FUNGAL GROWTH DURING MALTING OF BARLEY

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Fungi were isolated and identified in two samples of winter two-row barley (SSK3 and SSK6) harvested in 2003, Kragujevac location, during micromalting. Fungi were isolated and identified in barley before the micromalting, after the 1st, 2nd and 3rd day of steeping, the first day and after the germination, after kilning and after malt degermination. The total fungi count was followed in both barley samples, during the mentioned phases. The total count of fungi was also determined in the steeping water; and the isolation and identification was performed after the steeping process. Change of the total count of fungi during barley micromalting was exponential. During barley micromalting nine fungi genera were isolated: Phoma, Alternaria, Fusarium, Aspergillus, Cladosporium, Geotrichum, Scopulariopsis, Aureobasidium and Mucor. The most frequent genera were: Phoma, Alternaria and Fusarium. In water for steeping, five genera were identified: Geotrichum, Fusarium, Phoma, Cladosporium and Mucor. The most frequent genera was Phoma.

KEYWORDS: Barley; micromalting; fungi

INTRODUCTION

Barley is the raw material for production of malt, which is one of the basic elements for the production of very popular drink – beer.

Due to the nutritive value, barley is very convenient for the growth of various microorganisms, first of all different fungi (1, 2).

The conditions of malting may promote the activation of fungal spores which are present on/in barley seed and growth of fungi, resulting in the synthesis of very toxic secondary metabolites – mycotoxins (3).

Although several studies demonstrated that the composition of mycopopulations qualitatively changes during malting, it is difficult to discern any consistent pattern of change.
(4-8). The process variability has a pronounced effect on the growth of species for which growth conditions are optimal.

The activity and growth of mycological contamination during malting depends on:
- initial contamination of barley,
- interaction between the species of microbial population,
- characteristics of barley grains, like moisture content and availability of nutritive components,
- process parameters, like temperature, aeration, use of additives, such as SO₂ (9).

Additional contamination is possible due to specific mycopopulation which may be present in the micromalting plant (9).

The barley may be contaminated by fungi during growing in the field, transport and storage, resulting in yield decrease, lower average seed dimensions, decreased nutritive value, colour loss, change of smell and taste (10 – 13).

During the development on barley grains, the toxigenic fungal species may synthesize mycotoxins which are accumulating in the grains. Due to their thermal stability, these toxins may be transmitted into the final product – beer, over the malting process, wort production and pasteurization (14).

The fungal spores develop during and after the malting process causing:
- inhibition of barley grains germination,
- increase of α-amylase activity,
- gushing beer (9, 15, 16) and
- hydrolysis of beer barley proteins (17).

Having in mind the mentioned facts, and especially the ability of mycotoxins production, the aim of this work was to investigate the change of composition of fungi in the isolated mycopopulations during micromalting and of their total count.

EXPERIMENTAL

Two samples of two-row winter barley (SSK3 and SSK6), harvested in 2003, on the Kragujevac location, sampled from the storehouse in the same year, were micromalted in “Seeger” type plant, Germany, according to standard Kuhn’s method (18).

The total count of fungi per 1 g or 1 mL was determined during the micromalting process. The fungi were isolated and identified in barley, semiproducts of malting, final malt and steeping water.

Samples were taken before the micromalting process, after the first, second and third day of steeping, after the first day and at the end of germination (before the kilning), after kilning and after malt degermination.

Water samples were taken from the steeping vessel before the process and after the 1st, 2nd and 3rd day of steeping. The total count of fungi per 1 g or 1 mL was determined by dilution method according to Koch (1).

Identification of fungal species was performed according to Ellis (19, 20), Nelson et al. (21) and Samson and van Reenen-Hoekstra (22).
RESULTS AND DISCUSSION

Mycological status of steeping water

The total count of fungal isolates varied from very small values (before the malting process), over maximal value \(2.0 \times 10^3\) per 1 mL after the 1st day, sharp decrease after the 2nd day \(3.5 \times 10^2\) per 1 mL and increase the 3rd day \(1.5 \times 10^3\) per 1 mL, followed by significant change of mycopopulation (Figure 1).

![Graph showing change of total count of fungal isolates in steeping water](image)

**Fig. 1.** Change of total count of fungal isolates in steeping water (during the steeping process)

The isolated fungi in water for steeping most probably originated from the steeping vessel, from or immediately under the barley grain surface. Therefore, the great number of fungal isolates in the steeping water after the first day is most probably the result of washing off of fungi from the surface of grains. The total count of fungal isolates decreased to only 17.5% after the second day of steeping. The increase of total count of fungal isolates, determined the third day, may be a consequence of activation of fungal spores that were immediately under barley surface, due to better steeping of grains.

Only one fungal species, *Geotrichum candidum*, was found in the water before the steeping process, and the count was very low. *G. candidum* is very often found in dairy products, but was also identified on the equipment used for processing of plants and plant products (23). Therefore, *G. candidum* probably originates from the steeping vessel surface.

After the first day of steeping, *Fusarium sporotrichioides* and *Phoma glomerata* were identified. The same species were identified after the 2nd day of steeping, together with *F. verticillioides*. A significant decrease of total count of fungi was noted (only 17.5% compared to the first day), however, the ratio of the particular genera in total mycopopulation was almost identical. A significant change of composition of the mycopopulation was observed after the third day of steeping. The total count of fungal species increased again (75% of the total count determined the 1st day), and *F. sporotrichioides* was identified. Increased counts of *Cladosporium sphaerospermum* and *Mucor hiemalis* were also
observed. The ratio of *Fusarium* species in the total mycopopulations increased slightly. The most frequent species was *Phoma glomerata* (Fig. 2).

**Mycological status of SSK3 barley sample during micromalting**

The growth of the total fungal count during the micromalting process (from the first to the seventh day) was exponential. After the kilning, the count of fungi species decreased by 30%, and after the malt degeneration by another 50%.

The growth rate of the total fungal count to the seventh day of micromalting of SSK3 barley sample was exponential (Fig. 3), and the growth exponent is very close to 1 (1.025). The function of the total fungal count depending on the day of micromalting and the coefficient of correlation of the function are:

\[
\text{Total fungal count of SSK3} = 22.97 \times e^{1.025 \times \text{day of micromalting}}, \quad \rho = 0.7781
\]

In contrast to Fig. 3, where it seems that the growth of fungi is homogenous to the seventh day of micromalting, the dynamics as a consequence of alternating domination of certain fungi genera is obvious in Fig. 4. *Phoma* genus, with 80.4% frequency, was clearly dominant the first day of steeping. The intensive increase of *Alternaria* species the second day, decreased the growth rate of *Phoma* genus. However, a more intensive growth of *Phoma* genus and smaller decrease of *Alternaria* genus count was noticeable the third day. The growth of *Fusarium* and *Alternaria* genera inhibited the further growth of *Phoma* genus the fourth day. By the seventh day, the increase of *Geotrichum* genus inhibited the further growth of *Fusarium* genus. The increase of *Alternaria* and *Geotrichum* genera continued, however, without domination of either genus. Before the start of the kilning (the seventh day), a sharp increase of *Phoma* genus was observed, suppressing the growth of all other genera. The genus *Phoma* was represented with 90.5% in all mycopopulations on the day of kilning (Fig. 4).
**Fig. 3.** Change of total count of fungi isolates during micromalting of SSK3 sample

**Fig. 4.** Mycological status of most frequent fungi during micromalting of SSK3 barley sample
Mycological status of SSK6 barley sample during micromalting

The exponential growth of the total fungal count was observed in this sample too, from the first to seventh day of micromalting. The growth rate of the total fungal count was also exponential (Fig. 5), with the growth exponent (0.96) approaching 1. The function of total fungal count depending on the day of micromalting and the coefficient of correlation of that function are as follows:

$$\text{Total fungal count SSK6} = 35.04 \times e^{0.96 \times \text{day of micromalting}}, \rho = 0.7638$$

![Fig. 5. Change of the total fungal count during micromalting of SSK6 sample](image)

The kilning conditions are unfavorable for further fungal growth, so the total count of fungi in the barley samples SSK3 and SSK6 decreased after kilning. The moisture content of green malt decreased, while an elevated temperature affected the inactivation of enzymes, resulting in a decrease of metabolic activities, growth inhibition and dying of a great number of fungi. The further decrease of the total fungal count was affected by malt degeneration, as a certain number of fungi was removed with the germ.

An remarkable change of the dynamics of domination of some fungal genera was observed in the barley sample SSK6. The initial conditions in this sample were the most favourable for the growth of *Phoma* genus. However, *Fusarium* and *Alternaria* genera were represented in all phases of micromalting. The highest growth and domination in this sample showed *Fusarium* genus. The growth of *Alternaria* and *Fusarium* genera was identical by the second day. The growth of *Cladosporium* and *Mucor* genera inhibited the growth of *Alternaria* genus, however, not affecting the growth of *Fusarium* genus. After the fourth day, simultaneous growth of *Cladosporium*, *Mucor* and *Alternaria* genera was recorded, whereas the *Fusarium* genus was dominant. The *Alternaria* genus was identified in this sample also after kilning (79.34% in the whole population) (Fig. 6).
The data obtained in this study on the frequency genera *Alternaria* and *Fusarium* in both barley samples are in accordance with the literature data obtained by researchers in different countries. Mycological studies on 260 samples of wheat, barley and oat showed similar results in relation to the frequencies of these two genera in Norway (24). A high frequency of *F. poae* (approximately 20%) was determined in barley grain in the U.S.A. (25) and Czech Republic (26), as well as, in oat grain in Canada (27). The frequency of *Alternaria* spp. (up to 72%) and *Fusarium* spp., especially *F. poae* (53%), in barley, i.e. wheat grain, was determined by Bočarov-Stančić et al. (13), i.e. Balaž et al. (28) and Dopuđa and Lević (29), respectively.

In this study, nine fungi genera were isolated during barley micromalting, viz.: *Phoma, Alternaria, Fusarium, Aspergillus, Cladosporium, Geotrichum, Scopulariopsis, Aureobasidium* and *Mucor*. The same genera were isolated from South African barley during malting by Gyllang and Martinson (30) and Ackermann (31). Gyllang and Martinson (30) also identified in barley and during the malting process the following genera: *Penicillium, Rhizopus* and *Helminthosporium*. Ackermann (31) also reported the presence of following the genera: *Pyrenopora, Rhizopus, Penicillium* and *Epicoccum*. According to Haikara et al. (32) *Alternaria alternata* is the most common field fungus on the malting barley and a prominent species during malting. This study confirms that *Alternaria alternata* is the most common fungus on the malting barley and during micromalting.

The total fungal count in malt for SSK3 sample was $1.0 \times 10^4$ per 1 g, and for SSK6 sample $2.9 \times 10^4$ per 1 g, and this is in accordance with the Rulebook on microbiologic safety of foodstuffs at the market (Official Bulletin SRJ 26/93) (33). In malts produced
from both barley varieties, four fungi genera were isolated and identified: Alternaria, Fusarium, Cladosporium and Phoma. These results are in accordance with the results obtained by Noots et al. (9) and Gyllang and Martinson (30).

CONCLUSIONS

Five genera and seven fungal species (Geotrichum candidum, Fusarium sporotrichioides, F. verticillioides, F. poae, Phoma glomerata, Cladosporium sphaerospermum and Mucor hiemalis) were isolated and identified in the steeping water. The most dominant species was Phoma glomerata.

The fungal growth during micromalting was exponential and the specific growth rate of fungi was similar in both barley samples.

Eight fungi genera were isolated from SSK3 barley sample during micromalting: Alternaria, Fusarium, Aspergillus, Cladosporium, Phoma, Geotrichum, Scopulariopsis and Aureobasidium. The most dominant genera were Phoma and Alternaria.

Five fungi genera were isolated from SSK6 barley sample during micromalting: Alternaria, Fusarium, Cladosporium, Phoma and Mucor. The most dominant genera were Alternaria and Fusarium.

The total fungal count in malt for SSK3 sample was $1.0 \times 10^4$ per 1 g, and for SSK6 sample $2.9 \times 10^4$ per 1 g.

REFERENCES


33. Rulebook on microbiologic safety of foodstuffs at the market (Official Bulletin SRJ 26/93).

### РАЗВОЈ ГЉИВА ТОКОМ МИКРОСЛАДОВАЊА ЈЕЧМА

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У два узорка озимог дворедог јечма (ССК3 и ССК6) жетве 2003, са локалитета Крагујевца, вршено је изоловање и идентификација гљива током процеса микросладовања. Изоловање и идентификација гљива вршена је у јечму непосредно пре микросладовања, 1., 2., и 3. дана мочења, 1. дана клијања, након клијања, након сушења и након отклицавања. Током ових фаза праћена је и промена укупног броја гљива за оба узорка јечма. У води за мочење је такође одређиван укупан број гљива, њихово изоловање и идентификација током мочења. Промена укупног броја плесни током микросладовања била је експоненцијална. Током микросладовања јечма изоловано је девет родова плесни: *Phoma*, *Alternaria*, *Fusarium*, *Aspergillus*, *Cladosporium*, *Geotrichum*, *Scopulariopsis*, *Aureobasidium* и *Mucor*. Најзаступљенији родови су били: *Phoma*, *Alternaria* и *Fusarium*. У води за мочење идентификовано је пет родова: *Geotrichum*, *Fusarium*, *Phoma*, *Cladosporium* и *Mucor*. Најзаступљенији род је био *Phoma*.

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