From Field Barley to Malt:
Detection and Specification of Microbial Activity for Quality Aspects

Iris NOOTS, Jan A. DELCOUR and Chris W. MICHIELS*

Department of Food and Microbial Technology, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, B-3001 Hevelee, Belgium

* Corresponding author: Tel: 32-16-321634; Fax: 32-16-321997; email: Iris.Noots@agr.kuleuven.ac.be.

ABSTRACT: Barley grain carries a numerous, variable and complex microbial population that mainly consists of bacteria, yeasts and filamentous fungi and that can partly be detected and quantified using plating methods and microscopic and molecular techniques.

The extent and the activity of this microflora are determined by the altering state of the grain and the environmental conditions in the malt production chain. Three ecological systems can be distinguished: the growing cereal in the field, the dry barley grain under storage and the germinating barley kernel during actual malting.

Microorganisms interact with the malting process both by their presence and by their metabolic activity. In this respect, interference with the oxygen uptake by the barley grain and secretion of enzymes, hormones, toxins and acids that may affect the plant physiological processes have been studied. As a result of the interaction, microorganisms can cause important losses and influence malt quality as measured by brewhouse performance and beer quality. Of particular concern is the occurrence of mycotoxins that may affect the safety of malt.

The development of the microflora during malt production can to a certain extent be controlled by the selection of appropriate process conditions. Physical and chemical treatments to inactivate the microbial population on the barley grain are suggested. Recent developments however aim to control the microbial activity during malt production by promoting the growth of desirable microbial cultures, selected either as biocontrol agents inhibiting mycotoxin-producing moulds or as starter cultures actively contributing to malt modification. Such techniques may offer natural opportunities to improve the quality and safety of malt.

KEY WORDS: Barley, Microflora, Malt quality, Malt safety, Biocontrol, Starter cultures

I. INTRODUCTION

Barley is not only an important feed crop, it is also the predominant raw material for the production of malt for brewing purposes. Although barley physiology is of utmost importance for malt quality, the impact on malt quality of the natural microbial population throughout the complete production chain should not be ignored. Many studies have indicated that the microbial processes influence malt characteristics. In order to understand the microbial metabolic activity during malt

production and its interaction with the barley grain, insight in the changing microbial ecology during barley growth, storage and malting is needed.

The impact of the total microbial population as well as of defined microflora members on malt quality aspects can be investigated by selective suppression or artificial contamination during experimental malting or by examining specific activities of isolates of the microbial population. As most studies were conducted with laboratory scale malting trials, little is known about the activity of the microbial population in commercial malting systems.

We here review the microflora of hulled barley, methods used for the detection and quantification of microorganisms occurring on barley, the microbial flow and evolution in the production cycle and the microbial activity as a quality and safety factor. Finally, we discuss attempts to control the microbial processes during malt production with special emphasis to the addition of selected microbial cultures with a view to optimise malt quality and safety.

II. GENERAL OVERVIEW

The microflora of hulled barley is predominantly mesophilic and psychotrophic and consists of viruses, bacteria, filamentous fungi, yeasts, slime moulds and protozoa which occur within or beneath the husk, in the exterior layers of the barley kernel. Psychotrophic bacteria, actinomycetes and aerobic sporulating bacteria are nearly always present on cereal grains.[1] Filamentous fungi on barley and on cereals in general can be divided in two groups: field fungi that occur on or in grains until the time of harvest, and storage fungi which contaminate grains after harvest.[2] Field fungi generally do not grow below a water activity (a_w) of 0.90, corresponding to 20-25% moisture, whereas storage fungi are often xerophilic and able to grow down to a water activity of 0.80 (18% moisture) or even 0.68 (14% moisture).[1] The classification into field and storage species is applicable in temperate climates, but in warmer regions some species usually considered as storage fungi may even invade the developing grain.[3] In addition to the typical field and storage fungi, other filamentous fungi occurring on barley can be classified as mucoracous fungi associated with storage and yeast-like fungi.[1] Research on barley microflora has mainly focussed on filamentous fungi which are of most concern in view of barley and malt quality aspects.

Several authors studied the distribution of the microbial population over the structural components of the barley grain by microscopic techniques. Scanning electron microscopy revealed that a low

number of microorganisms are present on the surface of barley after harvest.[5] This contamination probably results from soil and dust during harvesting and handling the grain mass[6]. In comparison with the amount of microorganisms present on the outer surface of the husk, a high number of microorganisms occur on the inner surface below the husk, with the bacteria clustered as randomly distributed micro-colonies with up to 200 cells.[5] The fungal hyphae on the barley grain predominantly occur in the furrow and at the top of the grain, in openings between the husk and the pericarp and on areas of the kernel where the tissue is damaged.[7] After staining isolated husk with cotton blue, a rich fungal development was detected on the inner side of the husk, especially in close relation to the veins.[8] Fungal mycelium was detected in 44.3% and 59.2% of the area of the lemma and in 67.3% and 70.3% of the area of the palea, isolated from two different barley samples and stained with acid fuchsin.[9] The total dry weight of mycelium in individual grains was estimated to be 1.5 to 1.9 µg.[9] As this estimation was based on observations of mycelium in the husk only, the actual value is probably higher.[9] In the interior of barley grain tissues, hyphae were observed in the parenchyma layer of the lemma and palea and within the pericarp layer of the caryopsis.[10] The occurrence of clear entities in the pericarp totally occupied by microorganisms, mainly bacteria, was reported.[11] The aleurone layer and starchy endosperm appeared to be free of mycelium,[10] although the latter was detected in the endosperm of barley kernels that were discoloured by abundant development of fungi[7]. Studies of the distribution of microorganisms in whole barley grains by mechanical dehusking confirmed the predominant occurrence on the outer layers of the kernel.[12]

Fungal species that develop on the surface of individual barley grains have characteristic linear growth rates that change with water activity and temperature.[13] Observations of the growth of *Aspergillus flavus*, *Fusarium poae*, *Hyphopichia burtonii* and *Penicillium verrucosum* strains on barley showed that the germ tubes grow initially on the surface of the barley grain apparently without penetrating the grain tissue.[13] This implies that nutrients are required until the germ tubes or hyphae reach a point where they can penetrate the grain surface, for instance through cracks or other damages caused by the development of the seed germ. In the absence of such nutrients, hyphae may be able to extend some distance but only with limited branching.[13]

When during the initial stages of mycelial development on the barley husk external supplied nutrients are absent, microbial metabolism depends on respiratory substrates derived from the husk. If successful, fungi have to be able to degrade the major polysaccharide components of the cell walls[14],

i.e. cellulose and hemicelluloses containing hexose, pentose and uronic acid residues.[15] *In vitro* assays with fungal isolates from barley grains belonging to 23 different genera revealed that arabinoxylan degradation is more common than carboxymethylcellulose degradation.[15,16] This suggests that xylanase activity may be of greater importance than cellulase activity in making sugars available for growth on the husk.[15,16] Furthermore, the release of small sugars from the cell walls by adapted species may allow poorly adapted species to grow in mixed communities.[15] The extent of mould development also depends on the sensitivity of the barley variety for penetration by the mycelium.[17]

As a positive correlation exists between the total count of bacteria and the growth of filamentous fungi on barley grains, it is suggested that, when hyphae are growing in the kernel, bacteria are able to penetrate and to degrade nutrients released by the fungi.[18] On the other hand, the development of a specific group of the microbial population may be stimulated by selective suppression of other groups, due to a decreased substrate competition.[19]

Only a single study has addressed the interactions between different fungi developing on the barley grain surface.[13] Distinct interaction patterns were observed between the studied fungal strains (*Aspergillus flavus*, *Fusarium poae*, *Hyphopichia burtonii* and *Penicillium verrucosum*), but growth of the competing species was never affected before hyphae made contact. It is suggested that colonisation of barley grains by fungi is directed to capture as much of the grain resources as possible. The success of colonisation depends on the amount of inoculum, but also on growth rate and branching. Therefore, these characteristics, rather than antagonism, were identified as important determinants in the outcome of interactions between the studied grain fungi. For the tested species, inhibition of spore germination was not found.[13]

III. TECHNIQUES FOR DETECTION AND QUANTIFICATION

The study of the barley microflora aims to characterise the microbial population present on the grain, including non-viable microorganisms that are an indication of past contamination, as well as the spatial distribution of the microorganisms over the structural components of the barley kernel. It should be remarked in this context that the microflora of each individual kernel is unique so that for given barley samples only average results are obtained.

In addition to conventional plating methods and direct microscopy, molecular techniques based on highly specific nucleic acid or antibody technology, or on the chemical detection of specific cell wall

components or metabolites are increasingly applied for the detection and quantification of certain groups of the barley microflora. In particular, methods are developed to detect metabolites of microbial origin that are important in relation to malt safety. Furthermore, the combination of these molecular techniques with microscopy opens exciting new opportunities for the *in situ* observation, identification and quantification of almost any member of the microbial population.

A. Surface-disinfection

In order to reduce the superficial contaminants and fast growing surface-borne saprophytes on barley without killing the internal microflora, the grains may be surface-disinfected.[6,20,21,22] Subsequent analysis by plating provides indications of the degree to which moulds occur below the husk and have penetrated in the grain.

The most commonly used surface disinfection procedure is immersion in sodium hypochlorite (1%) during 1 minute.[20,21,23-26] The destruction of contaminants on the grain surface with this agent depends on the contact that can be established between the microorganisms and the disinfectant, the condition of the kernels, the pH and the concentration of the diluted sodium hypochlorite and the exposure time.[22] The efficiency of surface disinfection is limited at high levels of surface contamination.[22] Other disinfecting agents used include mercuric chloride (0.1%),[27,28] calcium hypochlorite (2%),[29] ethanol (80%)[30] or a sequential combination of different agents[18,22,29,31-34].

B. Plating methods

For mycological analyses of cereal grains, both direct and dilution plating have been used. The degree of mould contamination detected may vary with the applied technique[35]. For the analysis of barley kernels, direct plating is, in most cases, superior to dilution plating[30]. However, it must be emphasised that a complete characterisation of the types and numbers of filamentous fungi present on a grain can not be accomplished by any single plating method.

For direct plating, agar-plate or blotter tests are used. In the agar-plate test kernels are placed on a solid mycological agar medium. Pretreatments such as soaking of the grain[30] and the use of selective plating media and specific cultivation conditions allow selecting for different groups of the total mycoflora. An overview of different selective or non-selective plating media that have been used for microbiological analysis of barley is given in Table 1. Selective media for the isolation of specific

moulds occurring on barley have been much less developed than for bacteria, and are at present only available for *Fusarium* and for xerophilic moulds. In the blotter test, on the other hand, grains are placed on water-saturated sterile filter paper. This test is regarded as the best general method to estimate the type and extent of fungal contamination,[20,21,23,50,51] as no additional nutrients are provided and the fungi detected are those that can grow on the barley grain[21]. However, this is not completely true, since the cellulolytic activity of some isolates of the barley grain (cfr. *infra*) may result in a degradation of cellulose from the filter paper. Nevertheless, the range of saprophytes observed is markedly smaller than that detected in agar-plate tests[6]. The scarcity of nutrients in the blotter test stimulates spore production and thus enables identification of the moulds based on the microscopic characteristics of their spore structures. However this requires a high degree of skill.[21,50] The results of agar-plate or blotter tests can be expressed as Mould Frequency Index (MFI) values, that indicate the mean number of different mould types per kernel.[35,50,52] It is suggested to use this parameter to compare the efficacy of the different direct plating methods.[50] Direct plating has only limited quantitative value as it does not supply information about the real level of mould contamination in grains.

The use of dilution plating for quantification of filamentous fungi is also problematic. The mould genus to be distinguished as well as the extraction procedure, mostly including homogenisation steps[4,5,19,23-26,30,37,38,44,47,48,52-54], strongly influence the results. On the basis of an equal biomass this technique yields much higher counts for fungi that either readily fragment into viable pieces (e.g. *Geotrichum* and *Verticillium*) or produce large numbers of spores, than for purely mycelial and weakly sporulating species.[10,35,52] Whereas dry barley can be homogenised efficiently, there are analytical problems inherent to steeped grain. The difficulty to detect a mould that forms less than 12% of the total numbers by dilution plating,[35] may explain that a lower diversity of species is found in comparison with direct plating[30]. Furthermore, while dilution plating may indicate that the total mould contamination of barley kernels does not differ substantially, the number of different mould types per kernel after direct plating may vary widely.[35]

Bacteria and yeasts, as unicellular organisms, are usually counted after dilution plating. Slime moulds (e.g. *Physarum polycephalum*[24]) and protozoa (e.g. unspecified ciliated protozoa[24]) can be detected after direct plating of barley grains.

In conclusion, as plating techniques for mycological analyses only provide an estimate of the contaminating mycelium, the results obtained by these techniques should be interpreted with care. They give no assessment of the total amount of mycelium and the extent to which fungi have invaded the grain, nor do they detect dead fungi, that are a sign of past contamination and that, if detected, could be a warning for the potential presence of mycotoxins. For the quantitative measurement of the fungal contamination and for the detection of important mould genera in relation to microbial safety, molecular techniques have been developed. The internal mycelium within individual grains and the distribution of other microflora constituents can be studied by direct microscopic methods.

C. Direct microscopy

For the localisation of the microbial population on and in the barley grain, microscopic investigation of tissue slides, sometimes combined with specific staining, can be used. The total amount of fungal mycelium in the husk can be estimated after staining with a saturated solution of acid fuchsin in lactophenol[9,55] or cotton blue in lactophenol[8], while the fungal mycelium in deep tissues can be localised after cryo-sectioning and phenol-acetic-aniline blue staining[10]. Different ways have been proposed to estimate the total amount of mycelium in the kernel based on microscopic analysis of only a limited number of sections or fields, for instance from the total length of the mycelium in the husk,[9] or from the number of hyphal units in serial sections of individual barley grains[10]. Scanning electron microscopy has been applied to study the occurrence and distribution of the microflora in different sections of barley kernels.[5,7,11,13,43]

D. Molecular techniques

Several structural components of the fungal cell walls and metabolites secreted during development of fungi on the grain have been proposed as a chemical index of fungal biomass. These include chitin as a major constituent of the walls of fungal spores and mycelia. However, the chitin assay has low sensitivity and reproducibility[56]. In addition, the exoskeleton of insects and mites, that frequently infest cereal grains, also contains chitin. Ergosterol, the principal sterol produced by fungi has been used to measure the mould growth during barley storage[41] and malting[43]. It is suggested to use this cell wall component, possibly together with the *Fusarium* metabolite deoxynivalenol, in the

screening of barley and malt for microbial safety.[41,57] Furthermore, free fatty acids appear to be an objective measurement of fungal damage in stored grains.[1]

The occurrence of specific mould genera or species can be analysed by nucleic acid-based or immunological methods. In barley, *Fusarium* contamination has been quantified using ELISA or latex agglutination technology based on the detection of extracellular polysaccharides released in the substrate[51]. This was claimed to be a sensitive and selective method for the determination of the *Fusarium* infection on either single kernels or larger samples of barley at harvest and during malting.[39] ELISA is much slower than the latex agglutination method, but about then times more sensitive.[58] By including a pre-enrichment step, the amount of viable *Fusarium* can also be estimated.[39] Immunoassays were also developed for the detection of mycotoxins produced on barley and occurring in malt and beer.[59-62] Individual species of *Alternaria*, *Aspergillus* and *Penicillium* in the husk of barley grains could be detected using immunofluorescent staining.[9,55] Correlations between the results obtained by immunofluorescent staining and plating methods in terms of the percentage of contaminated grains were genus dependent.[55] PCR methods have been developed for detection of *Fusarium graminearum* and *F. culmorum*[63] and trichotecene producing *Fusarium* species in general[64] in brewing cereals. They are rapid and relatively easy to use, but do not supply any information about the actual production of harmful metabolites by the detected contaminants.

IV. MICROBIAL FLOW IN THE PRODUCTION CYCLE

Barley malt production involves steeping in water, germinating and finally kilning. However, cultivation, harvest and storage of the cereal are here considered as part of the production cycle (Figure 1), as the microbiological status of the barley grain is to a large extent influenced by the conditions prior to the actual malting process. In general, the microbiological status at any stage of the process can be considered as a result of contamination and growth, and/or inactivation of these contaminants during the preceding steps. The microflora developing during cultivation[65], storage[65,66] and malting[65,66,67], as well as factors influencing growth of fungi on barley[66] were reviewed earlier.

A. Growth of barley in the field

1. Field microflora

The field microflora consists of those microorganisms found on barley prior to harvest and is related to the microflora of soil, vegetation and the air.[67] It consists of saprophytic and parasitic organisms. The latter may lead to substantial losses by causing blights or blemishes, or by producing mycotoxins.[68,69] Especially Gram negative bacteria numerically dominate the field microflora.[35,52,65] The most common and abundant bacterial species during growth is *Erwinia herbicola*.[23] Yeasts are usually the next most abundant components, although filamentous fungi may exceed their numbers during the later stages of ripening.[65]

Of the field fungi, two-thirds are associated with the bractioles of the barley kernel and one-third with the caryopsis.[65] *Alternaria*[6,23,33-35,45,52,70], *Aureobasidium*[6,18,35,52], *Cladosporium*[6,18,23,35,52,70], *Epicoccum*[35,52,70], *Fusarium*[23,33,52] and *Helminthosporium*[33,34,70] are described as dominant field fungi for barley, but there are important geographic and climatic influences. The storage fungi, mainly *Aspergillus* and *Penicillium* species, are absent or occur in low numbers during growth[24,71,72] and on barley immediately after harvest[23]. Most of the *Penicillium* species isolated from barley kernels before harvest are more characteristic for storage. However, there are several exceptions of species that can invade barley before harvest, such as *Penicillium pedemontanum*, *Eupenicillium* species and others.[72] Storage *Penicillum* species tend to have lower a_w minima for growth.[73]

2. Growth conditions

The contamination of the barley grain by microorganisms originating from soil, vegetation, air, rain, insects, bird droppings etc. starts at ear emergence and continues throughout the growing season. For the microorganisms trapped between the caryopsis and the bracteoles, the conditions for growth are apparently more favourable than for those on the outer surface of the grain.[35] During the subsequent ripening stages of the grain, the number of microorganisms increases tremendously.[24,67] The time between emergence of the ears and harvest is well correlated with the extent of fungal contamination.[18] This does not explain the higher contamination levels in ‘winter’ than in ‘spring’ barley[70], because in another study the total count of bacteria and moulds in ‘winter’ barley was lower than that in ‘spring’ barley[47].

As temperature and moisture content play an important part in the deposition of microorganisms on barley kernels and have a profound effect on the colonisation, the type and the abundance of the microflora is to a large extent determined by climatic conditions.[23,67,74] The occurrence of certain types

of fungi is also brought in relation with the geographical region,[33,75] but more extensive studies are needed to draw correct conclusions.

Furthermore, the types and numbers of contaminating fungi depend upon characteristics of the barley variety.[23] Firstly, lodging of crops, a variety dependent characteristic, frequently leads to profuse mould growth. Lodged barley dries more slowly after rain, is more contaminated by soil, ripens prematurely and is more damaged. A moist-warm microclimate persists on the soil in the vicinity of the ears, which provides optimal conditions for the development of microorganisms.[65,70,72] Lodging was indicated as the cause of abundant development of certain *Penicillium* species.[72] Differences in *Fusarium* and *Cladosporium* contamination in barley varieties were also attributed to lodging.[23] Secondly, there are varietal differences in resistance to infection by *Fusarium* in barley that have been related to differences in occurrence of polyphenols in the testa layer.[74] Finally, because *Fusarium* can contaminate the barley kernels by growing from the soil through the stem to the ears, varieties with long stems are also more resistant to infection.[18]

The degree of microbial contamination of barley is further determined by cultivation practices such as the use of crop protective agents in general and fungicides[18,65,74] in particular, the percentage of acreage planted to susceptible crops[76], the frequent successive planting of the same cereal crops on the same land[70,76], leaving of plant debris on the field as a result of limited soil tillage[76] and the overuse of nitrogen fertiliser, that also leads to lodging[70].

B. Harvest and storage of barley

1. Harvesting conditions

In comparison with hand-trashing, combine harvesting leads to higher levels of contamination of kernels, especially by xerophilic penicillia.[71] Possible sources of the superficial contamination at this stage are the throwing up of spore-laden dust from field soil during harvesting, vegetative parts of the plants and residues in the combine-harvester and in storage bags.[71]

The types and numbers of contaminating microorganisms also depend upon the weather conditions at harvesting time.[23] Heavy rainfall immediately before harvest tears open kernels at the furrow and leads to development of mainly *Epicoccum nigrum* and *Botrytis cinerea*.[69]

The seed microflora is most numerous and vigorous immediately after harvest.[77] Sometimes, harvest can be delayed to reduce moisture levels in order to limit mechanical damage to the grain and

to keep drying costs low.[70] This practice results in higher microbial numbers, partly because the delay in drying allows mould sporulation on the kernels and results in secondary cross-contamination[71].

2. Storage microflora

The field fungi, which may have invaded the barley kernel before harvest, have little importance during storage because they do not grow below a water activity of 0.90.[26] The stored grain at low a_w is no longer physiologically active and specific associations with fungi, as they exist in the field are lost. Storage fungi are saprophytes with low specificity, whose ability to develop is mainly determined by a_w and temperature.[78]

The storage microflora comprises xerophilic species of the genus *Aspergillus* [particularly the species with a perfect state belonging to *Eurotium* (the so-called *Aspergillus glaucus* group)] and to a lesser extent *Penicillium* species.[6,35,52,81] With increasing moisture content, barley is preferably invaded by xerophilic species in the order *Aspergillus restrictus*, *Eurotium repens*, *E*. *amstelodami* and *E*. *rubrum*.[79] Less xerophilic *Aspergillus* species such as *Aspergillus candidus*, *A*. *ochraceus* and *A*. *flavus* grow at elevated moisture content, while growth of *Penicillium* species is favoured at even higher moisture content[72,80] by low temperature[80,81]. Initially *Penicillium brevicompactum* develops, followed by *Penicillium verrucosum* and other species.[72] Above 20% moisture, *Penicillium* species outnumber *Aspergilli*.[66] Other fungi that also develop at elevated moisture contents belong to the genera *Absidia*, *Rhizopus* and *Mucor*[35,52,80]. The most xerophilic storage fungus is *Wallemia sebi*, which can grow at a_w 0.69. It is suggested that the development of this mould can initiate the growth of *Aspergillus* and *Penicillium* during storage.[81] Self-heated barley is characterised by a distinct occurrence of *Eurotium*[66] or *Absidia* species[52]. After long periods of self-heating, thermo-tolerant species such as *Absidia corymbifera* and *Aspergillus fumigatus* may develop.[52]

3. Storage conditions

The composition of the microbial population of barley changes during storage depending on the initial microflora composition, the storage time and environmental conditions. The storage conditions that influence the development of the microorganisms are moisture content and temperature of the grain mass, aeration, inclusion of chaff and other materials such as broken grains and weed seeds and the occurrence of insects and mites.[31,65]

Under appropriate storage conditions, bacteria, yeasts and field fungi are not active due to the low moisture content and generally decrease in numbers[23,25,26,81]. Survival of field fungi during storage may depend on the origin of the cereal, the degree of infection, the genus and species and the competition within the species.[34] Although the vegetative forms of moulds progressively die with storage time, the spores on the grain are likely to remain viable for a variable time.[35,81] On dry stored barley *Fusarium* does not survive as long as *Alternaria*,[24,34] the spores of which may remain viable for a number of years[35]. This explains that on correctly stored barley mainly *Alternaria* species are detected.[66] The reduction in the number of *Bacillus* during storage is small compared to that of other bacteria and this is also attributed to the stability of the spores.[23]

The most important parameter for mould development in stored cereals is the minimum water activity permitting germination and growth. It is common practice to dry malting grade barley to a moisture content below 13%. Temperature also is important as, at constant moisture content of the grain, the water activity increases with temperature. For instance, the maximum tolerable moisture content for stable storage of barley at a temperature of 10°C is 14.5%[81] and the higher the storage temperature, the lower the moisture content needed to avoid fungal growth. The rapid development of storage fungi on barley kept at a temperature of 20-30°C and a moisture content of 16% was demonstrated.[26] However, barley varieties of equal water content may also differ in microbiological stability because different varieties have different water sorption isotherms.[65,68] Furthermore, not only does the moisture content of individual kernels in a grain bulk vary, different parts of the kernels also have different capacities to absorb moisture.[82] This may explain that during storage the embryo rather than the endosperm was invaded by *Eurotium* species.[82] Another hypothesis is that, as the non-reducing sugars sucrose and raffinose as well as lipids are found predominantly in the embryo and the aleurone, these parts of the barley grain would provide a suitable nutrient source for the growth of *Eurotium* species.[82]

When *Aspergillus* and *Penicillium* species develop, their metabolic activity is responsible for release of water by respiration. They can cause elevated temperatures in the grain mass. When the grain mass is aerated insufficiently, these factors initiate a chain reaction of outgrowth of other microorganisms that are less xerophilic and more thermophilic. This microbial proliferation results in further heating and so-called hot and smelly barley.[65] In some cases, thermophilic fungi such as *Thermoascus aurantiacus* may raise the temperature to 60-65°C. This results in grain darkening[1].

The ratio of field fungi to that of storage fungi has been proposed as an objective tool to judge the conditions under which the grain was stored.[52]

C. Malt production

The natural contamination of barley kernels at the start of the malting process is a result of both the field conditions under which the crop is grown and the post-harvest history of the grain, as explained above. Bacteria and fungi detected on barley, barley malt intermediates or malt are listed in Table 2.

Three major steps can be distinguished in a malting process: steeping, germination and kilning. (Figure 1). The activity and evolution of the microbial population during the different stages of the malting process depend on the initial barley contamination, the interactions between the members of the population, the varying substrate characteristics such as moisture content and availability of nutrients, the process conditions such as temperature and aeration and the use of additives such as sulphur dioxide. Furthermore, additional contamination may occur by a specific microflora developing in a malting plant.

During the steeping stage, the moisture content of the grains is elevated up to 42-44% by soaking in water. This results in conditions that are favourable for the multiplication of the contaminants present on the grain. Aerobic, heterotrophic bacteria, mycelial fungi and yeasts develop and, in general, the total number of microorganisms further increases during germination. It decreases as a result of kilning. The progressive increase in microbial population from dry barley to green malt is also attributed to the release of readily metabolisable components as a result of enzymic activity in the germinating barley kernels.[5] Although several studies demonstrate that the composition of the mould microflora qualitatively changes during malting,[4,23,35,68,81] it is difficult to discern any consistent pattern of change. The process variability has a pronounced effect on the microbial proliferation and those species for which growth conditions are optimal, dominate.

Steeping is the critical stage at which grain microorganisms start to proliferate. It is to be expected that bacteria and yeasts multiply and mould mycelium develops and that dormant spores are activated, producing new growth that can extend from kernel to kernel.[35] Vegetative cells in general start growing shortly after the grain is wetted, while spores only grow after a lag period of variable length.[90] A rapid proliferation is enhanced by steep-aeration.[54] Superficial contaminants can be washed from the

kernels, some to be deposited on other kernels.[4,35,54] As a consequence of the microbial proliferation a dense coverage of bacteria, yeasts and fungal spores is observed on steeped grains, in particular on damaged kernels, and a large number of microorganisms occur in the steep water.[5] The onset of microbial activity during steeping is also reflected by oxygen uptake activity associated with the husk and steep liquor where the availability of nutrients leads to further microbial proliferation.[90]

The viable counts of bacteria and yeasts reach a maximum during germination[5,23,35]. Growth of fungi also takes place particularly during germination. Using ergosterol as a measure of mould growth, it was found that the growth rate was slow during the second day of germination, but increased substantially during the third day.[43] Temperature and moisture conditions in the grain bed influence the microbial activity. For instance, *Aspergillus amstelodami* and *Aspergillus fumigatus* were detected in deeper parts of the barley layer in the germination box where the temperature rose from 14°C at the start of germination to more than 30°C.[46,91] High cast barley moisture content decreases the efficiency of grain bed drying, resulting in enhanced microbial proliferation and lactate production.[45]

Kilning temperature and procedure strongly influence the microbial load of the obtained malt. A large increase in ergosterol was observed during kilning[43] and scanning electron microscopy revealed an abundant branching of fungal hyphae below and on the surface of the husk of malt[5], suggesting that mould growth may be accelerated during the initial phase of kilning.[43] However, while specific genera such as *Mucor* and *Rhizopus* may proliferate at this stage[4,23,81], total counts of viable moulds and yeasts were reduced considerably on the final malt[4]. Many microcolonies of bacteria were detected on the surface of malt kernels, but only a negligible number was found below the husk.[5] Dominating groups of viable filamentous fungi on malt are the storage fungi *Aspergillus* and *Penicillium*, but also *Mucor* and *Rhizopus*.[46,92] In most cases, the number of different mould types per kernel was higher in barley than in malt.[35] During kilning, stress and nutrient limitation can modify the microbial metabolism. For instance, some lactobacilli may oxidise lactate formed earlier during steeping and germination to yield formic, acetic and succinic acids during glucose limitation.[45] A significant reduction in lactic acid concentration indeed was measured after kilning, but the levels of other organic acids showed little change,[93] and it seems likely that the volatile acids formed from lactate are removed during kilning so that they do not influence the final malt pH.[45]

The evolution of the barley microflora during malting may also be strongly influenced by the type of malting equipment.[4] Saladin boxes favour the growth of mesophilic bacteria, which are predominant

at all stages of the malting process,[4] and yeasts,[35] while floor malting favours the development of bacteria and moulds[35].

An important factor contributing to the development of the microorganisms on barley during malt production, is the specific microflora occurring on the malting equipment which contaminates the grains. Lactic acid bacteria and *Geotrichum candidum* were predominant contaminants during steeping and germination in industrial malthouses,[44] and the development of the yeast-like mould *Geotrichum candidum* is considered as typical during germination in Saladin boxes[4,81]. This microorganism was first detected on the malting barley after the first steep and by the end of the second air rest, but contaminated all kernels of the green malt after germination.[4] The spread of *Geotrichum candidum* from kernel to kernel during malting may be positively affected by aeration and be at the expense of another yeast-like species, *Aureobasidium pullulans*. At the end of germination[81] and during the early stages of kilning[4], rapidly growing mucoraceous types take over from *Geotrichum candidum* and begin to proliferate.

V. MICROBIAL ACTIVITY AS A QUALITY FACTOR IN MALT PRODUCTION

Barley malt production predominantly involves the mobilisation of enzymes in the starchy endosperm in order to modify it such that during brewing a high amount of fermentable sugars can be obtained without difficulty. The influence of microbial activity on barley and malt characteristics can be assessed by investigating the metabolic activities of isolates of the barley microflora[15,16,87,94,95,96], by (selective) suppression of the indigenous microflora by addition of antibiotics and/or disinfectants[7,11,19,27,29,48,54,75,77,87] or by inoculation before or during the malting process[7,19,48,49,85,89,91,92,97]. When barley is inoculated during flowering, microorganisms have a much longer time to develop and to secrete metabolically active compounds than when the grain is only inoculated during the malting process.[89] Inoculation at this stage also differs from natural contamination or inoculation at flowering in that the microorganisms are applied externally, while natural or artificial contamination at flowering allows the microorganisms to settle within or beneath the husk.[49,85,92] In most of the studies, the natural contamination of the barley grain is not eliminated before inoculation, but is supplemented with a pure culture of a contaminant. Therefore, the precise role of the natural microbial contamination is hardly understood from such experiments. Further, as microbial activities during malting depend on the process conditions, the experimental malting conditions also should be considered. The influence of the microbial activity on barley[44,65,66], malt[44,67] and beer[44,65] properties was already reviewed earlier.

Next to insects, the second most important cause of barley losses during storage is microbial spoilage by moulds.[70,81] Already in 1956 it was demonstrated that storage moulds and *Fusarium moniliforme* contributed to the loss of viability of barley stored at high moisture content (25%).[31] Later studies confirmed that kernels naturally or artificially contaminated with *Fusarium*[43,89] or invaded by storage fungi[26] show a decreased germination. A reduced malt yield caused by *Fusarium* species[49,89] has been explained by losses during steeping and germination[49] or by increased rootlet growth[49,87] resulting in larger cleaning loss.

Microbial contamination also alters typical physical characteristics of the barley kernels such as their colour. Highly stained kernels are more contaminated by yeasts[24] and certain genera of filamentous fungi[24,82,84]. Red or black discoloration indicate the presence of *Fusarium* and *Alternaria* respectively. Discoloration but also shrivelling of the kernels are mainly associated with the occurrence of field fungi.[33,82,]

More important in relation to malt quality is that the microbial metabolism influences the barley and malt composition both by chemical and biochemical interactions. The effects on malt analytical data presumably result from interference with barley respiration and from the secretion of metabolites and enzymes. As the microbial population of barley influences the final quality of malt, it may also influence the brewhouse performance of malt and the quality of the obtained beer.

A. Interactions with the malting process

1. Interference with barley respiration

The barley microflora appears to influence the rate of barley germination and may play a role in postharvest dormancy.[24] As the microorganisms in and on the husk begin to respire during barley steeping,[29,90] they may come in competition with the grain tissue for oxygen. This competition is believed to be at least partly responsible for grain dormancy, in addition to environmental conditions during germination and endogenous factors within the grain.[29] Control of the population of microorganisms on the grain surface layer indeed enhanced germination.[29]

Water sensitivity, or the inability of barley to germinate in an excess of water, is also related to both endogenous factors of the grain as well to its microbial load.[27,32,54,65] Water sensitivity occurred under conditions that favour bacterial and mould growth and was prevented when the microbial factor was eliminated by disinfectants.[27] The hypothesis that microorganisms are responsible for water sensitivity suggests that water sensitive seeds contain a large, complex microbial population[27,77] and more in particular field microorganisms that occur within the lemma, palea and pericarp-testa[65]. In excess of water, such as during steeping, this microflora may inhibit barley germination by physically impeding the entry of oxygen[77] and by competition for oxygen[27,77]. Some microorganisms may also attack the embryos[77].

2. Secretion of metabolites and enzymes

Successful malting includes the requirement of a favourable balance of the plant hormones gibberellic acid (GA_3), abscisic acid (ABA) and indole-3-acetic acid (IAA) in the germinating barley kernel. A limited number of fungal isolates of barley were found to produce GA_3 and ABA *in vitro*. The production of IAA was common for both fungi and bacteria. It was shown that microbial GA_3 and ABA production *in vivo* occurs in negligible amounts, while bacterial IAA production may be of physiological

significance in imbibed grains.[38] Some malt quality aspects obtained after artificial contamination with *Fusarium* species correspond to the effect of the addition of gibberellic acid.[87]

The production of highly toxic compounds by storage and field fungi (Table 3, Figure 2) and their occurrence in beer has been reviewed.[59] The toxins secreted by storage fungi are seldom detected in malt and beer. Their production can be prevented by the selection of appropriate storage conditions in contrast to that by field fungi. The latter are therefore an important threat to the safety of cereal products. *Fusarium* species are regarded as the most important mycotoxin producing field fungi. The most prevalent mycotoxins produced by this genus are 12,13-epoxy-Δ9-trichothecenes of which T-2 toxin, deoxynivalenol (DON), diacetoxyscirpenol (DAS), nivalenol (NIV), fusarenon-X and their derivatives may occur on barley.[59] Besides thrichothecenes, also the *Fusarium* oestrogenic toxin, zearalenone (ZER), is found on barley. The production of these mycotoxins mainly depends on the *Fusarium* species and the climatic conditions during growth in the field.[59] The production of mycotoxins during malting was studied for DON. The final DON concentration in malt was found to be approximately the same[81] or lower[81,88] than the concentration detected in the barley. DON was uniformly distributed throughout kernel size fractions.[43] During steeping DON levels were reduced,[43,88,98] probably due to washing out or removal of dirt or mould particles. During subsequent germination and the early stages of kilning DON levels increased again.[43,98] In another study, a sharp increase in DON level was only found during the first step of kilning.[88] On average, 80% of the DON content in malt was formed after steeping.[88] The two species shown to be responsible for DON production in barley and malt are *Fusarium graminearum*[18,43,98,99], predominant in warmer regions, and *F. culmorum*[18,99], predominant in temperate climate zones. A non-*Fusarium* toxin that frequently contaminates barley is the nephrotoxic ochratoxin A. Production of this toxin was first demonstrated in *Aspergillus ochraceus*. However, this species occurs mainly in tropical regions, and the responsible organism in contaminated barley was later demonstrated to be *Penicillium verrucosum*.[100,101] As all these toxic compounds are heat-stable, it is to be expected that they are not affected by wort boiling during brewing.[59] However, they may partially be removed with the spent grains. This holds in particular for toxins with low water solubility such as ZER[43]. The above mycotoxins are not only a threat to human health, several of them are also phytotoxins interfering with the malting process. Trichothecenes are highly toxic inhibitors of protein synthesis. When barley was artificially contaminated with DON and DAS, these toxins inhibited rootlet and coleoptile growth and affected the *de novo* synthesis of α-

amylase during germination. They also inhibited synthesis of proteolytic enzymes that led to lower levels of α-amino nitrogen in wort and malt. The phytotoxicity of DON was found to be lower than that of DAS and T2-toxin.[94]

There are indications that microbial population associated with the barley kernel influences amylolytic, hemi-cellulolytic as well as proteolytic enzyme activities during malting, although the significance of these microbial enzymes in the malt modification process was not demonstrated. Inoculation of *Fusarium* species during steeping[49,85,87] increased α-amylase activity of malt[49,85,87] and diastatic power[85,87], while inoculation during flowering resulted in a decrease in both α-amylase activity of malt and diastatic power[89]. Fungi isolated from barley showing amylase, β-glucosidase and β-xylosidase activities, may actively take part in the degradation of the starchy endosperm of barley and metabolise oligo- en disaccharides released by the degradation of cell wall components.[96] The degradation of β-glucan, the major cell wall component, may also be influenced by microbial activity as the microbial population contributes to the total β-glucanase content[11,80]. Fungal isolates with cellulase activity were demonstrated to be able to dissolve barley β-glucan.[95] More specifically, endo-1,4-β-glucanase[75] and β-glucan solubilase[95] activities appeared to be associated with microbial contaminants and the specific activity of a β-glucan solubilase, possibly originating from barley contaminating microorganisms, was further investigated.[102] It is suggested that microorganisms in the husk contribute to cellulase levels by direct synthesis of cellulase or by secretion of phytohormones or their analogues.[75] Increased protease activity after natural or artificial contamination may also result from different mechanisms and/or factors: some activity may already have been present in the inoculum,[49] some may result from the growth of the inoculated microorganisms during malting,[49,85] the synthesis of germinating barley proteases may be enhanced[49,85] or the release from a bound form may be stimulated by phytohormone secretion by the applied fungi[49,85].

B. Influence on ultimate malt quality : brewhouse performance and beer quality

Microbial contaminants may influence malt modification, brewhouse performance as measured by mash filtration and wort nitrogen content, pH, colour and fermentation, and properties of the obtained beer such as nitrogen content, pH, colour and flavour. One of the major malt-related problems is gushing in beer caused by fungi. The safety of beer may be affected by the occurrence of mycotoxins.

Malt extract difference, or the difference between the extract contents of malt after fine and coarse grinding, was reduced with increased activity of indigenous microorganisms.[19] *Aspergillus*[92] and *Rhizopus*[92] species inoculated during steeping and *Fusarium* species inoculated during flowering[89] or steeping[49,87], were also found to increase the extract and/or to reduce the extract difference. For *Fusarium*, this effect coincided with a decreased β-glucan content of the wort[89] and/or an intensified protein degradation[87]. In agreement with this finding, malt samples artificially contaminated with *Fusarium* after steeping or carrying a natural *Fusarium* contamination, showed an increased β-glucanase and protease activity.[7] Sufficient β-glucan degradation in malt is important to prevent mash filtration problems.

However, large numbers of bacteria on barley kernels may cause a retardation in mash filtration.[48] In this respect, strains of *Pseudomonas*, *Leuconostoc* and *Flavobacterium* species are harmful, while enterobacteria and lactic acid bacteria did not affect mash filtration.[48] It is suggested that the problems result from the production of bacterial capsules and slime such as dextran.[48] Dextran is produced from sucrose, which is the major soluble carbohydrate present in barley and malt.

Reduction of the natural microbial activity resulted in reduced wort proteinaceous nitrogen content.[19] Artificial contamination with yeasts[85], *Aspergillus*[85,92], *Hormodendrum*[85], *Mucor*[85] or *Rhizopus*[92] species or bacteria such as *Pseudomonas*[85] during steeping and with *Fusarium* [49,87] species during flowering[89] or steeping[49,85] increased wort proteinaceous nitrogen.

The fermentation of wort was improved by the inoculation of *Fusarium* species during flowering,[89] while *Aspergillus fumigatus* and *Rhizopus oryzae* added during steeping of barley[92] did not markedly affect the metabolism of the yeast.

The process-dependent organic acid profile and the final concentration of these acids in malt are important factors of variation in wort and beer pH.[45,93] Fluctuations in these factors can lead to inconsistent brewhouse performance and can occur due to both anaerobic respiration of the grain and metabolism of the barley microflora[93]. Organic acids, in particular lactic acid, are produced by microorganisms at all stages of the malting process.[45] It is even suggested to use malt and wort pH to demonstrate a direct link between the developing microflora and wort quality.[45] In another study, a low wort pH was attributed to intensive growth of lactic acid bacteria during malting of split barley kernels.[48]

Increased wort colour mainly seems related to increased activity of indigenous moulds and yeasts.[19] Addition of *Aspergillus*[92], *Rhizopus*[92] or *Fusarium*[49,87] species during steeping altered the

colour of wort and beer prepared from the resulting malts. Malt produced from barley inoculated with *Fusarium* species during flowering resulted in decreased pH of both wort and beer as well as in intensified wort and beer colour.[89] Changes in wort colour by *Fusarium* species may be caused by pigments associated with the added mycelium[49] or by an increased amount of melanoidins as a result of improved modification[49,87].

Inoculation with *Aspergillus fumigatus* during malting resulted in a pronounced roughness and staling flavour of the beer produced from this malt, while *Rhizopus oryzae* inoculation produced no special off-flavours.[92] Sour off-flavours in the mash were attributed to the intensive growth of lactic acid bacteria during malting of split barley kernels.[48]

While secondary gushing is due to faulty production processes or to improper treatment of the bottled beer, primary gushing seems to be related to the quality of the malt.[87] Gushing of beer appears to be associated with malt prepared from barley from late-harvest areas and wetter growing seasons and could therefore be more directly related to the growth of micro-organisms on the barley kernels under moist conditions.[65,87] As a consequence, malt-related gushing is a result of gushing factors already present on barley, although they may also be produced during malting.[88] A minimal quantity of gushing inducing malt appears to be required to cause the phenomenon.[88] The gushing inducing potential of different moulds isolated from 'wheathered' barley was compared by adding concentrated culture filtrates, to beer.[36] While in this study[36] gushing inducing factors are considered that are not generated during growth of the mould on barley, studies with malt obtained after artificial contamination during cultivation or malting give a better indication of the gushing potential of natural contaminants. From these studies *Fusarium* species appear as a major cause of malt-related gushing. Growth of *Fusarium* species during malting, either after inoculation during flowering or steeping, or as a consequence of a naturally occurring contamination, resulted in malt that induced decreased gas stability in the finished beer.[49,57,85,87,89] Significant correlations exist between the intensity of gushing and levels of DON in barley and malt[57], ergosterol in malt[57] and ZER in malt[89]. *Fusarium graminearum* is regarded as a main cause of gushing[57,69,88,98], while high contamination with *Fusarium avenaceum* may be important as well[69]. The infestation of kernels measured by direct or dilution plating has low predictive value for gushing potential.[57,88] As gushing factors were formed before DON during the germination of contaminated barley, it is suggested they are formed independently.[88] Although the factor or mechanism responsible for *Fusarium* related gushing is not yet known, [57] a relationship

between high wort nitrogen of malt treated with some Fusaria and the gas instability of the beers produced from these malts[85], as well as the involvement of a protein factor concentrated on the husk of infected barley or malt[98] are suggested. Furthermore, it is assumed that the gushing inducing factors are formed through interaction between the viable *Fusarium* mycelium and the germinating barley kernels.[87,88] Zearalenone sulphate, a derivative of ZER demonstrated in barley, has been proposed as another possible cause of gushing.[15] Inoculation of *Aspergillus* species during steeping also resulted in decreased gas stability.[85] *Aspergillus fumigatus* and *Aspergillus amstelodami* caused gushing and may be of significance for gushing when they develop during the germination period at temperatures higher than 30°C.[46,91,92] *Rhizopus oryzae*, added during steeping, did not cause gushing[92]. No relation between gushing and the amount of split kernels was found.[69]

VI. CONTROL OF MICROBIAL LOAD AND ACTIVITY

As discussed above, microbial activity may cause substantial losses and influence malt safety and quality, as measured by the microbial status of the malt and by wort and beer quality. Therefore, optimisation of malt production and malt quality necessarily implies that the natural microbial contamination and activity are controlled. To this end, control measures that do not harm the barley physiological processes and that are safe have to be selected.

Control of the microbial activity can, to a limited extent, be accomplished by selection of appropriate conditions during storage and malt production. γ-Irradiation or the use of chemical agents have been proposed for inactivation of the microbial population of barley. Major drawbacks of these treatments are that they are unacceptable to customers and that mycotoxins are not destroyed. There have been attempts to maintain the natural contamination of barley at a level that does not further cause quality defects by the addition of selected microbial cultures. Whereas the use of microorganisms to the advantage of quality malt production was already proposed in 1962[49], the next logical step, a procedure to regulate malt quality by the application of starter cultures, was formulated only in 1985[19]. The use of microbial cultures to suppress the development of toxigenic fungi with a hypothetical additional advantage to degrade or to metabolise mycotoxins was suggested in 1990.[80] A better understanding of the complex microbial ecology of the malting grain may lead to a more efficient exploitation of the microbial potential during malt production. Another approach, that is not further

discussed here, is the selection or genetic construction of barley varieties with increased resistance to fungal infection.

A. Process conditions

Although environmental conditions are unpredictable and uncontrollable before harvest, appropriate storage and malting conditions can be selected in order to restrict the development of the natural contaminants on the barley grain. During storage, temperature and moisture conditions have to be directed not only at prevention of development of storage fungi and decrease of viability of field fungi, but also at preservation of grain germination and prevention of pests caused by insects. The viable field microflora of barley mainly consisting in *Alternaria* (79%) and *Fusarium* (27%), may be reduced or eliminated during storage.[26] Barley highly contaminated with *Alternaria* (75%) and *Fusarium* (85%) was stored under different temperature and aeration conditions and the effect of storage on the viability of these field fungi and on the production of DON by *Fusarium* was investigated.[42] Under all tested conditions, the DON levels of the barley did not change but the viability of especially *Fusarium* was reduced.[42] At higher storage temperature, the ability of *Fusarium* to produce mycotoxins was reduced with storage time.[42] Experiments with one barley variety show that storage of *Fusarium* contaminated barley resulted in a substantial loss of the ability to produce gushing active malt and in a slight decrease of DON formation during malt production.[88] On-farm storage of contaminated barley during longer periods was proposed as a procedure to reduce the level of viable *Fusarium* organisms on barley.[42] Storage of barley under high temperature (40°C) and low moisture content (7.2-8.8%) resulted in a decrease of the viability of the microflora and the water sensitivity of the grain.[32]. Although warm storage is also recommended in other studies[77,90], it is assumed that the population can afterwards multiply to about the same level as that found in control samples.[90] Next to temperature and moisture conditions, the gas composition also influences the microbial activity during storage. Increased levels of carbon dioxide and nitrogen during storage inhibit the development of filamentous fungi.[15]

During malting, the adverse effects of microbes may be reduced by initially steeping the grain without aeration.[90] The use of air rests between immersions is effective for maintaining grain viability during steeping, ensuring subsequent even germination.[14] Changing the liquor with air rests limits the accumulation of microbes and their respiration, presumably by minimising nutrient supplies.[90] An

increased temperature during steeping and germination results in an increased development of bacteria, lactic acid bacteria and filamentous fungi.[47] Although the inhibition of microorganisms by germination at low temperature (5°C)[77] is unfeasible, microbial activity during this process step may be limited by efficient temperature control. For instance, intensified control of the temperature during germination, together with cleaning of the equipment, eliminated the problem of *Aspergillus* species induced gushing.[46]

B. Physical and chemical treatments

Steeping in dilute solutions of inorganic acids such as sulphuric acid,[29,103] phosphoric acid[103] and hypochloric acid[103] improves barley germinative capacity. It is suggested that these acids exert their beneficial effect by antagonising the microorganisms[29,77,90,103]. As a result of addition of sulphur dioxide to the kiln air stream, decreased microbial counts can be recorded[47].

Addition of sodium hypochlorite during steeping results in a reduction of the mould contamination.[47] Sterile barley is obtained by rather severe treatment with this agent, nor with silver nitrate or mercuric chloride.[11] When applied in rather high concentrations, disinfectants such as sodium hypochlorite and mercuric chloride as well as fumigants such as methylbromide or propylene oxide adversely affect seed germination.[28] Sodium hypochlorite in concentrations up to 0.1% does not affect seed germinative energy[47]. Disinfecting with formaldehyde during steeping kills Fusaria on grain, but results in an abnormal earthy smell during germination and a retardation of acrospire growth when this agent is not washed off in further steeping water.[87]

With γ-irradiation, complete sterilisation of barley can be accomplished. A 2 kGy dosage resulted in a greater than 4 log reduction in the number of viable fungi, as measured by dilution plating.[97] After treatment with a dose of 4 kGy most of the *Alternaria*, *Fusarium* and *Epicoccum* species were eliminated, while a dose of 12 kGy was required to kill the *Bacillus* species, yeasts and *Aureobasidium pullulans*.[28] Resistance to irradiation differed among the various fungi tested.[86] Although up to a dose of 8 kGy a slight improvement of germination was detected, the increase in dose to 15 kGy resulted in a gradually decrease of germination.[28] Irradiation doses of 1.25 and 2.50 kGy significantly increased the malt yields but considerably decreased the α-amylase activity.[105] In general, the best control of microorganisms was attained with a γ-irradiation dose of 12 kGy with little effect on seed germination, although the subsequent growth was retarded.[28] However, the measurement of germinative energy of

irradiated barley is too insensitive to predict the effect of irradiation on malt quality,[86] as most of the quality characteristics of malt produced from irradiated barley were negatively influenced by this treatment.[86,104] Nevertheless, it is suggested that, at a low irradiation dose and under specific circumstances, the quality of malt may be improved as a result of the decreased microbial load.[86] Furthermore, irradiation, but also fumigation for purposes of insect disinfestation, may introduce perturbations in the microbiology of the stored grain system and upset the competitive balance between mycotoxigenic species and the background microbial flora.[97] Also, malting of partially sterilised grain in a contaminated environment may result in recontamination or in a rapid development during the malting process of strains that were found to be more resistant to γ-irradiation, such as *Fusarium* species[86].

B. Microbial cultures

The addition of selected microbial cultures to malting barley was first described in 1959 in a modified malting process for the biological acidification of malt.[106] This was accomplished by steeping the germinated cereal in an acid steep liquor, that preferably contained a *Lactobacillus delbrückii* strain. The beneficial effect of this process was that a more favourable pH for the action of amylolytic and proteolytic enzymes was obtained.[107] The influence of culture broths or cell suspensions of *Lactobacillus plantarum* and *Pediococcus pentosaceus* on the indigenous barley microflora was tested during malting on laboratory[37] and pilot[108] scale. Especially the culture broths may be used as natural control agents as they reduced the total bacterial count and *Fusarium* contamination.[37,108] It is suggested that the inhibitory effect is due to the production of antimicrobial compounds by the starter cultures.[37] In the case of heavily contaminated barley, only a minor effect was observed.[108] The selective activity of these lactic acid bacteria allowed the development of other microflora constituents of barley, including yeasts.[37] Depending on the strain added, malt and beer quality was positively affected in terms of increased malt yield[37,108] and modification[37], decreased wort viscosity[37,108] and β-glucan content[37,108], increased mash and wort filterability[108] and improved enzyme balance[108]. Malt prepared with lactic acid bacteria resulted in a lower wort pH (0.1 unit), a reduced wort colour (may be due to restriction of *Fusarium* contamination), and a beer with sound organoleptic properties and with increased attenuation and slightly elevated nitrogen content.[108] Addition of *Lactobacillus plantarum* and *Lactobacillus acidophilus* to steep water resulted in a decreased formation of DON and ZER by the

natural *Fusarium* contaminants.[99] Lactic acid cultures used for inhibiting the growth of undesirable microbial contaminants were described[109], as was a process for improving the properties of malted cereals with *Lactobacillus plantarum* and *Pediococcus pentosaceus*[110]. In another study, a *Lactobacillus plantarum* strain isolated from an industrial malthouse had no significant inhibitory effect on the microflora and only moderately inhibited the production of mycotoxins.[44]

The addition of spores of *Geotrichum* strains with selected properties[44,111], to steeping water during malting of barley, artificially contaminated with *Fusarium*, resulted in the inhibition of undesirable microorganisms such as yeasts and filamentous fungi and in a stimulation of lactic acid bacteria, while the aerobic, mesophilic bacteria were not affected.[44] Also, during trials with this culture on industrial scale (30-200 Ton), the amount of kernels contaminated with *Fusarium* species and the level of DON and ZER in the obtained malt decreased.[112] The antifungal effect was attributed to competition.[112] During malting, *Geotrichum candidium* was detected on all analysed barley kernels.[44] The increased filtration rate of the wort from the obtained malt is attributed to the inhibition of polysaccharide synthesising microorganisms.[44,112] No significant variation in traditional malt quality parameters is found in laboratory scale experiments[44].

Next to studies with biocontrol agents, applications of starter cultures that actively contribute to barley germination and/or malt modification are described. An unspecified, β-glucanase producing starter culture increased green malt β-glucanase activity and improved malt quality parameters such as extract difference, viscosity and filtration time.[11,80] Inoculation under well specified conditions with selected fungal starter cultures during laboratory and industrial scale malting trials, gave rise to a largely increased hemicellulolytic activity of the obtained malt and improved malt modification parameters.[113,114] It was shown that the selected starter cultures deliver enzymes in the starchy endosperm, that contribute to the modification of the cell wall material.[114] The addition of a pure culture of a bacterial strain described as *Pseudomonas herbicola* during the steeping process, resulted in a better quality of the malt and the reduction of the germination period by one day.[115]

VII. CONCLUSIONS

Malt quality is dictated by brewing related specifications including an optimal and homogenous modification of the starchy endosperm, and absence of detectable amounts of toxic or other harmful metabolites of microbial origin.

Although the total microbial load is significant as a quality factor, particular *Fusarium* and *Aspergillus* species have been identified as the most harmful contaminants of which the metabolic activities have to be controlled. The best option is to prevent contamination of barley by these genera. This is theoretically possible during storage and malting, but unfeasible during growth under unpredictable climatic conditions. If malt is to be produced from barley carrying important microbial contaminants, which may develop during the production process and interfere with malt quality, the application of control measures can be considered. The decision on the need for control measures depends on the availability of accurate techniques to estimate past and future microbial metabolic activities important in relation to malt quality.

Whereas acids may be used to regulate the pH of the grain environment, the application of disinfecting agents is not advisable. A limited dose of irradiation may eliminate harmful contaminants, but has to be combined with measures to prevent barley from recontamination.

Biocontrol seems promising as a technique to reduce or inhibit growth, vitality and metabolic activity of natural contaminants on barley. It is the oldest form of food preservation as it has been used for many centuries in traditional food fermentations and is increasingly applied during processing of products such as vegetables, beverages, dairy, and meat and fish products.[116]

The development of lactic acid bacteria and *Geotrichum* on malting barley is probably favoured by malting conditions. Although the effects of these biocontrol agents on the occurrence of *Fusarium* on barley are measured by techniques with limited value from a quantitative point of view, the reduction of DON and ZER levels in the obtained malt indicates that they influence the metabolic activity of *Fusarium* species. The precise mechanism of the interaction between these biocontrol agents and the barley contaminants was not demonstrated so far. The effect depends on the strain used and most probably also on the type and extent of contamination on the barley.

Cultures with positive effects on the grain metabolism and modification may be used in a procedure to make quality malt from barley with physiological deficiencies. Their optimal application requires further insight in the interactions between the plant and microbial metabolic processes during malt production.

The above regulation of malt quality aspects implies a solid state process wherein the natural contamination on malting barley is supplemented with a selected microbial culture. Although this technique may have high potential, effects of the increased microbial load and microbial metabolism on

quality aspects, interactions of the culture with the malting grain and fate in the malt-beer chain deserve further attention. The heterogeneity of the raw material, the natural contamination and the process conditions probably imply difficulties to achieve a homogenous action on the grain mass by the added microbial culture. The effectiveness of these cultures may be enhanced by combined actions to reduce the natural microbial contamination.

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TABLE 1
Media used for Detection of Barley Grain Microorganisms by Direct and/or Dilution Plating

Medium	T	Additives	Detected microorganisms
Acid Potato Dextrose Agar	A		fungi[25], field fungi[26,33,34]
	B		field fungi and yeasts[25]
Czapek Agar	A		filamentous fungi[36]
Czapek-Dox Agar	A	Neomysin + triton X-100 + 2,4-dichlorophenoxy acetic acid	*Fusarium* species[23]
	A	Iprodione + dicloran	*Fusarium* species[37,38,39,40]
Dichloran Chloramphenicol Peptone Agar	A		*Alternaria*[41,42], *Fusarium* species[41,42,43]
Glucose Yeast Extract Agar	B	Cycloheximide	Bacteria[5]
MacConkey Agar	B		*Escherichia coli*[23]
Malt Extract Agar	A		filamentous fungi and yeasts[44]
	B		filamentous fungi and yeasts[44]
Malt Salt Agar	A		filamentous fungi[36]; storage fungi[4,20,21,23,26,31,45]
			Aspergillus and *Penicillium* species[6,23,50]
			Aspergillus glaucus group[6,46]
			Alternaria and *Cladosporium*[50]
			Eurotium species and *Aspergillus restrictus*[30]
	B		filamentous fungi[47], storage fungi[4,24,31]
de Man Rogosa Sharp	B		lactic acid bacteria[23,37,44,47,48]
	B	Cycloheximide	lactic acid bacteria[5]
Oxytetracycline Glucose Agar	B		yeasts[44]
Oxytetracycline Glucose Yeast Extract Agar	B		total count filamentous fungi and yeasts[19]
Pentachloronitrobenzene Agar	A		*Fusarium* species[30]
Plate Count Agar	B		total bacterial count[23,37,44,45,48]
	B	Cycloheximide	bacteria[38]
	B	Ampicillin	fungi[38]
	B	Violet red	total Gram negative count[19]
Potato Dextrose Agar	A		fungi[31,49], field fungi[20], bacteria[6,31]
	A	(Benzyl)penicillin + streptomycin	field fungi[4], *Fusarium* species[50]
	A	Pentachloronitrobenzene + neomycin sulphate	*Fusarium* species[20,21]
	B		bacteria[24], yeasts and fungi[45]
	B	Ampicillin	fungi[38]
	B	Benzylpenicillin	yeasts and filamentous fungi[5]
	B	Benzylpenicillin + streptomycin	field fungi[4]
Raka-Ray Agar	B		lactic acid bacteria[45]
Rose Bengal Streptomycin Agar	B		yeasts and filamentous fungi[24,26]
Tomato juice Agar	B		bacteria[25]
Tryptone Soya Agar	A	Cycloheximide	bacteria and actinomycetes[6]
			Bacillus species and actinomycetes[4]
	B		aerobic spore formers[37,48], total bacterial count[4,19]
			actinomycetes and *Bacillus* species[4]
Wort Agar	B	Sodium propionate	yeasts[23]
Yeast Malt Agar	B		yeasts, filamentous fungi[37]

Legend: T: plating technique: A: direct plating of whole kernels, B: dilution plating.

TABLE 2
Microorganisms detected on Barley(B), Barley Malt Intermediate(I) or Malt(M)

	B	I	M		B	I	M
Bacteria[1]							
Gram negative							
Alcaligenes[5]	-	+	-				
Clavibacterium				*Clavibacterium iranicum*[5]	+	+	+
Enterobacter				*Enterobacter agglomerans*[38,48]	+	-	+
Erwinia				*Erwinia herbicola*[5,12,23,35]	+	+	+
Escherichia				*Escherichia coli*[23]	+	+	+
Flavobacterium[48]	+	-	+				
Pseudomonas[23]	+	+	+	*Pseudomonas fluorescens*[5,38,48]	+	+	+
Xanthomonas				*Xanthomonas campestris*[35]	+	-	-
Gram positive							
Actinomycetes[4,6,23,35]	+	+	+				
Arthrobacter				*Arthrobacter globiformis*[5]	+	+	+
Bacillus[18,23,28,48]	+	+	+	*Bacillus cereus*[38]	+	-	-
				Bacillus cereus var. *Mycoides*[6]	+	-	-
Corynebacterium				*Corynebacterium fasciens*[38]	+	-	-
Lactobacillus[5]	+	+	+	*Lactobacillus acidophilus*[48]			
				Lactobacillus agilis[45]	-	+	-
				Lactobacillus alimentarius[48]			
				Lactobacillus casei[48]	-	-	+
				Lactobacillus casei subsp. *pseudoplantarum*[48]	+	-	-
				Lactobacillus casei subsp. *rhamnosus*[45]	-	+	-
				Lactobacillus delbrueckii subsp. delbrueckii[45]	-	+	-
				Lactobacillus gasseri[45]	-	+	-
				Lactobacillus plantarum[45,48]	-	+	-
Leuconostoc				*Leuconostoc mesenteroides* subsp. *mesenteroides*[48]	+	-	+
Micrococcus[23]	+	+	+				
Pediococcus				*Pediococcus pentosaceus*[48]	+	-	+
Streptomyces[6]	+	-	-				
Thermoactinomyces				*Thermoactinomyces vulgaris*[12]	+	-	-
Fungi[2]							
Ascomycota							
Alternaria[18,21,23,25,26,33,34,35,36,37,40,41,42,46,47,51,55,81,84,85,]	+	+	+	*Alternaria alternata* (syn.[3] *Alternaria tenuis*)[4,5,28,30,35,38,52,53,69,70]	+	+	+
				Alternaria infectoria[38]	+	-	-
				Alternaria tenuis[6,12,15,46]	+	-	-
				Alternaria tenuis sensu[33,34]	+	-	-
Arthrinium[30]	+	-	-	*Arthrinium phaeospermum*[6]	+	-	-
Aspergillus[10,21,23,24,35,36,40,4	+	+	+	*Aspergillus candidus*[4,5,6,12,16,23,30,35,71]	+	+	+

[1] Exceptionally coliforms, *Escherichia coli* and fecal streptococci are found.[16]
[2] Classification as described in Ainsworth & Bisbey's dictionary of the fungi[83]
[3] Synonym mentioned in this list

6,47,55,81,84,85,86							
				Aspergillus clavatus[5,30]	+	+	-
				Aspergillus flavus[5,6,9,13,15,16,23,28,30,31,38,46,71,82]	+	+	+
				Aspergillus fumigatus[5,6,9,12,16,30,35,46,82]			
				Eurotium (*Aspergillus glaucus* group)[4,5,6,9,12,23,31,35,52,71]	+	+	+
				E. amstelodami[6,12,30,46]			
				E. chevalieri[6,30]			
				E. repens[6,12,15,30]			
				E. rubrum[6,30]			
				Aspergillus nidulans[6,12,16,30]	+	-	-
				Aspergillus niger[6,30,31,47,71]	+	-	-
				Aspergillis ochraceus[30,82]	+	-	-
				Aspergillus restrictus group[30]	+	-	-
				Aspergillus sydowii[6]	+	-	-
				Aspergillus tamarii[71]	+	-	-
				Aspergillus terreus[5,6,16,36,82]	+	-	+
				Aspergillus ustus[30]	+	-	-
				Aspergillus versicolor[6,23,30,35]	+	+	+
Botrytis[18,81]	+	-	-	*Botrytis cinerea*[4,5,6,15,30,46,69]	+	-	+
Candida[24,38,85]	+	-	-	*Candida catenula*[5]	-	+	+
				Candida vini[5]	+	-	-
Chaetomium[23,30]	+	-	-	*Chaetomium globosum*[6,15]	+	-	-
				Chaetomium indicum[6,12,16]	+	-	-
Chrysosporium[5]							
Cladosporium[4,6,18,21,23,35,36,37,46,51,52,53,81]	+	+	+	*Cladosporium cladosporioides*[5,6,12,15,30,35,53,38]	+	-	-
				Cladosporium herbarum[6,12,30,53,46,69]	+	-	-
				Cladosporium macrocarpum[5,70]	+	-	-
Cochliobolus				*Cohliobolus sativus* (syn. *Drechslera sorokiniana*, *Helminthosporium sativum*, *Helminthosporium sorokinianum*)[4,5]	+	+	-
Curvularia				*Curvularia harvey*[30]	+	-	-
				Curvularia lunata[30]	+	-	-
				Curvularia spicifera[6,15]	+	-	-
Debaryomyces				*Debaryomyces hansenii*[5]	-	+	-
Didymella				*Didymella exitialis*[69]	+	-	-
Drechslera[30,51]	+	-	-	*Drechslera australiensis*[30]	+	-	-
				Drechslera biseptata[30]	+	-	-
				Drechslera dematioidea[30]	+	-	-
				Drechslera halodes[30]	+	-	-
				Drechslera sorokiniana[38,69]	+	-	-
				Drechslera teres[69]	+	-	-
				Drechslera state of *Cochliobolus miyabeanus*[30]	+	-	-
				Drechslera state of *Cochliobolus sativus*[30]	+	-	-
				Drechslera state of *Cochliobolus spiciferus*[30]	+	-	-
				Drechslera state of *Cochliobolus victoriae*[30]	+	-	-
				Drechslera state of *Pyrenophora avenae*[30]	+	-	-
				Drechslera state of *Pyrenophora chaetomioides*[30]	+	-	-
Eupenicillium[72]	+	-	-				
Fusarium[4,18,21,23,24,26,28,33,34,35,36,38,39,41,42,46,47,48,52,81,84,]	+	+	+	*Fusarium acuminatum*[30]	+	-	-

85,86,87,88							
				Fusarium avenaceum[5,6,12,15,18,40,51,52,69,89]	+	-	+
				Fusarium chlamydosporum[30]	+	-	-
				Fusarium culmorum[6,7,15,23,28,40,51,52,69,89]	+	-	-
				Fusarium dimerum[5]	+	-	-
				Fusarium equiseti[30,40,69]	+	-	-
				Fusarium graminearum[7,15,18,30,33,41,42,43,51,69,85,88]	+	-	-
				Fusarium lateritium[6]	+	-	-
				Fusarium moniliforme[30,31,85]	+	-	-
				Fusarium nivale[6,12,15]	+	-	-
				Fusarium oxysporum[7,30,69]	+	-	-
				Fusarium poae[5,6,13,15,18,30,40,41,69]	+	-	-
				Fusarium sambucinum[30,51]	+	-	-
				Fusarium scirpi[30]	+	-	-
				Fusarium solani[30]	+	-	-
				Fusarium sporotrichiodes[6,18,41,51,69]	+	-	-
				Fusarium subglutinans[30]	+	-	-
				Fusarium tricinctum[18,40,51,69,89]	+	-	-
Geotrichum[81]	-	+	-	*Geotrichum candidum*[4,5,30,52]	+	+	+
Gonatobotrys[30]	+	-	-	*Gonatobotrys simplex*[69]	+	-	-
Hansenula				*Hansenula polymorpha*[5]	+	-	-
Hyphopichia				*Hyphopichia burtonii*[13]	+	-	-
Hypocrea				*Hypocrea pulvinata*[38]	+	-	-
Kloeckera				*Kloeckera apiculata*[5]			
Microdochium				*Microdochium bolleyi*[69]	+	-	-
				Microdochium nivale v. majus[69]	+	-	-
				Microdochium nivale v. nivale[69]	+	-	-
Neocosmospora[30]	+	-	-				
Nigrospora[85]	+	-	-	*Nigrospora oryzae*[30]	+	-	-
Paecilomyces[30]	+	-	-				
Papularia[23]	+	-	-	*Papularia arundinis*[6]	+	-	-
Penicillium[4,5,6,7,10,12,21,23,30,35,36,40,46,47,52,55,69,71,75,81,84,85,86]	+	+	+	*Penicillium brevicompactum*[5,72]	+	-	-
				Penicillium canescens[72]	+	-	-
				Penicillium capsulatum[72]	+	-	-
				Penicillium chrysogenum[5,6,31,38,72]	+	-	-
				Penicillium citrinium[72]	+	-	-
				Penicillium claviforme[5,72]	+	-	-
				Penicillium commune[38]	+	-	-
				Penicillium concentricum[72]	+	-	-
				Penicillium corylophilum[38]	+	-	-
				Penicillium cyclopium[6,9,31]	+	-	-
				Penicillium digitatum[6]	+	-	-
				Penicillium expansum[5,6,72]	+	-	-
				Penicillium frequentans	+	-	-
				Penicillium funiculosum[72]	+	-	-
				Penicillium granulatum[72]	+	-	-
				Penicillium griseofulvum[72]	+	-	-
				Penicillium hordei[72]	+	-	-
				Penicillium janthinellum[72]	+	-	-
				Penicillium melinii[72]	+	-	-
				Penicillium nigricans[72]	+	-	-
				Penicillium oxalicum[72]	+	-	-
				Penicillium pedemontanum[72]	+	-	-
				Penicillium piceum[6,16,72]	+	-	-
				Penicillium pulvillorum[72]	+	-	-
				Penicillium purpurogenum[72]	+	-	-

				Penicillium raciborskii[72]	+	-	-
				Penicillium roquefortii[72]	+	-	-
				Penicillium rugulosum[72]	+	-	-
				Penicillium sorghina var. *corymbiferum*[72]	+	-	-
				Penicillium sorghina var. *ochraceum*[72]	+	-	-
				Penicillium urticae[72]	+	-	-
				Penicillium variabile[72]	+	-	-
				Penicillium verrucosum[13,72]	+	-	-
				Penicillium verrucosum var. *cyclopium*[72]	+	-	-
Phoma[6,18,30]	+	-	-	*Phoma herbarum*[5]	+	-	+
				Phoma sorghina[30]	+	-	-
Pithomyces[30]	+	-	-				
Pyrenophora				*Pyrenophora teres*[4,5]	+	+	+
Saccharomyces[24]	+	-	-				
Scopulariopsis[30]	+	-	-	*Scopulariopsis brevicaulis*[6,16]	+	-	-
Septoria[18,51]	+	-	-	*Septoria nodorum*[38]	+	-	-
Sordaria[30]	+	-	-	*Sordaria fimicola*[6]	+	-	-
Stemphylium[21,23,30,36,69,85]	+	-	-	*Stemphylium consortiale*[6,15]	+	-	-
Talaromyces				*Talaromyces emersonii*[72]	+	-	-
				Talaromyces thermophilus[72]	+	-	-
Thermoascus				*Thermoascus crustaceus*[6,12,16]	+	-	-
Thielavia[30]	+	-	-	*Thielavia sepedonium*[16]	+	-	-
Torulopsis[35]	+	-	-	*Torulopsis candida*[75]	+	-	-
Trichoderma[30,85]	+	-	-	*Trichoderma viride*[6,15,38]	+	-	-
Williopsis				*Williopsis californica*[38]	+	-	-
Basidiomycota							
Cryptococcus[12,35]	+	-	-	*Cryptocoocus albidus*[38]	+	-	-
Rhizoctonia[85]	+	-	-				
Zygomycota							
Absidia[4,35,52]	+	+	+	*Absidia corymbifera*[5,6,12,16,52]	+	-	+
				Absidia ramosa[16]	+	-	-
Mucor[4,7,10,21,23,30,35,37,46,81,85]	+	+	+	*Mucor hiemalis*[5,6]	+	-	-
				Mucor mucedo[69]	+	-	-
				Mucor pusillus[6,16]	+	-	-
Rhizopus[4,7,21,23,35,36,46,51,85]	+	+	+	*Rhizopus microsporus*[16]	+	-	-
				Rhizopus oryzae[30,92]	+	-	+
				Rhizopus stolonifer[6,69,75]	+	-	-
Syncephalastrum				*Syncephalastrum racemosum*[6,16,30,71]	+	-	-
Thamnidium				*Thamnidium elegans*[6]	+	-	-
Mitosporic fungi							
Acremoniella[21,23]	+	-	-	*Acremoniella atra*[6]	+	-	-
Acremonium[18,30]	+	-	-	*Acremonium strictum* (syn. *Cephalosporium acremonium*)[69]	+	-	-
Arthrobotrys				*Arthrobotrys superba*[46]	+	-	-
Aureobasidium[18,35,81]	+	-	-	*Aureobasidium pullulans*[4,5,6,12,15,28,30,35,38,52,69,75]	+	+	-
Cephalosporium[6,15,21,23,37]	+	+	+	*Cephalosporium acremonium*[6]	+	-	-
Doratomyces[23]	+	-	-				
Epicoccum[18,21,23,35,46,84]	+	+	+	*Epicoccum nigrum*[6,12,15,30,38,53,69,70]	+	-	-
				Epicoccum purpurascens (syn. *Epicoccum nigrum*)[4,5,28,35,40,52]	+	+	+

Gliocladium				*Gliocladium roseum*[4]	-	+	-
Harzia				*Harzia acremonioides* (syn. *Acremoniella* atra)[69]	+	-	-
Helminthosporium[21,23,24,25,26,33,34,37,36,84,85]	+	+	+	*Helminthosporium graminearum*[15]	+	-	-
				Helminthosporium sativum[6,15,46,70]	+	-	-
				Helminthosporium sorokinianum[33,34]	+	-	-
				Helminthosporium teres[6,46]	+	-	+
Hormodendrum[85]	+	-	-				
Monilia[85]	+	-	-				
Papulaspora[30]	+	-	-				
Rhodotorula[12,24,35,53]	+	-	-	*Rhodotorula glutinis*[38,75]	+	-	-
				Rhodotorula mucilaginosa[5]	+	-	+
Sclerotium[85]	+	-	-				
Septonema[85]	+	-	-				
Spicaria[85]	+	-	-				
Sporobolomyces[35]	+	-	-	*Sporobolomyces roseus*[5,6,12,38,53]	+	-	-
Thermomyces				*Thermomyces lanuginosus*[6,12,16]	+	-	-
Thielaviopsis[85]	+	-	-				
Torula				*Torula herbarum*[6]	+	-	-
Trichosporon				*Trichosporon beigelii*[5]			
Trichothecium[21,23]	+	-	-	*Trichothecium roseum*[4,6,15,30,46]	+	+	+
Ulocladium[18,30,40]	+	-	+	*Ulocladium atrum*[69]	+	-	-
Verticillium[12,18,35,53,69]	+	+	+	*Verticillium lecanii*[4,5,52]	+	+	-
Wallemia				*Wallemia sebi*[81]	+	-	-

FIGURE 1
Malt Production Chain

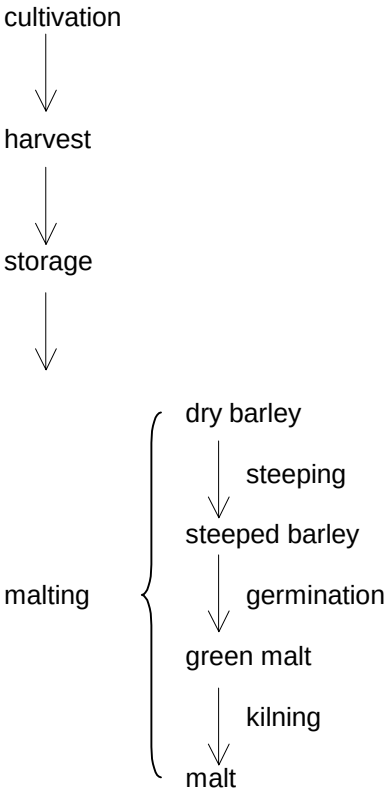
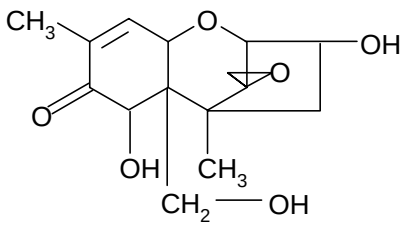
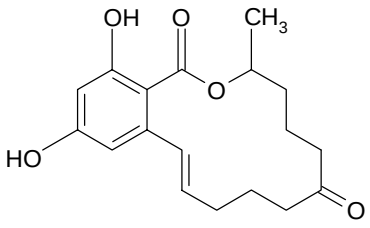


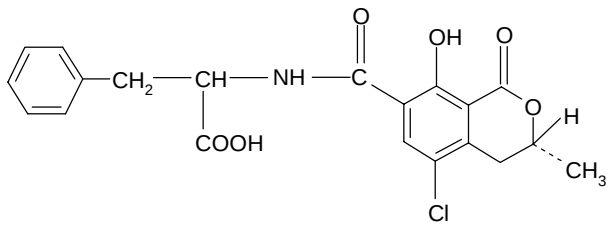
FIGURE 2
Structure of Trichotecene, Zearalenone and Ochratoxin A



12,13-epoxy-Δ9-trichotecenes



zearalenone



ochratoxin A

TABLE 3
Important mycotoxins detected in barley and malt, and producing microorganisms

Fungal species	Mycotoxin
Storage fungi	
Aspergillus flavus	aflatoxin B1[59]
Penicillium species	citrinin[59]
Aspergillus ochraceus, *Penicillium viridicatum*, *Penicillium verrucosum*	ochratoxin A[4,59,81]
Field fungi	
Fusarium graminearum, *Fusarium culmorum*	deoxynivalenol (DON) [59]
Fusarium graminearum, *Fusarium culmorum*, *Fusarium sporotrichioides*, *Fusarium poae*	diacetoxyscirpenol (DAS) [59]
Fusarium sporotrichioides, *Fusarium poae*	T-2 toxin[59]
Fusarium graminearum, *Fusarium culmorum*	zearaleneone (ZER) [59]