Improvement of Malt Modification by Use of *Rhizopus* VII as Starter Culture

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The development of a selected starter culture on malting barley and its effects on malt quality aspects were studied. Application of *Rhizopus* sporangiospores in a malting process resulted in increased β-glucanase and xylanase contents of the malting barley and improved starchy endosperm cell-wall degradation. Activation of the sporangiospores and optimization of the inoculation procedure led to a further increase in enzyme levels and to larger and more consistent impacts on cell-wall modification. Whereas the main effect of the starter culture on β-glucan degradation was observed during malting, a further decrease in β-glucan during mashing suggests that the microbial enzymes that survived the kilning step were active during mashing. Other quality aspects that were influenced by the starter culture activity were protein modification, wort color, and wort pH. The level of microbial enzymes produced was related to the amount of barley kernels infected with the starter culture.

Keywords: *Barley malt; cell wall modification; starter culture*

INTRODUCTION

The addition of microbial cultures during malting to improve malt quality aspects is regarded as a recent development in malting technology. However, as early as 1959, Dixon (*1*) claimed a procedure for the biological acidification of malt by the addition of lactic acid bacteria during malting. Haikara et al. (*2*), Haikara and Mattila-Sandholm (*3*), Haikara and Laetila (*4*), and Laetila et al. (*5*) used lactic acid bacteria as biocontrol agents, and Boivin and Malanda (*6*−*8*) investigated the use of *Geotrichum* as a biocontrol agent. These microbial cultures are believed to improve malt safety by controlling the development of the natural barley microflora. Another application was the addition of a *Pseudomonas herbicola* culture during steeping to improve barley germination (*9*). Bol et al. (*10*) describe the use of an unspecified β-glucanase-producing strain during malting, which resulted in an increased enzymatic activity of green malt and improved analytical data for fine−coarse extract difference, wort viscosity, and wort filtration.

The present study is part of a research effort that focuses on solving deficiencies in malt cell-wall modification by use of starter cultures which produce cell-wall-degrading enzymes during the malting process. Malt cell-wall modification is an important quality parameter, as it predicts how malt performs in the brewhouse in terms of extract yield and rate of wort separation from spent grains (*11*). For an optimal performance, evenly well-modified grain of low residual β-glucan content is preferred. The rate of modification depends not only on the rate of moisture distribution through the kernel and structural features of the starchy endosperm, but also on the rate of synthesis and extent of release of enzymes into the starchy endosperm (*12*). Besides the level of the major malt cell-wall component β-glucan, which is regarded as the best indicator of malt quality (*13*), malt friability, fine−coarse extract difference, and wort viscosity are functional measures that indicate the extent of cell-wall modification. In an initial phase of the research, fungal strains from different origins that develop on nongerminating sterilized barley and that contribute to cell-wall modification by delivering cell-wall-degrading enzymes in the starchy endosperm were identified (*14*). The main objective of the present study was to investigate the development and activity of one of these microbial cultures (a *Rhizopus* strain) in interaction with the natural barley microflora and the barley physiological processes during malting, and to study its effect on malt cell-wall modification. The contribution of the starter culture to malt modification was optimized, and malt quality aspects other than cell-wall modification were evaluated as well.

MATERIALS AND METHODS

Chemicals and Media. β-Glucazyme and xylazyme AX tablets were obtained from Megazyme (Bray, Ireland). Dichloran glycerol agar (DG18), potato dextrose agar (PDA), sabouraud dextrose agar (SDA), and tryptone soya broth (TSB) were purchased from Unipath (Hampshire, UK), and agar was obtained from Life Technologies (Paisley, UK). Calcofluor white, chloramphenicol, fast green, and tris(hydroxymethyl)-aminomethane (TRIS) were from Sigma-Aldrich (Bornem, Belgium), and glycerol and sodium hypochlorite came from Merck-Eurolab (Leuven, Belgium). Tryptone soya agar supplemented with 100 ppm pimaricine (TSAp), oxytetracycline gentamicine yeast extract agar (OGYE), malt salt agar (MSA), and pentachloronitrobenzene agar (PCNB) were prepared as described before (*14*).

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Barley. Malting-grade barley samples Escourgeon (1996, French harvest), Pastoral (1996, French harvest), Plaisant (1994, 1996, and 1997, French harvest), and Stander (1997, American harvest) were used, as well as the barley varieties Balkan, Cassandra, Gamelan, Milonga, Spinet (1996, Belgian harvest), and Cassandra (1997, Belgian harvest).

γ-Irradiation of Barley. Barley samples (1.0 kg) were treated in a Gammacell 2200 (IBAMediris, Fleurus, Belgium).

Cultivation of Starter Culture and Preparation of Inoculum. The strain *Rhizopus* VII (*14*) was used. Fungal inoculum was prepared by flooding a 7-day-old culture (grown at 28 °C on a PDA slant in the dark) with sterile physiological saline [0.9% (w/v) sodium chloride]. The mycelium was gently rubbed with a sterile spatula, and the suspension was washed and centrifuged (1600*g*, 15 min) twice. The pellet was resuspended in sterile physiological saline. The sporangiospore (further referred to as spore) count of the suspension was determined microscopically using a Thoma counting chamber. Spores (5×10^7) were transferred into 20-mL sterile, acidified (pH 4.0) TSB, incubated during 5−6 h in a shaking water bath at 42 °C, harvested by centrifugation (1600*g*, 15 min), washed and suspended in sterile physiological saline, and are further referred to as activated spores.

Malting. Steeping and germination were carried out on a laboratory scale using separate steeping vessels and germination drums to avoid contact between samples while using the starter culture. Two different malting processes were conducted.

In process A, barley (2.0 kg) was steeped with tap water (water/barley ratio of 1.5:1) in a fermentor (BioFlo III, 5.0-L working volume; New Brunswick Scientific Co., Edison, NJ) in which a perforated plate was placed. Steeping was carried out by immersion (wet stages: 6 h at 13 °C; 5 h at 14 °C, 2 h 30 min at 16 °C; air rest stages: 17 h at 20 °C; 15 h 30 min at 20 °C). Germination was in cylindrical drums (diameter,10 cm; length, 30 cm) with perforated lids at a temperature of 18 °C for 4 days. Air was supplied by natural diffusion. The drums were slowly rotated on an electronically controlled roller system (Tecnomara, Zurich, Switzerland). Thus, every 2 h the containers were rolled for 15 min at 1 rpm.

In process B, barley (5.0 kg) was steeped with tap water (water/barley ratio of 1.5:1). Steeping was by immersion (wet stages: 7 h at 15 °C; 5 h at 14 °C, 8 h at 18 °C; air rest stages: 13 h at 14 °C; 8 h at 18 °C; 1 h at 20 °C) in a steeping unit consisting of three vessels (10.0-L working volume; authors' design). Germination was in cylindrical drums (diameter, 30 cm; length, 30 cm; authors' design) at a temperature of 18 °C during 4 days. The drums were rotated for 10 min at 3 rpm every 3 h.

The germinated barley, further referred to as green malt, was kilned in a micromalting unit (Joe White, Perth, Australia) using seven temperature steps (3 h at 62 °C, 2 h at 65 °C, 2 h at 68 °C, 2 h at 73 °C, 1 h at 78 °C, 2 h at 80 °C, and 6 h at 86 °C). The total processing time was 160 h for process A and 156 h for process B. In process A steeping was 46 h, in process B it was 42 h. In both malting processes, germination was for 96 h and kilning was for 18 h.

Enzymatic Activities in Barley and Malt Extracts. The β-glucanase and xylanase activities were determined using Megazyme methods (use of β-glucazyme and xylazyme AX substrates, respectively) as described previously (*14*). Enzyme activities are expressed as extinction values per gram of dry weight malting barley or malt (E_{590}/g dw) or as extinction values per mL wort (E_{590}/mL).

Barley, Malt, and Wort Analyses. Barley was analyzed for microbial populations [colony forming units (CFU) per gram] by serial dilution plating using TSAp and OGYE as enumeration media for bacteria and fungi, respectively. Grain infection by filamentous fungi was assessed by directly plating 100 grains, surface disinfected with 80% (v/v) ethanol for 1 min and rinsed twice with sterile deionized water, onto MSA for storage fungi, PCNB for *Fusarium*, and PDA supplemented with 100 ppm chloramphenicol for field fungi. Filamentous fungi were presumptively identified to the genus level by microscopic analysis using keys described by Pitt and Hocking (*15*). Colonization of the kernels by the starter culture was defined as the amount of kernels infected with the starter culture, and was evaluated by plating 100 kernels, surface disinfected with sodium hypochlorite (0.1%) for 1 min and rinsed twice with sterile deionized water, on DG18. The percentage of infected grains was determined by visual inspection after incubation.

Acrospire lengths were determined by classifying kernels into 6 categories, i.e., those having no acrospire (0) and those having acrospire lengths of 0−25% (0−1/4), 25−50% (1/4−1/2), 50−75% (1/2−3/4), and >100% (>1) of the kernel length. Moisture content was assessed from loss of weight upon drying for 150 min at 150 °C. Unless indicated otherwise, Congress-wort preparations as well as malt and wort analyses were performed according to Analytica-EBC (*16*). More in particular, total and soluble protein content of malt was determined by the Kjeldahl method, malt modification and homogeneity were determined by the Calcofluor method, wort β-glucan content was measured fluorimetrically, and wort color was evaluated colorimetrically. Viscosity of the Congress-wort was measured using a Delta-viscosimeter (Haake, Karlsruhe, Germany). Wort filtration volume was determined with a filter-funnel filtration (Schleicher & Schuell no. 597 1/2) after a standard laboratory Congress-wort preparation. The difference between the extracts resulting from fine and coarse malt meals was measured as an additional parameter indicating the degree of malt modification.

To monitor β-glucan degradation during malting and mashing, β-glucan contents in barley, malt, wort, and spent grain samples were measured enzymatically using the McCleary method (*17*, *18*). To this end, mashing was stopped at specific times, and filtration of the mash was for 30 min. Part of the resulting wort was frozen in liquid nitrogen for later determination of enzymatic activities and part of it was boiled (10 min) to inactivate the enzymes before subsequent β-glucan measurement.

Statistical Analyses. Experiments were carried out at least in duplicate, and average results are presented. Where more replicates are available, 95% confidence intervals (Students' t values) are shown. Significant differences (at a $p = 0.05$ level) are indicated by an asterisk (*).

RESULTS AND DISCUSSION

Inoculation of Barley with *Rhizopus* VII and Initial Observations. For initial experiments, the 6-row winter barley variety Plaisant (1994) was used in malting process A. In a first instance, inoculation with the *Rhizopus* strain was carried out after steeping by immersion of the barley in a spore suspension of the starter culture (10^4 spores per g of air-dry barley). Inoculation after steeping was preferred based on the assumption that addition of the inoculum during steeping would lead to at least partial losses of the inoculum with the steeping water. Furthermore, no strong development of the starter culture during steeping was expected because of the variable substrate and environmental conditions during this process step. Literature data indeed suggest that growth of fungi on malting barley takes place particularly during germination (*19*−*21*). Regular, as well as sterilized barley obtained by γ-irradiation but still displaying (reduced) germination (*14*), were used. Acrospire lengths during germination reflected the reduced germination capacity: after 4 days germination the majority of the nonirradiated kernels (∼52%) had acrospire lengths in the category 3/4−1 of the kernel length, whereas the majority of the irradiated kernels (∼70%) had acrospire lengths in the category 1/4−1/2 of the kernel length. Inoculation led to increased β-glucanase and xylanase activities of the resultant malt, especially for the malt obtained from irradiated barley. The results indicate that the starter culture

Table 1. EBC Analytical Data of Malt and Congress-Wort, and Enzymatic Activities of Malt, Obtained from Irradiated (10 kGy) and Nonirradiated Barley (Plaisant, 1994 harvest), That Was Not Inoculated (Control) or Inoculated by Submersion After Steeping with 10^4 Spores of *Rhizopus* VII per g of Air-Dry Barley or 10^4 Activated Spores of *Rhizopus* VII per g of Air-Dry Barley

	irradiated barley			nonirradiated barley		
	control	*Rhizopus* VII	*Rhizopus* VII (activated spores)	control	*Rhizopus* VII	*Rhizopus* VII (activated spores)
EBC Analytical Data						
Malt						
extract (%)	78.3	79.2	79.2	80.2	80.6	80.4
fine−coarse extract difference (%)	2.0	2.0	1.3	0.7	1.1	0.4
friability (%)	56	56	60	83	82	85
unmodified grains(%)	3.9	3.6	2.4	0.3	0.3	0.1
Calcofluor modification (%)	47.0	48.3	55.5	87.0	89.2	89.8
Calcofluor homogeneity (%)	59.5	58.2	48.2	67.7	70.3	76.5
total protein (%)	10.5	10.6	10.6	10.1	10.2	10.0
soluble protein (%)	3.0	3.5	4.0	4.5	4.6	4.8
Kolbach index (%)	28.7	33.2	37.3	44.1	45.1	48.0
Congress-wort						
pH	6.19	6.02	5.95	6.00	5.96	5.87
turbidity (EBC units)	9.3	5.3	4.2	1.2	1.1	0.9
color (EBC units)	3.2	3.1	3.9	3.6	3.4	4.0
viscosity (kg/ms)	2.23	1.78	1.73	1.55	1.52	1.52
β-glucan content (mg/mL)	716	441	261	123	91	46
filtration volume (mL)	245	290	320	240	275	295
Enzymatic Activities						
β-glucanase activity (E_{590}/g dw)	4.2	69.0	114.3	11.3	12.8	33.2
xylanase activity (E_{590}/g dw)	0.21	3.66	8.76	0.60	0.72	1.23
diastatic power (WK units)	238	238	254	347	349	356

produced enzymes during development on malting barley and that these enzymes (partly) survived kilning. Table 1 indicates that, as a result of the starter culture activity, malt Calcofluor modification was slightly improved, whereas wort β-glucan content and viscosity were decreased. The wort filtration volume increased. In further work, a similar malting process was conducted, but the spores of the starter culture were activated before addition to the steeped barley. For certain species of *Rhizopus*, it is indeed known that the spores do not contain sufficient endogenous nutrients for germination (*22*−*24*). Appropriate nutritional conditions (including the presence of specific amino acids, glucose, and phosphate in the medium) combined with adequate physical conditions result in activation of the spore germination. The activation of spores prior to inoculation was an important breakthrough in the optimization of the process, as the metabolic activity of the starter culture during malting was largely improved and resulted in more consistent effects (Table 1). As the addition of activated spores resulted in an enhanced level of enzyme activity, the β-glucan content of the wort decreased to a low level, and malt friability, fine−coarse extract difference, and wort viscosity, as well as wort filtration, improved. The starter culture produced a lower level of enzymatic activities on nonirradiated barley in comparison to the levels produced on irradiated barley. Interactions with the natural barley microflora or with the barley physiological processes, or altered barley surface characteristics, may have influenced the starter culture metabolic activity on nonirradiated barley.

A further optimization was the inoculation of activated spores of the starter culture early in the malting process. The evolution of the β-glucanase (Figure 1a) and xylanase (Figure 1b) activities was monitored after inoculation with activated spores (10^4 per g of air-dry barley) either during the first wet stage of steeping or after steeping, and compared to the evolution of these enzymatic activities during malting of noninoculated barley. The microbial enzymes were predominantly produced during the germination step. Contrary to earlier assumptions, a significantly higher increase in enzyme production was observed when the starter culture was inoculated during the first wet stage of steeping. It is therefore reasonable to conclude that the inoculated spores adhere to and develop on the barley kernels already under steeping conditions.

These observations suggested that the functional quality of malt could be improved by inoculation of the starter culture according to an optimized procedure.

Effect on Quality Aspects of Malt obtained from Poor Malting Quality Barley. A 2-row winter barley variety (Cassandra, 1997 harvest) resulting in poorly modified malt was used for detailed studies. This variety was selected after comparison of the quality of malt produced from 7 barley varieties (Cassandra, Milonga, Spinet, Balkan, Gamelan, Plaisant, and Pastoral) by the same standard malting process. The variety Cassandra was of poor malting quality in terms of poor cell-wall modification, high Congress-wort viscosity, and β-glucan content. The barley was malted (process B) with addition of 10^4 activated spores per g of air-dry barley during the first wet stage of steeping, and the quality of the resultant malt was compared with that of malt obtained from noninoculated barley (further referred to as control malt). The development of the starter culture on malting barley certainly did not negatively affect barley germinative properties. Within the same malting trial, the acrospire lengths measured after one and four days of germination were higher for inoculated barley than for noninoculated barley. With the exception of Congress-wort turbidity and filtration volume, EBC analytical results indicated improved quality when the starter culture was added (Table 2). Significant differences were observed for malt fine−coarse extract difference, friability, and Calcofluor modification, as well as for wort β-glucan content and viscosity. Although the modification indices for malt obtained by a regular malting process directly relate to the malt enzyme content (*12*),

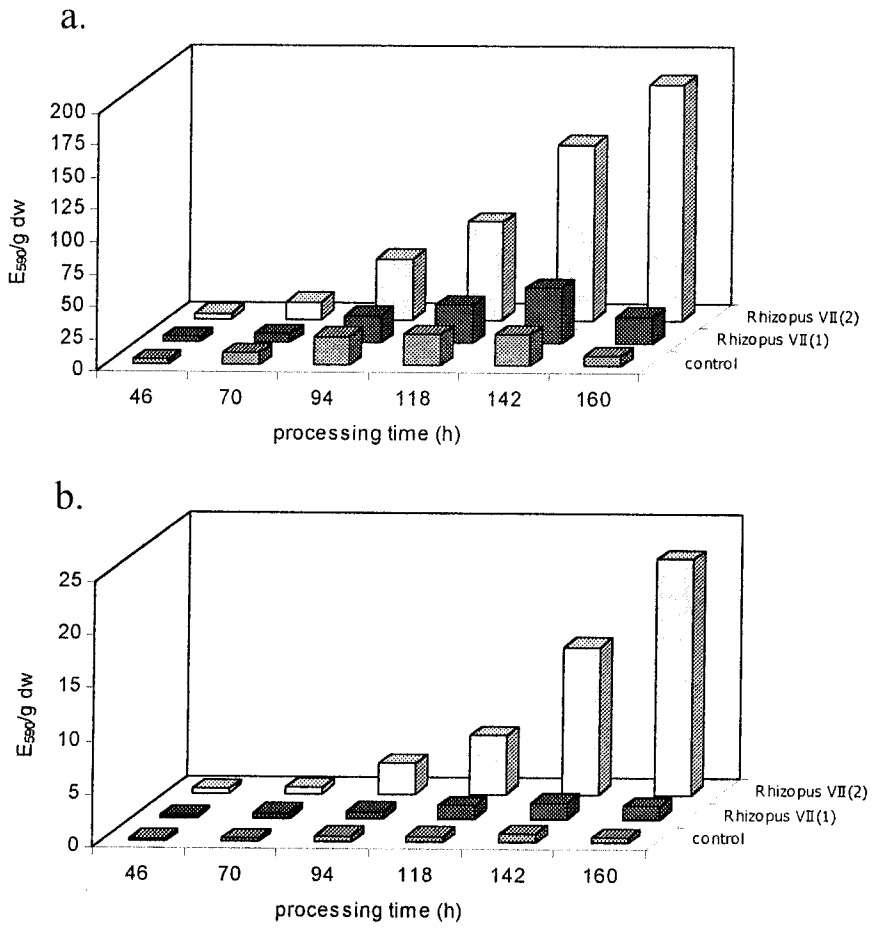

Figure 1. Evolution of β-glucanase (a) and xylanase (b) activities during malting of noninoculated (control) barley (Plaisant, 1994 harvest), and barley inoculated with activated spores of *Rhizopus* VII (10^4 per g of air-dry barley) after steeping (1) or during the first wet stage of steeping (2).

Table 2. EBC Analytical Data of Malt and Congress-Wort, and Enzymatic Activities of Malt, Obtained from Barley (Cassandra, 1997 harvest) that Was Not Inoculated (Control) or Inoculated During the First Wet Stage of Steeping with Activated Spores of *Rhizopus* VII (10^4 per g of Air-Dry Barley)

	control	*Rhizopus* VII (activated spores)
EBC Analytical Data		
malt		
extract (%)	79.7 (± 1.0)	80.7 (± 0.2)
fine−coarse extract difference (%)	3.3 (± 0.5)	2.0 (± 0.5)*
friability (%)	48 (± 1)	54 (± 4)*
unmodified grains (%)	12.3 (± 4.1)	9.1 (± 3.5)*
Calcolfuor modification (%)	76.8 (± 6.2)	83.5 (± 5.0)*
Calcofluor homogeneity (%)	41.1 (± 3.1)	51.2 (± 7.1)*
total protein (%)	11.9 (± 0.0)	11.9 (± 0.1)
soluble protein (%)	4.7 (± 0.4)	5.5 (± 0.4)*
Kolbach Index (%)	39.2 (± 3.4)	46.2 (± 3.0)*
Congress-Wort		
pH	5.87 (±0.03)	5.70 (± 0.13)
turbidity (EBC units)	7.6 (± 0.6)	8.7 (± 5.3)
color (EBC units)	4.1 (± 1.0)	5.1 (± 0.7)
viscosity (kg/ms)	1.88 (± 0.14)	1.63 (± 0.04)*
β-glucan content (mg/mL)	701 (± 143)	217 (± 163) *
filtration volume (mL)	257.5 (± 54.5)	176.0 (± 40.5)
Enzymatic Activities		
β-glucanase activity (E_{590}/g dw)	3.2 (± 1.0)	152.6 (± 40.9)*
xylanase activity (E_{590}/g dw)	0.84 (± 0.21)	15.39 (± 6.78)*
diastatic power (WK units)	186.5 (± 5.1)	193.17 (± 9)

this was obviously not the case for malt obtained from the inoculated barley (further referred to as starter malt). The β-glucanase activity of the starter malt increased by a factor of 48, whereas the xylanase activity increased by a factor 18. As arabinoxylans play a role in determining wort viscosity (*25*, *26*), and have been associated with lower filtration rates (*27*) and beer haze (*28*), it is likely that the xylanase also positively contributes to brewhouse performance. The high enzymatic activities obtained with the starter culture are expected to be especially useful when significant proportions of nonstarch polysaccharide-containing adjuncts are used in brewing. Although laboratory-scale filtration offers only an estimate of the wort separation characteristics (*12*), further experiments are needed to understand the negative effect on laboratory-scale wort filtration observed when using this barley variety. The presence of fine particles might have caused the negative effect, as the turbidity of the wort obtained from inoculated barley increased.

Not only cell-wall degradation, but also protein modification, increased as reflected by the Kolbach index.

Table 3. β-Glucan Content (as % of Barley Dry Weight) of Barley (Cassandra, 1997 harvest) and Malt, Wort, and Spent Grains Obtained from Such Barley That Was Not Inoculated (Control) or Inoculated During the First Wet Stage of Steeping With Activated Spores of *Rhizopus* VII (10^4 per g of Air-Dry Barley)

	β-glucan (%)	
	control	*Rhizopus* VII (activated spores)
barley	3.87 (± 0.13)	3.87 (± 0.13)
malt	1.36 (± 0.06)	0.99 (± 0.03)*
wort	0.45 (± 0.02)	0.01 (± 0.00)*
spent grains	0.26 (± 0.03)	0.11 (± 0.03)*

Table 4. Microbial Population of the Barley Samples Cassandra (1997 harvest), Plaisant (1996 and 1997 harvests), and Stander (1996 harvest)

	Cassandra	Plaisant 1996	Plaisant 1997	Stander
		% kernels contaminated		
Alternaria[a]	26 (± 4)	2 (± 2)	24 (± 8)	83 (± 2)
Aspergillus[b]	0 (± 0)	61 (± 9)	2 (± 2)	11 (± 2)
Arthrinium[a]	0 (± 0)	0 (± 1)	1 (± 1)	5 (± 5)
Drechslera[a]	0 (± 0)	0 (± 0)	48 (± 8)	0 (± 0)
Epicoccum[a]	1 (± 1)	0 (± 0)	4 (± 2)	1 (± 1)
Fusarium[c]	0 (± 0)	0 (± 0)	2 (± 1)	26 (± 5)
Mucorales[b]	0 (± 0)	1 (± 1)	0 (± 1)	3 (± 1)
		log CFU/g barley		
bacteria	5.47 (± 0.02)	6.52 (± 0.08)	7.23 (± 0.16)	7.13 (± 0.18)
yeasts	–	–	3.65 (± 0.12)	4.47 (± 0.02)

[a] PDA + chloramphenicol. [b] MSA. [c] PCNB.

The increased Kolbach index indicates an increase in protease activity which resulted in additional solubilization of nitrogenous substances during malting and/or in the laboratory mash. The increased levels in soluble nitrogen in wort obtained from the starter malt may, to a certain extent, be related to the decreased pH of the wort. On one hand, the pH of wort resulting from starter malt was lower, which might have been due to acid production by the starter culture, and no pH adjustment was applied during analytical mashing. On the other hand, as a negative correlation between wort soluble nitrogen content and the initial pH of the brewing water (5.4−6.4) or pH of the wort was observed (results not shown), the increased levels in wort soluble nitrogen content can partially be explained by the lower wort pH. As wort color relates to the color of the malt, which, for a given barley sample, depends on the malt amino acid and sugar content (*12*), the increased wort color is due to an improved overall modification.

In conclusion, the combination of the metabolic activities of a selected *Rhizopus* strain with barley physiological processes provides the basis for a production process for malted cereals with improved modification. In addition to the effects on cell-wall modification, the starter culture has impact on malt enzyme level, protein conversion, and general wort characteristics.

***β*-Glucan Degradation during Malting and Mashing.** The relative importance of microbial enzymes in *β*-glucan degradation during malting and mashing was investigated by measuring the *β*-glucan content of barley, malt, final wort, and spent grains. Malting losses were taken into account. The *β*-glucan content of starter malt was significantly lower than that of control malt (Table 3). During malting of inoculated barley, with the assay method used, 74.5% of the barley *β*-glucan was found to be degraded, while the corresponding figure for the noninoculated barley was 65.0%.

A study of the localization of the enzymes in the malt kernels provided further evidence for the degradation of starchy endosperm cell walls during malting with microbial enzymes. Milling with a break and reduction roll system showed that significant levels of microbial *β*-glucanase and xylanase activities were present in the endosperm tissue (results not shown). For nongerminating, sterilized barley it was demonstrated before that the enzymes were not only secreted by the starter culture during malting, but that they were also delivered in the starchy endosperm (*14*).

The enzymatic activities in the mash from starter malt were higher than in the mash resulting from control malt (Figures 2a and 2b). The microbial *β*-glucanase activity was inactivated at temperatures between 58 and 70 °C, whereas the microbial xylanase to a large extent survived mashing at 70 °C. The residual *β*-glucan level in malt, as well as solubilization and degradation of *β*-glucan during mashing, determine the wort *β*-glucan content. In contrast to the increase in the concentration of soluble *β*-glucan during mashing of control malt, the concentration of soluble *β*-glucan did not increase during mashing of the starter malt (Figure 2c). During mashing with starter malt, 22.5% of the barley *β*-glucan was degraded, but the corresponding figure for the control malt was 17.0% (Table 3). Although the starter culture enzymes predominantly

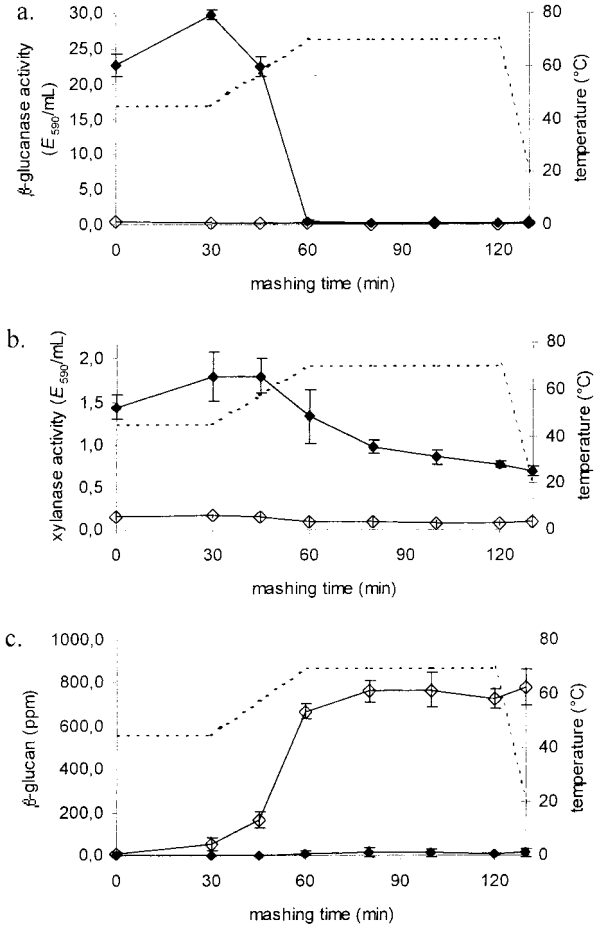

Figure 2. Evolution of *β*-glucanase (a) and xylanase (b) activities, and soluble *β*-glucan content (c) during mashing of malt obtained from noninoculated Cassandra (1997 harvest) barley (◇), and Cassandra (1997 harvest) barley inoculated with activated spores of *Rhizopus* VII (10^4 per g of air-dry barley) (◆). The temperature profile of the mash is also indicated (– – –).

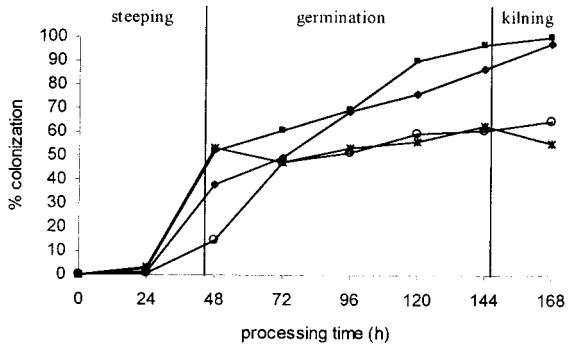

Figure 3. Evolution of barley colonization by *Rhizopus* VII during malting of the barley samples Cassandra (○), Plaisant 1996 (■), Plaisant 1997 (*), and Stander (◆).

contributed to β-glucan degradation during malting, the enzymes further enhanced β-glucan degradation during mashing or the β-glucan products formed by the microbial enzymes were a better substrate for the malt enzymes. The results also demonstrate that β-glucan was not only degraded, but also solubilized from the barley endosperm cell walls by the starter culture enzymatic activities.

Barley Colonization by the Starter Culture in Interaction with the Natural Barley Microflora. Colonization of various barley samples by the starter culture was assessed as a frequency of kernel infection during malting (process B) after inoculation with the starter culture (10^4 activated spores per g of air-dry barley) during the first wet stage of steeping. Colonization of these barley samples by the starter culture was different, especially during germination (Figure 3). For Stander and Plaisant (1996 harvest) the colonization increased during germination: at the end of the malting process, the starter culture was detected on >90% of surface-disinfected malt kernels. No visible development of the starter culture was detected during malting. For the Cassandra and 1997 Plaisant samples, the colonization remained approximately constant during germination and kilning. A majority (50 to 60%) of surface-disinfected malt kernels were infected with the starter culture. The β-glucanase activities of the resultant malts were in agreement with the extents of colonization: 153, 354, 162, and 392 (E_{590}/g dw) for Cassandra, Plaisant (1996 harvest), Plaisant (1997 harvest), and Stander, respectively. As the natural barley microflora might influence the development of the starter culture, the microbial populations of the barley samples were analyzed (Table 4). Field fungi (*Alternaria* and *Drechslera*) were dominant in the Cassandra and 1997 Plaisant samples. The 1996 Plaisant sample was contaminated with the storage fungus *Aspergillus*, and Stander (1997 harvest) was highly contaminated with field fungi, especially *Alternaria* and *Fusarium* species. The Cassandra and 1996 Plaisant samples also carried lower levels of yeasts and bacteria than the other barley samples. Although the natural barley microflora is likely to have an impact on the development of the starter culture and on the homogeneity of its action on the grain mass, additional factors are probably also involved, because the poor colonization of the starter culture for the Cassandra and 1996 Plaisant samples could not be obviously explained by differences in microbial load of the barley. Nevertheless, the results show that on a laboratory scale, the starter culture grows and produces enzymes on and in malting barley samples carrying various types of microflora. The contribution of the starter culture to modification may probably be improved further through knowledge of the mechanisms of interaction between the starter culture and the barley kernels.

ABBREVIATIONS USED

CFU, colony-forming units; DG18, dichloran glycerol agar; dw, dry weight; MSA, malt salt agar; OGYE, oxytetracycline gentamicine yeast extract agar; PCNB, pentachloronitrobenzene agar; PDA, potato dextrose agar; SDA, sabouraud dextrose agar; TSAp, tryptone soya agar supplemented with 100 ppm pimaricine; TSB, tryptone soya broth.

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