mash pH (5.55/5.54) compared to the reference mash pH (5.37) before mashing-off at 78°C ($P < 0.05$). All mashes were iodine normal after 10 min (reference) and 20–30 min (40% white/red sorghum) at 70°C, respectively. The lautering rate decreased considerably from 0.60 kg wort/min to 0.40 kg wort/min by substituting 40% barley malt with hammer-milled white or red sorghum. For this reason, brewing with high levels of sorghum adjunct necessitates the use of a mash filter to reduce wort separation times.

The first wort pH (20°C) and extract increased from 5.54 to 5.71/5.70 ($P < 0.05$) and from 14.3% w/w to 15.1% w/w ($P > 0.05$), respectively, when using 40% white/red sorghum. In contrast, its viscosity (calculated to 12.0% w/w extract) and TSN content (Figure 1) decreased from 1.798 mPa·s to 1.676/1.672 mPa·s ($P < 0.05$) and from 1,008 mg/L to 712/695 mg/L ($P < 0.001$), respectively, by replacing 40% barley malt with white/red sorghum adjunct. The use of 40% red sorghum caused not only significantly higher ($P = 0.01$) total polyphenol contents in first wort (210 mg/L) compared to that of 40% white sorghum (149 mg/L) (Figure 2) but also significantly increased ($P < 0.05$) color values (21.6 EBC units and 16.2 EBC units, respectively). These findings correspond approximately to those obtained in previous work *(20)*.

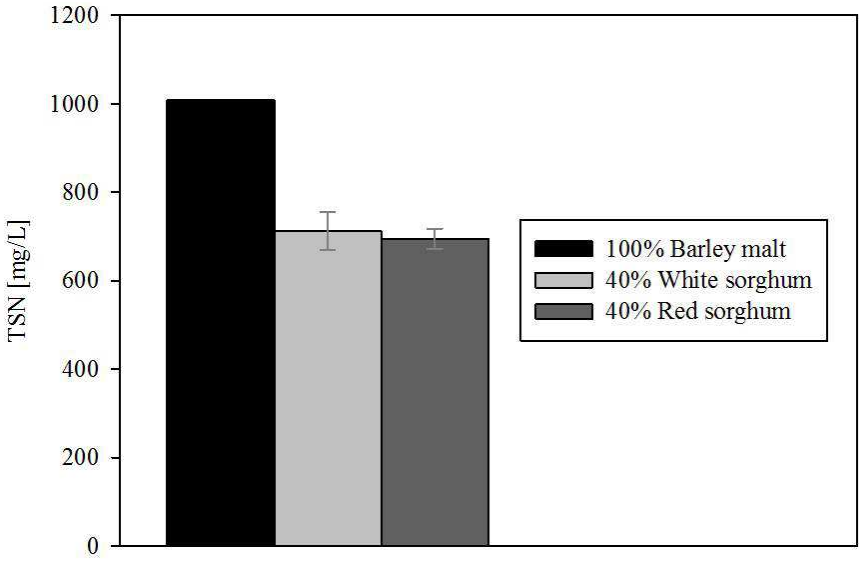


Figure 10–1. Impact of 40% white and red sorghum adjunct on first wort TSN content.

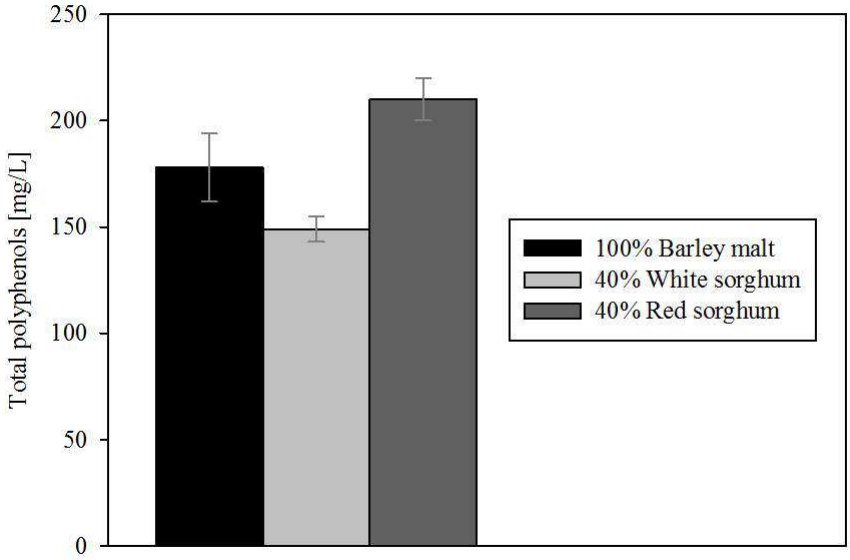


Figure 10–2. Impact of 40% white and red sorghum adjunct on first wort total polyphenol content.

The preboil wort pH (20°C) was slightly higher than the first wort pH increasing from 5.62 (reference) to 5.80/5.78 (40% white/red sorghum) ($P > 0.05$). In terms of viscosity (calculated to 12.0% w/w extract), a considerable increase by 15.3–21.1% in comparison to first wort was observed (reference 2.073 mPa·s; 40% white/red sorghum 1.972/2.025 mPa·s; $P < 0.05$), which is the result of an extensive extraction of viscosity-altering substances such as β-glucans, nitrogenous compounds, and

dextrins (starch) from spent grains *(23–25)*. The amount of TSN in preboil worts was approximately 31–88 mg/L higher than that in first worts as a consequence of sparging (reference 712 mg/L; 40% white/red sorghum 474/459 mg/L; $P < 0.001$). Preboil worts produced with 40% white sorghum had considerably lower color values/total polyphenol contents (11.9 EBC units/104 mg/L) than those produced with 100% barley malt (17.0 EBC units/124 mg/L) or 40% red sorghum (17.4 EBC units/155 mg/L).

Finally, cold worts containing 40% white/red sorghum adjunct had a significantly higher pH (20°C) than the reference wort (5.64/5.63 and 5.48, respectively; $P < 0.05$), which is in accordance with the literature *(10)*. The final wort viscosity (calculated to 12.0% w/w extract) decreased significantly from 1.879 mPa·s to 1.788/1.787 mPa·s ($P < 0.05$) by substituting 40% barley malt with white/red sorghum (Figure 3), most likely due to their lower β-glucan contents. However, the color values/total polyphenol contents ranging from 11.4 EBC units to 13.8 EBC units and from 148 mg/L to 188 mg/L, respectively, were not significantly different ($P > 0.05$). Furthermore, the TSN content of cold worts was significantly reduced from 805 mg/L (100% barley malt) to 509/501 mg/L when using 40% white/red sorghum adjunct ($P < 0.001$). In agreement with these TSN results, the FAN content of cold worts decreased significantly from 143 mg/L to 102 mg/L and 98 mg/L, respectively, by replacing 40% barley malt with white or red sorghum ($P < 0.01$) (Figure 4). According to the literature, recommended FAN values are 200–240 mg/L based on all-malt worts (12.0% w/w) *(26)*. This is, however, in strong contrast to different experimental studies stating that 85–130 mg/L FAN are sufficient for optimal yeast growth and fermentation *(27,28)*.

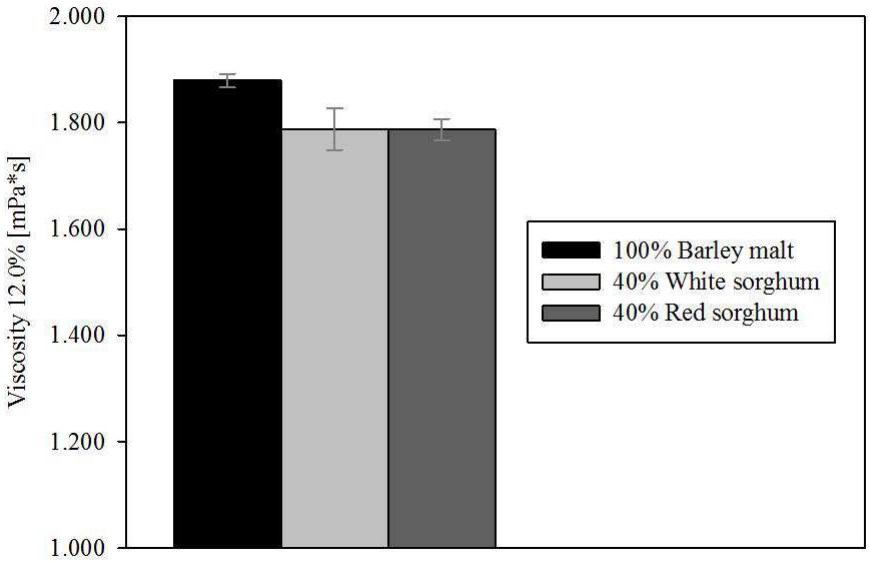


Figure 10–3. Impact of 40% white and red sorghum adjunct on cold wort viscosity (12.0% w/w).

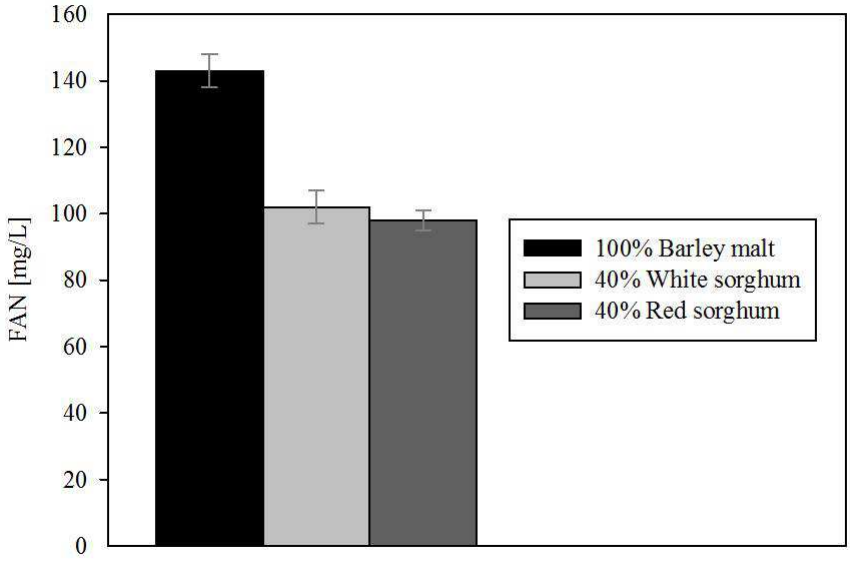


Figure 10–4. Impact of 40% white and red sorghum adjunct on cold wort FAN content.

The total amino acid content of cold worts (Table 1) decreased significantly from 115.75 mg/100 mL to 82.18 mg/100 mL (29.0% reduction) and 79.47 mg/100 mL (31.3% reduction), respectively, when replacing 40% barley malt with unmalted white or red sorghum ($P < 0.01$). The use of 40% red sorghum resulted in significantly lower levels of aspartic acid, glutamic acid, asparagine, serine, glutamine, threonine, arginine, and lysine ($P < 0.001$). Those amino acids (class A)

are assimilated immediately after the yeast cells contact the wort *(29)*. However, 40% white sorghum worts provided significantly higher levels of aspartic acid ($P < 0.001$), glutamic acid ($P < 0.01$), and asparagine ($P = 0.001$) in comparison to 40% red sorghum worts. The levels of histidine, valine, leucine ($P < 0.001$), methionine, and isoleucine ($P < 0.01$) (class B), assimilated more slowly than class A amino acids *(29)*, also decreased significantly when substituting 40% barley malt with red sorghum, even though the valine levels were significantly higher than those provided by white sorghum ($P < 0.001$). Both sorghum types caused significantly lower levels of glycine, alanine, tyrosine, tryptophan, and phenylalanine (class C) compared to the reference ($P < 0.001$). Those amino acids are not utilized until class A amino acids have disappeared from the wort *(29)*. Furthermore, the level of γ-aminobutanoic acid (GABA), which is not a constituent of proteins *(30)*, was significantly reduced by replacing 40% barley malt with white/red sorghum ($P < 0.01$); however, white sorghum worts contained higher levels of GABA than red sorghum worts ($P < 0.05$).

Table 10–1. Impact of white and red sorghum adjunct on wort amino acid composition [mg/100 mL].[a]

Amino acid	100% Barley malt	40% White sorghum	40% Red sorghum
Aspartic acid	6.34 ± 0.19	6.18 ± 0.36	5.35 ± 0.19
Glutamic acid	10.70 ± 0.13	8.54 ± 0.59	7.80 ± 0.12
Asparagine	7.99 ± 0.00	7.87 ± 0.38	7.12 ± 0.17
Serine	4.85 ± 0.01	3.44 ± 0.21	3.30 ± 0.18
Glutamine	2.28 ± 0.04	1.62 ± 0.01	1.52 ± 0.17
Histidine	5.63 ± 0.33	3.56 ± 0.73	3.32 ± 0.02
Glycine	1.47 ± 0.09	1.04 ± 0.03	1.09 ± 0.08
Threonine	4.37 ± 0.24	2.97 ± 0.19	2.93 ± 0.06
Alanine	4.46 ± 0.03	3.06 ± 0.20	3.04 ± 0.24
Arginine	15.00 ± 0.85	10.70 ± 0.43	10.25 ± 0.61
γ-Aminobutanoic acid	2.58 ± 0.08	2.41 ± 0.07	2.15 ± 0.27
Tyrosine	6.69 ± 0.39	4.18 ± 0.19	4.25 ± 0.21
Valine	7.89 ± 0.68	4.66 ± 0.08	4.93 ± 0.11
Methionine	0.97 ± 0.05	0.82 ± 0.37	0.63 ± 0.24
Tryptophan	2.42 ± 0.11	1.63 ± 0.12	1.70 ± 0.12
Isoleucine	1.75 ± 0.34	0.95 ± 0.33	1.19 ± 0.16
Phenylalanine	10.34 ± 0.21	6.76 ± 0.63	6.69 ± 0.44
Leucine	13.69 ± 0.99	7.99 ± 0.49	8.26 ± 0.28
Lysine	6.33 ± 0.42	3.80 ± 0.25	3.95 ± 0.13
Total amino acids	115.75 ± 4.42	82.18 ± 4.84	79.47 ± 2.64

[a] Data are means of duplicate determinations on each of two replicate brews ± 95% two-tailed Student's *t*-values for 3 degrees of freedom.

The total fermentable sugar content of cold worts increased from 74.4 g/L (100% barley malt) to 75.1 g/L (40% white sorghum) and 85.1 g/L (40% red sorghum), respectively ($P > 0.05$) as a result of the higher starch contents of both sorghum types. It should be mentioned here that the extract content of the reference worts (11.8% w/w) was slightly lower ($P > 0.05$) than that of 40% white/red sorghum worts (12.1/12.0% w/w). The substitution of 40% barley malt with sorghum adjunct caused reduced contents of glucose, fructose, and sucrose as well as increased contents of maltose ($P > 0.05$). In addition, 40% white/red sorghum worts contained significantly more maltotriose (12.2/13.8 g/L; $P < 0.05$) compared to the reference worts (11.0 g/L). The total fatty acid content and composition of cold worts produced with 40% white or red sorghum were similar to those of 100% barley malt worts ($P > 0.05$) (Table 2). Palmitic (hexadecanoic), stearic (octadecanoic), oleic (9-octadecenoic), linoleic (9,12-octadecadienoic), and linolenic (9,12,15-octadecatrienoic) acids accounted for 90.0–96.2% of total fatty acids in wort, which is in agreement with the literature *(31)*. Unsaturated fatty acids are essential for optimal yeast growth and metabolism due to their involvement in building up a functional yeast plasma membrane (exchange of molecules between cytoplasm and external environment of cell) *(12,32)*.

Table 10–2. Impact of white and red sorghum adjunct on wort fatty acid composition [mg/100 mL].[a]

Fatty acid	100% Barley malt	40% White sorghum	40% Red sorghum
Caproic acid (C 6:0)	0.02 ± 0.00	0.03 ± 0.01	<0.02 ± 0.00
Caprylic acid (C 8:0)	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Capric acid (C 10:0)	<0.02 ± 0.00	<0.02 ± 0.00	<0.02 ± 0.00
Lauric acid (C 12:0)	<0.02 ± 0.00	<0.02 ± 0.00	<0.02 ± 0.00
Myristic acid (C 14:0)	<0.02 ± 0.00	<0.02 ± 0.00	<0.02 ± 0.00
Palmitic acid (C 16:0)	0.20 ± 0.02	0.18 ± 0.01	0.20 ± 0.01
Stearic acid (C 18:0)	0.08 ± 0.03	0.06 ± 0.01	0.06 ± 0.01
Oleic acid (C 18:1)	0.04 ± 0.03	0.05 ± 0.00	0.05 ± 0.02
Linoleic acid (C 18:2)	0.08 ± 0.04	0.06 ± 0.01	0.08 ± 0.01
Linolenic acid (C 18:3)	0.08 ± 0.04	0.07 ± 0.01	0.09 ± 0.01
Saturated fatty acids	0.33 ± 0.06	0.32 ± 0.01	0.30 ± 0.06
Single unsaturated fatty acids	0.04 ± 0.03	0.05 ± 0.00	0.05 ± 0.02
Polyunsaturated fatty acids	0.16 ± 0.09	0.13 ± 0.01	0.17 ± 0.02
Total fatty acids	0.53 ± 0.12	0.50 ± 0.02	0.52 ± 0.05

[a] Data are means of duplicate determinations on each of two replicate brews ± 95% two-tailed Student's *t*-values for 3 degrees of freedom.

After 10 days of fermentation, the cold wort extract content was reduced by 58.7–59.3% to 4.8–5.0% w/w, corresponding to 3.2–3.4% w/w apparent extract content. The alcohol content and ADF of the reference beers (4.5% v/v; 72.8% w/w) were higher ($P < 0.05$) than those of 40% white or red sorghum beers (4.4% v/v; 71.1% w/w and 71.2% w/w, respectively). Young or 'green' beers produced with 40% white/red sorghum adjunct also had lower pH and color values at the end of the main fermentation (4.30/4.34; 8.7/8.4 EBC units) compared to 100% barley malt beers (4.39; 9.8 EBC units). The decrease in color values by 1.6–5.4 EBC units during fermentation is due to the pH drop by 1.09–1.34, causing a decoloration of anthocyanins and a loss of polyphenols as a result of adsorption by yeast cells *(12,33,34)*. It has been found that the polyphenol content of cold worts was reduced by 19.7–26.4% after 10 days of fermentation. Young or 'green' beers brewed with 40% red sorghum exhibited significantly higher ($P < 0.001$) total polyphenol contents (151 mg/L) than those brewed with 100% barley malt (120 mg/L) or 40% white sorghum (111 mg/L). The cold wort TSN content was decreased by 40.4–41.9% within 10 days of fermentation to 480 mg/L (reference) and 302/291 mg/L (40% white/red sorghum; $P < 0.001$), respectively. The protein profiles of young or 'green' sorghum beers were similar to that of the reference (Figure 5) revealing proteins with a molecular weight of 9.8–18.0 kDa (foam-positive low-molecular-weight fraction) and 36.2–36.7 kDa (foam-positive high-molecular-weight fraction), respectively *(35)*. However, the protein peaks/bands in the electropherogram/gel-like image of 40% white or red sorghum beers were less pronounced than those in the electropherogram/gel-like image of 100% barley malt beers. This can be explained by the lower protein contents of both sorghum types and the poorer solubility/extractability of unmodified high-molecular-weight proteins in aqueous systems.

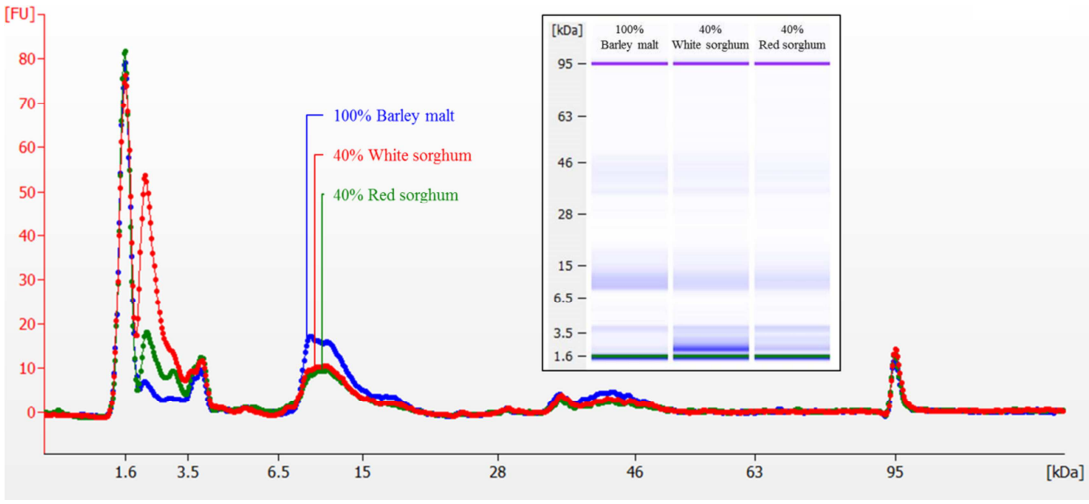


Figure 10–5. Impact of 40% white and red sorghum adjunct on young or 'green' beer protein profile.

Furthermore, a reduced lag phase (early log phase) of yeast cells was observed when using 40% white sorghum, whereas the replacement of 40% barley malt with red sorghum caused a delayed log phase. The latter can be explained by the significantly lower level of class A amino acids in 40% red sorghum worts compared to that in 100% barley malt worts as mentioned above. After the main fermentation, the number of yeast cells remaining in young or 'green' beers ranged from 5.9×10^6 (40% white sorghum) to 7.3×10^6 (40% red sorghum; $P > 0.05$). These yeast cell numbers correspond to the increase in final beer ADF by 4.7% w/w (40% white sorghum) and 6.2% w/w (40% red sorghum), respectively. As a consequence of this, the use of 40% red sorghum had no significant impact on the ADF of final beers (77.4% w/w; $P > 0.05$) when compared to the reference (77.9% w/w), whereas the use of 40% white sorghum caused a significantly lower ADF (75.8% w/w; $P < 0.001$). Beers produced with 40% white sorghum adjunct had significantly higher apparent extract contents (2.9% w/w) than those produced with 40% red sorghum (2.7% w/w; $P < 0.05$) or 100% barley malt (2.6% w/w; $P < 0.001$). As a result, they had lower alcohol contents (4.8% v/v) compared to 40% red sorghum beers (5.0% v/v) or the reference beers (4.9% v/v). These findings correspond to those obtained in previous work *(20)*. In terms of filtered beer color, no significant differences could be determined between the 100% barley malt beers (5.7 EBC units) and the 40% white/red sorghum beers (5.1 EBC units/5.0 EBC units). However, the

pH of the reference beers (4.38) was significantly higher than that of the 40% red sorghum beers (4.28; $P = 0.01$).

The foam stability of final beers decreased significantly from 282 s to 261 s ($P < 0.05$) and 241 s ($P < 0.001$), respectively, when replacing 40% barley malt with white or red sorghum adjunct (Figure 6). These findings can be explained by the significantly reduced levels of TSN and high-molecular-weight proteins in 40% white/red sorghum beers. Besides, the high polyphenol content in 40% red sorghum worts may have caused higher losses of foam-positive proteins during wort boiling *(35)*.

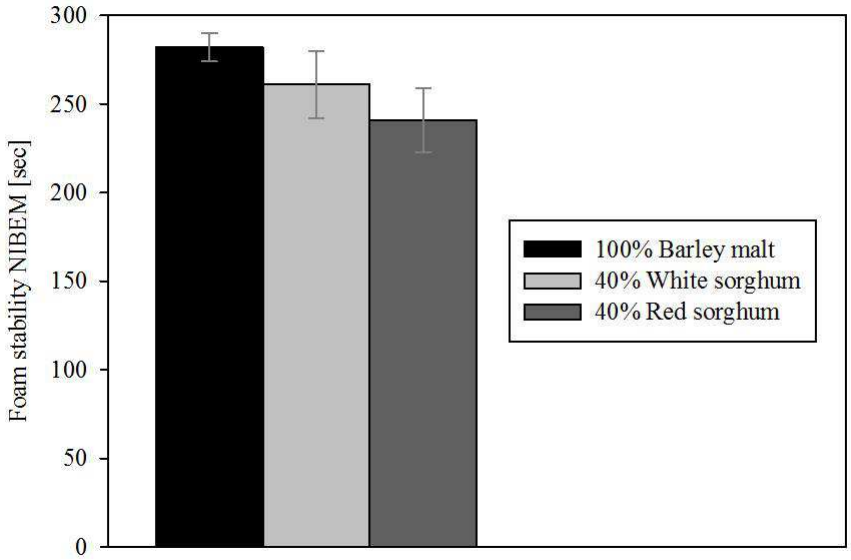


Figure 10–6. Impact of 40% white and red sorghum adjunct on beer foam stability.

Beers brewed with 40% white/red sorghum adjunct contained very low levels of FAN (4.5/4.0 mg/L) in comparison to the reference beers (42.5 mg/L; $P < 0.001$). Hence, up to 94–100 mg/L FAN was assimilated by yeast cells during fermentation. These results confirm previous findings and literature reports stating that FAN contents of 100 mg/L or even less are sufficient for optimal yeast growth and an efficient fermentation process *(14,20,28)*. The total amino acid content of final beers was reduced by 70.3% to 34.42 mg/100 mL (100% barley malt) and by 95.8/96.4% to 3.44/2.87 mg/100 mL (40% white/red sorghum), respectively, compared to that of cold worts. During fermentation, 79.2% (reference) and 96.6/97.2% (40% white/red sorghum) of class A amino acids, 86.3% (reference) and 96.9/97.5% (40% white/red sorghum) of class B amino acids, as well as 57.1% (reference) and 92.3/92.7% (40%

white/red sorghum) of class C amino acids were assimilated by the yeast. All amino acid levels in beer were lower than those in the respective wort except for tryptophan (class C) being constant and GABA showing a 2.9-fold increase to 7.38 mg/100 mL in 100% barley malt beers. The latter was 97.9% (0.05 mg/100 mL) and 99.1% (0.02 mg/100 mL), respectively, lower in 40% white or red sorghum beers compared to wort levels. GABA was found to be the major amino acid excreted by yeast cells utilizing it as a source of carbon. It builds up in the intracellular pool and is later released into the extracellular environment during autolysis. The GABA concentration depends on the sources of nitrogen available during growth, pH, and the concentration of solutes in the medium *(30)*. The remaining sugar content of the final beers (maltose, maltotriose) ranged from 1.4 g/L (40% white sorghum) to 2.2 g/L (40% red sorghum) ($P > 0.05$; reference 2.0 g/L). Hence, 97.4–98.1% of total fermentable sugars were metabolized by yeast cells during fermentation.

All beers contained beside caproic (hexanoic), caprylic (octanoic), and capric (decanoic) acids, rising from 0.70–1.00 mg/L (cold worts) to 7.12–8.33 mg/L (7.8–11.9-fold increase) as a result of fatty acid synthesis and release by yeast cells during fermentation *(31)*, also isovaleric (3-methylbutanoic) acid. However, the total concentration of those short/medium-chain fatty acids tended to be lower in 40% white (9.61 mg/L) or red (9.12 mg/L) sorghum beers (reference 10.53 mg/L). Isovaleric, caproic, caprylic, and capric acids are odor-active compounds whose thresholds range from 0.7 mg/L to 20 mg/L contributing with rancid, cheesy, sweaty, or oily notes to the sensory properties of beer *(12,36)*. Furthermore, the replacement of 40% barley malt with white or red sorghum reduced the acetaldehyde concentration in final beers from 11.55 mg/L to 8.55 mg/L ($P > 0.05$) and 8.40 mg/L ($P < 0.05$), respectively (Table 3). Acetaldehyde (flavor threshold 25 mg/L), the major aldehyde, contributes negative flavor attributes to beer (emulsion paint or green apple aroma). In general, aldehydes are much more flavor-active than their corresponding higher alcohols; the latter represent the major fraction of volatile compounds in beer *(37,38)*.

Higher alcohols are classified into aliphatic (*n*-propanol, isobutanol, 2-methylbutanol (active amyl alcohol), 3-methylbutanol (isoamyl alcohol)) and aromatic (e.g. 2-phenylethanol) higher alcohols. Aliphatic alcohols contribute to the alcoholic or solvent aroma of beer and impart a warm mouthfeel, while the aromatic alcohol 2-

phenylethanol confers positive sweet flavor notes to beer (rose or floral aroma) *(37,38)*. The concentration of isobutanol (flavor threshold 80–100 mg/L) and amyl alcohols (2- and 3-methylbutanol; flavor threshold 50–60 mg/L each) *(38)* in final beers increased significantly by 10.5–12.5% when substituting 40% barley malt with white or red sorghum ($P < 0.05$) (Table 3), even though 100% barley malt worts contained significantly higher amino acid levels (valine, isoleucine, leucine) than 40% sorghum worts. These findings confirm those of Bajomo and Young *(14)*, who found that beers produced with 100% unmalted sorghum and industrial enzymes (wort FAN content 51 mg/L) showed higher levels of isobutanol and amyl alcohols, in particular 3-methylbutanol, compared to commercial beers. In *Saccharomyces cerevisiae* yeast cells, two different metabolic pathways are involved in the formation of isobutanol, 2-methylbutanol, and 3-methylbutanol: 1) Ehrlich pathway – catabolism of valine, isoleucine, and leucine; 2) anabolic pathways implicated in *de novo* synthesis of branched-chain amino acids through their biosynthetic pathway from glucose *(39,40)*. At low concentrations of assimilable nitrogen (FAN), the biosynthetic pathway predominates, whereas at high FAN concentrations the Ehrlich pathway becomes prominent as a result of amino acid feedback inhibition of key enzymes in the biosynthetic pathway. As a consequence of this, the longer a fermentation proceeds in the absence of nitrogen, the greater is the production of higher alcohols *(41,42)*.

Esters are the most important fermentation-derived aroma compounds in beer (very low flavor thresholds) and subdivided into acetate esters and short/medium-chain fatty acid (C 4:0–C 10:0) ethyl esters. The first group includes ethyl acetate (fruity, solvent-like), isoamyl acetate (fruity, banana), 2-phenylethyl acetate (roses, honey), isobutyl acetate (fruity, banana) and the second group ethyl butyrate (papaya, apple), ethyl caproate (apple, aniseed), ethyl caprylate (apple), as well as ethyl caprate (soapy, chemical) *(12,37,38,43,44)*. The total ester concentration in final beers brewed with 40% white (9.66 mg/L) or red (9.38 mg/L) sorghum adjunct tended to be lower than that in reference beers (10.09 mg/L), with isoamyl acetate being significantly lower ($P < 0.001$) (Table 3). It has been reported that flavor-active acetate ester production is reduced with low wort FAN and glucose levels *(44)*. The concentration of diacetyl (butterscotch-like aroma; flavor threshold 0.10–0.15 mg/L

(12,45)) in final beers was not affected by replacing 40% barley malt with white or red sorghum adjunct (Table 3).

Table 10–3. Impact of white and red sorghum adjunct on beer aroma compounds [mg/L].[a]

Aroma compound	100% Barley malt	40% White sorghum	40% Red sorghum
Acetaldehyde	11.55 ± 2.20	8.55 ± 2.61	8.40 ± 0.59
n-Propanol	15.85 ± 0.10	16.20 ± 0.39	16.15 ± 0.29
Isobutanol	22.90 ± 0.59	25.30 ± 0.78	25.45 ± 0.88
Amyl alcohols	69.30 ± 0.59	77.35 ± 0.49	77.95 ± 2.65
2-Phenylethanol	38.90 ± 7.50	34.00 ± 2.60	36.90 ± 2.90
Ethyl acetate	8.90 ± 0.59	8.65 ± 0.49	8.35 ± 0.10
Isoamyl acetate	0.60 ± 0.00	0.50 ± 0.00	0.50 ± 0.00
2-Phenylethyl acetate	0.12 ± 0.00	0.09 ± 0.02	0.11 ± 0.02
Isobutyl acetate	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Ethyl butyrate	0.07 ± 0.00	0.06 ± 0.00	0.07 ± 0.01
Ethyl caproate	0.11 ± 0.01	0.10 ± 0.00	0.09 ± 0.00
Ethyl caprylate	0.22 ± 0.01	0.20 ± 0.02	0.19 ± 0.02
Ethyl caprate	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01
Total diacetyl	0.21 ± 0.11	0.21 ± 0.20	0.18 ± 0.03
Total 2,3-pentanedione	0.07 ± 0.04	0.08 ± 0.06	0.06 ± 0.02
Acetoin	4.70 ± 0.80	4.00 ± 2.60	4.20 ± 0.30

[a] Data are means of duplicate determinations on each of two replicate brews ± 95% two-tailed Student's *t*-values for 3 degrees of freedom.

Furthermore, the total organic acid content of 40% white (733.0 mg/L) or red (697.9 mg/L) sorghum beers was slightly higher compared to that of 100% barley malt beers (682.3 mg/L). Acetic acid accounted for 37.1–40.2% of the total organic acid content followed by citric acid (30.5–33.8%), lactic acid (15.3–18.4%), pyruvic acid (3.2–9.2%), oxalic acid (3.1–4.7%), formic acid (0.7–1.0%), and fumaric acid (0.6–0.9%). Oxalic/formic acid showed a 1.5–1.6-fold ($P < 0.01$), pyruvic acid a 2.4–3.0-fold ($P < 0.05$), and fumaric acid a 1.4–1.5-fold ($P > 0.05$) increase when substituting 40% barley malt with sorghum adjunct, whereas lactic acid decreased by 7.6–10.4%. Beers brewed with 40% white sorghum contained higher levels of citric (243.5 mg/L; $P < 0.01$) and acetic acid (277.5 mg/L; $P > 0.05$) than those brewed with 40% red sorghum (213.0 mg/L and 259.0 mg/L, respectively). Organic acids contribute not only to sourness but also to bitterness and astringency of beer *(36,46)*. The free DMS concentration in final beers decreased slightly from 15 μg/L to

11 µg/L and 13 µg/L, respectively, when replacing 40% barley malt with white or red sorghum adjunct. DMS (odor threshold 30 µg/L) confers generally undesirable flavor notes described as ‘cooked sweet corn/cabbage’ to beer *(12,47)*. In addition, the mineral content of final beers, also affecting taste and flavor, was within the range given in the literature *(35,48,49)*. The chloride (135.0–144.0 mg/L), sulfate (65.6–74.3 mg/L), and magnesium (70.0–78.9 mg/L) levels slightly decreased when replacing 40% barley malt with white or red sorghum, whereas the potassium (468.0–500.5 mg/L) and zinc (0.01–0.04 mg/L) levels slightly increased. Chloride, for example, gives beer a mellow palate and fullness, while sulfate enhances its dry character *(48)*.

Fresh beers brewed with 40% white or red sorghum exhibited lower levels of heat indicators (γ-nonalactone) and oxygen indicators (3-methylbutanal, 2-phenylethanal) resulting in a 17.6–22.4% reduction of staling components compared to fresh reference beers (Table 4). In consequence of the forced-aging process (for details see below), heat indicators (2-furfural, γ-nonalactone) showed a 2.2–2.3-fold increase, oxygen indicators (3-methylbutanal, 2-phenylethanal) a 1.1–1.4-fold increase, and staling components (3-methylbutanal, 2-furfural, 5-methylfurfural, 2-phenylethanal, γ-nonalactone) a 1.9–2.0-fold increase in the aged beers. The substitution of 40% barley malt with white or red sorghum adjunct reduced heat indicators by 6.3–12.7%, oxygen indicators by 28.6–37.1%, and staling components by 12.8–19.5% in forced-aged beers. These findings were reflected in the sensory quality of the final beers (Table 5) analyzed according to the DLG scheme (5-point scale; 1 = dislike extremely, 5 = like extremely). The replacement of 40% barley malt with white/red sorghum adjunct had no significant adverse effect on the sensory properties of fresh beers receiving an overall score of 4.0 out of 5.0. These results confirm literature reports stating that the quality of lager-type beers brewed with 40–50% unmalted sorghum was comparable to that of 100% barley malt beers *(6,10)*. Furthermore, beers brewed with 40% adjunct, in particular white sorghum, exhibited an acceptable flavor stability receiving an overall score of up to 3.8 out of 5.0 after the forced-aging process (Table 5). For this purpose, the bottled beers were shaken overhead at 70 rpm for 24 hours at room temperature (simulated transportation) and afterward stored for 4 days at 40°C to simulate 3–4 month of aging. With respect to the quality of bitterness, fresh 40% red sorghum beers achieved equally good scores than 40%

white sorghum or reference beers; however, the corresponding forced-aged beers received slightly lower scores.

Table 10–4. Impact of white and red sorghum adjunct on fresh/aged beer aging indicators [μg/L].[a]

Aging indicator	100% Barley malt		40% White sorghum		40% Red sorghum	
	Fresh	Aged	Fresh	Aged	Fresh	Aged
2-Methylbutanal[b,c]	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0
3-Methylbutanal[b,c]	9 ± 3	11 ± 3	7 ± 4	9 ± 4	8 ± 1	9 ± 0
2-Furfural[a,c]	16 ± 0	59 ± 7	16 ± 0	53 ± 5	15 ± 0	56 ± 7
5-Methylfurfural[c]	<5 ± 0	6 ± 2	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0
Benzaldehyde[b,c]	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0
2-Phenylethanal[b,c]	21 ± 5	22 ± 4	13 ± 0	13 ± 2	12 ± 0	13 ± 0
Succinic acid diethyl ester[c]	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0
Nicotinic acid ethyl ester	6 ± 0	8 ± 0	6 ± 0	8 ± 0	6 ± 1	7 ± 0
Phenylacetic acid ethyl ester[c]	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0
2-Acetylfuran[c]	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0
2-Propionylfuran[c]	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0	<5 ± 0
γ-Nonalactone[a,c]	40 ± 3	67 ± 10	37 ± 7	66 ± 17	32 ± 11	54 ± 10
[a] Heat indicators	56 ± 3	126 ± 17	53 ± 7	118 ± 22	47 ± 11	110 ± 17
[b] Oxygen indicators	29 ± 8	35 ± 12	18 ± 9	25 ± 7	20 ± 1	22 ± 0
[c] Staling components	85 ± 5	164 ± 2	70 ± 2	143 ± 15	66 ± 10	132 ± 17

[a] Data are means of duplicate determinations on each of two replicate brews ± 95% two-tailed Student's *t*-values for 3 degrees of freedom.

Table 10–5. Impact of white and red sorghum adjunct on fresh/aged beer sensory quality [5-point scale].[a]

DLG criteria	100% Barley malt		40% White sorghum		40% Red sorghum	
	Fresh	Aged	Fresh	Aged	Fresh	Aged
Aroma	3.8 ± 0.2	3.3 ± 0.6	3.7 ± 0.0	3.4 ± 0.0	3.7 ± 0.2	3.4 ± 0.5
Purity of taste	3.8 ± 0.2	3.3 ± 0.6	3.7 ± 0.0	3.4 ± 0.0	3.7 ± 0.2	3.4 ± 0.5
Fullness of body	4.3 ± 0.0	4.4 ± 0.0	4.3 ± 0.0	4.4 ± 0.0	4.3 ± 0.0	4.4 ± 0.0
Carbonation	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.0
Quality of bitterness	4.1 ± 0.0	3.9 ± 0.2	4.1 ± 0.1	3.8 ± 0.2	4.1 ± 0.2	3.7 ± 0.2
Overall score	4.1 ± 0.1	3.7 ± 0.3	4.0 ± 0.0	3.8 ± 0.0	4.0 ± 0.1	3.7 ± 0.3

[a] Data are means of duplicate determinations on each of two replicate brews ± 95% two-tailed Student's *t*-values for 3 degrees of freedom.

The gluten content of beers produced with 40% white (168 ppm; $P < 0.05$) or red (98 ppm; $P < 0.001$) sorghum adjunct was significantly reduced compared to that of 100% barley malt beers (233 ppm) (Figure 7). According to the Codex Alimentarius (Alinorm 08/31/26), 40% red sorghum beers were even very low in gluten (gluten content above 20 and up to 100 ppm), most likely as a result of the higher polyphenol content in the respective worts as mentioned above.

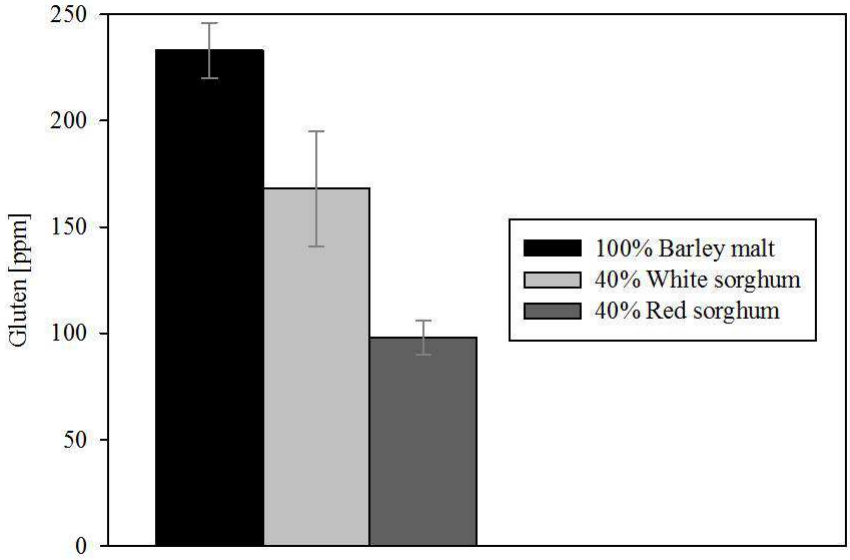


Figure 10–7. Impact of 40% white and red sorghum adjunct on beer gluten content.

Conclusion

In the present study, the impact of 40% white Nigerian and red Italian sorghum on wort and beer quality applying optimized levels of exogenous enzymes was determined and compared. The substitution of 40% barley malt with each of those sorghum types caused significantly lower assimilable nitrogen contents in worts amongst others. In terms of fermentation performance, however, no significant differences between 40% unmalted sorghum and 100% barley malt trials were observed proving that approximately 100 mg/L FAN is sufficient for optimal yeast growth. The concentrations of total fatty acids, acetaldehyde, total esters, acetoin, free DMS, and aging indicators (γ-nonalactone, 3-methylbutanal, 2-phenylethanal) in fresh beers decreased when using 40% white or red sorghum adjunct, whereas those of higher alcohols (isobutanol, amyl alcohols) and total organic acids increased. However, the sensory analysis performed by a panel of 10 professional tasters according to the DLG scheme revealed no significant differences between 40% white or red sorghum and 100% barley malt beers. In addition, the forced-aging process simulating 3–4 month of aging revealed an acceptable flavor stability of 40% white Nigerian and red Italian sorghum beers. In conclusion, this study successfully demonstrated the use of 40% European grown sorghum as brewing adjunct. However, more research is needed with regard to breeding of sorghum cultivars particularly suitable for beer production.

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Chapter 11

Overall discussion and conclusion

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The brewing industry worldwide is facing an ever increasing challenge to become more cost effective, while at the same time maintaining or improving product quality. Brewing with unmalted oats and sorghum, also referred to as adjuncts, is not only cost saving but also innovative. Within this Ph.D. project, the quality/processability of mashes, worts, and beers produced with various types and levels of oats or sorghum was evaluated and optimized applying industrial enzymes. The objective of the first study was to develop a highly precise rheological method for monitoring changes in mash viscosity or, more precisely, mash consistency during the mashing process (Chapter 4). For this purpose, the controlled stress rheometer Physica MCR 301 equipped with a defined paddle-shaped rotor was used; this rotor enables mash particles to be kept in suspension throughout the rheological measurement. During mashing, the degradation of high-molecular-weight substances such as β-glucan, protein, and starch by endogenous and/or exogenous enzymes leads to continuous changes in mash consistency *(1,2)*. The determination of consistency changes in mashes is of great importance when brewing with cereal adjuncts (e.g. oats, sorghum) particularly with regard to quality control *(3)* as well as process and enzyme optimization. By means of statistical tools, it has been shown that the precision of the new rheological method is significantly better compared to that of the established Rapid Visco Analyser (RVA). Mash consistency curves were described using regression or approximation functions in order to determine characteristic curve points mathematically correct. Thus, the start and end point of starch gelatinization and liquefaction could be well defined. According to a RVA method described by Mitteleuropäische Brautechnische Analysenkommission (MEBAK) *(4)*, the gelatinization of starch starts when the viscosity increases by 24 mPa·s within 1 s. However, the automatic determination of the gelatinization temperature by a RVA and Thermocline for Windows software can result in inaccuracies with a coefficient of variation CV = 16.4%. Noisy baselines may lead to misinterpretations, which is why MEBAK recommends the manual interpretation of data. Such problems can be eliminated by the use of regression functions mentioned above. The new rheological method applying a Physica MCR rheometer has proven to be highly precise, gives reproducible and thus reliable results, and is suitable for mash systems containing different cereal adjuncts such as oats or sorghum.

In another study, eight different oat cultivars were compared in terms of their suitability as brewing adjuncts (Chapter 5). These oat cultivars, 7 husked (Lutz, Buggy, Galaxy, Scorpion, Typhon, Ivory, Curly) and 1 huskless or naked (NORD 07/711), were chosen because of their low contents of husk, β-glucan, protein, fat, and/or high starch contents. It has been found that all oat cultivars had significantly higher contents of fat, β-glucan, protein, and ash as well as negligibly low enzyme activities compared to barley malt. However, the naked oat cultivar contained considerably less β-glucan as well as more protein and starch than the 7 husked oat cultivars. These findings are largely in agreement with data published in the literature *(5–11)*. When replacing barley malt with unmalted oats in brewing, high amounts of unmodified high-molecular-weight β-glucans and proteins are brought into the mash, increasing its consistency. The application of the new rheological method using a Physica MCR rheometer also revealed that the gelatinization of oat starch caused significantly higher peak consistencies compared to that of barley malt starch; this is due to differences in morphology and size of starch granules *(12,13)*. Nevertheless, the use of 40% naked oats resulted in a final mash consistency similar to that obtained with 100% barley malt. With regard to wort quality/processability, the substitution of 20% or 40% barley malt with hammer-milled oats caused significantly higher pH values, β-glucan contents, and viscosities (based on 12% w/w extract) as well as significantly lower filtration rates, polyphenol contents, color values, total soluble and free amino nitrogen concentrations. Mashing with up to 40% naked oats resulted in constant extract yields, whereas the use of husked oats led to significant extract losses. The best results in terms of nitrogenous compounds in worts were achieved with naked oats as well as the husked oat cultivars 'Lutz' and 'Scorpion'. Especially interesting are the findings concerning wort viscosity: The naked oat cultivar with the lowest β-glucan content caused the highest viscosity (lowest filtration rate); the husked oat cultivar 'Lutz' with the highest β-glucan content caused the lowest viscosity (highest filtration rate). It has been reported that naked oats contain more water-soluble and less water-insoluble β-glucan than husked oats *(14)*. Besides, a high level of extracted β-glucan does not necessarily involve a high viscosity (and vice versa) since larger fragments contribute more to viscosity than smaller but more numerous fragments *(15)*. In view of all these results, the oat cultivars 'NORD 07/711' (naked oats) and 'Lutz' (husked oats) have proven to be particularly interesting and promising as brewing adjuncts.

The objective of a further study was to investigate the limitations of endogenous barley malt enzymes and the benefits of the application of industrial enzymes in high-gravity brewing, substituting 10–40% barley malt with the oat cultivar 'Lutz' (Chapter 6). On the basis of preliminary rheological tests was found that it is impossible to reduce the mash consistency of 40% oat mashes to a similar level as obtained with 100% barley malt by extending the cytolytic/proteolytic mash rest. However, this reduction in mash consistency could be achieved by the addition of Ondea® Pro (2.0 g/kg oats) to 40% oat mashes. This enzyme cocktail proved to be especially suitable for brewing with unmalted oats, containing the following enzyme activities: Pullulanase (declared enzyme 637 U/g), α-amylase, endoprotease, β-glucanase, xylanase, and lipase. Mashing with up to 40% unmalted oats resulted in complete saccharification after 10 min at 72°C without enzyme addition. Furthermore, very high positive correlations between mash consistency and oat concentration were determined. With regard to wort quality, the β-glucan content increased ($P < 0.001$) whereas the free amino nitrogen content decreased ($P < 0.05$) with increasing adjunct level. Significant adverse effects on wort extract and viscosity (based on 12% w/w extract) were observed when using 20% or more oats. In addition, the substitution of 30% and 40% barley malt with unmalted oats caused significantly higher filtration times and wort pH values. This increase in pH is likely the result of a lower concentration of buffer substances such as peptides/polypeptides with aspartate and glutamate residues in oat-containing worts *(16)*. The application of Ondea® Pro led to considerably reduced wort β-glucan concentrations and thus viscosities as well as increased free amino nitrogen and extract contents. Recommended values for free amino nitrogen in high-gravity worts (18–24% w/w) are 250–280 mg/L *(17,18)*; those concentrations have still been reached in worts produced with up to 20% oats without the addition of exogenous enzymes. Nevertheless, the use of Ondea® Pro has the potential to considerably improve the filterability and fermentability of high-gravity worts produced with 30% or more oats. Side effects of the addition of this enzyme mix to oat-containing mashes were lower wort pH values (higher buffering potential) and increased wort colors (excessive formation of Maillard products) due to an extensive protein degradation. However, the substitution of up to 20% barley malt with unmalted oats in high-gravity brewing can easily be realized without the application of industrial enzymes.

In another study, the impact of various levels of unmalted oats (10–40%; cultivar 'Lutz') on the quality and processability of mashes, worts, and beers brewed at pilot-plant scale (60 L) was evaluated (Chapter 7). Oats were milled using a hammer mill in order to improve extract yields and prevent pipeline blockages (high husk volume). For mashing, a common infusion process was applied (30 min at 50°C; 40 min at 65°C; 20 min at 72°C; 5 min at 78°C, mashing-off) since oat starch has a somewhat lower gelatinization temperature than barley malt starch (see Chapter 6). It has been found that brewing with up to 40% hammer-milled oats at pilot-plant scale is technically feasible without the addition of industrial enzymes. However, the reported positive effects of intact oat husks on lautering/filtration performance *(19–23)* do not occur. Nevertheless, hammer milling versus roller milling results in lower final mash β-glucan contents (higher solubility/extractability, higher enzyme susceptibility) *(24)* and lower wort viscosities (findings of preliminary brewing trials). The substitution of 40% barley malt with hammer-milled oats caused a 97-fold increase of β-glucan in the final mash. Besides, a very high positive correlation between final mash β-glucan content and preboil wort viscosity (based on 12% w/w extract) was determined. As a result, the use of 20% or more hammer-milled oats led to significantly reduced lautering rates; the use of 10% oats had no significant impact on the processability of mashes/worts. Furthermore, a positive effect of oats on yeast growth could be observed, which might have been stimulated by higher zinc and lipid contents in oat-containing worts *(20,21,25,26)*. In terms of beer quality, the replacement of up to 40% barley malt with unmalted oats resulted in acceptable values for lager-type beers based on 12% w/w original extract *(27–29)* as regards alcohol, apparent residual extract, apparent degree of fermentation, pH, and color, even without exogenous enzyme addition. However, the foam stability of beers produced with 20% or more oats was significantly lower compared to that of 100% barley malt beers as a consequence of insufficient amounts of total soluble nitrogen *(30)* and high-molecular-weight proteins, respectively. In contrast, the sensory quality of oat beers improved with increasing adjunct level; 30% and 40% oat-containing beers were rated higher in terms of aroma and purity of taste than all-malt beers. These oat beers exhibited considerably lower concentrations of heat indicators/staling components (2-furfural, γ-nonalactone) and acetaldehyde; their content of higher alcohols (*n*-propanol, isobutanol) was lower, their ester content (ethyl acetate, isoamyl acetate) higher compared to 100% barley malt beers.

In a further study, the advantages and limitations of the use of commercially available oat and sorghum flours for beer production were determined, substituting 10–90% barley malt (Chapter 8). Brewing with commercial flours has the potential to reduce mashing times due to the high solubility/extractability of very finely milled cereals and improve brewhouse efficiency *(31,32)*. Mashing was carried out using the common infusion process mentioned above (see Chapter 7). Sorghum flour contained significantly less fat, β-glucan, and protein as well as more starch, polyphenols, and ash than oat flour; in addition, it had a considerably higher starch gelatinization temperature and a different protein profile compared to oat flour. In comparison with barley malt, both commercial flours exhibited lower levels of protein, higher levels of starch and fat, as well as negligibly low enzyme activities. These findings are largely in agreement with literature reports *(8,33,34)*. When replacing barley malt with oat flour in brewing, the extract content of worts steadily increased with increasing adjunct concentration; however, the use of more than 50% sorghum flour caused a rapid decrease in wort extract due to an insufficient starch degradation. Hence, the substitution of barley malt with sorghum flour is limited to 50% when applying a normal infusion mashing process (no pregelatinization of sorghum starch) without the addition of exogenous enzymes. All mashes containing up to 90% oat flour and up to 50% sorghum flour, respectively, showed iodine normality after 10–15 min at 72°C as well as a good filterability. Furthermore, the use of up to 50% commercial oat flour did not result in higher wort viscosities (based on 12% w/w extract), indicating that the β-glucanase activity in 50% barley malt was still sufficient for the degradation of β-glucan comprised in 50% oat flour. Worts produced with 10–70% commercial oat flour revealed a very good or good fermentability, those containing 30–50% sorghum flour caused a lower alcohol production. Equivalent factors were introduced in order to determine the limitations of the use of commercial oat and sorghum flours in brewing from an economic point of view. These are a measure of the amount of flour adjunct required to substitute a defined amount of barley malt without causing relevant changes in wort extract. It has been found that the use of up to 70% oat flour and up to 50% sorghum flour, respectively, is not only technically feasible but also economically beneficial. For example, when aiming at an adjunct concentration of 10%, only 870 kg of oat flour is needed to replace 1000 kg of barley malt without extract losses.

The objective of another study was to compare white Nigerian and red Italian sorghum as brewing adjuncts as well as to optimize the application of industrial enzymes to brewery mashes containing 10–40% of each sorghum type (Chapter 9). For mashing, a slightly modified double infusion process used in the brewing industry was chosen. First, sorghum mash was cooked to gelatinize starch (5 min at 55°C; 30 min at 90°C) and then mixed with barley malt mash to enzymatically convert gelatinized starch into fermentable sugars (5 min at 60°C; 30 min at 70°C; 5 min at 78°C, mashing-off). It is recommended to add protease and heat-stable α-amylase to the sorghum mash and β-glucanase to the total mash. In general, high levels of exogenous enzymes improve both quality and processability of sorghum mashes/worts. However, a balance between product quality and production costs has to be established. Both unmalted sorghum types were characterized by higher starch contents, lower β-glucan and protein contents, as well as negligibly low enzyme activities compared to barley malt. These findings are in agreement with data published in the literature *(35–40)*. Nevertheless, red sorghum caused a significantly higher mash consistency than white sorghum because of its higher proportion of vitreous starch (higher intrinsic viscosity, higher gelatinization temperature (*41*)). The addition of industrial enzymes to sorghum-containing mashes was optimized by applying the new rheological method using a Physica MCR rheometer. It has been found that the consistency of sorghum mashes increased enormously with increasing adjunct level (no enzyme addition). The use of heat-stable α-amylase (Hitempase 2XP; 1.0 g/kg sorghum) as recommended caused a decrease in mash consistency to a large extent. However, 50% of the recommended heat-stable α-amylase dose was sufficient for brewing with up to 40% white or red sorghum. Besides, the addition of protease (Bioprotease N120MG; 0.13 g/kg sorghum) to sorghum mashes and β-glucanase (Bioglucanase TX; 0.25 g/kg malt) to total mashes as recommended had no significant effect on mash consistency or wort quality. Worts produced with 40% hammer-milled sorghum (white/red) and optimized enzyme levels (Hitempase 2XP; 0.5 g/kg sorghum) exhibited significantly lower viscosities (based on 12% w/w extract), filtration rates, total soluble and free amino nitrogen contents as well as significantly higher pH values. Furthermore, the substitution of 30% or more barley malt with white sorghum resulted in significantly reduced wort polyphenol contents; in contrast, the use of 30% or more red sorghum caused significantly increased polyphenol concentrations.

In the last study, the impact of 40% white Nigerian and red Italian sorghum on the quality of worts and beers brewed at pilot-plant scale (60 L) was evaluated and compared (Chapter 10). Mashing was performed using the double infusion process mentioned above (see Chapter 9), applying optimized levels of industrial enzymes (Hitempase 2XP; 0.5 g/kg sorghum; see Chapter 9). Final worts produced with 40% white or red sorghum revealed higher concentrations of total fermentable sugars, in particular maltose ($P > 0.05$) and maltotriose ($P < 0.05$). Their fermentability was comparable to that of 100% barley malt worts, despite very low free amino nitrogen contents of around 100 mg/L. According to the literature, recommended values for free amino nitrogen are 200–240 mg/L based on all-malt worts (12% w/w) *(42)*. Beers brewed with 40% red sorghum had a significantly reduced foam stability compared to 100% barley malt beers. The use of 40% white sorghum had a less adverse effect on beer foam. It is likely that the high polyphenol content in red sorghum worts caused higher losses of foam-positive proteins during wort boiling *(27)*. Furthermore, 40% sorghum beers received similarly good scores in terms of sensory quality and flavor stability than all-malt beers. The substitution of 40% barley malt with unmalted sorghum (white/red) resulted in considerably reduced levels of staling components (γ-nonalactone, 3-methylbutanal, 2-phenylethanal) and acetaldehyde. In addition, 40% sorghum beers exhibited significantly increased contents of higher alcohols (isobutanol, 2- and 3-methylbutanol) as well as lower ester contents (isoamyl acetate) compared to 100% barley malt beers. This is in contrast to oat beers containing less higher alcohols and more esters than all-malt beers. It has been reported that flavor-active acetate ester production is reduced with low wort free amino nitrogen and glucose levels *(43)*. Besides, the longer a fermentation proceeds in the absence of nitrogen, the greater is the production of higher alcohols *(44,45)*. The gluten content of beers brewed with 40% white or red sorghum was significantly reduced compared to that of 100% barley malt beers. According to the Codex Alimentarius (Alinorm 08/31/26), 40% red sorghum beers were even very low in gluten (gluten content above 20 ppm and up to 100 ppm), most likely as a result of the higher polyphenol content in the respective worts (see above). Hence, European grown red sorghum has great potential to be used as brewing adjunct. In summary, this Ph.D. thesis lays the foundation for the successful use of unmalted oats and sorghum in brewing.

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Chapter 12

Appendix

List of Publications

- Schnitzenbaumer B, Kerpes R, Titze J, Jacob F and Arendt EK (2012) Impact of various levels of unmalted oats (*Avena sativa* L.) on the quality and processability of mashes, worts, and beers. Journal of the American Society of Brewing Chemists, 70:142-149.

- Schnitzenbaumer B, Arendt EK and Titze J (2013) Statistical comparison of a new rheological method for defining changes in mash consistency during mashing with the established Rapid Visco Analyser. Journal of Cereal Science, 57:39-46.

- Schnitzenbaumer B and Arendt EK (2013) A comparative study of oat (*Avena sativa*) cultivars as brewing adjuncts. European Food Research and Technology, 236:1015-1025.

- Schnitzenbaumer B and Arendt EK (2013) Effect of unmalted oats (*Avena sativa* L.) on the quality of high-gravity mashes and worts without or with exogenous enzyme addition. European Food Research and Technology, DOI:10.1007/s00217-013-2105-8.

- Schnitzenbaumer B, Karl CA and Arendt EK (2013) A comparison of white Nigerian and red Italian sorghum (*Sorghum bicolor*) as brewing adjuncts based on optimized enzyme additions. Journal of the American Society of Brewing Chemists, 71:248-257.

- Schnitzenbaumer B, Karl CA, Jacob F and Arendt EK (2013) Impact of unmalted white Nigerian and red Italian sorghum (*Sorghum bicolor*) on the quality of worts and beers applying optimized enzyme levels. Journal of the American Society of Brewing Chemists, 71:258-266.

- Schnitzenbaumer B, Kaspar J, Titze J and Arendt EK (2013) Implementation of commercial oat and sorghum flours in brewing. European Food Research and Technology, DOI:10.1007/s00217-013-2129-0.

List of Presentations

- Schnitzenbaumer B, Ryan LAM and Arendt EK (2010) The impact of various levels of unmalted oats on the quality and processability of mashes and worts. 2nd International Symposium for Young Scientists and Technologists in Malting, Brewing and Distilling. Freising-Weihenstephan, Germany, May 2010. *Oral presentation*.

- Schnitzenbaumer B, Ryan LAM and Arendt EK (2011) Development, application and validation of a new rheological method for defining changes in mash viscosity during mashing with oats. 40th Annual UCC Food Research Conference. Cork, Ireland, April 2011. *Oral presentation*.

- Schnitzenbaumer B, Kerpes R, Jacob F and Arendt EK (2011) Impact of various levels of unmalted oats on the quality and processability of mashes, worts and beers. American Society of Brewing Chemists Annual Meeting. Fort Myers, Florida, U.S.A., June 2011. *Oral presentation*.

- Schnitzenbaumer B, Titze J and Arendt EK (2012) A comparative study of oat (*Avena sativa* L.) cultivars as brewing adjuncts. 41st Annual UCC Food Research Conference. Cork, Ireland, April 2012. *Oral presentation*.

- Schnitzenbaumer B, Titze J and Arendt EK (2012) A comparative study of oat (*Avena sativa* L.) cultivars as brewing adjuncts. World Brewing Congress. Portland, Oregon, U.S.A., July 2012. *Oral presentation*.

- Schnitzenbaumer B, Titze J and Arendt EK (2012) Optimization of the application of commercial enzymes in sorghum mashes. World Brewing Congress. Portland, Oregon, U.S.A., July 2012. *Poster presentation*.

- Schnitzenbaumer B, Karl C and Arendt EK (2012) A comparison of white and red sorghum as brewing adjuncts based on optimised enzyme additions. 3rd International Symposium for Young Scientists and Technologists in Malting, Brewing and Distilling. Nottingham, UK, October 2012. *Oral presentation*.

- Schnitzenbaumer B, Karl CA, Jacob F and Arendt EK (2013) Impact of white Nigerian and red Italian sorghum on the quality and processability of worts and beers. 34th Congress of the European Brewery Convention. Luxembourg, May 2013. *Poster presentation.*