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ORIGINAL PAPER

In vitro degradation of wheat gluten fractions by *Fusarium graminearum* proteases

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Abstract *Fusarium* spp. infection of cereal grain is a common problem, which leads to a dramatic loss of grain quality. The aim of the present study was to investigate the effect of *Fusarium* infection on the wheat storage protein gluten and its fractions, the gliadins and glutenins, in an in vitro model system. Gluten proteins were digested by *F. graminearum* proteases for 2, 4, 8 and 24 h, separated by Osborne fractionation and characterised by chromatographic (RP-HPLC) and electrophoretic analysis (SDS-Page). Gluten digestion by *F. graminearum* proteases showed in comparison with gliadins a preference for the glutenins whereas the HMW subfraction was at most affected. In comparison with a untreated control, the HMW subfraction was degraded of about 97% after 4 h incubation with *Fusarium* proteases. Separate digestion of gliadin and glutenin underlined the preference for HMW-GS. Analogue to the observed change in the gluten composition, the yield of the proteins extracted changed. A higher amount of glutenin fragments was found in the gliadin extraction solution after digestion and could mask a gliadin destruction at the same time. This observation can contribute to explain the frequently reported reduced glutenin amount parallel to an increase in gliadin quantity after *Fusarium* infection in grains.

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Keywords Gluten · Gliadin and glutenin fractions · Peptides · Serine and trypsin protease

Abbreviations

FHB	Fusarium head blight
DON	Deoxynivalenol
HMW-GS	High molecular weight glutenin subunits
LMW-GS	Low molecular weight glutenin subunits
NIV	Nivalenol
RP-HPLC	Reverse-phase high-pressure liquid chromatography
SDS-Page	Sodium dodecyl sulphate
rpm	Revolutions per minute
TCA	Trichloric acid
TDI	Tolerable daily intake
Tris	Tris(hydroxymethyl)-aminomethane
TFA	Trifluoric acid

Introduction

Fusarium graminearum (teleomorph: *Gibberella zeae*) is the most relevant *Fusarium* species in Europe causing Fusarium head blight (FHB) in wheat and barley [1, 2]. Besides *F. graminearum* and *F. culmorum,* a number of other *Fusarium* spp. are also known to cause FHB [1, 3]. *Fusarium graminearum* produces trichothecene mycotoxins such as nivalenol (NIV) and deoxynivalenol (DON) [4]. High concentrations of *Fusarium* toxins in the diet lead to adverse effects on human and animal health. Typical symptoms are vomiting, diarrhoea, internal bleeding of the intestines at high doses with an impairment of the immune function and the inhibition of protein synthesis [5–7]. These toxic effects make the reduction of trichothecene

content in food and feed essential; therefore, the European Union has limited the maximum concentrations of DON tolerated in products destined for human food (TDI: 1 μg DON kg^{-1} bodyweight/day; maximum concentration in unprocessed crop: 1,250 μg kg^{-1}) [*Commission Regulation (EC) No. 856/2005*. 2006] and for animal nutrition (crop and crop products: 8,000 μg kg^{-1} at 12% moisture content) [*Commission Regulation (EC) No. 576/2006*. 2006].

The viscoelastic properties of the storage protein fractions (gluten) in wheat are essential for the use and the processing quality of bread and bakery products [8]. Gluten-forming proteins absorb water and form a network during dough making which is able to retain gas during the baking process and results in an elastic crumb structure [9, 10]. For analyses of gluten proteins, the classical extraction procedure is the one described by Osborne [11]. The stepwise separation of the proteins in salty water, alcohol and buffer solution under reducing conditions results in an albumin/globulin fraction, a gliadin fraction and glutenin fraction, respectively, which differ in their functional contribution during wheat processing. Wieser et al. [12] established the separation of gliadins and glutenins into subfractions (gliadins: ω-, α- and γ-gliadins; glutenins: ω-glutenin, high molecular weight glutenin (HMW-GS) and low molecular weight glutenin (LMW-GS) by RP-HPLC.

Fusarium infection and the consequent production of proteases by the fungus lead to gluten degradation, thus changing dough consistence and resistance to extension, which results in a loss of dough functionality and loaf volume [9]. Recent studies focusing on the influence of *Fusarium* infection on grain proteins have documented either no or just a moderate impact on total protein content and supposed a dependence of protein degradation on the degree of infection with *Fusarium* spp. [13, 14]. However, an influence on the proportion and composition of the protein fractions such as an increase in gliadin and a reduction in glutenin content has also been observed [14, 15]. Fungal proteases produced by *Fusarium* spp. include trypsin-, serine- or subtilisin-like proteases as well as protein phosphatases, serine peptidases, aminopeptidases and alkaline proteinases. They are part of the exoproteome of the fungus and degrade proteins in both wheat and barley grains [16–18]. *Fusarium* proteases are mainly reported to be trypsin-like serine proteases that cut the proteins at the lysine or arginine amino acid [16, 19]. A study that investigated the ability of *Fusarium* proteases from *F. graminearum*, *F. avenaceum* and *F. poae* to degrade gliadin in an in vitro trail observed a degradation of gliadin just after *F. poae* treatment but not after *F. graminearum* treatment [20]. An earlier study explained the reduction of the glutenin fractions and the increase in the quantity of the gliadin fraction after *Fusarium* infection with the differing impact of the fungus on protein synthesis during maturation stages [14]. However, a further study did not show any alterations in the protein synthesis behaviour during grain maturation and only a belated polymerisation of glutenins was observed [21]. Therefore, the true nature of protein alteration by *Fusarium* spp. is still a matter of discussion.

The present study contributes to a better understanding of the degradation of storage proteins by *Fusarium* spp. An in vitro experiment was performed in which the effect of proteases produced by *F. graminearum,* the main causal agent of *Fusarium* head blight in small cereal grain, on gluten-forming proteins was investigated. It was of interest if *Fusarium* proteases prefer the degradation of particular subfractions of gluten. Therefore, the separate digestion of gliadin and glutenin by fungal proteases was initiated. The quantification and characterisation of the liberated products were performed by RP-HPLC and SDS-Page. The present study can contribute to the fundamental understanding of protein degradation observed in earlier studies resulting from *Fusarium* infection and the potential of protease action during wheat flour processing [13–15].

Materials and methods

Protein extraction procedure

The extraction procedure was adapted with modifications from the method of Wieser et al. [12]. Commercially available gluten from wheat (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was used for extraction of gliadin and glutenin. The crude gluten was washed twice with a fivefold quantity of an albumin and globulin-extracting solution [65 mM Na_2HPO_4, 2 mM KH_2PO_4, with 400 mM NaCl, pH 7.6] for 30 min to remove any leftover of albumin and globulin. In each extraction cycle, the sample was centrifuged for 20 min at 1,700 *g* and the supernatant was discarded. The salt was removed by washing the pellet for 30 min with distilled water and centrifuged for 20 min at 1,700 *g* and the supernatant was discarded again. All of the steps were performed at room temperature (25 °C).

Gliadin was extracted from the gluten as described above. The gluten pellet was suspended for three times with a fivefold quantity of the extraction solution containing 60% ethanol (v/v) by homogenisation of the pellet for 1 min at 24,000 rpm with an ultra turrax (IKA® Werke GmbH & Co. KG, Staufen, Germany). The suspension was shaken vigorously for 30 min and centrifuged at 1,700 g for 20 min at room temperature. The gliadin-containing supernatants were combined and filtered (MN 616¼ 150 mm; MACHERY-NAGEL GmbH & Co. KG, Düren,

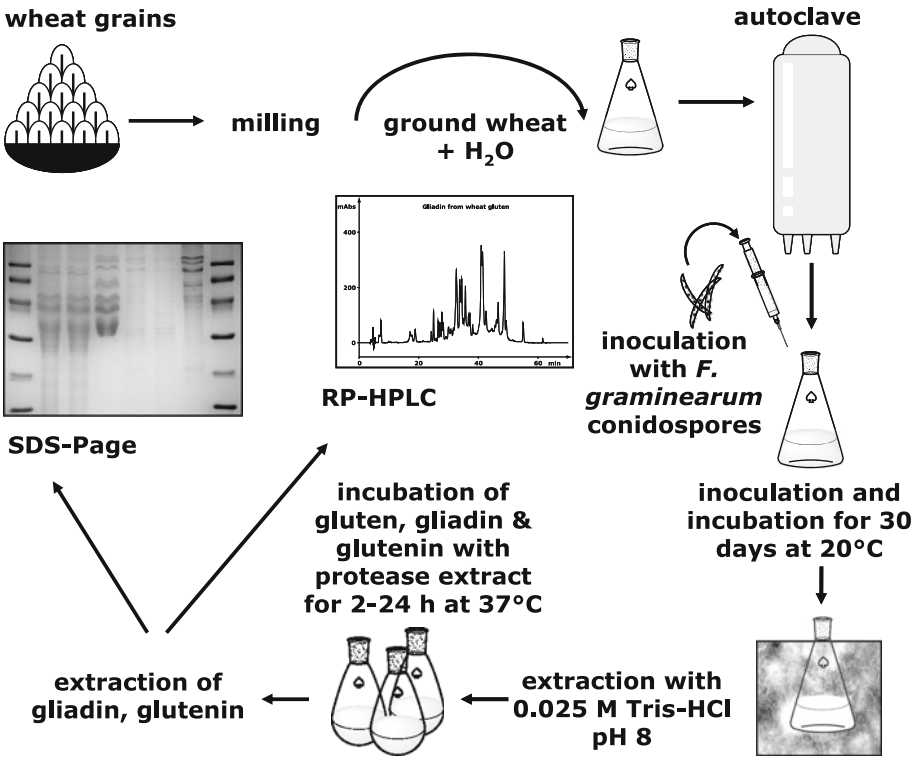

Fig. 1 Model system for the isolation of *F. graminearum* protease extract and degradation of wheat gluten and gluten fractions. Wheat grains were milled, mixed with water and autoclaved. *Fusarium graminearum* (FG 142, 143, 144) macro conidiospores (0.5×10^6 ml^{-1}) were added to sterile medium and incubated for 30 days at 20 °C. *Fusarium* proteases were extracted with Tris–HCl and used for incubation and degradation of the total gluten as well as gliadin and glutenin separately over different time periods. Proteins and peptides were isolated and detected by RP-HPLC and SDS-Page

Germany). The ethanol was evaporated at 40 °C for 24 h from the gliadin and the residue was freeze-dried (CHRIST Gefriertrocknungsanlagen, EPSILON 2-40, Osterode, Germany), homogenised by pestle under liquid nitrogen (N_2) and stored at −20 °C.

Glutenin was extracted twice with a fivefold quantity of the extraction solution [containing 50% 1-propanol (v/v) mixed with 50% of 2 mol/l urea, 0.05 mol/l Tris/HCl (pH 7.5) and 1% dithioerythritol] by homogenisation of the pellet for 1 min at 24,000 rpm with an ultra turrax. The suspension was shaken vigorously for 30 min at 60 °C and centrifuged for 20 min at 5,214 *g* at room temperature. The glutenin-containing supernatants were combined, filtered and dialysed for 24 h in a cellulose acetate tube against distilled water. The residue was freeze-dried, homogenised by pestle under liquid N_2 and washed again three times with 60% (v/v) ethanol to remove any gliadin residue. Finally, the pellet was washed with ice-cold (−20 °C) acetone, dried under N_2 and stored at −20 °C.

Preparation of *Fusarium graminearum* protease solution

Fusarium graminearum isolates (FG 142, 143, 144) were DON-producing strains and were obtained the reference stock of the Division of Plant Pathology and Crop Protection at the Department of Crop Science of the Georg-August-University, Göttingen. Macro conidiospore production was conducted on autoclaved wheat straw suspension which represented a nutrient deficient medium for 7 days at 20 °C containing 9 g straw, 300 ml distilled water and 60 mg streptomycin (Carl Roth GmbH + Co. KG, Karlsruhe, Germany). From each isolate grown on oatmeal agar, five discs were punched out with a cork borer and added to the straw suspension. Macro conidiospores quantification was accomplished using a Fuchs-Rosenthal counting chamber (depth: 0.2 mm 1/16 mm^3). For protease production, 1 ml of macro conidiospore suspension (0.5×10^6 ml^{-1}) from each isolate was added to an aseptical medium containing 5 g of wheat seeds (*Triticum aestivum*, L.) milled to less than 0.5 mm particle size (Retsch ZM 100, Haan, Germany) in 30 ml of distilled water in a 250 ml Erlenmeyer flask which was autoclaved twice for 15 min at 121 °C (Fig. 1). Each isolate was incubated two times for 30 days at 20 °C at day/night frequency. After incubation, *F. graminearum* proteases were extracted from the media for 16 h with 40 ml sterile Tris–HCl buffer (25 mmol, pH 8 [16]) by gentle shaking at 4 °C. Afterwards, the extracts were filtered over a hair sieve, centrifuged for 15 min at 3,830 *g* and combined in equal parts. The extract mixture was used to minimise differences in protease production that depend on the isolate and to ensure an adequate protease production. For control, medium without macro conidia was incubated and extracted under the same conditions. All extracts were stored at −20 °C for further analyses (Fig. 1).

Protein incubation and extraction

Two sets of experiments were performed: (1) Proteolysis of gluten by *Fusarium* protease, followed by extraction of the gliadins and glutenins and investigation of them by RP-HPLC and SDS-Page. (2) Proteolysis of the isolated and purified gliadins and glutenins by *Fusarium* protease, and investigation of them by the above-mentioned methods.

Proteolysis

Each of 20 mg gluten, gliadin and glutenin were weighed in 2 ml reaction tubes. For proteolysis, each of 1 ml of protease extract was added to the tubes. All samples were gentle shaken at 37 °C. For gluten samples, the incubation durations were 4 and 24 h, whereas for gliadin and glutenin samples incubation durations of 2, 4 and 8 h, respectively, were applied (Fig. 1). The proteolysis was stopped by adding 1 ml of ice-cold (−20 °C) acetone with 20% trichloroacetic acid (TCA) to the reaction mixtures. Thereafter, the solutions were vortexed for 30 s and the samples were stored for 12 h at −20 °C.

Protein extraction after proteolysis

The samples were centrifuged for 20 min at −9 °C and 20,000 *g*. The supernatants from the separately incubated gliadin and glutenin fractions were collected and stored at −20°C for further RP-HPLC determination of the peptides. The pellets were washed with ice-cold acetone at −20 °C for 30 min, again centrifuged at the same conditions given above. Finally, the pellets were dried under N_2. For gliadin extraction, the respective pellet was treated twice with 1 ml of 60% (v/v) ethanol. Then, the pellet was homogenised with a hand mixer at 7,000 rpm (Xenox S.A. Xenox—Motorised Hand Tool, Wecker, Luxemburg), shaken for 20 min at 20 °C and centrifuged for 20 min at 20 °C and 20,000 *g*. The resulting supernatant was divided into two aliquots—one for RP-HPLC and the other for SDS-Page. The gluten and glutenin pellets were extracted twice with the glutenin extraction solution [containing 50% 1-propanol (v/v) mixed with 50% of 2 mol/l urea, 0.05 mol/l Tris/HCl (pH 7.5) and 1% dithioerythritol]. The samples were homogenised with a hand mixer at 7,000 rpm (Xenox S.A. Xenox—Motorised Hand Tool, Wecker, Luxemburg), shaken for 30 min at 60 °C under N_2 and the obtained extracts were centrifuged at 7,000 g for 20 min at 20 °C. The supernatants were divided into two aliquots according to the gliadin sample.

Sample preparation for SDS-Page

To a 1 ml sample solution from the gliadin and glutenin extract, 1 ml ice-cold (−20 °C) acetone with 20% TCA was added. The samples were stored for 12 h at −20 °C. Afterwards, the samples were centrifuged for 20 min at −9 °C and 20,000 *g*. The obtained pellets were washed with ice-cold acetone and dried under N_2.

RP-HPLC

A dual pump mode Shimadzu 10A system (Duisburg, Germany) with a PerfectSil 300 C8 column 300 × 4.5 mm, 300 Å, 5 µm (MZ-Analysentechnik GmbH, Mainz, Germany) was used. The flow rate was 1 ml/min and detection was performed at 220 nm with a column temperature of 50 °C. The two eluents were A = 0.1% TFA in distilled water and B = acetonitrile. The gradient was applied under the following conditions: 100% eluent A, 0 min; 76% eluent A, 5 min; 50% eluent A, 50 min; 10% eluent A, 51 min; 10% eluent A, 56 min; 100% eluent A, 57–70 min (regeneration/equilibration). The injection volumes of the samples were as follows: 100 µl for the peptide fraction, 50 µl for the samples from gliadin and glutenin proteolysis and 200 µl for the ethanol-soluble glutenins. The content of each of 20 mg of gluten, gliadin and glutenin used for proteolysis was used to quantify the proteins using the AUC (area under the curve).

SDS-Page

A mini SDS-Page (Bio-Rad Laboratories GmbH, Munich, Germany) was used and the separation according to the method of Laemmli performed [22]. The separation conditions of the gel were for the separating gel 14% T and the stacking gel 10% T. The samples were dissolved in a sample buffer with the following composition: 4% sodium dodecyl sulphate 'SDS', 12% glycerol, 0.61% Tris–HCl, 5% mercaptoethanol, 1% dithioerythritol and 0.01% Coomassie Brilliant Blue R 250 with pH 6.8. The protein pellets were dissolved in 1 ml sample buffer for gliadin and glutenin and in 200 µl sample buffer for destructed glutenins soluble in 60% (v/v) ethanol. The protein solutions were treated for 5 min in an ultrasonic bath, heated at 90 °C for 3 min. To each slot, 10 µl of the sample solution was applied. A standard of 10 µl of calibration proteins (Amersham Bioscience Europe GmbH, Freiburg, Germany) dissolved in 300 µl sample buffer were added to each gel as molecular weight markers.

Statistical analysis

Generally, the analyses were repeated at least three times and evaluated by their means and standard deviations.

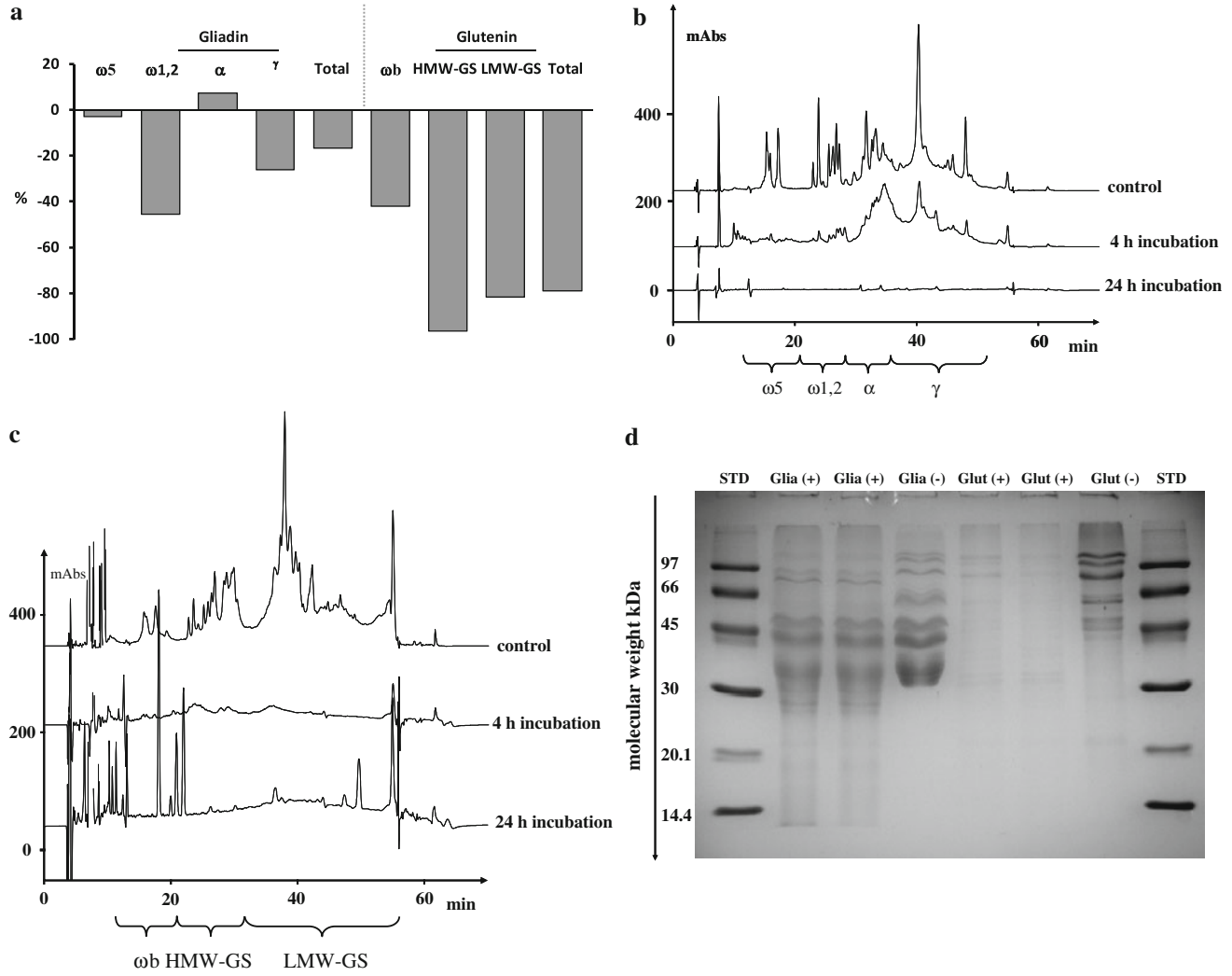

Fig. 2 **a** Degradation of gliadins and glutenins as well as subfractions extracted from gluten after 4 h incubation with *F. graminearum* proteases as % peak area in comparison with control. **b** RP-HPLC results of degraded wheat gliadin subfractions (ω5-, ω1,2-, α-, γ-gliadin) extracted from gluten after 4 and 24 h of incubation with *F. graminearum* proteases in comparison with control, (mAbs, micro absorption units). **c** RP-HPLC results of degraded wheat glutenin subfractions (ωb-, HMW-, LMW-glutenin) extracted from gluten after 4 and 24 h of incubation with *F. graminearum* proteases in comparison with control, (mAbs, micro absorption units). **d** Results of SDS-Page showing the degradation of wheat gliadin and glutenin subfractions extracted from gluten after a 4 h incubation with *F. graminearum* proteases. Key: *STD* Standard, *Glia (+)* protease-degraded gliadin, *Glia (−)* control gliadin, *Glut (+)* protease-degraded glutenin, *Glut (−)* control glutenin

Results and discussion

Digestion of wheat gluten by *F. graminearum* protease

An incubation of gluten with *Fusarium* proteases for 4 h led to a reduction of about 17% in gliadins and 80% in glutenins in comparison with the control (Fig. 2a). After 24 h, the chromatograms showed complete absence of gliadins and the loss of typical glutenins fractions (Fig. 2b, c). A 4 h incubation with *Fusarium* proteases led to a stronger degradation of the glutenin fraction compared to the gliadins (Fig. 2a–d), indicating a preferred digestion of glutenins by the proteases. The more distinct reduction of glutenins in comparison with gliadins has also been ascertained in naturally and artificially infected samples from field trials [14, 15]. Contradictory to these results, another study did not find a degradation of gliadin after incubation with *F. graminearum* proteases which may be explained with different in vitro conditions applied [20]. Within the gliadins, the most affected subfractions were ω1.2-gliadins which were reduced about 45% and γ-gliadins which were reduced about 26% in comparison with the control (Fig. 2c). At the same time, an increase in α-gliadin about 7% could be observed. We assume that the

differences in the breakdown of the gliadin subfractions may result from a change in solubility of digested gluten fractions which has been discussed in an earlier study [15].

The strongest impact of fungal proteases on glutenin subfractions was observed for HMW-GS (Fig. 2b, c), which decreased about 97% after 4 h incubation. LMW-GS decreased about 82% and ωb glutenins about 42%. These results underline the high susceptibility of glutenins to the digestion by proteases from *F. graminearum*. These proteases have been reported to be mainly trypsin-like serine proteases that cut the proteins at the lysine or arginine amino acid [16, 19]. A possible explanation for the primary destruction of HMW-GS in comparison with LMW-GS is their relatively higher quantity of lysine or arginine. In literature, lysine contents between 0.7 and 1.4% for HMW-GS and between 0 and <1% for LMW-GS were reported, while no difference in arginine content between the two fractions was described [23–25]. Therefore, we assume that lysine is the crucial amino acid responsible for the observed higher breakdown of HMW-GS compared to LMW-GS. HMW-GS are, when compared to other glutenin subfractions, predominantly responsible for the elastic properties of the dough and strongly positively associated to a high baking volume [10]. Therefore, the breakdown of HMW-GS by *Fusarium* proteases may contribute to explain the observed decrease in dough quality and baking performance after *Fusarium* infection of wheat [9].

Digestion of purified gliadin and glutenin by *F. graminearum* protease

The incubation of just gliadins with *Fusarium* proteases resulted in a decrease in total gliadins over 2, 4 and 8 h

Table 1 Degradation of gliadin (Fig. 2b) and glutenin (Fig. 3b) subfractions remaining after incubation with *F. graminearum* protease in % peak area in comparison with control; for abbreviations of the subfractions, see 'Abbreviations'

Protein	Subtraction	Incubation time (h)		
		2	4	8
Gliadin	ω5	88	89	86
	ω1,2	78	91	94
	α	76	88	94
	γ	75	88	94
	Total	77	88	94
Glutenin	ωb	0	0	10
	HMW-GS	39	47	58
	LMW-GS	13	28	43
	Total	16	30	45

Table 2 Peptide release from purified gliadin, glutenin and the amounts of degraded glutenins soluble in the gliadin extraction solution after incubation with *F. graminearum* proteases in comparison with control. Results are presented as means ± SD ($n = 3$)

Protein	Control	Incubation time (h)		
	0	2	4	8
		Peak area × 10^{-6}		
Gliadin	2.9 ± 1.8	28.4 ± 1.7	54.9 ± 1.3	84.0 ± 3.9
Glutenin	0.7 ± 0.4	44.4 ± 0.2	67.4 ± 10.9	89.2 ± 2.7
Glutenins soluble in gliadin fraction	45.5 ± 3.2	66.6 ± 29.2	71.6 ± 0.5	74.4 ± 7.7

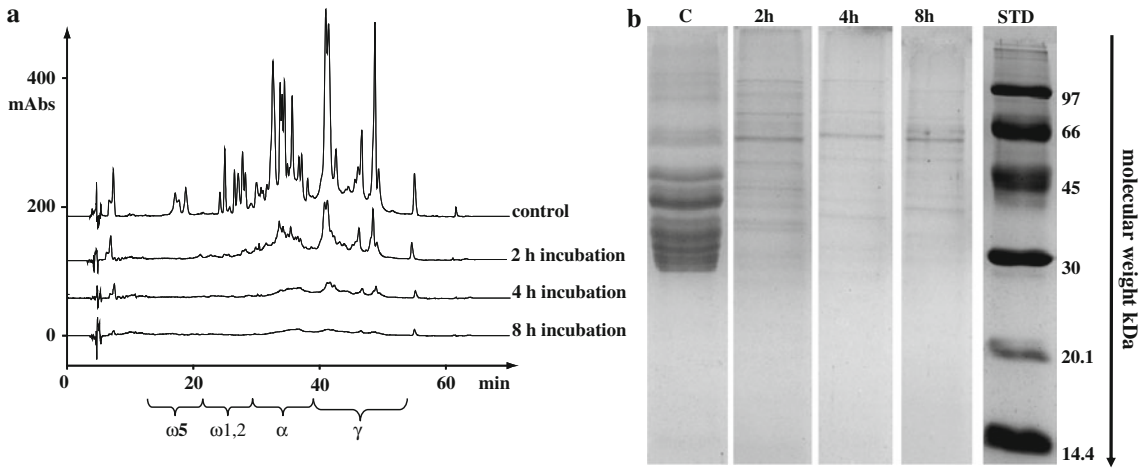

Fig. 3 **a** RP-HPLC results of degraded gliadin subfractions (ω5-, ω1,2-, α-, γ-gliadins) after incubation with *F. graminearum* proteases in comparison with control, (mAbs, micro absorption units). **b** SDS-Page results of gliadin degradation after incubation with *F. graminearum* proteases for 2, 4 and 8 h (*STD* standard, *C* control)

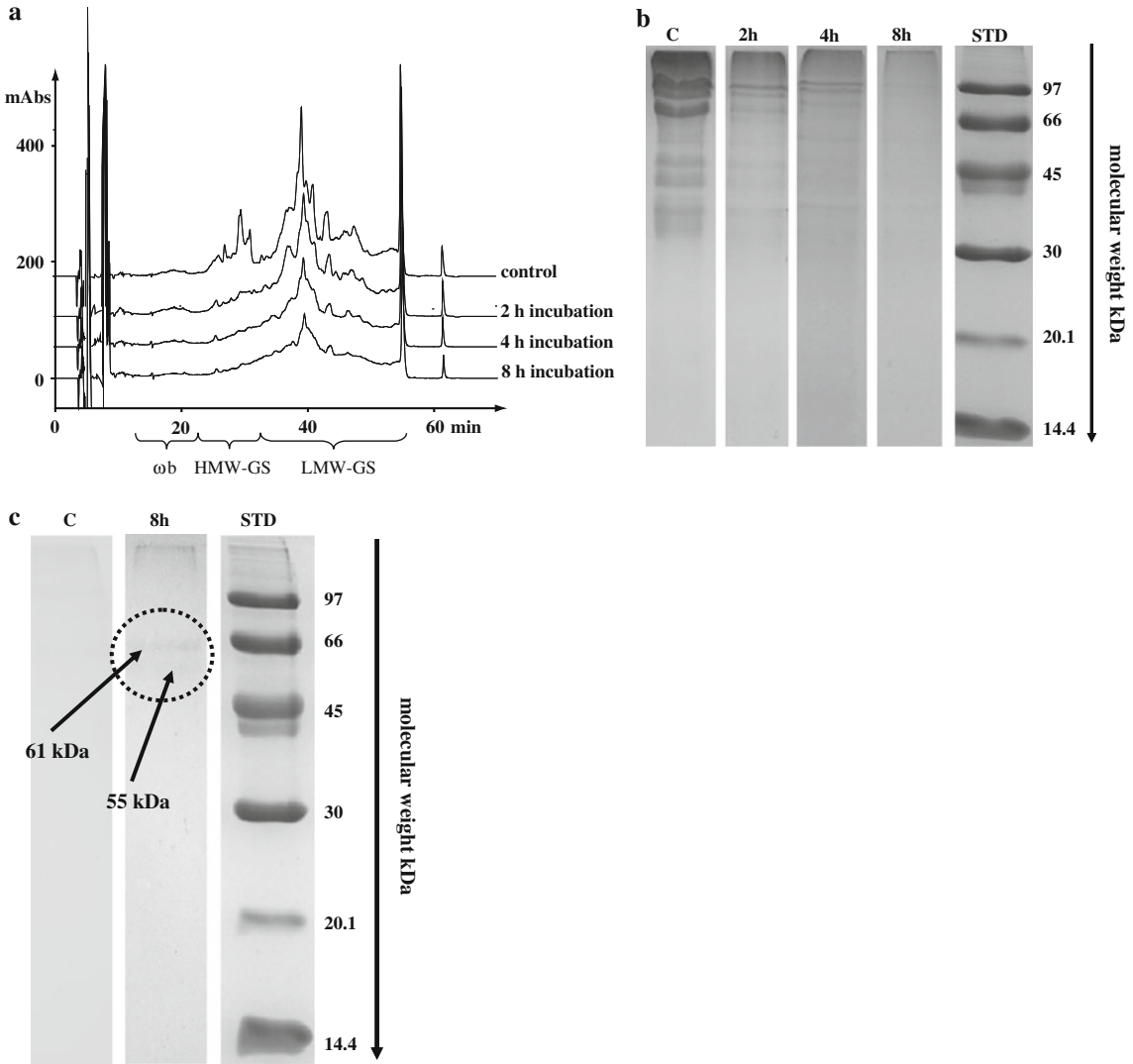

Fig. 4 **a** RP-HPLC results of degraded glutenin subfractions (ωb-, HMW-, LMW-glutenin) after incubation with *F. graminearum* proteases in comparison with control, (mAbs, micro absorption units). **b** SDS-Page results of glutenin degradation after incubation with *F. graminearum* proteases for 2, 4 and 8 h (*STD* standard, *C* control). **c** SDS-Page results of glutenins soluble in the gliadin extraction solution containing 60% (v/v) ethanol after an 8 h incubation period with *F. graminearum* proteases in comparison with control (*STD* standard, *C* control)

about 77, 88 and 94%, respectively. All gliadin subfractions were decreased in a range of 88–91% (Table 2; Fig. 3a, b). Obviously, if only gliadins acted as substrate for the fungal proteases, a stronger destruction of gliadins took place compared to degradation of total gluten (Table 1; Figs. 2a–c and 3a, b). These results confirm the hypothesis that besides glutenin digestion, gliadin degradation also takes place in infected wheat grains [15]. This can be confirmed by the increasing formation of peptides from gliadin detected after 2, 4 and 8 h of incubation in comparison with the control (Table 2). The breakdown of gliadins by *Fusarium* proteases shows the potential of these proteases to degrade all of the gliadin fractions completely (Figs. 2a, 3a) and documents the eligibility of these enzymes as a prospective degrading tool with regard to coeliac disease [26, 27].

The degradation of purified glutenins occurred to a lower extent when compared to purified gliadins. After 2, 4 and 8 h incubation with proteases, total glutenin was reduced about 16, 30 and 45%, respectively. Again, HMW-GS were affected the most and reduced about 47% after 4 h of incubation, whereas LMW-GS decreased about 28% (Table 1; Fig. 4a, b). Glutenins which were soluble in gliadin extraction solution increased with incubation time (Table 2). The higher solubility of protein fractions resulting from glutenin digestion could additionally be

confirmed by SDS-PAGE where two fragments with a molecular weight of 61 and 55 kDa after an 8 h incubation time could be detected (Fig. 4c). These results confirm that fragments resulting from glutenin digestion by fungal proteases are most likely extracted with the gliadin fraction in samples. Because these fractions are co-detected with the gliadin fraction by RP-HPLC, the destruction of gliadins is masked. Higher gliadin content and lower glutenin content after *Fusarium* infection were described in earlier studies and can now be explained with the masking of gliadin destruction by glutenin fragments [14, 15]. This aspect has yet not been considered so far in the discussion of altered gliadin and glutenin contents in *Fusarium* infected wheat samples from field trials [14]. Nevertheless, further experiments are necessary to analyse the degradation of gliadins and glutenins by fungal proteases. Low abundant subfractions could be identified with 2D-Page and MALDI-TOF–MS and provide a better understanding of the consequent allocation of the subfractions of gliadins and/or glutenins. Additionally, it can be an interesting biochemical approach to identify basic mechanisms in interaction between proteases and the substrate, wheat storage proteins, by an in vitro test system with *Fusarium* proteases. Therefore, the present study is the initial point for further analysis of wheat storage proteins by *Fusarium* proteases with biochemical methods.

Conclusion

The present study revealed a preference of *F. graminearum* proteases for the digestion of glutenins rather than gliadins during gluten digestion. Within the glutenin subfractions, the HMW-GS subfraction was the strongest affected one. This effect probably results from the specificity of the *Fusarium* proteases. The separated digestion of gliadin and glutenin by *F. graminearum* proteases confirmed former results from field samples, i.e. a clear effect on both gliadin and glutenin fractions. The formation of a protein fraction with higher solubility in the gliadin extraction solution after incubation with proteases was also observed. This result indicates that fragments from purified glutenin can be falsely allocated to gliadin subfractions and lead to the conclusion that the amount of gliadins increases over time during incubation with *F. graminearum* proteases. Therefore, the allocation of wheat gluten subfractions according to their solubility in different solvents and their conventional analysis by RP-HPLC may lead to false conclusions, especially if wheat grain had been exposed to *Fusarium* infection. This underlines the need for application of more advanced methods for characterisation of gliadins and glutenins.

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