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Impact of *Fusarium culmorum* on the Polysaccharides of Wheat Flour

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To assess the effects of *Fusarium* infection on the polysaccharides of winter wheat grain (*Triticum aestivum* L.), grain samples obtained from plants artificially inoculated with *Fusarium culmorum* were analyzed. Microscopy revealed obvious damage to the starch granules in the seriously infected samples. The *Fusarium* infection had no analytically detectable influence on the starch and total insoluble dietary fiber content of the wheat grain. There were significantly positive relationships between α-amylase activity, cellulase activity, total soluble dietary fiber content, pentosan content, and degree of infection quantified by an enzyme-linked immunosorbant assay, which would indicate the importance of fungal enzymes. A distinct higher Hagberg falling number (FN) was determined in the seriously infected samples, while the viscosity and sucrose content of the flour decreased. However, the addition of a liquid medium contaminated with *F. culmorum* led to a significant decrease in the FN. Depending on the type of buffer used, the α-amylase of *F. culmorum* demonstrated its maximum activity between pH 5.5 and pH 7.0 at 30−50 °C. Remarkably, this fungal α-amylase showed a thermostable characteristic and was active over a wide range of temperatures, from 10 to 100 °C. This type of thermostability suggests that the α-amylase of *F. culmorum* may damage starch granules throughout the processing of wheat flour, thereby inducing weak dough properties and unsatisfactory bread quality.

KEYWORDS: ***Fusarium culmorum*; polysaccharides; viscosity; fungal α-amylase; wheat**

INTRODUCTION

Fusarium head blight (FHB) is presently one of the most important fungal diseases affecting small grain cereals, e.g., wheat, barley, and maize (*1*, *2*). *Fusarium* causes root, stem, and ear rot, with severe reductions in crop yield, often estimated at between 10 and 30% (*3*). In addition, an outbreak of FHB is often accompanied by mycotoxin contamination, such as with the very stable trichothecene deoxynivalenol, which is reduced during neither milling nor baking (*2*). The *Fusarium* infection and grow can have an effect on the content of nutrients of the grain as well as the processing quality. Bechtel et al. (*4*) characterized *Fusarium graminearum* as an aggressive invader destroying starch granules, storage proteins, and cell walls. *F. graminearum* infection can cause significant compositional changes in carbohydrates and proteins (*5*). Generally, most of the researchers working on the effects of FHB have concentrated mainly on the change in gluten protein quality. Wheat infected by *Fusarium culmorum* exhibited inferior baking quality because of a degradation in the wheat gluten proteins (*6*). *Fusarium*-infected Canadian red hard spring wheat showed weak dough properties and unsatisfactory bread quality (*7*). Moreover, it was observed that FHB reduced the gluten functionality (strength) of durum wheat (*8*). In this context, changes caused by *Fusarium* infection in the expansion of dough during the baking phase are generally attributed to changes in both protein quantity and quality. However, only a few reports in the literature have focused on the impact of FHB on polysaccharides, such as the starch and pentosans of wheat and the relationship between quality degradation and the character of fungal amylase.

Actually, on a purely quantitative basis, the role of wheat starch in the unique breadmaking ability of wheat flour should not be underestimated. Starch has several distinct functions in the breadmaking process. It sets a balance with gluten protein, lipids, water, and other compounds during the formation of dough, thereby contributing to the dough's optimal viscoelastic properties (*9*). Starch is the substrate for the cereal amylases that produce fermentable sugars for the germination of yeast and at least also for fermentation, and it represents a reservoir for water absorption during the baking process (*10*). Water soluble pentosans are able to absorb water up to nine times of their own weight and water insoluble pentosans up to five times of their own weight (*11*). The objective of the present study was to investigate the effects of *Fusarium* infection on the polysaccharides in winter wheat and to characterize the fungal α-amylase produced by *F. culmorum*. It was considered that

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Table 1. Relationships between *Fusarium* Infection (FPE), Polysaccharide Content,[a] Water Extract Viscosity, and Hagberg FN of Wheat Flour ($n = 9$)

FPE (μg g^{-1})[a]	grade of infection	starch (%)	total dietary fiber (%)		pentosans (%)	viscosity (mPa s)	FN (s)
			soluble	insoluble			
7.29	light	63.8	0.9	11.5	6.7	3.0	320
11.07		65.7	1.6	10.5	7.6	2.9	343
11.34		64.6	1.3	11.3	6.8	2.5	372
12.69	moderate	65.1	1.8	10.3	7.7	1.7	329
14.58		62.9	1.7	11.1	7.8	2.5	340
16.20		63.9	1.7	10.8	6.9	1.7	345
25.92	serious	63.6	2.6	10.4	7.9	1.6	387
39.15		64.6	2.0	10.5	8.1	1.6	382
49.95		65.1	2.9	10.9	8.6	1.4	391
r		0.184	0.844**	–0.309	0.811**	–0.756*	0.798**

[a] On the basis of dry matter; *r*, coefficient of correlation; *$p < 0.05$; **$p < 0.01$.

such a characterization may contribute to the explanation of why and to what extent *Fusarium* spp. may influence the processing quality of winter wheat.

MATERIALS AND METHODS

Wheat Samples. Cultivar Hanseat was commercially grown and harvested in 1999 at the field station Reinshof, University of Göttingen (Germany). To obtain an infection of the wheat plants with *F. culmorum*, the wheat spikes were sprayed artificially with a *F. culmorum* conidiospore suspension (2.5×10^5 spores mL^{-1}) at the beginning of flowering. Twenty-seven samples from different field plots were divided into three groups according to their degree of infection as determined by a DAS-ELISA (double antibody sandwich enzyme-linked immunosorbent assay) (*12*) and expressed in *Fusarium* protein equivalent concentrations (FPE in μg g^{-1}): light infection, FPE < 12 μg g^{-1}; moderate infection, 12 μg g^{-1}< FPE < 20 μg g^{-1}; and serious infection, FPE > 20 μg g^{-1}. Three samples were selected from each of these groups for the present study as shown in **Table 1**.

The tested seeds were ground to produce whole kernel flour by a falling number (FN) mill (Laboratory Mill 120, Perten, Germany) with a sieve of 0.8 mm. All of the samples were stored at 4 °C, except for those used in the enzyme tests, which were stored at −20 °C.

Microscopic Investigation. One drop of iodine stock solution, 0.22 g mL^{-1}, prepared according to International Association of Cereal Science and Technology (ICC) no. 108 (*13*), was added to a drop of a 2% suspension of the wheat flour sample on a microscope slide. After it was stained for 1 min, the slide was carefully covered with a cover glass and observed under a Leica DMR microscope (Leica, Germany).

Analytical Tests. The starch content was determined by the polarimetric method with flour samples dissolved in hydrochloric acid according to ICC no. 123/1 (*13*). The wheat proteins were removed by precipitating them with wolframatophosphoric acid. The rotation angle of the filtrate at 589 nm was used to calculate the starch content. The total dietary fiber was measured using the method described in ref *14* in combination with enzymatic and gravimetric methods (*15*, *16*). The protein content was determined according to the DUMAS method (*17*). An enzyme reagent kit from Merck KGaA (BIOQUANT total dietary fiber) was used to convert the starch into a paste and to break it down, as well as to digest the protein. The pentosan content was measured using the method of Dörfer (*18*). A xylose solution with a concentration of 10 mg mL^{-1} was used to make the standard curve with a concentration gradient of 0, 6, 8...24% of xylose. The pentosan content in the samples was calculated by comparing the different extinctions at 670 and 580 nm of the standard solutions.

The water extract viscosity was measured according to the method described in Dusel et al. (*19*). A rapid visco analyzer (RVA) (Newport Scientific, Australia) was applied to detect the changes in the viscosity in relation to the cellulase activity and to assess the starch pasting properties (ICC no. 162) (*13*). Sodium carboxymethyl cellulose (high viscosity, Sigma, Germany) was used as the substrate.

The Hagberg FN was determined according to the standard procedures as described in ICC no. 107 (*13*) using the Falling Number system FN1200 (Perten, Germany). Extracellular α-amylase activity was determined with the enzyme test developed by Wirth and Wolf (*20*).

The mono- and oligosaccharides were extracted from the flour samples with 80% ethanol (*21*) and then measured by reversed-phase high-performance liquid chromatography (RP-HPLC) using a refractive index detector (198.00, Knauer, Germany). The following HPLC system was used for the analysis of the samples: degasser Well-Chrom K-5004 (Knauer, Germany); pump, Elcho Maxi Star K-1000 (Knauer, Germany); autosampler, Pharmacia LKB 2157 (Uppsala, Sweden); column compartment, Thermostat Jetstream 2 (Knauer, Germany); integrator, Mega 2 (Shimadzu, Japan); precolumn, LiChro-CART 4-4 (LiChrosorb 100 NH2, 5 μm, Merck, Germany); and separation column, LiChro-CART 250-4 (LiChrospher 100 NH2, 5 μm, Merck). An acetonitrile/water solution (80/20, v/v) was used as the eluent. The separation of the mono- and oligosaccharides was achieved at a flow rate of 1 mL min^{-1} and a temperature of 20 °C. The detection limit was 250 ppm.

Preparation of Fungal Cultures. Ten pieces of agar inoculated with *F. culmorum* at 25 °C for 4 days were transferred under sterile conditions into 300 mL Erlenmeyer flasks containing 100 mL of 2% whole kernel flour suspension filtrate with a pH of 5.5 that had been autoclaved at 121 °C for 15 min. These flasks were vibrated at a speed of 110 rpm at 25 °C in a dark climatic chamber. For the selection of the fungal cultures with the highest α-amylase activity, these cultures were examined daily using the enzyme test according to Wirth and Wolf (*20*). Finally, the 4 day old inoculated fungal culture with the highest α-amylase activity was selected and used for the determination of the enzyme's optimum temperature and pH. The filtrate of the selected *F. culmorum* culture was diluted into a 1:10 (v/v) solution using one of two buffers: Buffer 1, 0.1 M sodium acetic acid with 0.02% sodium azide (*20*), and buffer 2, Megazyme buffer, ICC no. 303 (*13*). Buffer 2 consists of 1 M sodium malic acid, 1 M sodium chloride, 40 mM calcium chloride, and 0.1% sodium azide. The pH values of the diluted fungal culture were adjusted to 11 different gradients in the range of 3.5−8.5. These solutions were then added to the Remazol-Brilliant-Blue substrate in a microtiter plate ($96 \times 350\mu$L, Costar, United States). Analogous to Wirth and Wolf (*20*), these mixtures were incubated in a water bath at 40 °C for 2 h. The extinctions were determined as the relative activity of fungal amylase at 592 nm (Spectra, SLT, Austria). Comparable assays were conducted also at a temperature of 25 °C. A supernatant was collected from the filtrate after it was centrifuged at 10000 rpm for 15 min at 4 °C. The quantity of supernatant actually used in the FN determination was calculated to give the equivalent of the corresponding activity of α-amylase from *F. culmorum* according to a standard curve obtained from a commercial *Aspergillus oryzae* fungal α-amylase (39 U mg^{-1}, solid body, Sigma, Germany).

Statistical Analysis. The calculations of the mean values and standard deviations were performed using a Microsoft Excel 2000 program and all pairwise multiple comparison procedures (one-way analysis of variance, and Tukey's test) of the SigmaStat 2.03 program. The significant differences are given in the corresponding tables and figures (*$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$). The *P* values of <0.05 were considered significant. The relationships between the polysaccharide content, enzyme activity, and degree of infection (in FPE) were examined using the Pearson product moment correlation analysis.

RESULTS AND DISCUSSION

Microscopic Research. Wheat starch granules are normally lens-shaped and polyhedral (*10*). After they were stained with the iodine solution, the granules could be very easily seen under the microscope (**Figure 1**). Many more destroyed and broken granules were observed in the seriously infected samples in comparison to the lightly infected ones, indicating a substantial structural alteration in the endosperm. Nightingale et al. (*5*) also found that in severely infected areas within the kernel, the starch granules had begun to show symptoms of amylolytic degrada-

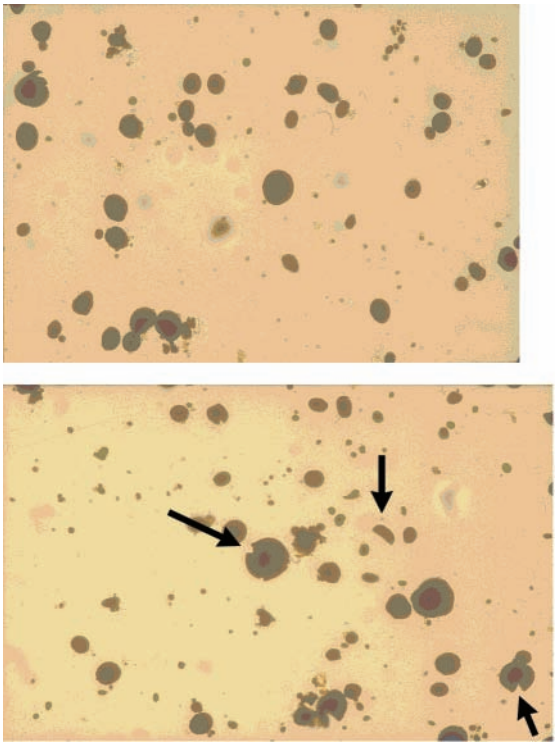

Figure 1. Light microscope pictures (200×) of wheat starch granules in two samples infected with *F. culmorum* (iodine staining with a concentration of 0.22 g mL^{-1}). (**Top**) Lightly infected—intact starch granules of different sizes are recognizable. (**Bottom**) Seriously infected—arrows show damaged and/or destroyed starch granules.

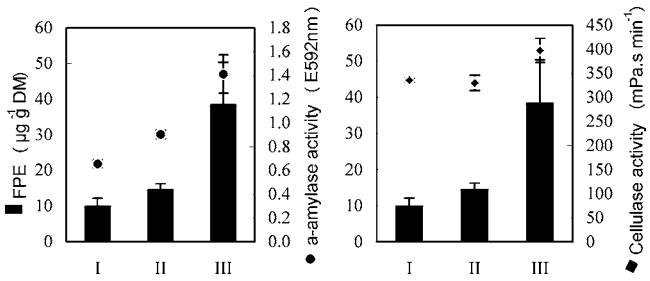

Figure 2. Relationship between the degree of *Fusarium* infection and fungal enzymes in wheat flour: α-amylase (**left**) and cellulase activity (**right**). Infection grade: I, light; II, moderate; and III, serious. Bars represent the mean values of three samples with the same degree of infection; error bars represent standard deviation.

tion, a finding that was in agreement with the conclusions of other authors (*4*, *22*). The results in the present study appeared to substantiate this too, as it was suggested that the starch granule destruction reflected the fungal infection. Furthermore, Hayman et al. (*23*) observed that starch granule size was at least one of the factors that affected the crumb structure of bread.

α-Amylase and Cellulase Activity. As shown in **Figure 2**, there were close positive relationships between the α-amylase activity, cellulase activity, and degree of *Fusarium* infection. The correlation coefficients between the degree of infection and the α-amylase and cellulase activity were 0.904 and 0.940, respectively ($p < 0.001$, $n = 9$). Similar results have also been reported by other authors (*24*, *25*). However, the differences between light and moderate infection grade were not significant but between light and serious infection ($p < 0.05$, $n = 3$). α-Amylase hydrolyzes starch to dextrins, maltose, and glucose to provide fermentable sugars for yeasts (*10*). In view of the increases in each of α-amylase and cellulase, it is to be expected

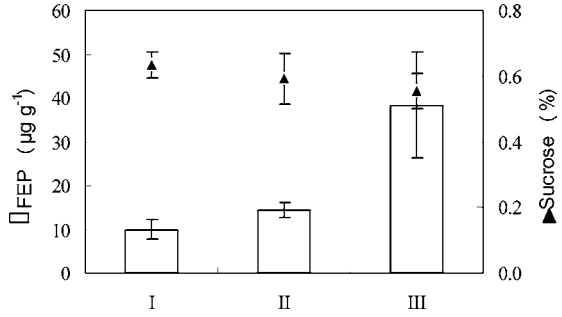

Figure 3. Relationship between the degree of *Fusarium* infection and sucrose content of wheat flour contaminated with *F. culmorum.* Infection grades: I, light; II, moderate; and III, serious. Bars represent the mean values of three samples with the same degree of infection; error bars represent standard deviation.

that an infection with *Fusarium* could considerably influence the quality of wheat polysaccharides, such as starch and cellulose, and so would affect the success of wheat processing.

Starch Content. The *Fusarium* infection did not significantly change the wheat grain's starch content as shown in **Table 1**. This lack of change is in agreement with the results of Dexter et al. (*7*), in which, as compared with the least damaged samples, the starch content of three from four tested Canadian red spring wheat cultivars declined; only in one cultivar was the starch content slightly increased. This suggests that although a *Fusarium* infection can alter the size and shape of starch granules (see **Figure 1**), and furthermore probably shortens and/or breaks the amylose or amylopectin molecules, this is not reflected by an actual change in the amount of starch present.

Mono- and Oligosaccharides. Only the sucrose content of the samples is shown in **Figure 3**, because the fructose, glucose, galactose, arabinose, xylose, maltose, and raffinose contents were lower than the detection limit. The sucrose content was negatively related to the degree of infection, whereas the differences between light and serious infection were significant ($p < 0.05$, $n = 3$). In comparison to the lightly infected samples, the sucrose content of the moderately and seriously infected ones decreased by about 6.8 and 12.5%, respectively. The observed decrease in sucrose could not be correlated with the activity of any starch-degrading enzymes such as α-amylase (see **Figure 2**), which normally lead to an increase in the mono- and/or oligosaccharide concentration. Sugars such as glucose and sucrose are the most important source of nutrients for fungi and bacteria (*26*). The low quantity of mono- and oligosaccharides found in the present samples can presumably be attributed to the presence of *F. culmorum*.

Total Dietary Fiber. According to the description of the method used, the total dietary fiber content in the samples consisted of a large number of complex, nonstarch polysaccharides (NSPs) and lignin. None of these substances (cellulose, hemicelluloses, pectins, hydrocolloids, resistant starch, and lignin) can be split by human digestive enzymes. In the present study, the *Fusarium* infection led to a significant increase in soluble dietary fiber and a slight decrease in insoluble dietary fiber (see **Table 1**). The coefficients of correlation between these two types of dietary fiber and the degree of infection were 0.844 ($p < 0.01$) and 0.309 ($p > 0.05$), respectively. The decrease in insoluble dietary fiber and increase in soluble dietary fiber were in good agreement with the results of Matthäus et al. (*25*), who also determined the NSP in wheat kernels (cv. Ritmo) contaminated by *F. culmorum*. These authors concluded that the fungus could possibly change the insoluble high molecular NSP into NSP with a lower molecular weight, whereby the solubility of

Table 2. Viscosity[a] of Wheat Flour Infected with *F. culmorum*[b]

sample	peak viscosity (mPa s)	breakdown (mPa s)	pasting temp (°C)
I	1210 a ± 10	457 a ± 10	78 a ± 1.4
II	1248 a ± 20	474 a ± 22	78 a ± 0.4
III	827 b*** ± 16	135 b*** ± 2	69 b*** ± 0.9

[a] Mean standard deviation obtained from determination of three samples. Within columns, values followed by different letter are significantly different (***$p < 0.001$). [b] Infection grades: I, light; II, moderate; and III, serious.

the NSP would increase. These findings suggested that the fungus produces cell wall-degrading enzymes, such as cellulase, xylanase, and glucanase, in order not only to invade into the plant cell but also to use the polysaccharides as a carbon source for its own growth (*24*). These results could be also supported by the work of Kang and Buchenauer (*27*), who observed that the fungal hyphae grew during the colonization of the wheat spikes and caused severe damage of the host tissue. This led to a significant reduction in the cell wall components cellulose, xylan, and pectin.

Pentosan Content. Pentosans are a major part of the NSPs in wheat flour (*28*). In the present study, there was a close positive correlation between pentosan content and *Fusarium* infection ($r = 0.811$, $p < 0.01$) as shown in **Table 1**. This distinct increase in pentosan content confirmed the results of the dietary fiber investigation. The increase in pentosan content and soluble dietary fiber may be one of the reasons for the increase in water absorption ability of wheat flour observed by Meyer et al. (*6*) during their analysis of wheat flour quality and by Pawelzik et al. (*24*) during their investigation on dough properties.

Viscosity. As shown in **Table 1**, a statistically significant decline in viscosity in the seriously infected samples occurred. As compared to the lightly infected samples, the relative viscosity of the most seriously infected samples decreased by about 53.5%. Udy (*29*) studied the viscosity of flour water extracts and concluded that 95% of the intrinsic viscosity was due to the polysaccharides and 5% to the soluble protein, while the other soluble components did not influence the viscosity. In the present study, the decline in viscosity confirmed that the polysaccharides were being degraded by *F. culmorum*. Similar results have been also reported by Schwarz et al. (*30*) in barley infected with *F. graminearum* and *Fusarium poae*, and by Matthäus et al. (*25*) in wheat infected with *F. culmorum*. These authors also agreed that the lower viscosity of the infected samples could be due to damage to the starch caused by the fungal infection and colonization. They also concluded that this damage might be due to the presence of fungal amylolytic enzymes and higher activities of endoxylanase and cellulase.

The quality of numerous wheat foods (e.g., noodles, gravies, and thickeners) is related, among others, to the pasting properties of the flour (*31*). The RVA method was used in the present study to access the effects of *F. culmorum* on the pasting properties of wheat starch. As shown in **Table 2**, as compared to the lightly infected sample, the peak viscosity and its breakdown clearly increased in the seriously infected ones, while that of the samples with a moderate degree of infection only slightly increased. Remarkably, the pasting temperature of the seriously infected starch was 9 °C lower than that of other two samples. Because the viscosity examined was that of the whole wheat flour, the lower pasting temperature would not have been only related to the higher α-amylase activity (*10*) but also to the higher cellulase activity as already shown in **Figure 3**. Similarly, during the research into the effects of fungal

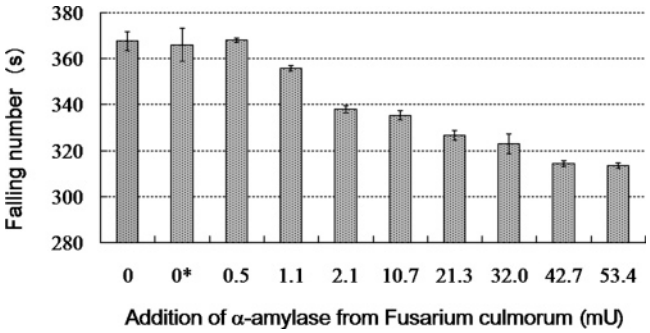

Figure 4. Effect of α-amylase from *F. culmorum* on the FN of wheat flour. Key: 0, no medium, no *F. culmorum*; 0*, medium plus *F. culmorum*. Error bars represent standard deviation.

α-amylase on the amylograms of low protein flour done by Maninder et al. (*32*), an obvious decrease in peak viscosity (from 28.3 to 45.0%) and gelatinization temperature (from 59.50 to 57.25 °C) was obtained after the addition of α-amylase from *A. oryzae*. The decrease in pasting temperature in our seriously infected samples probably indicated that the starch pasting had already started before the viscosity analysis was undertaken. This premature pasting therefore caused longer and more intensive starch granule degradation by the amylolytic enzymes (*33*). During swelling and gelatinization, the starch granules in wheat flour dough strongly absorb free water and some of the water that is liberated by the heat denaturation of gluten. Gelatinized starch in the dough, along with gluten, forms the membranes of the gas cells in the breadcrumb (*10*). Consequently, the change in timing of starch pasting associated with severe *F. culmorum* infections can be expected to be able to change crumb structure and crust formation.

Hagberg FN. Remarkably, in the present study, there was a close positive relationship between FN and α-amylase activity as a consequence of the *Fusarium* infection ($r = 0.798$, $p < 0.01$; **Table 1** and **Figure 2**). Similar results have been also described in earlier studies (*6*, *24*). The FN method determines the viscosity of the gelatinized starch at nearly 100 °C during a relatively short period of heating after enzymatic decomposition (*13*). The results suggest that during the FN determination, the activity of fungal α-amylase could not be fully in evidence. One may assume that the fungal amylase in the original flour samples was inactive during the FN determination.

Effect of Fungal Amylase on Hagberg FN. To clarify the effect of fungal amylase on the Hagberg FN, filtrate from a medium inoculated with *F. culmorum* was added during the FN determination. This resulted in an obvious decrease in the FN (**Figure 4**). The addition of 53.4 mU fungal amylase caused a significant decrease in FN by 17.6%. These results suggest a serious discrepancy between fungal enzymes that occur in the original matrix (wheat kernel) and added enzyme from the same fungus species. The reasons for these different results need to be clarified. It could be possible that in the kernel the enzyme is bound with other compounds and therefore not active. Moreover, inhibitors of α-amylase from wheat have been identified (*34*), which could affect the fungal enzyme activity in the kernel. It could be also possible that *Fusarium* enzymes other than α-amylase were present in the medium filtrate and that they were responsible for the degradation of the flour pasting, because the Hagberg FN actually reflects not only the viscosity of starch but also that of other compounds, as hemicellulose and cellulose, which are as well present in whole flour.

Precharacterization of Fungal α-Amylase from *F. culmorum*. Bechtel et al. (*4*) observed under the light microscope

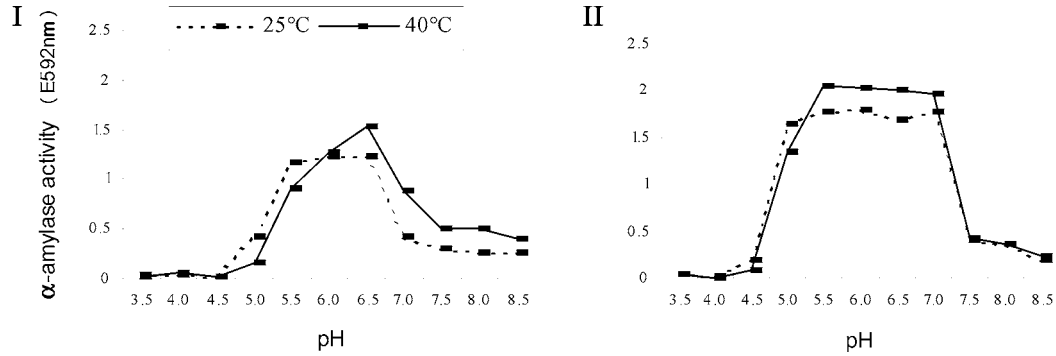

Figure 5. Effect of the pH value on *F. culmorum* α-amylase activity at 25 and 40 °C in two different buffers. I, buffer 1 (0.1 M sodium acetic acid with 0.02% sodium azide); II, buffer 2 (1 M sodium malic acid, 1 M sodium chloride, 40 mM calcium chloride, and 0.1% sodium azide).

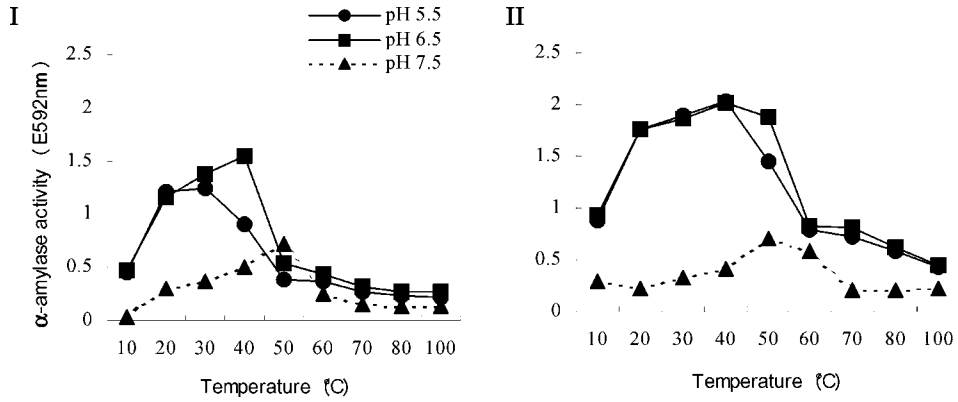

Figure 6. Effect of temperature on *F. culmorum* α-amylase activity at pH 5.5, 6.5, and 7.5 in two different buffers. I, buffer 1 (see **Figure 5**); II, buffer 2 (see **Figure 5**).

that shrivelled wheat kernels exhibited the greatest modification when infected with *F. graminearum*; indeed, numerous hyphae could be found throughout the kernel. In our seriously infected kernels, the starch granules showed a large degree of apparently enzymatic degradation as well as being associated with numerous hyphae. The characterization in terms of pH and temperature requirements of the fungal α-amylase from *F. culmorum* could serve as an explanation why the fungal infection is closely related with the degradation in wheat quality. As shown in **Figure 5**, the α-amylase from *F. culmorum* showed a wide range of activity from pH 5.0 to 8.5, with an optimum at pH 5.5−6.5 in buffer 1 and between 5.0 and 7.0 in buffer 2. To assess the comparable activity of *F. culmorum* α-amylase at pH 5.5−7.0, the enzyme filtrate was further diluted in buffer 1 at a ratio of 1:20 and in buffer 2 of 1:80. The highest activity for both temperatures occurred at pH 6.5 in buffer 1 and at 6.0 in buffer 2. As a consequence, the pH values 5.5, 6.5, and 7.5 were chosen for determining the optimal temperature for this fungal α-amylase. *F. culmorum*'s α-amylase was found to be thermostable at the three different pH values chosen (5.5, 6.5, and 7.5); indeed, it remained active up to 100 °C (**Figure 6**). The temperature range tested was from 10 to 100 °C, with a maximum activity in both buffers at the ratio of 1:10. The temperature optimum was dependent on the pH value, being 30, 40, and 50 °C, respectively. Generally, the α-amylase activity was much lower at pH 7.5 than that at either pH 5.5 or 6.5. After the additional dilution of both extracts, the optimum temperature at pH 5.5 and 6.5 was 30−40 °C in buffer 1 and at 40 °C in buffer 2.

The production of bread with an appropriate volume requires a balanced amount of amylases in the flour, as these are responsible for a certain level of fermentable sugars. Because in flour such optimal conditions are often not present, enzyme preparations are added (*10*). A comparison of the present results with commonly used α-amylase preparations from different origins is shown in **Table 3**. *Fusarium* α-amylase possesses a comparatively lower optimal and higher inactivating temperature than amylases from *Aspergillus*, although the latter are in contrast thermoinstable (*35*). These results support former investigations that showed that the α-amylase of *F. culmorum* may degrade starch granules during the whole process of wheat flour preparation and baking, hence causing unsatisfactory bread quality related to volume, crumb structure, and crust color. This degradation of flour functionality and bread quality will be accentuated in those baking processes that include long fermentation periods because the α-amylase will have more time to cause detrimental effects.

Table 3. Characterization of α-Amylase from Different Origins

	origin			
criteria	cereal (malt) (*36*)	bacteria (*Bacillus subtilis*) (*33*, *36*)	fungi (*A. oryzae*, *Aspergillus niger*) (*33*, *36*)	present results (*F. culmorum*)
pH optimum	4.5	5.9–6.0	4.0–5.0	5.5–7.0
temp optimum (°C)	65–70	60–90	60–65	30–50
inactivating temp (°C)	85	95 (incomplete)	70–75	100 (incomplete)

In conclusion, our results reveal the complexity of *Fusarium* impact on the wheat polysaccharides and explain possible reasons for degradation of flour and bread quality.

ABBREVATIONS USED

DAS-ELISA, double antibody sandwich enzyme-linked immunosorbent assay; ICC, the International Association of Cereal Science and Technology; FHB, *Fusarium* head blight; FN,

Hagberg falling number; FPE, *Fusarium* protein equivalent; RVA, rapid visco analyzer; w/v, weight to volume; v/v, volume to volume.

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LITERATURE CITED

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