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Starch facilitates enzymatic wheat gluten hydrolysis

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Wheat gluten can be hydrolyzed by either using (vital) wheat gluten or directly from wheat flour. This study investigates the influence of the presence of starch, the main component of wheat, on enzymatic wheat gluten hydrolysis. Wheat gluten present in wheat flour (WFG) and vital wheat gluten (VWG) were hydrolyzed at constant protein concentrations, but subsequently 5.6 times higher amounts of wheat flour. Nevertheless, WFG hydrolysis at 40% total solids resulted in significantly higher degrees of hydrolysis (DH%) than VWG hydrolysis at 7.2% solids. This difference increased to up to 4.5% in 6 h and diminished again for longer reaction times. Possible differences in the gluten composition and the presence of albumins and globulins in wheat flour could not explain the difference in DH% because the addition of starch to VWG increased the rate of hydrolysis similarly. Instead, it was concluded that starch granules impede gluten aggregation, which facilitates the hydrolysis. At higher solid concentrations of up to 70% wheat flour, the positive effect of starch disappeared, because WFG hydrolysis was hindered by mass transfer limitations and lower water activities.

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1. Introduction

Wheat gluten, the major protein of wheat, is a high-value, modestly priced plant protein, available at increasing amounts (Day, 2011). Native vital wheat gluten is an excellent bread improver and also used to texturize foods (Agyare, Addo, & Xiong, 2009). However, native vital wheat gluten is scarcely water-soluble at neutral pH, which limits further applications in the food industry. Therefore, enzymatic hydrolysis is used as a mild process to increase the water solubility at neutral pH and to alter other functional properties, such as its foaming and emulsifying properties (Hardt, van der Goot, & Boom, 2013; Kong, Zhou, & Qian, 2007; Linarès, Larré, Lemeste, & Popineau, 2000). In practice, wheat gluten hydrolysates are used as savory flavoring agents, in sports nutrition, and for the production of bioactive peptides, among others (Bombara, Pilosof, & Añón, 1992; Giesler, Linke, Rabe, Appel, & Berger, 2013).

Hydrolysis of wheat gluten is performed using isolated (vital) wheat gluten (VWG), a by-product of the wheat starch production. Thus, a pre-process step is required to separate gluten from wheat flour in processes such as the Martin or the batter process. These separation processes consume copious amounts of water and often require 10–15 L of water per kg of dry matter (Schutyser & van der Goot, 2011), which has to be removed afterwards. Alternatively, wheat gluten can be hydrolyzed when still present in the wheat flour (WFG) (Bombara, Añón, & Pilosof, 1997; Bombara et al., 1992), thereby omitting the separation step, or separation can take place after gluten hydrolysis. Currently, mild proteolysis of wheat flour is performed in the baking industry with the aim to “weaken” the gluten network and to improve the handling properties of dough (Bombara et al., 1997; Wikström & Eliasson, 1998). Other possible applications of wheat flour hydrolysates are cereal-based drinks (Nielsen & Peterson, 2011).

Previously, we showed that vital wheat gluten can be hydrolyzed at solid concentrations of up to 60% at increased reactor productivity compared to the conventionally used concentration of 20% solids (Hardt et al., 2013). It is therefore interesting to investigate the role of the high starch content in wheat flour (around 80%, dry basis (Belitz, Grosch, & Schieberle, 2009)) on the hydrolysis process, since starch replaces water in WFG hydrolysis compared to VWG hydrolysis at constant protein concentration. Starch in wheat flour is present as starch granules. Since wheat starch granules absorb up to 50% of its dry weight of water (Goesaert et al., 2005), high starch concentrations might induce mass transfer limitations or reduce the water activity, which would reduce the enzyme activity. On the other hand, starch granules might also facilitate the hydrolysis: Native wheat gluten shows a

Abbreviations: DH%, degree of hydrolysis; SWG, self-washed gluten from Ibis wheat flour; VWG, vital wheat gluten; VWG + starch, vital wheat gluten plus added starch; WFG, gluten present in Ibis wheat flour.

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strong tendency to aggregate in the presence of water at neutral pH close to its isoelectric point, which then introduces limitations to the transfer of the enzyme and the hydrolysis products. While hydrated vital wheat gluten forms gluten aggregates of up to some centimeter length (Hardt et al., 2013), the gluten aggregates in the presence of wheat starch in wheat flour batters are millimeter-sized (Van der Borght, Goesaert, Veraverbeke, & Delcour, 2005) and thus presumably easier accessible for the protease because of the increased surface-to-volume ratio.

In this study, we therefore compare high solid wheat flour hydrolysis with vital wheat gluten hydrolysis. First, the influence of starch on wheat gluten hydrolysis is investigated and the molecular mass distributions and the protein solubility of the hydrolysates are analyzed. Second, the influence of varying wheat flour concentrations from 20% to 70% (*w*/*w*) on protein hydrolysis is studied.

2. Materials and methods

2.1. Materials

Ibis wheat flour with 12.6 ± 0.4% ($N \times 5.7$, Dumas method) crude protein content and 13.1 ± 0.5% water content (all by weight) was obtained from Meneba (Rotterdam, The Netherlands). Vital wheat gluten (Roquette) with 73.5 ± 1.3% crude protein content and 8.9 ± 0.6% water content was obtained from Barentz BV (Hoofddorp, The Netherlands). Wheat starch with 10.5 ± 0.7% water content was obtained from Sigma–Aldrich, Steinheim, Germany. Two commercial protease mixtures from *Aspergillus oryzae* (Flavourzyme ≥ 500 LAPU/g) and from *Bacillus licheniformis* and *Bacillus amyloliquefaciens* (Protamex ≥ 1.5 AU-NH/g) were purchased from Sigma–Aldrich, Steinheim, Germany. Flavourzyme is a mixture of endo- and exopeptidases with mainly exoproteolytic activity (Ashie, 2007) and Protamex is an endoprotease (Nielsen, 1996). Borosilicate glass beads with 1 mm diameter and glass beads with 5 mm diameter were obtained from Sigma–Aldrich, Steinheim, Germany. Milli-Q water was used in all experiments unless stated otherwise. All other chemicals were purchased from Sigma–Aldrich, Steinheim, Germany unless stated otherwise.

2.2. Gluten washing

Ibis wheat flour (200 g) was mixed with 120 g of tap water and allowed to rest for 15 min. Then, the dough ball was gently kneaded, placed in a 500-ml beaker full of tap water at ambient temperature, and allowed to stand for 5 min. This step was repeated three times. Afterwards, the remaining starch was continuously washed out under running tap water until the dough ball mass had approximately reduced to 16–18% of the initial mass. The dough ball was then freeze-dried, ground, and used for hydrolysis. The average crude protein and water content of the self-washed Ibis gluten were 86.6 ± 1.1% and 1.9 ± 0.2%, respectively.

2.3. Hydrolysis reaction

The hydrolysis reaction was carried out using 200-ml double-walled glass vessels connected to a water bath. Each experiment was conducted with a total mass of 150 g reaction mixture. Table 1 shows the process conditions used for hydrolysis at 5.8% protein concentration.

All hydrolysis reactions were performed at an enzyme-to-substrate ratio of 1:100 (*w*/*w*). Only the protein fraction was considered as substrate when calculating the enzyme-to-substrate ratio. The hydrolysis temperature was 50 °C. The enzyme was mixed with the water prior to addition to the substrate. The double-walled glass vessel was closed with a clasp to avoid evaporation of water and stirred using an overhead stirrer at 40 rpm. After reaction, the enzyme was inactivated by heating at 95 °C for 15 min in a water bath. The samples were then freeze-dried, ground and stored for further analysis. Hydrolysis reactions were conducted under floating pH conditions. Nevertheless, the pH remained above 4 for Flavourzyme and Protamex. The optimum pH range for Flavourzyme is stated to be between 4 and 8 and for Protamex between 6 and 8 (Nielsen & Olsen, 2002). Standard experiments with Flavourzyme were performed in quadruplicate and with Protamex in triplicate, except for all reactions with the self-washed gluten, which were performed in duplicate. Experiments investigating the influence of the protein concentration on hydrolysis were single experiments.

2.4. Degree of hydrolysis (DH%)

The DH% was measured by the o-phthaldialdehyde (OPA) method according to Nielsen, Petersen, and Dambmann (2001) with serine (L-Serine, 99%, Alfa Aesar, Ward Hill, USA) as a standard and with minor modifications: Hydrolysate was suspended in 12.5 mmol/l sodium tetraborate decahydrate plus 2% (*w*/*w*) sodium dodecyl sulfate (SDS). The amount of suspended hydrolysate was varied between 0.6 and 0.9 mg protein/ml to avoid spectrophotometer absorbance values above 1.5 for samples with a high DH%. The mass of the added wheat flour hydrolysate powder was approximately 5.5 times higher than for the vital wheat gluten hydrolysate powder because of the different starch contents. The suspended hydrolysate was mixed for 60 min and then centrifuged at 3900 × g for 15 min. The resulting supernatant was filtered with a 0.45-μm filter unit and used for analysis. The DH% was calculated using the following formula:

$$\mathrm{DH\%} = \frac{(\text{Serine-NH}_2 - \beta)/\alpha}{h_{tot}}$$

where

$$\text{Serine-NH}_2 = \frac{A_{\mathrm{hydr}} - A_{\mathrm{OPA}}}{\text{protein}\left[\frac{g}{l}\right]} \times \frac{mM\ \text{serine}}{A_{\mathrm{Serine}} - A_{\mathrm{OPA}}}$$

and Serine-NH_2 is meqv serine-NH_2 per g of protein. A_{hydr} is the absorbance of the hydrolysate sample, A_{OPA} is the absorbance of the blank OPA reagent, and A_{serine} is the absorbance of the serine standard. $h_{\mathrm{tot}} = 8.3$ and $\alpha = 1$ (Adler-Nissen, 1979). A value of 0.16 was measured for β (native wheat gluten). The calculated DH% was the mean of two determinations. The protein concentration ($N \times 5.7$) of the dried hydrolysates was determined using the Dumas method (*N* analyzer FlashEA 1112 series, Interscience, Breda, the Netherlands) with methionine as a standard.

Table 1
Sampling parameters for standard hydrolysis reactions.

Source	Added wheat flour or gluten (g)	Added water (g)	Added wheat starch (g)	Added enzyme (g)	Solid concentration	Protein concentration
Vital wheat gluten (VWG)	11.84	138.16	0	0.087	7.2%	5.8%
Wheat flour (WFG)	69.04	80.96	0	0.087	40%	5.8%
Vital wheat gluten + starch (VWG + starch)	11.84	83.17	54.99	0.087	40%	5.8%
Self-washed Ibis gluten (SWG)	10.05	139.95	0	0.087	6.6%	5.8%

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