Taylor & Francis
Taylor & Francis Group

Journal of the American Society of Brewing Chemists
The Science of Beer

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ujbc20

Enzymes from Cereal and *Fusarium* Metabolism Involved in the Malting Process – A Review

Cajetan Geißinger, Martina Gastl & Thomas Becker

To cite this article: Cajetan Geißinger, Martina Gastl & Thomas Becker (2021): Enzymes from Cereal and *Fusarium* Metabolism Involved in the Malting Process – A Review, Journal of the American Society of Brewing Chemists, DOI: 10.1080/03610470.2021.1911272

To link to this article: https://doi.org/10.1080/03610470.2021.1911272

Published online: 17 May 2021.

JOURNAL OF THE AMERICAN SOCIETY OF BREWING CHEMISTS
https://doi.org/10.1080/03610470.2021.1911272

ASBC Routledge Taylor & Francis Group

REVIEW ARTICLE

OPEN ACCESS Check for updates

Enzymes from Cereal and *Fusarium* Metabolism Involved in the Malting Process – A Review

Cajetan Geißinger, Martina Gastl and Thomas Becker

Chair of Brewing and Beverage Technology, Technical University of Munich (TUM), Freising, Germany

ABSTRACT

The enzymatic properties of brewing malts are an important and sometimes limiting factor in malt and beer production. In order to observe malt quality, multiple analytical tests were developed for determining the various enzymatic activities of brewing-related enzymes in brewing cereals and their corresponding malts. When using the prevailing detection methods, little or no attention has been paid to the microbiological status of the processed cereals. Infection of cereals with *Fusarium* spp. can lead to a deterioration in cereal and malt quality. This weakening in quality during cereal processing is mostly attributed to enzymatic degradations. The enzymes involved can be cereal-borne as well as fungal, in which case enzymes up- or down-regulation can be often ascribed to pathogenesis-related protein secretion or can be a result of host-pathogen interactions. Accordingly, when determining the enzyme activity of infested grain, an increase in enzyme activity is usually detected.

KEYWORDS

Enzymes; *Fusarium* spp; brewing cereals; barley; malting process

Introduction

Quality requirements for brewing cereals

Cereals used for brewing purposes such as barley (*Hordeum vulgare L.*) must meet basic quality characteristics. The term "quality characteristics" can be divided into three groups: food safety, processing quality, and product quality.[1] Food safety is mainly aimed at whether a product is saleable and implies, among other things, an absence of toxins or toxic metabolites in the product.[2] Processing quality can be impacted or influenced based on agricultural factors during plant growth or processing factors during malt and beer production since there are typically adequate amylolytic activities in the malt produced. Similarly most malts typically have well balanced cytolytic and proteolytic enzyme levels.[1] Processing quality merges into product quality, which implies quality factors such as the germinative energy of barley, the water or protein content of barley or malt, as well as visible discoloration that indicates weathering.[2] The minimum specifications of barley product quality for malting purposes are: a sufficient germinative energy higher than 95%,[3, 4] a protein content ranging from 9.5–11.5% dry basis,[4, 5] a moisture of ≥14.5%,[4, 6] and as few microbiological contaminants as possible.[1, 7]

Fusarium and FHB

The microorganisms found on the surface of barley are multifaceted. Over 200 different species of bacteria, yeasts, and fungi have been detected on the natural surface of barley,[8–10] whereby the quality damaging aspects of representatives of the genus *Fusarium* should be a focus of maltsters and brewers. In brewing cereals, the disease caused by *Fusarium* spp. is called Fusarium Head Blight (FHB). FHB is mainly caused by a complex of 17 species,[11] with the predominant species worldwide described as *F. graminearum*, *F. culmorum*, and *F. avenaceum*.[11–14] However, the complex of the FHB causal agents differs between the growing region, harvest year, and grain type.[11, 15, 16] FHB can lead to a deterioration in terms of the previously mentioned food safety, processing quality, and product quality as well as to strong yield losses in cereal crops. The yield losses are mostly based on agricultural aspects implicating economic losses.

Agricultural effects and economic losses due to FHB

Given an increased incidence of FHB, the yield losses caused by FHB has in extreme cases reached as high as 70% in various regions worldwide.[17–22] However, the main damage is caused by the mycotoxins produced by *Fusarium* and food safety is no longer assured in such cases. These mycotoxins lead to symptoms of disease in animal feed and deteriorate the brewing quality of the grain.[23] In addition, there is also a reduction in the germination capacity of the seed.[24] The *Fusarium* community causes damage to small grain cereals all over the world, including Northern Europe,[1, 12, 13, 17, 19, 25–27] Northern and Southern America,[18, 20, 21, 28] Canada,[28] Australia,[22, 29] and Asia.[28, 30] However,

CONTACT Martina Gastl Martina.Gastl@tum.de

the incidence of diseases caused by *Fusarium* is not uniform over the world.[31] In Australia for example, the weather conditions are not favorable for the growth of *Fusarium*, as high temperatures are not met by high humidity.[32] The world barley (*Hordeum vulgare L.*) harvest in 2018/19 alone was 140.6 million tons[33] on an area of 49.5 million ha. The selling price ranged 2017/2018 from €123.8–180.9 per ton in Europe.[34] In Europe, the market volume of brewing barley in 2018/19 amounted to €10 billion. A high incidence of FHB can therefore lead to massive economic losses.

Climatic conditions vs. Fusarium incidence

Fusarium infections are reported to be favored by warm and humid weather conditions. These weather conditions can promote the infestation intensity and growth of fungi, depending on the *Fusarium* spp.[35] Several authors have described the increasing frequency and severity of FHB epidemics due to advancing climate change in the Northern hemisphere, especially in Europe.[25, 36–40] For example, current German weather trends show that April 2018 was 5 °C above the annual average – the warmest April since weather records began in 1881. In addition, the month of May was exceptionally warm, with a monthly average of about 2.5 °C above the long-term average, a significant precipitation deficit of about 25% of the long-term average was recorded in both months.[41]

There appears to be a contradiction between the intensity of the infection and the changing weather conditions. Climate change in both Central Europe and other regions of the world is moving towards higher temperatures and less precipitation.[42–46] However, occasionally occurring heavy rainfall events can lead to higher *Fusarium* infections because rain splashes distribute the *Fusarium* spores from soil-borne inoculum.[42] In addition, high temperatures have been reported to correlate positively with the occurrence of *Fusarium culmorum* in spring and winter barley patterns.[45] However, the number of cases of *Fusarium* infections in winter and spring barley samples has been increasing for several decades.[42] In addition, it must be taken into account that the spectrum of *Fusarium* spp. found in barley samples did not change for decades,[42] although climatic conditions changed drastically. As a result, factors beyond that of climate change should be taken into account for the increasing numbers of cases of *Fusarium* infections in barley.

Enzymology and the malting process

Malting is the externally-controlled steeping, germination, and finally drying (kilning) of cereal grains, which causes physical and biochemical changes mainly in the endosperm.[47] An exemplary schematic of the malting process according Narziss and Back[30] is shown in Figure 1. Using the parameters of germination temperature, germination time, the humidity of germinating material, and the O_2/CO_2 ratio of the supplied air, maltsters are able to produce malt without brewers needing to add exogenous enzymes later in the brewhouse. During the malting process, a complex interaction of biochemical, physiological, and biological processes takes place in the grain.

The main objective of malting is both the production of hydrolytic enzymes and the degradation and depolymerization of certain structural components of the grains into soluble substances,[30, 48] but largely not starch (whereas ~5% of starch will be depolymerized for embryo respiration). The malting process can roughly be subdivided into three steps. First, the biological growth phase (steeping and germination); second, the biochemical solubilizing phase (germination); and, third, the drying phase (kilning).[30, 49] The first phase involves the physical uptake of water, through which the barley kernel passes from a largely inactive state of life into the active phase of root and leaf growth and the formation of enzymes,[48] which later undergo substance transformations during mashing.[50] This means that undissolved substances are transformed into soluble ones. During steeping, the total water content can reach up to 46%.[51]

In the second phase, enzyme formation continues, and enzymatic activity begins to dissolve substances of the endosperm. This mainly involves the degradation of skeletal stages (hemicelluloses) by cytolytic enzymes and proteinaceous substances by proteolytic enzymes.[51, 52]

The biochemical phase is complete when the endosperm has become easily attackable due to a friable structure. On the contrary, amylolytic effects should be kept low during malting (~4.5%) because these will result in undesirable malting losses.[51, 52]

Finally, there follows an enzyme-conserving drying process, i.e., the withering and kilning process. In the drying procedure, in which temperatures reach up to 85–105 °C (185–220 °F), the storability and the typical aroma of the malt is adjusted. After kilning, the malt reaches a water content of 3–5%.[51]

Fusarium and the malting process

During malting, microbes, such as *Fusarium* spp., can act as the biological part and attack the grains entailing host-pathogen interactions[51] since the growth conditions for fungal growth are favorable during steeping, germination, and the early-mid stages of kilning.[53–55] The result should – given healthy grains – be a homogeneous, friable malt with well-balanced enzyme activity. As a final control, the enzyme activity of the malt is usually measured in order to determine the product quality for downstream processing.[56–60] Enzyme activity is measured by means of enzyme kits and is an interaction of degraded substrate per time by an enzyme. However, these kits can only be used to determine the cleavage products of the enzymes, and enzyme activity is then calculated. An exact allocation of the respective enzymes is usually not possible because the cleavage products are often the same for various enzymes of cereal or microbial origin. Only methods that aim at the gene expression of the corresponding enzymes would be suitable for differentiating or classifying the origin of the enzymes (cereal or fungal). However, the influence of post-translational modifications cannot be taken into account thereby.

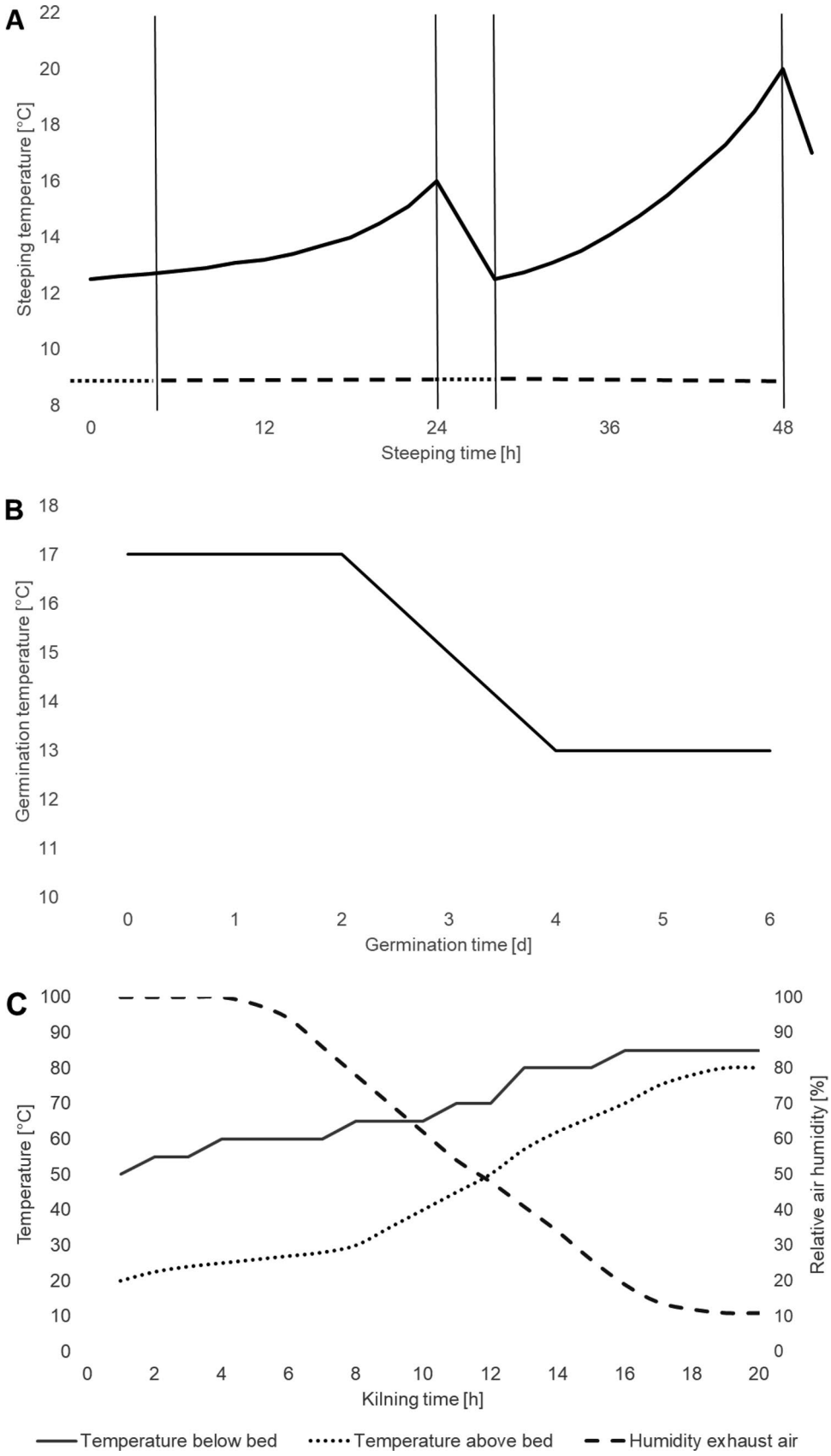

Figure 1. Exemplary schematic of the malting process according to Narziss and Back.[30]

Maltsters can use several options to reduce *Fusarium* growth by varying malting parameters. Intensive aeration during steeping and additional air rests, reduced germination times, and malting with a falling temperature program can reduce the growth of *Fusarium*.[61] Beyond the technological options for suppressing *Fusarium* growth,

there are a number of biological, chemical, or physical methods that have been shown to be effective in lowering the growth of *Fusarium*. The application of different yeasts or lactic acid bacteria has also been reported as a biological means of lowering *Fusarium* growth.[62, 63] The chemical treatment of grains has been performed by adding reducing agents while malting to lower the mycotoxin formation.[64, 65] Physical methods *have also applied* with promising *Fusarium* reducing effects.[66, 67] All of these methods result in both reduced *Fusarium* growth and reduced enzyme potential after malting.[62, 65, 66] However, if the various pathogens – in particular *Fusarium* spp. – infect the grains, the pathogens will add an enormous additional enzyme potential to the malting process. The additional enzyme load of pathogenic origin can act in a way similar to the corresponding cereal-borne enzymes[62] as well as lead to high solubilization properties of the malt.

This means that maltsters are often no longer able to handle the raw material properly. Until now, the enzyme potential of pathogens and cereals has always been considered separately, or they have been scientifically investigated independently of one another. Many publications exist that demonstrate the amylolytic, proteolytic, and cytolytic enzyme potential of *Fusarium* spp. isolated from cereals, mostly from wheat or barley for in vivo or in vitro studies.[10, 54, 68–86] In practice, the enzyme activity was mostly higher when malt was infected with *Fusarium*. Nevertheless, it is still not useful in practice to differentiate between enzyme potential that is cereal borne and enzyme potential that is of microbiological origin.

In addition to the minimum requirements for malt quality mentioned above, the malt should fulfill requirements for well-balanced enzymatic properties[50] and as little microbial contamination as possible. Enzymes acting as biocatalysts are indispensable to the production of malt and beer. On the one hand, these enzymes may be of cereal origin or of microbial origin, such as those induced by *Fusarium* spp. On the other hand, the interaction between cereal grain and fungus can lead to a synthesis of enzymes caused by this host-pathogen interaction. A selection of enzymes involved in this process is shown in Table 1. The enzymes that act during malting and brewing can roughly be divided into three groups: enzymes for amylolysis, for proteolysis, and for cytolysis. One enzyme action takes on a special position – a cytolytic acting 'enzymè – which is the β-glucan-solubilase. It was described as an enzyme that breaks down high molecular β-glucan from a protein matrix,[87, 88] has carboxypeptidase activity,[89] and is still measurable in mature barley kernels. However, it is also reported that its activity originates from a fungal cellulase, which was found on the surface of barley kernels and is therefore of fungal origin.[88]

Enzyme formation induced by gene expression

Enzyme activity and the amount of enzymes in barley and barley malt is regulated by gene expression before and during malting.[90–92] On the one hand, gene expression may be a result of induced phytohormones that regulate the growth and development of plants.[93] On the other hand, if pathogens attack a plant, they could lead to host-pathogen interactions and, consequently, again gene expression as a defense reaction from the plant/grain or from the pathogen itself and can result in pathogenesis-related (PR) proteins.[78] Additionally, the enzyme activity may be suppressed by specific enzyme inhibitors, which play a decisive role in plant development as well as in terms of host pathogen interactions. For instance, alpha amylase 2 (AMY-2), an isoform of alpha amylase, is inhibited by the endogenous barley α-amylase/subtilisin inhibitor (BASI). Additionally, BASI can protect the grain from an attack of secreted proteases from pathogens in its second function as a subtilisin inhibitor.[94]

Main enzymes acting during malting and brewing

Primarily worthy of mention in this context are the amylolytic enzymes that act during mashing to hydrolyze starch to fermentable sugars. Due to thermolability, the other malt enzymes of proteolytic or cytolytic classes act mostly during malting. The amylolytic enzymes involved thereby are mainly α-amylase, β-amylase, limit dextrinase, and α-glucosidase.[95, 96] Brewers like to summarize the effect of these four amylolytic enzymes under the quality characteristic of Diastatic Power (DP),[47] but specific enzymatic tests are currently available to determine the enzyme activity of these four enzymes. Cereal α-amylase (1,4-α-D-glucan glucanohydrolase) is a "de novo" enzyme, which means that formation and activation happens during the malting/germination process. The activity of the α-amylase can be significantly influenced by the malting process. Using a favorable O_2/CO_2 ratio (high O_2, low CO_2) and a high degree of steeping leads to a high level of α-amylase activity, but growth of *Fusarium* is also favored by good aeration and high humidity.

The β-amylase (1,4-α-D-glucan maltohydrolase), however, is still present in barley grain, but activity increases during germination while malting. An increase in β-amylases and further enzyme activity is based on the release of bound β-amylase in the endosperm by proteases[97] and due to the gene expression of isoforms of β-amylases occurring during the phases of malting.[90–92, 98, 99]

Both the α-amylase and the β-amylase enzymes are only able to cut the starch chains at α-1→4-bonds. The biggest difference between the two enzymes is that the α-amylase cleaves the starch inside (endo-enzyme), and the β-amylase only cleaves the starch outside (exo-enzyme) after every second glucose unit starting from the non-reducing end. Limit-dextrinase (endo-enzyme) is able to cut the starch at α-1→6-bonds on the inner side of the starch molecule. Alpha-glucosidase is the main endosperm enzyme catalyzing the breakdown of maltose to glucose. Alpha-glucosidase is not required for starch degradation during germination, but it has also been reported to attack starch granules.[100] to supply the seedling with nutrients during germination.

Table 1. Malting related enzymes from Barley and *Fusarium* spp.

Name	Alternative name	EC-number	Origin	Reaction catalyzed	Enzyme activity test	Source
Enzymes of amylolysis						
α-amylase	1,4-α-D-glucan glucanohydrolase	3.2.1.1	cereal, *Fusarium* spp.	Acts on starch, and related polysaccharides and oligosaccharides in a random manner; reducing groups are liberated in the alpha-configuration	Colorimetric Assay	https://enzyme.expasy.org/;[121]
β-amylase	1,4-α-D-glucan maltohydrolase	3.2.1.2	cereal, *Fusarium* spp.	Hydrolysis of 1,4-alpha-D-glucosidic linkages in polysaccharides so as to remove successive maltose units from the non-reducing ends of the chains	Colorimetric Assay	https://enzyme.expasy.org/;[140]
limit dextrinase	amylopectin1,6 glucosidase	3.2.1.142	cereal	Hydrolysis of 1,6-alpha-D-glucosidic linkages in alpha- and beta-limit dextrins of amylopectin and in amylopectin	Colorimetric Assay	https://enzyme.expasy.org/;[152]
α-glucosidase	1-4-α-D-glucan glucohydrolase	3.2.1.3	cereal	Hydrolysis of terminal 1,4-linked alpha-D-glucose residues successively from non-reducing ends of the chains with release of beta-D-glucose	Colorimetric Assay	https://enzyme.expasy.org/;[96]
Enzymes of cytolysis						
endo (1,4)-β-glucanase	cellulase, beta-1,4-endoglucan hydrolase	3.2.1.4	cereal, *Fusarium* spp.	Endohydrolysis of 1,4-beta-D-glucosidic linkages in cellulose, lichenin and cereal beta-D-glucans; Will also hydrolyze 1,4-linkages in beta-D-glucans also containing 1,3-linkages	Colorimetric Assay	https://enzyme.expasy.org/[164]
endo-(1,3)-β-glucanase	1,3-β-glucan endohydrolase	3.2.1.39	cereal, *Fusarium* spp.	Hydrolysis of 1,3-beta-D-glucosidic linkages in 1,3-beta-D-glucans; Very limited action on mixed-link 1,3/1,4-beta-D-glucans	Colorimetric Assay	https://enzyme.expasy.org/;[24, 161]
exo-β-glucanase	1,3-β-D-glucan glucohydrolase	3.2.1.58	cereal	Successive hydrolysis of beta-D-glucose units from the non-reducing ends of 1,3-beta-D-glucans, releasing alpha-glucose	Colorimetric Assay	https://enzyme.expasy.org/
endo-1,4-β-xylanases	glucuronoarabinoxylan endo-1,4-beta-xylanase	3.2.1.136	cereal, *Fusarium* spp.	Endohydrolysis of 1,4-beta-D-xylosyl links in some glucuronoarabinoxylans; high activity toward feruloylated arabinoxylans from cereal plant cell walls	Colorimetric Assay	https://enzyme.expasy.org/;[108]
Enzymes of proteolysis						
cysteine class proteases, e.g. EP-A and EP-B		3.4.22.-	cereal	Only specified due to protein class	SDS-PAGE	[184, 188]
metalloproteases		3.4.24.-	cereal, *Fusarium* spp.	Not specified	SDS-PAGE	[82, 184, 188, 199]
aspartic proteases, e.g. HvAP		3.4.23.-	cereal	Not specified	SDS-PAGE	[184, 188]
serine class proteases, e.g. SEP1		3.4.21.-	cereal	Not specified	SDS-PAGE	[184, 188]
carboxypeptidase		3.4.16–3.4.18	cereal, *Fusarium* spp.	Hydrolysis of C-terminal peptide bonds	Colorimetric Assay	www.uniprot.org;[198]
aminopeptidase		3.4.11.-		Hydrolysis of N-terminal peptide bonds		[185]

The second group of enzymes are the proteases. Depending on their protease class, proteases break down peptide bonds by hydrolysis at specifics regions inside the protein structure. Similar to the amylolytic enzymes, in the class of proteolytic enzymes there are two protease groups: exo-[101, 102] and endo-enzymes[103] to degrade proteins from outside or inside, respectively. Furthermore, barley proteases can be categorized into four protease classes, depending on their mechanism of catabolism:[103, 104] cysteine proteases, metalloproteases, aspartic proteases, and serine class proteases.[103]

The third group of enzymes relevant to malting and brewing are the cytolytic acting enzymes. The main representatives of this group of enzymes are the β-glucanases and xylanases. They are responsible for the degradation of cell walls and promote the degradation of beta-glucans, pentosans, and other structural substances during malting and mashing (cellulose, hemicellulose, and pectin).[105]

Intention of the review

The activity of enzymes and the expression of their genes changes from mature grain to malt as a result of phytohormone related processes in the kernels during malting.[106] Moreover, if *Fusarium* spp. infect barley, the enzyme levels could be influenced[107–109] both in terms of activity and in terms of gene expression during the malting procedure.[70, 88, 110–112] Depending on the time of infection, e.g., pre- or post-harvest, amylolytic activity can be increased or decreased, which was described in the early studies in *Fusarium*-related research.[113–116] In addition, cell wall degradation is influenced by a *Fusarium* infection[70, 117] due to released enzymes as they are endo-1,4-β-glucanase[111] and β-xylosidase.[118] In the proteolytic enzyme group, an increase in enzymatic activity has also been described in *Fusarium* infected samples.[70, 81]

Given that the unpredictable combination of cereal and pathogen-borne enzymes in malting cereals lead to problems

in processability, this review addresses a compact compilation of the main enzymes involved in malt preparation in relation to *Fusarium* infection. It covers both cereal and fungal enzymes and enzymes for which the origin cannot be analytically determined. Enzymes that can be of both cereal and microbial origin are of particular interest since they are the great unknown in the malting process that the maltster cannot control.

Enzymes in malting procedure

The enzyme activity described by the relevant literature mostly relates to cereal-borne enzymes. Enzymes of microbial origin have not often been considered but are still present in mature grain or malt. An exceptional case is β-glucan solubilase, which is reported to be a cereal cellulase.[88] This additional enzyme potential can significantly influence the processing quality of the malt.[54, 74] Enzymes that may be technologically relevant to maltsters and brewers and that may be associated with fungal infestations include, among others: α-amylase, β-amylase, β-1,3-glucanase, xylanases, as well as endo- and exo-proteases.

The following sections will characterize the context in which enzymes are native to cereals and which are native to fungi. The main hydrolases relevant to malting are also described, whereby the formation or increase in activity is one of the main objectives of malting. Enzyme inhibitors and their related impact in enzymatic activity are additionally considered. It should be noted that enzyme activity could also be increased by the action of enzymes from the metabolism of *Fusarium* spp. However, these enzymes are often genetically completely different and can be distinguished by molecular biological methods.

Amylolytic acting enzymes

In barley malt, four different enzymes are involved in degrading starch into fermentable sugars late in the brewing process. These four enzymes are: α-amylase, β-amylase, α-glucosidase, and limit-dextrinase.[119] An infection of cereals with *Fusarium* spp. can also contribute to starch degradation.[70, 120]

α-Amylase (1,4-α-D-glucan glucanohydrolase [E.C. 3.2.1.1])

Alpha-amylase is one of the most important starch debranching enzymes used in brewing technology.[121] This enzyme is an endo-enzyme that cleaves amylose and amylopectin from the inside out, at only α-1→4 bonds inside the molecule. The resulting cleavage products include oligosaccharides, maltotriose, and others. In mature barley and wheat tissue, no detectable α-amylase activity is measurable,[122] but in the early development stages and in immature grain, a small amount of α-amylase was found by Duffus and other researcher.[78, 123–126] However, the amount of α-amylase in mature barley grain is very low, and its activity is mostly undetectable using common analytical methods.[121] In the development of barley grains, α-amylase was found and was called "green α-amylase",[127] isoform low pI α-amylase (AMY-1), or high pI α-amylase (AMY-2). [128–130] In addition, two more genes for α-amylase were described earlier – AMY-3 and AMY-4.[126, 131] Authors describe AMY-3 to be a complex from AMY-1 and AMY-2,[128, 132] but it seems to be a complex of Barley α-Amylase Subtilisin Inhibitor (BASI) and AMY-2.[133, 134]

Specific inhibitors can inhibit amylases, but BASI indeed only inhibits AMY-2 and does not inhibit AMY-1. However, AMY-1 only plays a minor role in malting and brewing technology because the activity in malt is only described with 5% of the total α-amylase activity. The other 95% of the activity is attributed to AMY-2[30] and can presumably be inhibited by BASI. Muralikrishna and Nirmala, on the other hand, characterized the activity of AMY-2 with 60% of the total activity.[128] MacGregor et al. reported AMY-2 accounting for up to 80–90% of the total α-amylase activity in malt.[135, 136]

AMY-1 degrades starch independently of the presence of calcium.[128] AMY-2 is calcium-dependent[128] and is synthesized and activated de novo during malting. In contrast, Muralikrishna and Nirmala described both isoforms AMY-1 and AMY-2 to be synthesized de novo during germination. [128] In the early phase of plant development (germination), α-amylase (possibly AMY-1) ensures the supply of carbohydrates to the plant seedling. In a study by Hofer et al.,[78] α-amylase was found in developing stages after flowering in greenhouse-cultivated barley samples that were not infected with pathogens. The alpha-amylase found in this study in developing barley grain tissues was AMY-2,[78, 90, 137] whereby the similarity in the sequence of the two isoforms AMY-1 and AMY-2 was described as being 80%.[138]

Fusarium spp. can also contribute to amylolytic potential to the grain being processed. However, it should be noted that sequence similarities between cereal amylases and amylases from microorganisms are very low.[139] However, in the routine quality control of cereals, no molecular-biological methods are used to differentiate which organism the enzyme in question belongs to. Instead, only the amount of cleavage products per period of the enzymes is measured and is indicated as enzyme activity. In this way, a *Fusarium* infestation can make a hidden contribution to the enzyme potential.

These findings confirm the results of an earlier study[123] by Duffus, in which contamination by potential pathogens such as *Fusarium* spp. was not considered. In this early study, the author reported that α-amylase activity was detected in immature grain a few days after anthesis.[123]

β-Amylase (1,4-alpha-D-glucan maltohydrolase [E.C. 3.2.1.2])

In contrast to α-amylase, β-amylase is still present in the immature barley grain[140] and is not thought to be synthesized de novo during germination.[141] Beta-amylase is present in barley in three different forms – a free, a bound, and a latent type, with the free fraction accounting for up to 90% of the β-amylase activity in malted cereals.[97]

Beta-amylase hydrolyzes the 1,4-α-D-glucosidic linkages in polysaccharides and cuts maltose units from the non-reducing ends of the starch chains. In cereal plants, the activity of β-amylase starts to increase the free as well as the bound form of β-amylase, ten days after anthesis.[142] The free and the bound form of β-amylase is accumulated in the aleurone layer and the starchy endosperm.[143] However, the bound form has been described as being mainly inactive in barley kernels.[144, 145]

Two active isoforms of barley β-amylase have heretofore been known. Beta-amylase 1 (Bmy-1)[146] and Beta-amylase 2 (Bmy-2)[147] encoded by the genes Bmy-1 and Bmy-2 respectively. One recent study showed that gene expression of Bmy-2 was strongly increased during malting[99] in contrast to Bmy-1[148] However, native Bmy-1 contributes most to β-amylase activity.

Several authors have in recent years described α- and β-amylase as playing an important role in host-pathogen interactions due to a *Fusarium* spp. infection of various cereals.[149–151] For instance, Eggert et al. found an up-regulation of β-amylase as a result of an infection of emmer grains (*Triticum dicoccum*) by *F. culmorum* and *F. graminearum*[150] and described the biological function of the up-regulated β-amylase to not only be in charge of starch degradation. Beta-amylase acts also as a factor for the programmed cell death of grain cells.[150] Possibly, the β-amylase in that case came from the metabolism of the pathogens, and the function of the β-amylase was a targeted cell death of the grain. The necrotic barley cells became all the more accessible as a nutrient supply of *Fusarium*.

Limit dextrinase (1,6-alpha-glucanohydrolase [E.C. 3.2.1.33])

The third starch debranching enzyme in cereals is the limit dextrinase, which catalyzes the hydrolysis of 1,6-α-D-glycosidic linkages of the starch amylopectin.[132] Limit dextrinase activity is present but low in mature barley kernels,[152] and activity increases approximately six-fold during germination[153] induced by gibberellic acid,[154] but the activity of limit dextrinase in malt is reported to be halved from green malt to kilned malt.[154, 155] Limit dextrinase exists in a free, a latent, and a bound form. The bound form cannot hydrolyze amylopectin, but it can be activated through proteolysis by endogenous thiol proteases, by cysteine endopeptidases, or by the reduction of inhibitors by thioredoxin.[132, 156] In *Fusarium* infected samples, no increase of limit dextrinase was detected,[78] although *Fusarium* spp. are known to increase proteolytic activity in malt[24, 70] and can potentially activate the bound form of limit dextrinase.

Cytolytic acting enzymes

The cell walls of cereal grains are mainly composed of β-glucan and arabinoxylan (AX). However, lower amounts of cellulose, heteromannan, pectin, and xyloglucan are also located in the walls of barley or wheat.[157] The composition of this non-starchy polysaccharide differs, depending on the tissue (endosperm, embryo, husk, or aleurone layer). In barley endosperm, β-glucan accounts for approximately 75% of the total non-starch polysaccharides, and arabinoxylan accounts for 25% thereof.[158] In wheat tissue, the ratio is the opposite. In contrast, the aleurone layer of barley includes approximately 70% arabinoxylan and 25% β-glucan.[30, 159] Beta-glucan is a macromolecule, and its molecular weight is about 200–300 kDa.[160] The linkages between the glucose molecules are mainly β-1,4-glycosyl-bonds for approximately 70% and for 30% β-1,3-glycosyl-bonds.[160]

Glucanases

Three different glucanases are needed to degrade the β-glucan molecules – the endo-(1,4)-β-glucanase, the endo-(1,3)-β-glucanase, and the exo-(1,3)-β-glucanase. The hydrolysis from the degradation products (glycosides or oligosaccharides) to a single glucose unit is at least performed by β-glucosidase.[105, 161] The endo-(1,4)-β-glucanase is still present in mature barley grains,[70] whereas the endo-(1,3)-β-glucanase and exo-(1,3)-β-glucanases are only formed during germination.[161, 162] During germination, the activity of (1,3)-β-glucanase and exo-(1,3)-β-glucanase increases up to ten-fold.[30, 163] Due to the fact that exo-(1,3)-β-glucanase is not thermostable, it has a very low technological relevance for brewers in the mashing procedure. During germination, exo-(1,3)-β-glucanase is still active and can therefore influence cytolysis.

The role of endo-(1,3; 1,4)-β-glucanase in mature barley grain is not yet entirely clear. It may serve to improve the accessibility of starch by amylases through the cleavage of glucan, or the cleavage of glucan for seedling nutrition.[164] However, a fungal origin of a (1,4)-β-glucanase activity cannot be excluded.[165]

Apart from the endo-(1,4)-β-glucanase, barley β-glucanases are not synthesized before germination.[70, 166] The activity of β-glucanases detected in unprocessed barley is mostly of microbial origin. In cases where barley is infected with *Fusarium* ssp., activity of β-glucanases can be increased. However, *F. graminearum* (among others) is able to enhance the β-glucanase activity, but not *F. poae*.[70] This fungal (1,3)-β-glucanase is also described as a cellulase.[71]

The endo-(1,3)-β-glucanase plays a central role in degrading cell walls during malting.[105] Regarding cereals infected with pathogens such as *Fusarium* spp., it has been reported that endo-(1,3)-β-glucanase is up-regulated in response to the fungal attack, and (1,3)-β-glucanase activity increases rapidly.[24, 167] Fincher reports that an endo-(1,3)-β-glucanase is active against (1,3)-β-glucan in fungal cell walls.[105] The up-regulation of (1,3)-β-glucanase is, among other things, a plant defense reaction against biotic attack.[164] However, it should be noted that (1,3)-β-glucanase also acts on the plant´s own substrates and can thus contribute to the formation of signal molecules able to act as the plant´s triggers for further defense mechanisms.[168] The (1,3)-β-glucanase can also be detected in non-infected cereals, in which case it may be involved in normal developing processes during malting.[164] If pathogens attack the plant, (1,3)-β-glucanase is up-regulated as a Pathogenesis-Related Protein (PR). PRs are classified into 17 families, and (1,3)-β-glucanase is a

class 2 PR. The functions of PRs have been well-reviewed in a publication by Gorjanović.[164] The antimycotic function of a plant (1,3)-β-glucanase (as PR-2) is to hydrolyze the structural (1,3)-β-glucan present in the fungal cell walls. Glucan is most exposed at the apex of filamentous fungi hyphae. This leads to a weak cell wall and, afterwards, to cell lysis and cell death.[169] On the contrary, Kikot et al. described cell wall degrading enzymes that were produced in an extra-cellular manner from pathogenic fungi. However, it should be noted that these cell wall degrading enzymes can also be xylanases, chitinases, or pectinases.[69] Ultimately, it was not elucidated whether increasing activity of (1,3)-β-glucanase was only cereal-borne or also of fungal origin. Malt quality will always be affected in the presence of high glucanase activity.

Xylanase and arabinoxylan (AX)

Beyond β-glucan, AX is the main non-starch polysaccharide in barley. The structure of AX is complex, with many side chains on the xylose backbone. These are: L-arabinose, L-arabinose ester-linked with ferulic acid, D-galactose, 4-o-methyl-D-glucuronic acid, and acetyl groups. Seven enzymes are needed to degrade AX totally. These AX-degrading enzymes are: β-D-xylosidase, endo-1,4-β-xylanase, feruloyl esterase, arabinofuranosidase, acetylxylan esterase, α-D-glucuronidase, and α-D-galactosidase.[160, 170, 171] The degradation of AX during malting is relatively low in contrast to β-glucan because endo-xylanases are developed relatively late during germination. However, the AX digestion is important to the brewing process since it affects wort composition and processability as well as beer quality.[172]

Endo-xylanases (endo-1,4-β-xylanases) (EC 3.2.1.8) break down the xylan backbone of AX, the basic structure of AX, by hydrolyzing the β-1,4-xyloside bonds of xylans.[173–175] The previous literature describes many isoforms of barley xylanases secreted during malting,[176] but little knowledge has existed about xylanases during malting up to now. In mature barley, the activity of endo-xylanase is low and increases during germination.[177] The endo-1,4-β-xylanase has been described as an enzyme that is released when the precursor is proteolytically activated. Van Campenhout et. al. also discovered that the precursor of endo-1,4-β-xylanase in barley is an active enzyme,[178] and they isolated the xylanase of barley aleurone tissue.

If *Fusarium* ssp. contaminate barley plants, then xylanase activity can increase drastically. Sarlin et al. reported an increase of xylanase of endo-1,4-β-D-xylanase in barley malt when the samples were contaminated with *F. culmorum* and *F. graminearum,* respectively.[112] Dong et al. observed two different xylanases (xylanase I and xylanase II) from *F. graminearum*, which were isolated from wheat bran.[75] The same xylanases were found by Kanauchi et al. in barley malt that was not infected; the barley was even surface sterilized prior to malting.[177] In barley malt that was infected with *F. poae*, xylanase activity was either increased or decreased, depending on the barley variety. These results confirmed the findings of Schwarz et. al.[70] In contrast to Sarlin et al.,[112] however, xylanase activity in the work of Schwarz et al.[70] was also increased in samples infected with *F. poae*. Besides the increase of xylanase activity, often an increase of β-glucanase has been reported due to *Fusarium* infection,[24, 70, 112] in which the β-glucan amount in malt is drastically decreased.[70] This normally results in improving the filterability of wort and beer, but the Sarlin et al. paper describes a worse filtration rate in one sample of their study.[112]

The main proportion of the xylan backbone is degraded during mashing, but it can be inhibited by a specific inhibitor, which also develops during germination.[177, 179] Several xylanase inhibitors can inhibit xylanases. In wheat, the inhibitors are called Triticum Aestivum Xylanase Inhibitor (TAXI) and in barley (similar to wheat) Hordeum Vulgare Xylanase Inhibitor (HVXI).[173] In addition to these two inhibitor classes, a Xylanase Inhibiting Protein (XIP) has also been described.[180] Both XIP and TAXI are proteins in wheat and can inhibit microbial xylanases (e.g., from *F. graminearum*).[180]

Proteolytic acting enzymes

The third group of enzymes in barley and malt are the proteolytic enzymes. They degrade the proteins and can be classified according to the site of action in the protein. It is reported that several protease inhibitors inhibit the protease action,[181, 182] for example, they contain cereal protease inhibitors that may act against fungal attacks.[181, 183]

Proteases are hydrolases and hydrolyze peptide bonds at specific regions.[184] They can be divided into exopeptidases (hydrolyzing peptide bonds at the terminal end)[185] and endopeptidases (or proteinases) hydrolyzing internal peptide bonds of the protein.[184] The endo-cleaving proteases of barley and malt were well-reviewed by Jones.[103] Many of the endoproteinases are synthesized after germination.

A second classification can be made according the amino acid or the metal ion that is located at the active center of the enzyme. These class-specific proteases are cysteine proteases, aspartic proteases, serine proteases, and metalloproteases.

During malting, the proteins are made soluble by endopeptidases. Degradation of protein to amino acids or peptides follows temperature dependent from the action of exo-peptidases. The most important protease class are the cysteine class proteases. Metalloproteases act during mashing as they do during malting, whereas aspartic class proteases contribute less to protein solubilization. Serine class proteases are similarly not deeply involved in the hydrolysis of proteins during malting or mashing.[103]

According to the MEROPS database, 590 different peptidases or putative peptidases are known in *Hordeum vulgare* L..[186, 187] Concerning the three predominant *Fusarium* spp. (*F. graminearum*, *F. culmorum* and *F. avenaceum*), 560 different known and putative proteases have been described to date.[186, 188] These fungal proteases have been reported to increase proteinase activity in cereals.[81]

Mainly relevant to germination are 42 different proteases, and 27 proteases of this pool are cysteine proteases.[189, 190]

Proteinase activity is highly influenced by the fungal infection of the cereals. This has been shown in barley, wheat, and in emmer samples that were infected by *F. graminearum*, *F. culmorum*, and *F. avenaceum*.[23, 83, 150]

Cysteine endoproteases [E.C. 3.4.22]

Cysteine class proteases carry out their hydrolytic activity based on a cysteine residue located at the active site of the protease. This hydrolysis by means of cysteine class proteases is histidine-activated. In barley grain, the cysteine proteases are located in the endosperm; in the seedling, and during germination they are also located in the aleurone cells.[191] The maximum of cysteine class proteases activity has been described as being reached at the end of the germination period (day five).[104] The main representatives of the cysteine class proteases are two papain-like proteases: Endo-Protease A (EP-A).[192] and Endo-Protease B (EP-B).[193] These two proteases are synthesized in barley by the induction of gibberellic acid. Both EP-A and EP-B are the main proteases hydrolyzing barley hordein.[103] Three further cysteine class proteases have been described in addition to EP-A and EP-B: Malt Endopeptidase 1 (MEP-1), the 30 kDa proteinase from green malt,[194] and the 31 kDa proteinase from green malt.[195] MEP-1 is also a hordein hydrolyzing peptidase and was found in green malt, which was not kilned.[196] Despite some inconsistencies between the studies of Koehler and Ho[193] and Phillips and Wallace,[196] Jones presumed them to be the same enzyme.[103] In addition, it should be noted that the 30 kDa proteinase and the 31 kDa proteinase appear to be isozymes. These five enzymes are highly active in both barley and malt and contribute mainly to the degradation of storage proteins such as hordeins.[103] Most of these enzymes exhibit a high level of activity in green malt, and also after kilning. The activity is not lowered in the finished malt. On the contrary, the kilning process can even increase the activity of proteases.[104, 197] The studies by Jones et al. and Bell did not test whether the grains were infected by *Fusarium*. During kilning, the growth of *Fusarium* (expressed as a DNA amount) can increase during the kilning process.[78] It is possible that the increase of proteolytic activity is based on the increase of *Fusarium* biomass during the early stages of kilning.

Metalloproteases [E.C. 3.4.24.]

Simpson as well as Jones concluded in their reviews that metalloproteases are second to cysteine endoproteases,[103, 198] and relatively little knowledge exists about metalloproteases during malting.[103] Metalloproteases have mainly been found in the aleurone cells of malt and in the endosperm of green malt and could not be detected in the endosperm of kilned malt.[191] Their activity is very low in mature barley and during the first day of germination.[103] The activity of metalloproteases has been reported to increase from the first day of germination.[104] The maximum activity was measured at day two of germination.[103] Wrobel and Jones were the first to find five different high molecular weight metalloproteases in green cereal malt, which was germinated for four days.[199] These proteases were either zinc- or manganese-dependent and were reported to be responsible for protein hydrolysis in early malting stages.[199] Metalloproteases are able to hydrolyze hordein. Oliveira et al. showed in one trial that either malting or a *Fusarium* infection can increase the activity of metalloproteases.[82]

Aspartic proteases [E.C. 3.4.23.-]

Sarkkinen et al. purified an enzyme from barley which was previously described by Belozersky et al. in wheat seeds.[200, 201] This enzyme could be inhibited by the addition of pepstatin, which indicates that the enzyme is an aspartic endoprotease[202] and was later named Hordeum vulgare Aspartic Proteinase (HvAP).[203] HvAP was not found to solubilize barley hordeins since it was only found to be located in aleurone cells, and not in the endosperm.[103] The work of Zhang and Jones demonstrated some aspartic proteases in barley, which were relatively similar to one another and to HvAP. Four protease forms were found in barley and six forms in malt. Like HvAP, none the proteases described by Zhang and Jones hydrolyzed hordeins, but rather a globulin preparation.[103, 190] This indicates that aspartic proteases are not a contributing factor in the solubilization of storage proteins, but they might act against globulins during malting. An increased or decreased activity of aspartic protease in connection with a *Fusarium* infection has not been established to date. Therefore, the enzyme load of aspartic protease would have to be entirely cereal in origin.

Serine proteases [E.C. 3.4.21.]

To date, only two serine proteases have been purified from barley, but relatively little knowledge exists about their physiological role.[204] These two serine proteases are Serine Endoprotease 1 (SEP-1) and Hordolisin.[104, 205] The endoprotease SEP-1 was found in barley as well as in green malt after one day of germination, but only in the seedling and in very low amounts. The enzyme activity increased from days two to six of the germination period.[191] At day six of germination, SEP-1 was found in all of the green malt tissues apart from the starchy endosperm.[103] This suggests that SEP-1 is not even involved in hydrolyzation of storage proteins, rather more in mechanisms of regulatory and physiological manner. This includes also a hydrolytic degradation of β-amylase during germination.[206] A study by Fontanini et al. describes SEP-1 having lost its activity at temperatures higher than 70 °C.[204] This could indicate that SEP-1 activity should not be found in malt (which is kilned at temperatures of 80 °C and higher). In addition, the hordolisin does not degrade storage proteins in barley and malt (maybe the misleading trivial name should be changed!). Its contribution to the hydrolization of barley hordein is negligible,[103] and their specific serine-protease inhibitors may have inhibited the serine proteases in this case. A further study by Jones and Budde found that serine class proteases did not affect the release of soluble protein.[207]

Fungal proteases in general can have a wide range of temperatures at which they can be active. Wang et al.

reported this temperature range to be from 10–100 °C.[108] In addition, the pH level ranges widely, from 4.5–8.5. This was learned by analyzing wheat (*Triticum aestivum* L.) for baking purposes, which was infected with *F. culmorum*.[108]

Conclusion

During malting and brewing, multiple enzymes act as biocatalyzers to transform biomolecules. Maltsters and brewers have used the action of these enzymes for thousands of years - knowingly or unknowingly – to produce malt and malt-based beverages. Given the emergence of modern analytical capabilities, the effects of enzymes have become better understood and characterized in recent years. As a result, brewers and maltsters are now in a position to use natural and exogenous enzymes to specifically control the desired conversion processes of the various substrates. In the past, however, only the desired cereal borne enzymes have usually been investigated and taken into account (apart from artificially added technical enzymes and phytohormones). Despite many years of research and the continuous improvement of analytical capabilities, we still have relatively little knowledge about the input of contaminants with respect to enzymatic action. For example, we know that some *Fusarium* spp. can increase proteolysis and cytolysis in malt, but it is in most cases unclear, which enzymes are acting.

In respect to the predominant *Fusarium* species worldwide, which have been described as *F. graminearum*, *F. culmorum*, and *F. avenaceum*,[11–14] it has been reported that, for instance, a *F. graminearum* infection can result in significant changes in the composition of carbohydrates, proteins, and lipids.[107] Pawelzik et al. found (in baking cereals) a high correlation between *Fusarium* infection and the activity of fungal enzymes such as amylases, chitinases, cellulases, glucanases, xylanases, and proteases.[108, 208] The enzymes released by the fungus into the cereal pathogen system also depend upon the species. Are the changes of enzymatic behavior in *Fusarium* infected cereals originated only by the pathogen, or is it a result of host-pathogen interaction? This is the question that should be answered in upcoming years with the use of modern analysis techniques. In addition to the cereal enzymes that also occur and act in non-contaminated material, some of these are also considered to be pathogen-related proteins. The pathogen related proteins and their origins have been well-reviewed by Gorjanović.[164] Due to this mixing of the original cereal enzyme potential and the host-pathogen reaction, the real enzymatic input of the fungal enzymes still remains unknown.

The quality changing aspects, when cereals are infected with *Fusarium* spp. are known.[1, 2] The *Fusarium* spp. affecting the malt quality mainly are reported to be *F. culmorum*, *F. graminearum* and not to be forgotten *F. avenaceum* and *F. tricinctum*.[1, 51] Here some positive and negative effects are reported. Positive attributed effects include amongst others the production of hydrolytic acting enzymes contributing to the modification of malt: amylases, proteases, β-glucanases and xylanases.[209] However, some of the oversupplied enzymes can also affect the quality of the malt and beer negatively. Higher amylolytic activity may lead to a higher malting loss during malting as degraded starch serves to pathogens' nutrition.[1, 24] Higher proteolytic activity may lead to foam problems in beer and in addition with higher cytolytic activity during malting, to an overmodification of malt.[1, 24, 209]

In large, it is not possible to decipher the origin of the enzymes because enzymatic power is mostly analyzed by the decrease in the concentration of the substrate, which is converted into the product by the enzyme, or by the increase in the concentration of the product formed during the enzyme reaction by means of colorimetric methods. The activity of the fungal enzymes can be demonstrated by in vitro studies. However, does this approach reflect the natural processes in grain processing? Only in vivo studies can contribute to the clarification, whereby these studies must be carried out under controlled conditions, e.g., in greenhouse trials. Many hydrolases present in the grain are also contributed to by *Fusarium* ssp. However, the similarity in sequences between cereal and fungal enzymes can be completely different,[138] but the enzyme effect of both is identical. Some of the fungal hydrolases even appear not to be specific,[23] so it may be difficult to distinguish these proteases from cereal enzymes using the conventional methods mentioned above. Suitable, fast, and preferably cost-effective methods must then be developed for research and quality assurance. Molecular-biological methods would be suitable for this purpose. However, to identify the fungal enzymes requires knowledge of the gene sequences of the individual *Fusarium* ssp. The design of a (multiplex)-method for the detection of fungal enzymes would help to better assess the influence of *Fusarium* infection on quality in the future. The detection of enzymes would also be of great benefit in terms of early detection methods for pathogens during the growth of cereals. The development of such methods could help in the future to secure the supply of high quality and easily processable cereals in terms of the varieties of cereals currently grown (little resistance to plant diseases) and as a proactive action due to the climate change, which is already in full progress.

Acknowledgements

This research project was supported by the German Ministry of Economics and Technology (via AiF) and the WiFo (Wissenschaftsförderung der Deutschen Brauwirtschaft e. V.) project AiF 17221 N.

Literature cited

[1] Geißinger, C.; Hofer, K.; Habler, K.; Heß, M.; Hückelhoven, R.; Rychlik, M.; Becker, T.; Gastl, M. Fusarium Species on Barley Malt: Is Visual Assessment an Appropriate Tool for Detection? *Cereal Chem.* **2017**, *94*, 659–669. DOI: 10.1094/CCHEM-08-16-0212-R.

[2] Laitila, A. More Good than Bad: Microbes in Maltings. *Brewer & Distiller International* **2008**, *4*, 52–54.

[3] ASBC Methods of Analysis, Barley Method 3. Germinative Energy. Approved **1958**, rev. 1977. American Society of Brewing Chemists: St. Paul, MN, U.S.A.

[4] Jacob, F. Brautechnische Analysenmethoden - Rohfrucht, Gerste, Malz Hopfen Und Hopfenprodukte. *Selbstverlag Der Mitteleuropäischen Brautechnischen Analysenkommission (MEBAK)*. F. Jacob. Ed.; 2016.

[5] ASBC Methods of Analysis, Barley Method 7. Protein. Approved **1958**, rev. 1977 and 1990.

[6] ASBC Methods of Analysis, Barley Method 5. Moisture. Approved **1958**, rev. 1977.

[7] ASBC Methods of Analysis, Barley Method 2. Weathering and kernel damage. Approved **1958**, rev. 2003.

[8] Petters, H. I.; Flannigan, B.; Austin, B. Quantitative and Qualitative Studies of the Microflora of Barley Malt Production. *J. Appl. Bacteriol.* **1988**, *65*, 279–297. DOI: 10.1111/j.1365-2672.1988.tb01895.x.

[9] Flannigan, B., The Microbiota of Barley and Malt. In *Brewing Microbiology*; F. G. Priest and I. Campbell, Eds.; Springer US: Boston, MA, **2003**; pp 113–180.

[10] Sultan, A.; Frisvad, J. C.; Andersen, B.; Svensson, B.; Finnie, C. Investigation of the Indigenous Fungal Community Populating Barley Grains: Secretomes and Xylanolytic Potential. *J. Proteomics*. **2017**, *169*, 153–164. DOI: 10.1016/j.jprot.2017.03.009.

[11] Parry, D. W.; Jenkinson, P.; McLeod, L. Fusarium Ear Blight (Scab) in Small Grain Cereals—A Review. *Plant Pathol.* **1995**, *44*, 207–238. DOI: 10.1111/j.1365-3059.1995.tb02773.x.

[12] Bottalico, A.; Perrone, G. Toxigenic Fusarium Species and Mycotoxins Associated with Head Blight in Small-Grain Cereals in Europe. *Euro. J. Plant Pathol.* **2002**, *108*, 611–624. DOI: 10.1023/A:1020635214971.

[13] Nielsen, L. K.; Jensen, J. D.; Nielsen, G. C.; Jensen, J. E.; Spliid, N. H.; Thomsen, I. K.; Justesen, A. F.; Collinge, D. B.; Jørgensen, L. N. Fusarium Head Blight of Cereals in Denmark: species Complex and Related Mycotoxins. *Phytopathology* **2011**, *101*, 960–969. DOI: 10.1094/PHYTO-07-10-0188.

[14] Linkmeyer, A.; Götz, M.; Hu, L.; Asam, S.; Rychlik, M.; Hausladen, H.; Hess, M.; Hückelhoven, R. Assessment and Introduction of Quantitative Resistance to Fusarium Head Blight in Elite Spring Barley. *Phytopathology* **2013**, *103*, 1252–1259. DOI: 10.1094/PHYTO-02-13-0056-R.

[15] Wagacha, J. M.; Muthomi, J. W. *Fusarium culmorum*: Infection Process, Mechanisms of Mycotoxin Production and Their Role in Pathogenesis in Wheat. *Crop Prot.* **2007**, *26*, 877–885. DOI: 10.1016/j.cropro.2006.09.003.

[16] Shah, D. A.; Pucci, N.; Infantino, A. Regional and Varietal Differences in the Risk of Wheat Seed Infection by Fungal Species Associated with Fusarium Head Blight in Italy. *Eur. J. Plant Pathol.* **2005**, *112*, 13–21. DOI: 10.1007/s10658-004-6891-5.

[17] Tuşa, C.; Munteanu, I.; Capetti, E.; Pirvu, T.; Bunescu, S.; Sin, G.; Nicolae, H.; Tianu, A.; Caea, D.; Romaşcanu, O.; Stoica, V. Aspects of the Fusarium Attacks on Wheat in Romania. *Probleme de Protecţia Plantelor* **1981**, *9*, 15–31.

[18] Mihuta-Grimm, L.; Forster, R. L. Scab of Wheat and Barley in Southern Idaho and Evaluation of Seed Treatments for Eradication of Fusarium spp. *Plant Dis.* **1989**, *73*, 769–771. DOI: 10.1094/PD-73-0769.

[19] Perkowski, J.; Kiecana, I. Reduction of Yield and Mycotoxins Accumulation in Oats Cultivars after Fusarium Inoculation. *Cereal Research Communications*. **1997**, *25*, 801–803. DOI: 10.1007/BF03543853.

[20] Moschini, R.; Pioli, R.; Carmona, M.; Sacchi, O. Empirical Predictions of Wheat Head Blight in the Northern Argentinean Pampas Region. *Crop Sci.* **2001**, *41*, 1541–1545. DOI: 10.2135/cropsci2001.4151541x.

[21] Pereyra, S.; Vero, S.; Garmendia, G.; Cabrera, M.; Pianzolla, M. Diversity of Fungal Populations Associated with Fusarium Head Blight in Uruguay. In *The Global Fusarium Initiative for International Collaboration: A Strategic Planning Workshop Held at CIMMYT, El Batán, Mexico*. CIMMYT DF: Mexico, **2006**.

[22] Murray, G. M.; Brennan, J. P. Estimating Disease Losses to the Australian Wheat Industry. *Austral. Plant Pathol.* **2009**, *38*, 558–570. DOI: 10.1071/AP09053.

[23] Nightingale, M. J.; Marchylo, B. A.; Clear, R. M.; Dexter, J. E.; Preston, K. R. Fusarium Head Blight: Effect of Fungal Proteases on Wheat Storage Proteins. *Cereal Chem.* **1999**, *76*, 150–158. DOI: 10.1094/CCHEM.1999.76.1.150.

[24] Oliveira, P. M.; Mauch, A.; Jacob, F.; Waters, D. M.; Arendt, E. K. Fundamental Study on the Influence of Fusarium Infection on Quality and Ultrastructure of Barley Malt. *Int. J. Food Microbiol.* **2012**, *156*, 32–43. DOI: 10.1016/j.ijfoodmicro.2012.02.019.

[25] Parikka, P.; Hakala, K.; Tiilikkala, K. Expected Shifts in Fusarium Species' Composition on Cereal Grain in Northern Europe Due to Climatic Change. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* **2012**, *29*, 1543–1555. DOI: 10.1080/19440049.2012.680613.

[26] Torp, M.; Nirenberg, H. I. *Fusarium langsethiae* sp. nov. on Cereals in Europe. *Int J Food Microbiol.* **2004**, *95*, 247–256. DOI: 10.1016/j.ijfoodmicro.2003.12.014.

[27] Uhlig, S.; Jestoi, M.; Parikka, P. *Fusarium avenaceum* - The North European Situation. *Int. J. Food Microbiol.* **2007**, *119*, 17–24. DOI: 10.1016/j.ijfoodmicro.2007.07.021.

[28] Dinolfo, M. I.; Castañares, E.; Stenglein, S. A. Fusarium–Plant Interaction: state of the Art – A Review. *Plant Protect. Sci* **2017**, *53*, 61–70.

[29] Wegulo, S. N.; Baenziger, P. S.; Hernandez Nopsa, J.; Bockus, W. W.; Hallen-Adams, H. Management of Fusarium Head Blight of Wheat and Barley. *Crop Prot.* **2015**, *73*, 100–107. DOI: 10.1016/j.cropro.2015.02.025.

[30] Narziss, L.; Back, W. *Die Bierbrauerei: Band 1 - Die Technologie Der Malzbereitung*. Wiley-VCH: Weinheim, Germany, 2012.

[31] Backhouse, D.; Burgess, L. W. Climatic Analysis of the Distribution of *Fusarium graminearum, F. pseudograminearum* and *F. culmorum* on Cereals in Australia. *Austral. Plant Pathol.* **2002**, *31*, 321–327. DOI: 10.1071/AP02026.

[32] Burgess, L. W.; Klein, T. A.; Bryden, W. L.; Tobin, N. F. Head Blight of Wheat Caused by *Fusarium graminearum* Group 1 in New South Wales in 1983. *Austral. Plant Pathol.* **1987**, *16*, 72–78. DOI: 10.1071/APP9870072.

[33] Statista. *World Barley Production from* 2008*/2009 to 2017/2018*. 06.03.2018; Available from: https://de.statista.com/statistik/studie/id/29740/dokument/getreide-statista-dossier.

[34] Eurostat. *Verkaufspreis von Braugerste in der Europäischen Union nach Ländern in den Jahren* 2007 *bis 2017*. 06.03.2018; Available from: https://de.statista.com/statistik/daten/studie/457554/umfrage/verkaufspreis-von-braugerste-in-der-eu-nach-laendern/.

[35] Janssen, E.; Liu, C.; Fels, HJvd. Fusarium Infection and Trichothecenes in Barley and Its Comparison with Wheat. *World Mycotoxin Journal* **2018**, *11*, 33–46. DOI: 10.3920/WMJ2017.2255.

[36] Pautasso, M.; Döring, T. F.; Garbelotto, M.; Pellis, L.; Jeger, M. J. Impacts of Climate Change on Plant Diseases—Opinions and Trends. *Eur. J. Plant Pathol.* **2012**, *133*, 295–313. DOI: 10.1007/s10658-012-9936-1.

[37] Jeger, M. J.; Pautasso, M. Plant Disease and Global change-the importance of long-term data sets. *New Phytol.* **2008**, *177*, 8–11. DOI: 10.1111/j.1469-8137.2007.02312.x.

[38] Hernandez Nopsa, J. F.; Thomas-Sharma, S.; Garrett, K. A. Climate Change and Plant Disease. In *Encyclopedia of Agriculture and Food Systems*, N.K. Van Alfen, Ed. Academic Press: Oxford, **2014**; pp 232–243.

[39] Kriss, A. B.; Paul, P. A.; Madden, L. V. Variability in Fusarium Head Blight Epidemics in Relation to Global Climate Fluctuations as Represented by the El Niño-Southern Oscillation and Other Atmospheric Patterns. *Phytopathology* **2012**, *102*, 55–64. DOI: 10.1094/PHYTO-04-11-0125.

[40] Schwarz, P. B. Fusarium Head Blight and Deoxynivalenol in Malting and Brewing: successes and Future Challenges. *Trop. plant Pathol.* **2017**, *42*, 153–164. DOI: 10.1007/s40858-017-0146-4.

[41] Leppelt, T.; Janssen, W.; Koppe, C. *Trockenheit 2018 in Deutschland*. Deutscher Wetterdienst, **2018**.
[42] Hofer, K.; Hückelhoven, R.; Hess, M. Analysis of Archive Samples of Spring and Winter Barley Support an Increase in Individual Fusarium Species in Bavarian Barley Grain over the Last Decades. *J. Plant Dis. Prot*. **2019**, *126*, 247–254. DOI: 10.1007/s41348-019-00220-0.
[43] Dawson, I. K.; Russell, J.; Powell, W.; Steffenson, B.; Thomas, W. T. B.; Waugh, R. Barley: A Translational Model for Adaptation to Climate Change. *New Phytol*. **2015**, *206*, 913–931. DOI: 10.1111/nph.13266.
[44] Brisson, N.; Gate, P.; Gouache, D.; Charmet, G.; Oury, F.-X.; Huard, F. Why Are Wheat Yields Stagnating in Europe? A Comprehensive Data Analysis for France. *Field Crops Res*. **2010**, *119*, 201–212. DOI: 10.1016/j.fcr.2010.07.012.
[45] Linkmeyer, A.; Hofer, K.; Rychlik, M.; Herz, M.; Hausladen, H.; Hückelhoven, R.; Hess, M. Influence of Inoculum and Climatic Factors on the Severity of Fusarium Head Blight in German Spring and Winter Barley. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* **2016**, *33*, 489–499. DOI: 10.1080/19440049.2015.1133932.
[46] Hückelhoven, R.; Hofer, K.; Coleman, A.; Heß, M. Fusarium Infection of Malting Barley Has to Be Managed over the Entire Value Chain. *J. Plant Dis. Prot*. **2018**, *125*, 1–4. DOI: 10.1007/s41348-017-0101-0.
[47] Yousif, A. M.; Evans, D. E. Changes in Malt Quality during Production in Two Commercial Malt Houses. *J. Inst. Brew*. **2020**, *126*, 233–252. DOI: 10.1002/jib.609.
[48] Mäkinen, O. E.; Arendt, E. K. Nonbrewing Applications of Malted Cereals, Pseudocereals, and Legumes: A Review. *J. Am. Soc. Brew. Chem*. **2015**, *73*, 223–227. DOI: 10.1094/ASBCJ-2015-0515-01.
[49] Habte, T.; Habte, M.; Kumar, V. Nonstarch Polysaccharide Enzymes—General Aspects. In *Enzymes in Human and Animal Nutrition*, C.S. Nunes and V. Kumar, Eds.; Academic Press; **2018**. pp 183–209.
[50] Evans, D. E.; Li, C.; K.; Eglinton, J. The Properties and Genetics of Barley Malt Starch Degrading Enzymes. Chapter 6 in: *Genetics and Improvement of Barley Malt Quality*. G. Zhang and C. Li, Eds.; Springer: New York, **2010**; pp 143–189.
[51] Justé, A.; Malfliet, S.; Lenaerts, M.; De Cooman, L.; Aerts, G.; Willems, K.; Lievens, B. Microflora during Malting of Barley: Overview and Impact on Malt Quality. *Brew. Sci*. **2011**, *64*, 22–31.
[52] Guido, L.; Moreira, M. *Malting, in Engineering Aspects of Cereal and Cereal-Based Product*, G. Raquel and C. Paula, Eds.; CRC Press: New York, **2013**; pp 51–70.
[53] Vegi, A.; Schwarz, P.; Wolf-Hall, C. E. Quantification of Tri5 Gene, Expression, and Deoxynivalenol Production during the Malting of Barley. *Int. J. Food Microbiol*. **2011**, *150*, 150–156. DOI: 10.1016/j.ijfoodmicro.2011.07.032.
[54] Geißinger, C.; Whitehead, I.; Hofer, K.; Heß, M.; Habler, K.; Becker, T.; Gastl, M. Influence of *Fusarium avenaceum* Infections on Barley Malt: Monitoring Changes in the Albumin Fraction of Barley during the Malting Process. *Int J Food Microbiol*. **2019**, *293*, 7–16. DOI: 10.1016/j.ijfoodmicro.2018.12.026.
[55] Habler, K.; Hofer, K.; Geißinger, C.; Schüler, J.; Hückelhoven, R.; Hess, M.; Gastl, M.; Rychlik, M. Fate of Fusarium Toxins during the Malting Process. *J Agric Food Chem*. **2016**, *64*, 1377–1384. DOI: 10.1021/acs.jafc.5b05998.
[56] MacGregor, A. W. Changes in α-Amylase Enzymes during Germination. *J. Am. Soc. Brew. Chem*. **1978**, *36*, 1–5. DOI: 10.1094/ASBCJ-36-0001.
[57] Brennan, C. S.; Amor, M. A.; Harris, N.; Smith, D.; Cantrell, I.; Griggs, D.; Shewry, P. R. Cultivar Differences in Modification Patterns of Protein and Carbohydrate Reserves during Malting of Barley. *J. Cereal Sci*. **1997**, *26*, 83–93. DOI: 10.1006/jcrs.1996.0103.
[58] Pollock, J. R. A.; Pool, A. A. Enzymes of Barley and Malt III. The Latent β-Amylase of Barley. *J. Inst. Brew*. **1958**, *64*, 151–156. DOI: 10.1002/j.2050-0416.1958.tb06307.x.
[59] Longstaff, M. A.; Bryce, J. H. Development of Limit Dextrinase in Germinated Barley (*Hordeum vulgare* L.) (Evidence of Proteolytic Activation). *Plant Physiol*. **1993**, *101*, 881–889. DOI: 10.1104/pp.101.3.881.
[60] Schroeder, S. W.; MacGregor, A. W. Synthesis of Limit Dextrinase in Germinated Barley Kernels and Aleurone Tissues. *J. Am. Soc. Brew. Chem*. **1998**, *56*, 32–37. DOI: 10.1094/ASBCJ-56-0032.
[61] Böhm-Schraml, M. C. Fusarienwachstum Und Toxinbildung Während Der Mälzung — Entwicklung Von Selektivnährböden Zum Nachweis Toxinbildender Fusarien. *Technische Universität München*. TUM: Freising; **1995**.
[62] Laitila, A.; Kotaviita, E.; Peltola, P.; Home, S.; Wilhelmson, A. Indigenous Microbial Community of Barley Greatly Influences Grain Germination and Malt Quality. *J. Inst. Brew*. **2007**, *113*, 9–20. DOI: 10.1002/j.2050-0416.2007.tb00250.x.
[63] Tsitko, I.; Manninen, J.; Smart, K.; James, S.; Laitila, A. Management of Barley-Associated Bacterial Biofilms: A Key to Improving Wort Separation. *J. Inst. Brew*. **2018**, *124*, 325–335. DOI: 10.1002/jib.506.
[64] Wolf-Hall, C. E. Mold and Mycotoxin Problems Encountered during Malting and Brewing. *Int. J. Food Microbiol*. **2007**, *119*, 89–94. DOI: 10.1016/j.ijfoodmicro.2007.07.030.
[65] Kottapalli, B.; Wolf-Hall, C. E.; Schwarz, P. Evaluation of Gaseous Ozone and Hydrogen Peroxide Treatments for Reducing Fusarium Survival in Malting Barley. *J. Food Prot*. **2005**, *68*, 1236–1240. DOI: 10.4315/0362-028X-68.6.1236.
[66] Kottapalli, B.; Wolf-Hall, C. E. Effect of Hot Water Treatments on the Safety and Quality of Fusarium-Infected Malting Barley. *Int J Food Microbiol*. **2008**, *124*, 171–178. DOI: 10.1016/j.ijfoodmicro.2008.03.010.
[67] Kottapalli, B.; Wolf-Hall, C. E.; Schwarz, P. Effect of Electron-Beam Irradiation on the Safety and Quality of Fusarium-Infected Malting Barley. *Int J Food Microbiol*. **2006**, *110*, 224–231. DOI: 10.1016/j.ijfoodmicro.2006.04.007.
[68] Bakri, Y.; Jawhar, M.; Arabi, M. Enzymatic Activity of the Endophytic Fusarium Species Strains Isolated from Wheat. *Adv. Hortic. Sci*. **2014**, *28*, 129–132.
[69] Kikot, G.; Hours, R.; Alconada, T. Contribution of Cell Wall Degrading Enzymes to Pathogenesis of Fusarium graminearum: A Review. *J Basic Microbiol*. **2009**, *49*, 231–241. DOI: 10.1002/jobm.200800231.
[70] Schwarz, P. B.; Jones, B. L.; Steffenson, B. J. Enzymes Associated with Fusarium Infection of Barley. *J. Am. Soc. Brew. Chem*. **2002**, *60*, 130–134. DOI: 10.1094/ASBCJ-60-0130.
[71] Kikot, G. E.; Hours, R. A.; Alconada, T. M. Extracellular Enzymes of *Fusarium graminearum* Isolates. *Braz. Arch. Biol. Technol*. **2010**, *53*, 779–783. DOI: 10.1590/S1516-89132010000400005.
[72] Trivedi, L. S.; Rao, K. K. Production of Cellulolytic Enzymes by Fusarium Species. *Biotechnol. Lett*. **1981**, *3*, 281–284. DOI: 10.1007/BF00127395.
[73] Ortega, L. M.; Kikot, G. E.; Astoreca, A. L.; Alconada, T. M. Screening of *Fusarium graminearum* Isolates for Enzymes Extracellular and Deoxynivalenol Production. *J. Mycol*. **2013**, **2013**, 1–7. DOI: 10.1155/2013/358140.
[74] Yang, F.; Jacobsen, S.; Jørgensen, H.; Collinge, D.; Svensson, B.; Finnie, C. *Fusarium graminearum* and Its Interactions with Cereal Heads: Studies in the Proteomics Era. *Front Plant Sci*. **2013**, *4*, 37. DOI: 10.3389/fpls.2013.00037.
[75] Dong, X.; Meinhardt, S. W.; Schwarz, P. B. Isolation and Characterization of Two Endoxylanases from *Fusarium graminearum*. *J. Agric. Food Chem*. **2012**, *60*, 2538–2545. DOI: 10.1021/jf203407p.
[76] Voss-Fels, K. P.; Qian, L.; Gabur, I.; Obermeier, C.; Hickey, L. T.; Werner, C. R.; Kontowski, S.; Frisch, M.; Friedt, W.; Snowdon, R. J.; Gottwald, S. Genetic Insights into Underground Responses to *Fusarium graminearum* Infection in Wheat. *Sci. Rep*. **2018**, *8*, 13153 DOI: 10.1038/s41598-018-31544-w.
[77] Jin, X.; Feng, D.; Wang, H.; Wang, J. A Novel tissue-specific plantain beta-1,3-glucanase gene that is regulated in response to infection by *Fusarium oxysporum* fsp. cubense. *Biotechnol. Lett*. **2007**, *29*, 1431–1437. DOI: 10.1007/s10529-007-9403-9.

[78] Hofer, K.; Geißinger, C.; König, C.; Gastl, M.; Hückelhoven, R.; Heß, M.; Coleman, A. D. Influence of Fusarium Isolates on the Expression of Barley Genes Related to Plant Defense and Malting Quality. *J. Cereal Sci.* **2016**, *69*, 17–24. DOI: 10.1016/j.jcs.2016.02.005.

[79] Schmidt, M.; Horstmann, S.; De Colli, L.; Danaher, M.; Speer, K.; Zannini, E.; Arendt, E. K. Impact of Fungal Contamination of Wheat on Grain Quality Criteria. *J. Cereal Sci.* **2016**, *69*, 95–103. DOI: 10.1016/j.jcs.2016.02.010.

[80] Nielsen, L. K.; Cook, D. J.; Edwards, S. G.; Ray, R. V. The Prevalence and Impact of Fusarium Head Blight Pathogens and Mycotoxins on Malting Barley Quality in UK. *Int. J. Food Microbiol.* **2014**, *179*, 38–49. DOI: 10.1016/j.ijfoodmicro.2014.03.023.

[81] Pekkarinen, A.; Mannonen, L.; Jones, B. L.; Niku-Paavola, M. L. Production of Proteases by Fusarium Species Grown on Barley Grains and in Media Containing Cereal Proteins. *J. Cereal Sci.* **2000**, *31*, 253–261. DOI: 10.1006/jcrs.2000.0305.

[82] Oliveira, P. M.; Waters, D.; Arendt, E. The Impact of *Fusarium culmorum* Infection on the Protein Fractions of Raw Barley and Malted Grains. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 2053–2065. DOI: 10.1007/s00253-013-4696-1.

[83] Phalip, V.; Delalande, F.; Carapito, C.; Goubet, F.; Hatsch, D.; Leize, E.; Dupree, P.; Dorsselaer, A.; Jeltsch, J.-M. Diversity of the Exoproteome of *Fusarium graminearum* Grown on Plant Cell Wall. *Curr. Genet.* **2005**, *48*, 366–379. DOI: 10.1007/s00294-005-0040-3.

[84] Pekkarinen, A. I.; Sarlin, T. H.; Laitila, A. T.; Haikara, A. I.; Jones, B. L. Fusarium Species Synthesize Alkaline Proteinases in Infested Barley. *J. Cereal Sci.* **2003**, *37*, 349–356. DOI: 10.1006/jcrs.2002.0512.

[85] Paper, J. M.; Scott-Craig, J. S.; Adhikari, N. D.; Cuomo, C. A.; Walton, J. D. Comparative Proteomics of Extracellular Proteins in Vitro and in Planta from the Pathogenic Fungus *Fusarium graminearum*. *Proteomics* **2007**, *7*, 3171–3183. DOI: 10.1002/pmic.200700184.

[86] Pekkarinen, A. I.; Jones, B. L.; Niku-Paavola, M.-L. Purification and Properties of an Alkaline Proteinase of *Fusarium culmorum*. *Eur. J. Biochem.* **2002**, *269*, 798–807. DOI: 10.1046/j.0014-2956.2001.02697.x.

[87] Ballance, G. M.; Meredith, W. O. S. Purification and Partial Characterization of an Endo-β1,3-Glucanase from Green Malt. *J. Inst. Brew.* **1976**, *82*, 64–67. DOI: 10.1002/j.2050-0416.1976.tb03726.x.

[88] Yin, X. S.; MacGregor, A. W.; Clear, R. M. Field Fungi and β-Glucan Solubilase in Barley Kernels. *J. Inst. Brew.* **1989**, *95*, 195–198. DOI: 10.1002/j.2050-0416.1989.tb04626.x.

[89] Bamforth, C. W.; Moore, J.; McKillop, D.; Williamson, G.; Kroon, P. A. Enzymes from Barley Which Solubilize β-Glucan. *Proc. Eur. Brew. Conv. Cong. Maastricht*. IRL Press, Oxford; **1997**. pp 75–82.

[90] Lapitan, N. L. V.; Hess, A.; Cooper, B.; Botha, A.-M.; Badillo, D.; Iyer, H.; Menert, J.; Close, T.; Wright, L.; Hanning, G.; et al. Differentially Expressed Genes during Malting and Correlation with Malting Quality Phenotypes in Barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* **2009**, *118*, 937–952. DOI: 10.1007/s00122-008-0951-8.

[91] Watson, L.; Henry, R. J. Microarray Analysis of Gene Expression in Germinating Barley Embryos (Hordeum Vulgare L.). *Funct. Integr. Genomics*. **2005**, *5*, 155–162. DOI: 10.1007/s10142-005-0133-6.

[92] White, J. F. Gene Expression Analysis of Malting Barley. PhD Thesis, Southern Cross University: Lismore, NSW, **2009**.

[93] Dilworth, L. L.; Riley, C. K.; Stennett, D. K. Plant Constituents: Carbohydrates, Oils, Resins, Balsams, and Plant Hormones. In *Pharmacognosy*, S. Badal and R. Delgoda, Eds.; Academic Press: Boston, **2017**; pp 61–80.

[94] Vallée, F.; Kadziola, A.; Bourne, Y.; Juy, M.; Rodenburg, K. W.; Svensson, B.; Haser, R. Barley Alpha-Amylase Bound to Its Endogenous Protein Inhibitor BASI: Crystal Structure of the Complex at 1.9 a Resolution. *Structure* **1998**, *6*, 649–659. DOI: 10.1016/S0969-2126(98)00066-5.

[95] Sun, Z.; Henson, C. A. A Quantitative Assessment of the Importance of Barley Seed α-Amylase, β-Amylase, Debranching Enzyme, and α-Glucosidase in Starch Degradation. *Arch. Biochem. Biophys.* **1991**, *284*, 298–305. DOI: 10.1016/0003-9861(91)90299-X.

[96] Andriotis, V. M. E.; Rejzek, M.; Barclay, E.; Rugen, M. D.; Field, R. A.; Smith, A. M. Cell Wall Degradation is Required for Normal Starch Mobilisation in Barley Endosperm. *Sci. Rep.* **2016**, *6*, 33215 DOI: 10.1038/srep33215.

[97] Evans, D. E.; Wallace, W.; Lance, R. C. M.; MacLeod, L. C. Measurement of Beta-Amylase in Malting Barley (*Hordeum vulgare* L.). II. The Effect of Germination and Kilning. *J. Cereal Sci.* **1997**, *26*, 241–250. DOI: 10.1006/jcrs.1997.0120.

[98] Vinje, M. A.; Walling, J. G.; Henson, C. A.; Duke, S. H. Comparative Gene Expression Analysis of the β-amylase and hordein gene families in the developing barley grain. *Gene* **2019**, *693*, 127–136. DOI: 10.1016/j.gene.2018.12.041.

[99] Vinje, M. A.; Duke, S. H.; Henson, C. A. De Novo Expression of β-amylase2 (Bmy2) in Barley Grains during Micromalting. *J. Am. Soc. Brew. Chem.* **2020**, *78*, 126–135. DOI: 10.1080/03610470.2019.1705104.

[100] Stanley, D.; Rejzek, M.; Naested, H.; Smedley, M.; Otero, S.; Fahy, B.; Thorpe, F.; Nash, R. J.; Harwood, W.; Svensson, B.; et al. The Role of Alpha-Glucosidase in Germinating Barley Grains. *Plant Physiol.* **2011**, *155*, 932–943. DOI: 10.1104/pp.110.168328.

[101] Wallace, W.; Lance, R. C. M. The Protein Reserves of the Barley Grain and Their Degradation during Malting and Brewing. *J. Inst. Brew.* **1988**, *94*, 379–386. DOI: 10.1002/j.2050-0416.1988.tb04598.x.

[102] Sopanen, T.; Takkinen, P.; Mikola, J.; Enari, T.-M. Rate Limiting Enzymes in the Liberation of Amino Acids in Mashing. *J. Inst. Brew.* **1980**, *86*, 211–215. DOI: 10.1002/j.2050-0416.1980.tb06868.x.

[103] Jones, B. L. Endoproteases of Barley and Malt. *J. Cereal Sci.* **2005**, *42*, 139–156. DOI: 10.1016/j.jcs.2005.03.007.

[104] Bell, A. Analysis of the Barley Grain Protease Spectrum. PhD thesis, Heriot-Watt University, Edinburgh, **2012**.

[105] Fincher, G. B. Molecular and Cellular Biology Associated with Endosperm Mobilization in Germinating Cereal-Grains. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1989**, *40*, 305–346. DOI: 10.1146/annurev.pp.40.060189.001513.

[106] Bønsager, B. C.; Finnie, C.; Roepstorff, P.; Svensson, B. Spatio-Temporal Changes in Germination and Radical Elongation of Barley Seeds Tracked by Proteome Analysis of Dissected Embryo, Aleurone Layer, and Endosperm Tissues. *Proteomics* **2007**, *7*, 4528–4540. DOI: 10.1002/pmic.200700766.

[107] Boyacioğlu, D.; Hettiarachchy, N. S. Changes in Some Biochemical Components of Wheat Grain That Was Infected with *Fusarium graminearum*. *J. Cereal Sci.* **1995**, *21*, 57–62. DOI: 10.1016/S0733-5210(95)80008-5.

[108] Wang, J.; Wieser, H.; Pawelzik, E.; Weinert, J.; Keutgen, A.; Wolf, G. A. Impact of the Fungal Protease Produced by *Fusarium culmorum* on the Protein Quality and Breadmaking Properties of Winter Wheat. *Eur. Food Res. Technol.* **2005**, *220*, 552–559. DOI: 10.1007/s00217-004-1112-1.

[109] Wieser, H. Simple Determination of Gluten Protein Types in Wheat Flour by Turbidimetry. *Cereal Chem.* **2000**, *77*, 48–52. DOI: 10.1094/CCHEM.2000.77.1.48.

[110] Noots, I.; Delcour, J. A.; Michiels, C. W. From Field Barley to Malt: Detection and Specification of Microbial Activity for Quality Aspects. *Crit. Rev. Microbiol.* **1999**, *25*, 121–153. DOI: 10.1080/10408419991299257.

[111] Hoy, J. L.; Macauley, B. J.; Fincher, G. B. Cellulases of Plant and Microbial Origin in Germinating Barley. *J. Inst. Brew.* **1981**, *87*, 77–80. DOI: 10.1002/j.2050-0416.1981.tb03990.x.

[112] Sarlin, T.; Laitila, A.; Pekkarinen, A.; Haikara, A. Effects of Three Fusarium Species on the Quality of Barley and Malt. *J. Am. Soc. Brew. Chem.* **2005**, *63*, 43–49. DOI: 10.1094/ASBCJ-63-0043.

[113] Sloey, W.; Prentice, N. Effects of Fusarium Isolates Applied during Malting on Properties of Malt. *Proc. Am. Soc. Brew. Chem.* **1962**, *20*, 24–29. DOI: 10.1080/00960845.1962.12007060.

[114] Prentice, N.; Sloey, W. Studies on Barley Microflora of Possible Importance to Malting and Brewing Quality. I. The Treatment

of Barley during Malting with Selected Microorganisms. *Proc. Am. Soc. Brew. Chem.* **1960**, *18*, 28–33. DOI: 10.1080/00960845.1960.12006893.

[115] Gjertsen, P.; Trolle, B.; Andersen, K. Studies on Gushing Caused by Microorganisms, Specially Fusarium Spec. *Proc. Eur. Brew. Conv. Congr.* Elsevier, Amsterdam, **1965**; pp 428–438.

[116] Haikara, A. Malt and Beer from Barley Artificially Contaminated with Fusarium in the Field. *Proc. Eur. Brew. Conv. Congr. London*. IRL Press: Oxford, **1983**; pp 401–408.

[117] Angelino, S. A. G. F.; Bol, J. Impact of Microflora during Storage and Malting on Malt Properties. In *Proc. Chair J. De Clerck IV*. Leuven, **1990**; pp 1–14.

[118] Flannigan, B.; Sellars, P. N. Amylase, β-Glucosidase and β-Xylosidase Activity of Thermotolerant and Thermophilic Fungi Isolated from Barley. *Trans. British Mycolog. Soc.* **1977**, *69*, 316–317. DOI: 10.1016/S0007-1536(77)80053-3.

[119] Vinje, M. A.; Willis, D. K.; Duke, S. H.; Henson, C. A. Differential RNA Expression of Bmy1 during Barley Seed Development and the Association with β-Amylase Accumulation, Activity, and Total Protein. *Plant Physiol. Biochem.* **2011**, *49*, 39–45. DOI: 10.1016/j.plaphy.2010.09.019.

[120] Schwarz, P. B.; J. Y.; H. Impact of Fusarium Head Blight on Malting and Brewing Quality of Barley. In *Fusarium Head Blight of Wheat and Barley*, K.J. Leonard and W.R. Bushnell, Eds.; American Phytopathological Society: St Paul, Minnesota, USA, **2003**; pp 395–419.

[121] McCleary, B. V.; McNally, M.; Monaghan, D.; Mugford, D. C. Measurement of α-Amylase Activity in White Wheat Flour, Milled Malt, and Microbial Enzyme Preparations, Using the Ceralpha Assay: Collaborative Study. *J. AOAC Int.* **2002**, *85*, 1096–1102. DOI: 10.1093/jaoac/85.5.1096.

[122] Keßler, M. T. Analytical Evaluation of Starch Degradation in Barley and Barley Malt and Its Significance for the Brewing Process. Dissertation, TUM, Germany, **2006**.

[123] Duffus, C. M. α-Amylase Activity in the Developing Barley Grain and Its Dependence on Gibberellic Acid. *Phytochemistry* **1969**, *8*, 1205–1209. DOI: 10.1016/S0031-9422(00)85558-2.

[124] MacGregor, A. W.; LaBerge, D. E.; Meredith, W. O. S. Changes in Barley Kernels during Growth and Maturation. *Cereal Chem.* **1971**, *48*, 255–269.

[125] Georg-Kraemer, J. E.; Mundstock, E. C.; Cavalli-Molina, S. Developmental Expression of Amylases during Barley Malting. *J. Cereal Sci.* **2001**, *33*, 279–288. DOI: 10.1006/jcrs.2001.0367.

[126] Radchuk, V. V.; Borisjuk, L.; Sreenivasulu, N.; Merx, K.; Mock, H.-P.; Rolletschek, H.; Wobus, U.; Weschke, W. Spatiotemporal Profiling of Starch Biosynthesis and Degradation in the Developing Barley Grain. *Plant Physiol.* **2009**, *150*, 190–204. DOI: 10.1104/pp.108.133520.

[127] Derera, N. F. *Preharvest Field Sprouting in Cereals*. CRC Press, 2018.

[128] Muralikrishna, G.; Nirmala, M. Cereal α-Amylases—An Overview. *Carbohydr. Polym.* **2005**, *60*, 163–173. DOI: 10.1016/j.carbpol.2004.12.002.

[129] Rogers, J. C.; Milliman, C. Isolation and Sequence Analysis of a Barley Alpha-Amylase cDNA Clone. *J. Biol. Chem.* **1983**, *258*, 8169–8174. DOI: 10.1016/S0021-9258(20)82044-4.

[130] Khursheed, B.; Rogers, J. C. Barley Alpha-Amylase Genes. Quantitative Comparison of Steady-State mRNA Levels from Individual Members of the Two Different Families Expressed in Aleurone Cells. *J. Biol. Chem.* **1988**, *263*, 18953–18960. DOI: 10.1016/S0021-9258(18)37374-5.

[131] Rahmatullah, R. J.; Huang, J. K.; Clark, K. L.; Reeck, G. R.; Chandra, G.; Muthukrishnan, S. Nucleotide and Predicted Amino Acid Sequences of Two Different Genes for High-pI α-Amylases from Barley. *Plant Mol Biol* **1989**, *12*, 119–121. DOI: 10.1007/BF00017454.

[132] Stenholm, K. Malt Limit Dextrinase and Its Importance in Brewing. In *VTT Biotechnology and Food Research*; Technical Research Center of Finland: Espoo, Finland, **1997**.

[133] Munck, L.; Mundy, J.; Vaag, P. Characterization of Enzyme Inhibitors in Barley and Their Tentative Role in Malting and Brewing. *J. Am. Soc. Brew. Chem.* **1985**, *43*, 35–38. DOI: 10.1094/ASBCJ-43-0035.

[134] Sidenius, U.; Olsen, K.; Svensson, B.; Christensen, U. Stopped-Flow Kinetic Studies of the Reaction of Barley α-Amylase/Subtilisin Inhibitor and the High pI Barley α-Amylase. *FEBS Lett.* **1995**, *361*, 250–254. DOI: 10.1016/0014-5793(95)00187-E.

[135] MacGregor, A. W.; Bazin, S. L.; Izydorczyk, M. S. Gelatinisation Characteristics and Enzyme Susceptibility of Different Types of Barley Starch in the Temperature Range 48–72°C. *J. Inst. Brew.* **2002**, *108*, 43–47. DOI: 10.1002/j.2050-0416.2002.tb00121.x.

[136] MacGregor, A. W.; Marchylo, B. A.; Kruger, J. E. Multiple Alpha-Amylase Components in Germinated Cereal Grains Determined by Isoelectric Focusing and Chromatofocusing. *Cereal Chem.* **1988**, *65*, 326–333.

[137] Huang, J. K.; Swegle, M.; Dandekar, A. M.; Muthukrishnan, S. Expression and Regulation of Alpha-Amylase Gene Family in Barley Aleurones. *J. Mol. Appl. Genet.* **1984**, *2*, 579–588.

[138] Robert, X.; Haser, R.; Svensson, B.; Aghajari, N. Comparison of Crystal Structures of Barley α-Amylase 1 and 2: Implications for Isozyme Differences in Stability and Activity. *Biologia* **2002**, *57*, 59–70.

[139] Rogers, J. C. Conserved Amino Acid Sequence Domains in Alpha-Amylases from Plants, Mammals, and Bacteria. *Biochem. Biophys. Res. Commun.* **1985**, *128*, 470–476. DOI: 10.1016/0006-291X(85)91702-4.

[140] McCleary, B. V.; Codd, R. Measurement of β-Amylase in Cereal Flours and Commercial Enzyme Preparations. *J. Cereal Sci.* **1989**, *9*, 17–33. DOI: 10.1016/S0733-5210(89)80018-9.

[141] Guerin, J. R.; Lance, R. C. M.; Wallace, W. Release and Activation of Barley Beta-Amylase by Malt Endopeptidases. *J. Cereal Sci.* **1992**, *15*, 5–14. DOI: 10.1016/S0733-5210(09)80052-0.

[142] LaBerge, D. E.; Marchylo, B. A. Changes in Beta-Amylase Enzymes during Kernel Development of Barley and the Effect of Papain as an Extractant. *J. Am. Soc. Brew. Chem.* **1986**, *44*, 16–19. DOI: 10.1094/ASBCJ-44-0016.

[143] MacGregor, A. W.; Bhatty, R. S. *Barley: Chemistry and Technology*. American Association of Cereal Chemists. St. Paul, MN, **1993**.

[144] Bendelow, V. M. Inheritance of Free Beta-Amylase in Barley. *Can. J. Plant Sci.* **1964**, *44*, 550–554. DOI: 10.4141/cjps64-108.

[145] Hejgaard, J. Free and Protein-Bound β-Amylases of Barley Grain. Characterization by Two-Dimensional Immunoelectrophoresis. *Physiol. Plant.* **1976**, *38*, 293–299. DOI: 10.1111/j.1399-3054.1976.tb04006.x.

[146] Clark, S. E.; Hayes, P. M.; Henson, C. A. Effects of Single Nucleotide Polymorphisms in β-amylase1 Alleles from Barley on Functional Properties of the Enzymes. *Plant Physiol. Biochem.* **2003**, *41*, 798–804. DOI: 10.1016/S0981-9428(03)00118-9.

[147] Jung, W.; Skadsen, R. W.; Peterson, D. M. Characterization of a Novel Barley β-Amylase Gene Expressed Only during Early Grain Development. *Seed Sci. Res.* **2007**, *11*, 325–333.

[148] Hardie, D. G. Control of Carbohydrase Formation by Gibberellic Acid in Barley Endosperm. *Phytochemistry* **1975**, *14*, 1719–1722. DOI: 10.1016/0031-9422(75)85281-2.

[149] Perlikowski, D.; Wiśniewska, H.; Kaczmarek, J.; Góral, T.; Ochodzki, P.; Kwiatek, M.; Majka, M.; Augustyniak, A.; Kosmala, A. Alterations in Kernel Proteome after Infection with *Fusarium culmorum* in Two Triticale Cultivars with Contrasting Resistance to Fusarium Head Blight. *Front Plant Sci.* **2016**, *7*, 1217–1217. DOI: 10.3389/fpls.2016.01217.

[150] Eggert, K.; Zörb, C.; Mühling, K. H.; Pawelzik, E. Proteome Analysis of Fusarium Infection in Emmer Grains (*Triticum dicoccum*). *Plant Pathology* **2011**, *60*, 918–928. DOI: 10.1111/j.1365-3059.2011.02442.x.

[151] Yang, F.; Jensen, J. D.; Svensson, B.; Jørgensen, H. J. L.; Collinge, D. B.; Finnie, C. Analysis of Early Events in the Interaction between *Fusarium graminearum* and the Susceptible Barley (*Hordeum vulgare*) Cultivar Scarlett. *Proteomics* **2010**, *10*, 3748–3755. DOI: 10.1002/pmic.201000243.

[152] Mangan, D.; McCleary, B. V.; Cornaggia, C.; Ivory, R.; Rooney, E.; McKie, V. Colourimetric and Fluorimetric Substrates for the Assay of Limit Dextrinase. *J. Cereal Sci.* **2015**, *62*, 50–57. DOI: 10.1016/j.jcs.2014.12.006.

[153] Evans, D. E.; Redd, K.; Haraysmow, S. E.; Elvig, N.; Metz, N.; Koutoulis, A. The Influence of Malt Quality on Malt Brewing and Barley Quality on Barley Brewing with Ondea Pro, Compared by Small-Scale Analysis. *J. Am. Soc. Brew. Chem*. **2014**, *72*, 192–207. DOI: 10.1094/ASBCJ-2014-0630-01.

[154] Lee, W. J.; Pyler, R. E. Barley Malt Limit Dextrinase: Varietal, Environmental, and Malting Effects. *J. Am. Soc. Brew. Chem* **1984**, *42*, 11–17. DOI: 10.1094/ASBCJ-42-0011.

[155] Stenholm, K.; Home, S. *A* New Approach to Limit Dextrinase and Its Role in Mashing. *J. Inst. Brew.* **1999**, *105*, 205–210. DOI: 10.1002/j.2050-0416.1999.tb00020.x.

[156] Ross, H. A.; Sungurtas, J.; Ducreux, L.; Swanston, J. S.; Davies, H. V.; McDougall, G. J. Limit Dextrinase in Barley Cultivars of Differing Malting Quality: Activity, Inhibitors and Limit Dextrin Profiles. *J. Cereal Sci*. **2003**, *38*, 325–334. DOI: 10.1016/S0733-5210(03)00048-1.

[157] Burton, R.; Fincher, G. Evolution and Development of Cell Walls in Cereal Grains. *Front Plant Sci*. **2014**, *5*, 456. DOI: 10.3389/fpls.2014.00456.

[158] Fincher, G. B. Morphology and Chemical Composition of Barley Endosperm Cell Walls. *J. Inst. Brew.* **1975**, *81*, 116–122. DOI: 10.1002/j.2050-0416.1975.tb03672.x.

[159] Bacic, A.; Stone, B. Chemistry and Organization of Aleurone Cell Wall Components from Wheat and Barley. *Functional Plant Biol.* **1981**, *8*, 475–495. DOI: 10.1071/PP9810475.

[160] Fincher, G. B.; Stone, B. A. Cell Walls and Their Components in Cereal Grain Technologie. In *Advances in Cereal Science and Technologie*, Y. Pomeranz, Ed.; American Association of Cereal Chemists: St. Paul, MN, **1986**.

[161] Kanauchi, M.; Bamforth, C. W. The Relevance of Different Enzymes for the Hydrolysis of β-Glucans in Malting and Mashing. *J. Inst. Brew.* **2008**, *114*, 224–229. DOI: 10.1002/j.2050-0416.2008.tb00332.x.

[162] Stuart, I. M.; Loi, L.; Fincher, G. B. Development of (1->3,1->4)-beta-d-Glucan Endohydrolase Isoenzymes in Isolated Scutella and Aleurone Layers of Barley (Hordeum vulgare). *Plant Physiol.* **1986**, *80*, 310–314. DOI: 10.1104/pp.80.2.310.

[163] Preece, I. A.; MacDougall, M. Enzymic Degradation of Cereal Hemicelluloses II. Pattern of Pentosan Degradation. *J. Inst. Brew.* **1958**, *64*, 489–500. DOI: 10.1002/j.2050-0416.1958.tb01414.x.

[164] Gorjanović, S. A Review: Biological and Technological Functions of Barley Seed Pathogenesis-Related Proteins (PRs). *J. Inst. Brew.* **2009**, *115*, 334–360. DOI: 10.1002/j.2050-0416.2009.tb00389.x.

[165] Varghese, J. N.; Garrett, T. P.; Colman, P. M.; Chen, L.; Høj, P. B.; Fincher, G. B. Three-Dimensional Structures of Two Plant Beta-Glucan Endohydrolases with Distinct Substrate Specificities. *Proc Natl Acad Sci USA*. **1994**, *91*, 2785–2789. DOI: 10.1073/pnas.91.7.2785.

[166] Fincher, G. B. Cell Wall Metabolism in Germinating Barley. In *Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology*, P.R. Shewry, Ed.; CAB International: Wallingford, UK; **1992**. pp 413–437.

[167] Balasubramanian, V.; Vashisht, D.; Cletus, J.; Sakthivel, N. Plant β-1,3-Glucanases: their Biological Functions and Transgenic Expression against Phytopathogenic Fungi. *Biotechnol. Lett*. **2012**, *34*, 1983–1990. DOI: 10.1007/s10529-012-1012-6.

[168] van Loon, L. C.; Rep, M.; Pieterse, C. M. J. Significance of Inducible Defense-Related Proteins in Infected Plants. *Annu. Rev. Phytopathol*. **2006**, *44*, 135–162. DOI: 10.1146/annurev.phyto.44.070505.143425.

[169] Dahiya, N. Biotechnology: A New Tack on Fungal Resistant Crops. In *Advances in Fungal Biotechnology*, M. Rai, Ed.; I K International Publishing House Pvt: New Delhi, **2009**; pp 57–81.

[170] Lee, R. C.; Hrmova, M.; Burton, R. A.; Lahnstein, J.; Fincher, G. B. Bifunctional Family 3 Glycoside Hydrolases from Barley with alpha-L-Arabinofuranosidase and beta-D-Xylosidase Activity. Characterization, Primary Structures, and COOH-Terminal Processing. *J. Biol. Chem*. **2003**, *278*, 5377–5387. DOI: 10.1074/jbc.M210627200.

[171] Sun, A.; Faulds, C.; Bamforth, C. Barley Contains Two Cationic Acetylxylan Esterases and One Anionic Feruloyl Esterase. *Cereal Chem.* **2005**, *82*, 621–625. DOI: 10.1094/CC-82-0621.

[172] Mangan, D.; Cornaggia, C.; Liadova, A.; Draga, A.; Ivory, R.; Evans, D. E.; McCleary, B. V. Development of an Automatable Method for the Measurement of Endo-1,4-β-Xylanase Activity in Barley Malt and Initial Investigation into the Relationship between Endo-1,4-β-Xylanase Activity and Wort Viscosity. *J. Cereal Sci.* **2018**, *84*, 90–94. DOI: 10.1016/j.jcs.2018.10.003.

[173] Gusakov, A. V. Proteinaceous Inhibitors of Microbial Xylanases. *Biochemistry (Mosc)*. **2010**, *75*, 1185–1199. DOI: 10.1134/s0006297910100019.

[174] Simpson, D. J.; Fincher, G. B.; Huang, A. H. C.; Cameron-Mills, V. Structure and Function of Cereal and Related Higher Plant (1→4)-β-Xylan Endohydrolases. *J. Cereal Sci.* **2003**, *37*, 111–127. DOI: 10.1006/jcrs.2002.0488.

[175] Slade, A. M.; Høj, P. B.; Morrice, N. A.; Fincher, G. B. Purification and Characterization of Three (1→4)-β-d-Xylan Endohydrolases from Germinated Barley. *Eur. J. Biochem*. **1989**, *185*, 533–539. DOI: 10.1111/j.1432-1033.1989.tb15146.x.

[176] Benjavongkulchai, E.; Spencer, M. S. Purification and Characterization of Barley-Aleurone Xylanase. *Planta* **1986**, *169*, 415–419. DOI: 10.1007/BF00392139.

[177] Kanauchi, M.; Chijimi, A.; Ohnishi-Kameyama, M.; Bamforth, C. W. An Investigation of Two Xylan-Degrading Enzymes and a Novel Xylanase Inhibitor in Malted Barley. *J. Inst. Brew.* **2013**, *119*, 32–40. DOI: 10.1002/jib.64.

[178] Van Campenhout, S.; Pollet, A.; Bourgois, T. M.; Rombouts, S.; Beaugrand, J.; Gebruers, K.; De Backer, E.; Courtin, C. M.; Delcour, J. A.; Volckaert, G. Unprocessed Barley Aleurone endo-beta-1,4-xylanase X-I is an active enzyme. *Biochem. Biophys. Res. Commun*. **2007**, *356*, 799–804. DOI: 10.1016/j.bbrc.2007.03.066.

[179] Goesaert, H.; Debyser, W.; Gebruers, K.; Proost, P.; Van Damme, J.; Delcour, J. A. Purification and Partial Characterization of an Endoxylanase Inhibitor from Barley. *Cereal Chem.* **2001**, *78*, 453–457. DOI: 10.1094/CCHEM.2001.78.4.453.

[180] Igawa, T.; Tokai, T.; Kudo, T.; Yamaguchi, I.; Kimura, M. A Wheat Xylanase Inhibitor Gene, Xip-I, but Not Taxi-I, is Significantly Induced by Biotic and Abiotic Signals That Trigger Plant Defense. *Biosci. Biotechnol. Biochem*. **2005**, *69*, 1058–1063. DOI: 10.1271/bbb.69.1058.

[181] Jones, B. L. The Endogenous Endoprotease Inhibitors of Barley and Malt and Their Roles in Malting and Brewing. *J. Cereal Sci.* **2005**, *42*, 271–280. DOI: 10.1016/j.jcs.2005.06.002.

[182] Shewry, P. R. Enzyme Inhibitors of Seeds: Types and Properties. In *Seed Proteins*, P.R. Shewry and R. Casey, Eds.; Springer Netherlands: Dordrecht, **1999**; pp 587–615.

[183] Pekkarinen, A. I.; Jones, B. L. Purification and Identification of Barley (*Hordeum vulgare* L.) Proteins That Inhibit the Alkaline Serine Proteinases of *Fusarium culmorum*. *J Agric Food Chem*. **2003**, *51*, 1710–1717. DOI: 10.1021/jf026035v.

[184] García-Carreño, F. L. Protease Inhibition in Theory and Practice. *Biotechnology Education* **1992**, *3*, 145–150.

[185] Strelec, I.; Vukeli, B.; Vitale, L. Aminopeptidases of Germinated and Non-Germinated Barley. *Food Tech. Biotechnol* **2009**, *47*, 296–303.

[186] Rawlings, N. D.; Barrett, A. J.; Thomas, P. D.; Huang, X.; Bateman, A.; Finn, R. D. The MEROPS Database of Proteolytic Enzymes, Their Substrates and Inhibitors in 2017 and a Comparison with Peptidases in the PANTHER Database. *Nucleic Acids Res.* **2018**, *46*, D624–D632. DOI: 10.1093/nar/gkx1134.

[187] MEROPS. cited 2019 23.11.2019; Available from: https://www.ebi.ac.uk/merops/cgi-bin/speccards?sp=sp000152;type=peptidase.

[188] MEROPS. cited 2019 24.11.2019; Available from: https://www.ebi.ac.uk/merops/cgi-bin/organism_index?type=p;id=F.

[189] Diaz-Mendoza, M.; Dominguez-Figueroa, J. D.; Velasco-Arroyo, B.; Cambra, I.; Gonzalez-Melendi, P.; Lopez-Gonzalvez, A.; Garcia, A.; Hensel, G.; Kumlehn, J.; Diaz, I.; Martinez, M.

HvPap-1 C1A Protease and HvCPI-2 Cystatin Contribute to Barley Grain Filling and Germination. *Plant Physiol.* **2016**, *170*, 2511–2524. DOI: 10.1104/pp.15.01944.

[190] Zhang, N.; Jones, B. L. Characterization of Germinated Barley Endoproteolytic Enzymes by Two-Dimensional Gel Electrophoresis. *J. Cereal Sci.* **1995**, *21*, 145–153. DOI: 10.1016/0733-5210(95)90030-6.

[191] Zhang, N.; Jones, B. L. Development of Proteolytic Activities during Barley Malting and Their Localization in the Green Malt Kernel. *J. Cereal Sci.* **1995**, *22*, 147–155. DOI: 10.1016/0733-5210(95)90044-6.

[192] Koehler, S.; Ho, T.-H. D. Purification and Characterization of Gibberellic Acid-Induced Cysteine Endoproteases in Barley Aleurone Layers. *Plant Physiol.* **1988**, *87*, 95–103. DOI: 10.1104/pp.87.1.95.

[193] Koehler, S. M.; Ho, T.-H. D. A Major Gibberellic Acid-Induced Barley Aleurone Cysteine Proteinase Which Digests Hordein: Purification and Characterization. *Plant Physiol.* **1990**, *94*, 251–258. DOI: 10.1104/pp.94.1.251.

[194] Poulle, M.; Jones, B. L. A Proteinase from Germinating Barley: I. Purification and Some Physical Properties of a 30 kD Cysteine Endoproteinase from Green Malt. *Plant Physiol.* **1988**, *88*, 1454–1460. DOI: 10.1104/pp.88.4.1454.

[195] Zhang, N.; Jones, B. I. Purification and Partial Characterization of a 31-kDa Cysteine Endopeptidase from Germinated Barley. *Planta* **1996**, *199*, 565–572. DOI: 10.1007/BF00195188.

[196] Phillips, H. A.; Wallace, W. A Cysteine Endopeptidase from Barley Malt Which Degrades Hordein. *Phytochemistry* **1989**, *28*, 3285–3290. DOI: 10.1016/0031-9422(89)80332-2.

[197] Jones, B. L.; Marinac, L. A.; Fontanini, D. Quantitative Study of the Formation of Endoproteolytic Activities during Malting and Their Stabilities to Kilning. *J Agric Food Chem*. **2000**, *48*, 3898–3905. DOI: 10.1021/jf000458k.

[198] Simpson, D. J. Proteolytic Degradation of Cereal Prolamins—the Problem with Proline. *Plant Sci*. **2001**, *161*, 825–838. DOI: 10.1016/S0168-9452(01)00482-4.

[199] Wrobel, R.; Jones, B. L. Identification and Partial Characterization of High Mr Neutral Proteinases from 4-Day Germinated Barley Seed. *J. Cereal Sci.* **1993**, *18*, 225–237. DOI: 10.1006/jcrs.1993.1049.

[200] Sarkkinen, P.; Kalkkinen, N.; Tilgmann, C.; Siuro, J.; Kervinen, J.; Mikola, L. Aspartic Proteinase from Barley Grains is Related to Mammalian Lysosomal Cathepsin D. *Planta* **1992**, *186*, 317–323. DOI: 10.1007/BF00195311.

[201] Belozersky, M. A.; Sarbakanova, S. T.; Dunaevsky, Y. E. Aspartic Proteinase from Wheat Seeds: Isolation, Properties and Action on Gliadin. *Planta* **1989**, *177*, 321–326. DOI: 10.1007/BF00403589.

[202] Doi, E.; Shibata, D.; Matoba, T.; Yonezawa, D. Characterization of Pepstatin-Sensitive Acid Protease in Resting Rice Seeds. *Agric. Biolog. Chem*. **1980**, *44*, 741–747. DOI: 10.1080/00021369.1980.10864028.

[203] Kervinen, J.; Sarkkinen, P.; Kalkkinen, N.; Mikola, L.; Saarma, M. Hydrolytic Specificity of the Barley Grain Aspartic Proteinase. *Phytochemistry* **1993**, *32*, 799–803. DOI: 10.1016/0031-9422(93)85208-9.

[204] Fontanini, D.; Jones, B. L. SEP-1 - A Subtilisin-like Serine Endopeptidase from Germinated Seeds of *Hordeum vulgare* L. cv. Morex. *Planta* **2002**, *215*, 885–893. DOI: 10.1007/s00425-002-0823-4.

[205] Terp, N.; Thomsen, K. K.; Svendsen, I.; Davy, A.; Simpson, D. J. Purification and Characterization of Hordolisin, a Subtilisin-like Serine Endoprotease from Barley. *J. Plant Physiol.* **2000**, *156*, 468–476. DOI: 10.1016/S0176-1617(00)80161-3.

[206] Schmitt, M. R.; Marinac, L. Beta-Amylase Degradation by Serine Endoproteinases from Green Barley Malt. *J. Cereal Sci.* **2008**, *47*, 480–488. DOI: 10.1016/j.jcs.2007.06.002.

[207] Jones, B. L.; Budde, A. D. How Various Malt Endoproteinase Classes Affect Wort Soluble Protein Levels. *J. Cereal Sci.* **2005**, *41*, 95–106. DOI: 10.1016/j.jcs.2004.09.007.

[208] Pawelzik, E.; Permady, H.; Weinert, J.; Wolf, G. A. Untersuchungen Zum Einfluss Einer Fusarien-Kontamination Auf Ausgewählte Qualitätsmerkmale Von Weizen. *Getreide Mehl Und Brot* **1998**, *52*, 264–266.

[209] Laitila, A. Microbes in the Tailoring of Barley Malt Properties. PhD thesis, VTT Technical Research Centre of Finland, **2007**. http://urn.fi/URN.