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The Kinetics of β-Elimination of Cystine and the Formation of Lanthionine in Gliadin

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When gliadin, a mixture of wheat storage proteins containing only intramolecular disulfide (SS) bonds, is heated at high temperatures and preferably at alkaline pH, the SS bonds are cleaved by β-elimination reactions leading to decreased cystine levels and the generation of dehydroalanine (DHA) and free sulfhydryl (SH) groups. DHA and the free SH group of cysteine can further react to form the irreversible cross-link lanthionine (LAN). The kinetics of this reaction were studied by heating model systems containing gliadin at different pH values (pH 6.0, 8.0 and 11.0) at temperatures up to 120 °C. Multiresponse modeling was applied to simultaneously describe the course of the reaction partners, intermediates and products. The estimated kinetic parameters indicate that the reaction rate constant for the elimination reaction increases with temperature and pH. Moreover, the predominant reaction consuming the intermediary DHA is the cross-link with cysteine to form LAN following second-order reaction kinetics. The corresponding reaction rate constant is less dependent on temperature and pH. Use of the proposed kinetic model to estimate reaction product concentrations in cereal-based foods allowed us to conclude that the β-elimination reaction may be less important during, e.g., bread making, but may well contribute to gluten network formation during the production of soft wheat products. It may also well be relevant in the production of bioplastics made from gluten.

KEYWORDS: Gluten; cross-links; beta-elimination reaction; dehydroalanine; lanthionine

INTRODUCTION

The storage proteins of wheat consist of monomeric gliadin and polymeric glutenin. Together, they are referred to as gluten. Gluten plays a key role in structure and texture of different wheat-based food products and is, for instance, responsible for the bread making capabilities of wheat flour (*1*). Furthermore, wheat gluten and its fractions have a large potential for use in nonfood applications, such as adhesives, coatings and thermoplastic materials (*2*). During the processing and setting of gluten containing products, temperature plays a crucial role. Heat treatment of gluten proteins results in large aggregates due to (further) polymerization of glutenin and formation of gliadin−glutenin bonds (*3*). Besides high temperatures, also alkaline conditions and/or mechanical energy input strongly contribute to protein cross-linking (*4*, *5*). Gluten network formation has mainly been attributed to the formation of intermolecular disulfide (SS) bonds by oxidation of sulfhydryl (SH) groups of cysteine and/or SH−SS-exchange reactions between cysteine and cystine. Such reactions lead to glutenin aggregation at moderate heating, and also involve the gliadin fraction at higher temperatures (*6*−*9*). It has been demonstrated that gliadin becomes part of the glutenin network by SH−SS-exchange reactions, which are initiated by free SH groups in the glutenin fraction (*3*). However, not all reaction phenomena with respect to hydrothermal treatment of gluten or its fractions can be fully explained by this mechanism. Especially at alkaline pH, high temperatures and/or long exposure times, β-elimination of cystine in different proteins has been demonstrated to result in dehydroalanine (DHA) and cysteine formation (*10*). The impact of such β-elimination reactions on cross-linking of gliadin and gluten has been studied at pH 8.0 (*11*).

Gliadin consists of proteins containing α-, γ-, and ω-gliadins. The α- and γ-gliadins contain 3 and 4 intramolecular disulfide (SS) bonds respectively, while ω-gliadins lack cysteine residues (*12*). Due to β-elimination of intramolecular cystine, demonstrated at pH 8.0 and 130 °C, free SH groups are formed (*11*). On the other hand, under conditions of mild alkaline pH, DHA residues can react further with cysteine to form the DHA cross-link lanthionine (LAN). At higher pH values, lysinoalanine, a cross-link formed from DHA and lysine, can also be formed in gluten proteins (*11*, *13*). Such β-elimination reactions may well be relevant for several processes involving gluten as they form covalent nonreducible cross-links and new free SH groups which can participate in SH−SS-interchange reactions leading to formation of new intermolecular SS bonds.

In order to predict and control β-elimination of cystine and subsequent formation of irreversible cross-links, and to determine its relevance for different processes, kinetic parameters, such as rate constants and activation energies, are very useful. By applying multiresponse modeling, changes in the concentrations of the different reaction partners, intermediates and products are simultaneously taken into account. Purified gliadin is a good model to

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investigate β-elimination reactions from SS in gluten. In contrast to glutenin, unheated gliadin contains only intramolecular SS bonds and no free SH groups which themselves are intermediates in the β-elimination reactions.

The aim of the present study was to establish the kinetics of β-elimination reactions during hydrothermal treatment of gliadin at different pH in order to demonstrate their relevance for biotechnological processes.

MATERIALS AND METHODS

Materials. Vital wheat gluten [77.6% protein on dry matter (dm)] was obtained from Syral (Aalst, Belgium). Gliadin was extracted at room temperature by horizontally shaking 20.0 g of wheat gluten with 70% (v/v) ethanol (250 mL) for 20 min at 150 spm. After centrifugation (10 min, 20 °C, 10000*g*) the supernatant (gliadin fraction) was rotary evaporated to remove ethanol, freeze-dried, and ground in a laboratory mill (250 μm, IKA, Staufen, Germany). The gliadin fraction contained 78.0% protein dm.

Chemicals and Reagents. Urea, tetrasodium ethylenediamine tetraacetate (EDTA), HCl, and phenol were from VWR International (Leuven, Belgium). Sodium phosphate buffers were prepared with two salts, also obtained from VWR International: disodium hydrogen phosphate dodecahydrate and sodium dihydrogen phosphate dihydrate. 5,5′-Dithio-bis(2-nitrobenzoic acid) (DTNB), reduced glutathione, norleucine and the amino acid standard were from Sigma-Aldrich (Steinheim, Germany). Sodium dodecyl sulfate (SDS) was from Acros Organics (Geel, Belgium), and NaOH was from Mallinckrodt Baker (Deventer, The Netherlands). The LAN standard was from TCI Europe (Zwijndrecht, Belgium). All chemicals, solvents and reagents were at least of analytical grade.

Protein Content. Protein contents were determined in triplicate, using an adaptation of the AOAC Official Method 990.03 (*14*) to an automated Dumas protein analysis system (EAS variomax N/CN, Elt, Gouda, The Netherlands). A conversion factor of 5.7 was used to calculate protein from nitrogen content.

Hydrothermal Treatment of Gliadin. Gliadin samples (500 mg) were heated in 5.0 mL of sodium phosphate buffer (pH 6.0, 8.0 or 11.0; 50 mM) in hermetically sealed reaction tubes (glass, inside diameter = 27 mm, outside diameter = 34 mm, height = 100 mm). Heat treatment was performed in a thermostated oil bath set at 110, 120, or 130 °C. For the kinetic experiments, samples were taken at different heating times, which were chosen depending on the treatment temperature. After thermal treatment, samples were immediately cooled in an ice bath to stop any further reaction. The pH, measured after heating, remained unchanged. The reaction mixtures were then freeze-dried and subsequently ground in a laboratory mill (250 μm, IKA, Staufen, Germany). The obtained samples had protein contents ranging from 72.0 to 78.6% (db). During the heating and subsequent cooling phase, the temperature of the reaction mixture within the closed glass tubes was registered at regular time intervals (20 s) using a thermocouple (type K) connected to a data logger (model 2132, Eurotherm Controls, Leesburg, VA). The maximal temperatures reached under the experimental conditions were 103, 112, and 120 °C respectively (**Figure 1**). The temperature profiles in **Figure 1** were used for the kinetic parameter estimation (see also Kinetic Data Analysis). In what follows, the temperatures mentioned refer to the heating medium temperatures (oil bath) unless stated otherwise.

Free SH Group Determination. Free SH groups were determined colorimetrically after reaction with DTNB according to Ellman (*15*). Gliadin (1.0 mg) was suspended in 900 μL of sodium phosphate buffer (0.05 M, pH 6.5) containing 2.0% (v/v) SDS, 3.0 mol/L urea and 1.0 mM EDTA. Mixtures were shaken (60 min), DTNB reagent (0.1% w/v in sample buffer, 100 μL) was added and mixtures were further shaken (30 min). After 45 min, the extinctions of centrifuged (5 min, 11000*g*) samples were determined at 412 nm (path length 1.000 cm). Control samples containing either no DTNB or no gliadin were used to correct for background extinction of DTNB and gliadin. Extinction values were converted to levels of free SH groups using a calibration curve with reduced glutathione (*16*). All analyses were done in triplicate.

DHA Determination. Based on Patchornik and Sokolovsky (*17*), DHA was first converted to pyruvic acid by acid hydrolysis. Hydrolysis conditions were optimized for gliadin: samples (10.0 mg) were heated (90 min, 110 °C) in 500 μL of HCl solution (1.50 mol/L), and reaction mixtures were diluted and neutralized with dilute sodium hydroxide solution. The pyruvic acid level of unheated gliadin (typically 3.7 μmol/g protein) was considered as the control value, accounting for pyruvic acid formation during acid hydrolysis. Pyruvic acid concentration was then determined colorimetrically after centrifugation of the hydrolysates (3500*g*, 5 min, 6 °C) according to a Megazyme (Bray, Ireland) procedure. In the presence of reduced nicotinamide-adenine dinucleotide (NADH), D-lactate dehydrogenase converts pyruvic acid stoichiometrically into D-lactic acid. The consumption of NADH was measured by the decrease in extinction at 340 nm. All analyses were done in triplicate.

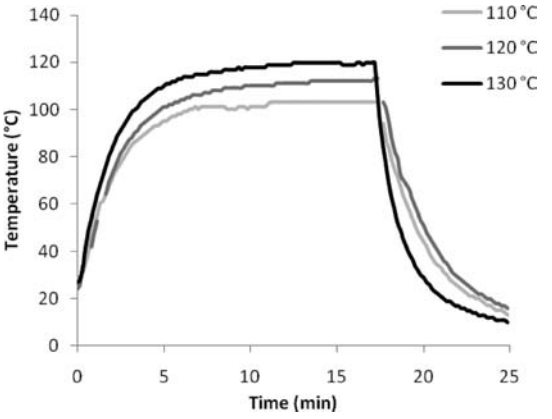

Figure 1. Example of temperature−time profiles of gliadin samples heated in closed glass tubes in an oil bath.

Amino Acid Analysis. Gliadin amino acids, including cystine and LAN, were liberated by acid hydrolysis and separated by high-performance anion-exchange chromatography with integrated pulsed amperometric detection (HPAEC-IPAD) as described by Rombouts et al. (*18*). Gliadin samples (7.0 mg of protein, db) were heated (24 h, 110 °C) in 1.0 mL of 6.0 mol/L HCl containing 0.1% phenol (as protective agent) and 1.5 mM norleucine (as internal standard). Samples were flushed with nitrogen prior to hydrolysis to prevent amino acid oxidation. Reaction mixtures were then evaporated (110 °C, 3 h), resuspended, diluted (200-fold) and filtered (Millex-GP, 0.22 μm, polyethersulfone, Millipore, Carrigtwohill, Ireland). Amino acids were separated on an AminoPac PA10 column (250 × 2 mm, Dionex Benelux, Amsterdam, The Netherlands), using a Dionex BioLC system (Sunnyvale, CA). Gliadin amino acid levels were calculated based on relative areas of amino acids and internal standard in sample hydrolysates and in standard solutions, and expressed on dry matter protein (μmol/g).

Kinetic Data Analysis. Previous research (*11*) suggested a reaction scheme (**Scheme 1**) to describe cystine degradation in wheat gliadin. The β-elimination of cystine was assumed to follow first order kinetics as also suggested by Volkin and Klibanov for a range of globular proteins (*19*), whereas the formation of LAN was considered to follow second order kinetics. The application of this reaction schedule onto the kinetic data obtained in this study was performed using multiresponse modeling. Four responses (i.e., cystine, DHA, free SH and LAN) were measured and modeled simultaneously according to the proposed reaction scheme. For each step in the proposed reaction scheme, a differential equation was set up describing the reaction rate:

$$\frac{d[\text{cystine}]}{dt} = -k_E[\text{cystine}] \quad (1)$$

$$\frac{d[\text{DHA}]}{dt} = k_E[\text{cystine}] - k_F[\text{DHA}][\text{cysteine}] \quad (2)$$

$$\frac{d[\text{cysteine}]}{dt} = k_E[\text{cystine}] - k_F[\text{DHA}][\text{cysteine}] \quad (3)$$

$$\frac{d[\text{LAN}]}{dt} = k_F[\text{DHA}][\text{cysteine}] \quad (4)$$

In eqs 1−4 [compound] represents the concentration of a particular compound, k is the reaction rate constant and t is the reaction time. Initially (t = 0), the concentrations of DHA, free SH and LAN are considered to be zero, whereas for the reactant the initial cystine concentration was considered.

Scheme 1. Cystine Degradation in Wheat Gliadin under Conditions of High Temperature and Alkaline pH[a]

$$\text{Cystine} \xrightarrow{k_E} \text{DHA} + \text{Cysteine} \xrightarrow{k_F} \text{LAN}$$

[a] Cystine, dehydroalanine (DHA), cysteine and lanthionine (LAN) refer to the amino acids in their protein-bound form. k_E, rate constant of the cystine elimination reaction; k_F, rate constant of the LAN formation reaction.

The effect of temperature on each reaction was expressed using the Arrhenius equation, in which the temperature dependence of a rate constant k is quantified by the activation energy E_a (J/mol) according to

$$k = k_{ref} \exp\left(\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T(t)}\right)\right) \quad (5)$$

In eq 5, R represents the universal gas constant (8.3143 J K^{-1} mol^{-1}), k_{ref} the reaction rate constant at reference temperature T_{ref} (K), and k the reaction rate constant at temperature T (K). Because heating of the samples resulted in nonisothermal conditions (**Figure 1**), the integrated effect of temperature should be taken into account. The rate constants in the differential eqs 1−4 can be substituted using eq 5. The resulting mathematical model then needs to be solved using numerical integration of the registered temperature−time profiles. The corresponding kinetic parameters, the reaction rate constants and the activation energies of the reactions, were estimated by nonlinear regression, using the determinant criterion by means of the software package Athena Visual Studio v11.0 (www.athenavisual.com). This fit criterion replaces the commonly used least-squares minimalization to comply with statistical demands for multiresponse modeling (*20*). The goodness-of-fit of the model is evaluated by scrutiny of residuals. In addition, the adequacy of the kinetic model has been evaluated graphically by using the parity plot, showing the relation between experimental and predicted values (*21*).

RESULTS AND DISCUSSION

Multiresponse Modeling of the Kinetics of Cystine Elimination and Related LAN Formation. In order to evaluate the proposed reaction mechanism (**Scheme 1**), different responses were quantified. By simultaneously incorporating the different responses in the mathematical model (including both the Arrhenius equation and the mechanism-based reaction scheme), multiresponse modeling could be applied. This technique has an advantage over single-response modeling, in that it generates insight into the actual reaction mechanism and provides more precise kinetic parameter estimates.

The concentrations of both the reactant cystine and the reaction product LAN, as well as those of the intermediate products DHA and cysteine, were quantified. **Figure 2** shows 4 measured responses for the gliadin model system as a function of time at pH 8.0 and 130 °C. The cystine concentration in gliadin decreased as a function of time during hydrothermal treatment due to β-elimination. In the process, 1 mol of cystine is converted into an equimolar mixture of cysteine and DHA, and these residues can react further to form 1 mol of LAN (**Scheme 1**). This is illustrated by the sum of cystine, DHA and LAN concentrations that remained constant as a function of time (**Figure 2**). As can be seen from **Figure 2**, the cysteine concentration, measured as the level of free SH, is considerably lower than the DHA concentration. Due to this large difference and the uncertainty on the data for free SH (see also the following paragraph), we first omitted the latter from the multiresponse modeling in order to obtain a reliable estimation of the other kinetic parameters. **Figure 3** shows the impact of time and temperature on cystine, DHA, and LAN levels at pH 8.0. The concentration of cystine decreased as a function of time. This was more pronounced at higher reaction temperatures (**Figure 3A**). The concentrations of the intermediary product DHA and the reaction product LAN increased as a function

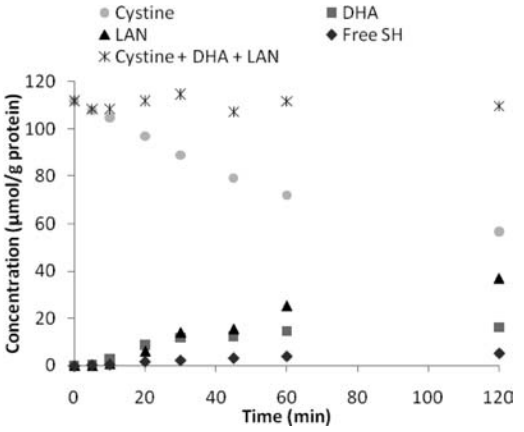

Figure 2. Time course of cystine, dehydroalanine (DHA), free SH, lanthionine (LAN) concentrations and the sum of cystine, DHA and LAN concentrations in a gliadin model system in sodium phosphate buffer (pH 8.0, 50 mM) during hydrothermal treatment in an oil bath at 130 °C.

of time, but apparently evolved toward a plateau in the case of DHA, indicating its consumption in other reaction(s). Moreover, a lag-phase was observed for these compounds, which was more extended in the case of LAN, indicating that its formation resulted from intermediate products. For both DHA and LAN, the enhancing effect of temperature on the reactions under consideration was clear (**Figures 3B** and **3C**). In **Figure 3**, the proposed model is shown by the full lines. For the three responses under consideration, the model described the experimental data (obtained at the three temperatures at pH 8.0) simultaneously and adequately. The good correspondence between experimental and predicted values for the different responses, as seen in **Figure 4** from the corresponding parity plots, confirms the adequacy of the model fit. Based on the registered temperature−time profiles and the measured concentrations of cystine, DHA, and LAN for each sample, the reaction rate constants k_E, k_F for cystine elimination and LAN formation and the corresponding Arrhenius equation activation energies E_{aE} and E_{aF} (**Table 1**),were estimated by nonlinear regression. The temperature sensitivity of the rate constant of cystine elimination was about ten times higher than the one of LAN formation, implying that temperature changes have a larger impact on the first reaction than on the latter one.

The kinetic model proposed for the pH 8.0 observations was also valid to simultaneously describe the cystine, DHA, and LAN concentrations for the gliadin model system at pH 6.0 heated in the same temperature range (results not shown). This pH is relevant for most cereal-based food systems. **Table 1** lists the corresponding kinetic parameters. As already expected based on the absolute values of the responses, a significantly lower reaction rate constant for both cystine elimination and LAN formation was obtained at pH 6.0 as compared to pH 8.0 (**Table 1**). Nevertheless, no significant ($\alpha = 0.05$) difference between the activation energies of each reaction at either pH 6.0 or 8.0 was observed.

The reaction rate constant based on the model from **Scheme 1** was used to calculate reaction rates and half-lives at different temperatures. When comparing the half-lives of cystine residues in gliadin at pH 6.0 (27 h) and 8.0 (4.4 h) at 110 °C to those reported by Volkin and Klibanov (*19*) for other proteins heated at 100 °C, about 10 and 1 h respectively, it can be concluded that the β-elimination of cystine in gliadin proceeds at a slower rate. A possible explanation for this might be the difference in solubility. In contrast to gliadin, the proteins studied by Volkin and Klibanov (*19*) were all soluble at the experimental pH. Moreover gliadin polymerized under these reaction conditions, while Volkin and Klibanov (*19*) created conditions to prevent protein interaction.

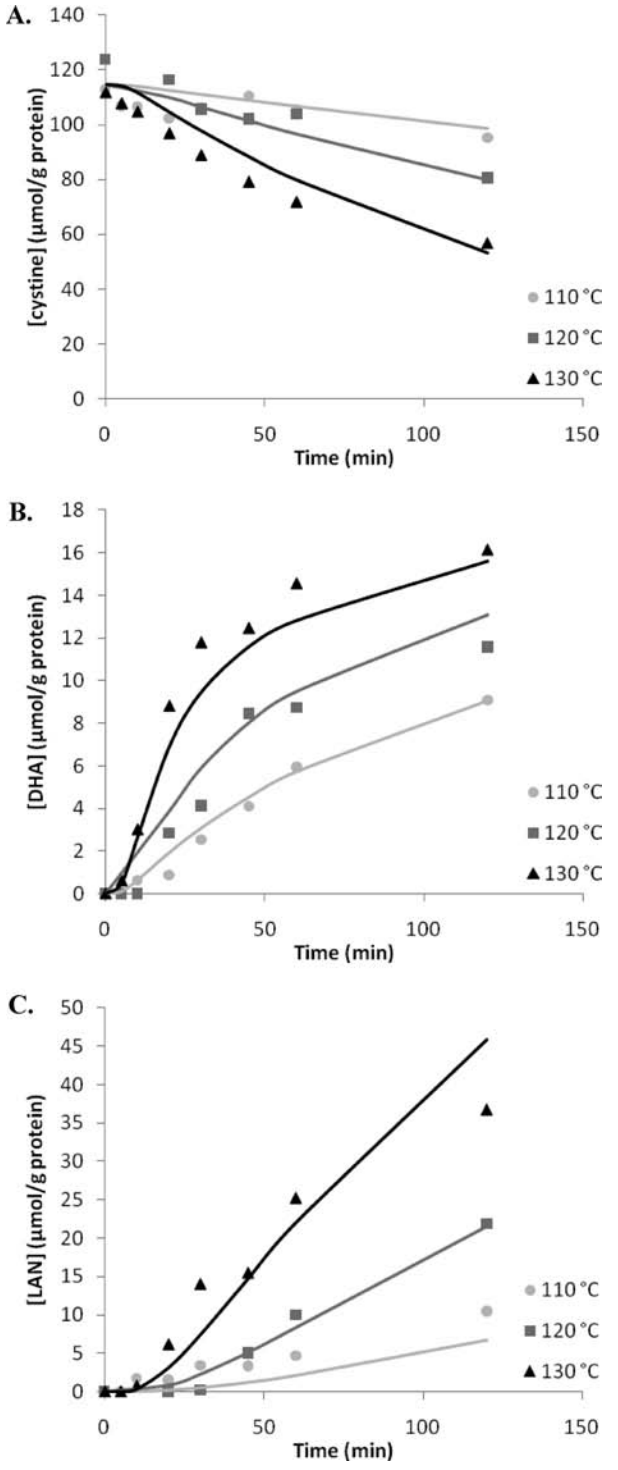

Figure 3. Time course of cystine (**A**), DHA (**B**), and LAN (**C**) concentrations in a gliadin model system in sodium phosphate buffer (pH 8.0, 50 mM) after hydrothermal treatment in an oil bath. The full lines represent the fit according to **Scheme 1**, while the experimental data are represented by the symbols.

Multiresponse Model To Evaluate the Reaction Mechanism of Cystine Elimination and Related LAN Formation. Although theoretically β-elimination reactions of cystine would result in formation of an equimolar mixture of free SH groups and DHA (**Scheme 1**), DHA levels in heated gliadin samples were systematically higher than free SH levels measured with Ellman's reagent (**Figure 2**). Indeed, when the kinetic model based on **Scheme 1** was used to predict the time course for the concentration of free SH,

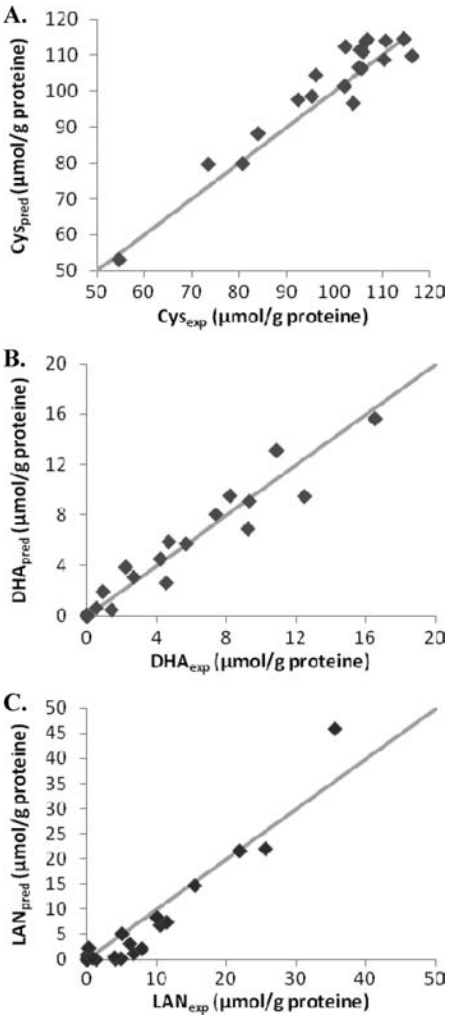

Figure 4. Parity plot of predicted versus experimental concentrations of cystine (**A**), DHA (**B**), and LAN (**C**) in gliadin heated in sodium phosphate buffer (pH 8.0, 50 mM). The full lines have a slope of 1.

Table 1. Estimated Kinetic Parameters (at a Reference Temperature of 120 °C) Based on Multiresponse Data Using **Scheme** *1* and Describing Cystine Elimination and Lanthionine Formation in a Gliadin Model System in Sodium Phosphate Buffer (50 mM) at pH 6.0 and 8.0 during Hydrothermal Treatment at Temperatures between 110 and 130 °C

	pH 8.0	pH 6.0
k_{Fref} ($\times 10^{-3} \cdot \text{min}^{-1}$)	6.77 ± 0.75^a	0.859 ± 0.11
k_{Eref} ($\times 10^{-3} \cdot g_{protein} \cdot \mu\text{mol}^{-1} \cdot \text{min}^{-1}$)	1.49 ± 0.38	0.0555 ± 0.015
E_{aF} (kJ/mol)	119 ± 21^a	88.2 ± 13
E_{aE} (kJ/mol)	12.3 ± 5.2	6.51 ± 4.1

[a] ±95% highest posterior density (HPD) interval.

there was a large discrepancy between model and experimental values (**Figure 5A**). Therefore, it is assumed that additional (unidentified) reaction(s) consuming cysteine, such as oxidation of free SH to intra- and/or intermolecular SS bonds (*22*) or to higher oxidation states, need to be taken into account to explain the lower concentrations of free SH. To verify this hypothesis, an additional reaction consuming cysteine was included in the reaction network (**Scheme 2**). Based on **Scheme 2**, the differential equation for the cysteine concentration (eq 3) can be replaced by

$$\frac{d[\text{cysteine}]}{dt} = k_E[\text{cystine}] - k_F[\text{DHA}][\text{cysteine}] - k_x[\text{cysteine}] \quad (6)$$

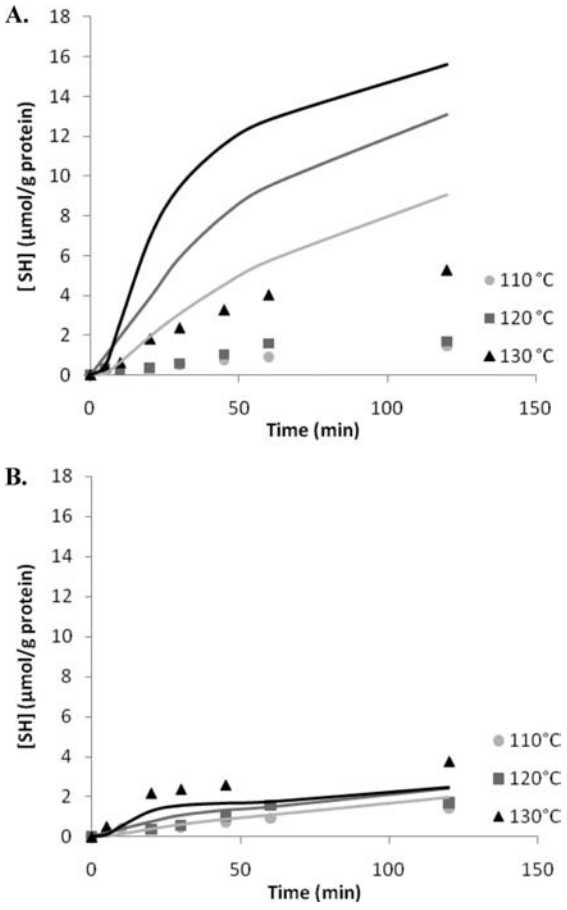

Figure 5. Time course of free SH concentrations in a gliadin model system in sodium phosphate buffer (pH 8.0, 50 mM) after hydrothermal treatment in an oil bath. The experimental data are represented by the symbols, while the full lines represent the fit according to **Scheme 1** (**A**) and **Scheme 2** (**B**).

Scheme 2. Cystine Degradation in Wheat Gliadin under Conditions of High Temperature and Alkaline pH with an Additional Reaction Consuming Cysteine[a]

$$\text{Cystine} \xrightarrow{k_E} \text{DHA} + \text{Cysteine} \xrightarrow{k_F} \text{LAN}$$
$$\downarrow k_x$$
$$\text{X}$$

[a] Cystine, dehydroalanine (DHA), cysteine and lanthionine (LAN) refer to the amino acids in their protein-bound form. The exact nature of the reaction(s) consuming cysteine is unknown and therefore is indicated with the symbol "X". k_E, rate constant of the cystine elimination reaction; k_F, rate constant of the LAN formation reaction; k_x, rate constant of the unknown reaction(s) consuming cystine.

As shown in **Figure 5B**, these assumptions strongly improved the correspondence between experimental and predicted values. Only for the cysteine concentration for samples heated at 130 °C, a less adequate model description was obtained, which could also be observed in the parity plot for cysteine concentrations (results not shown). This might be explained by color changes of the gliadins which were most obvious for the samples heated at 130 °C and pH 8.0. The colored pigments may have interfered with the colorimetric analysis and have led to less reliable data. Overall, this multiresponse analysis confirms the hypothesis that cysteine is partly consumed in other reactions. Similar observations of higher DHA than SH levels can be found in the literature for lysozyme heated at 100 °C and pH 8 (*19*). In the latter case the destruction of persulfide and other sulfhydryl species was put forward as possible explanation.

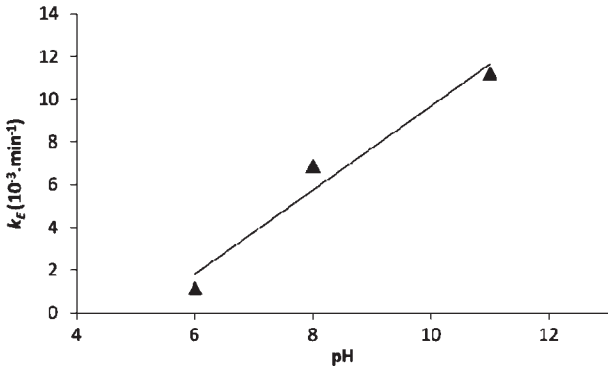

Figure 6. Relation between buffer pH and the rate constant for cystine elimination estimated under isothermal conditions in a gliadin model system in sodium phosphate buffer (50 mM).

Effect of pH on Reaction Rates of Cystine Elimination and Related LAN Formation. The gliadin model system was studied not only at pH 6.0 and 8.0 but also at pH 11.0 (heated at 130 °C). Under more alkaline conditions, the elimination of cystine and related formation of LAN were clearly enhanced (data not shown), as verified by estimating the rate constants of both reactions using the proposed kinetic model. In this case, isothermal conditions were assumed for the reactions under consideration. The temperature effect could not be included in the model, since the responses at pH 11.0 were only determined for samples heated at 130 °C. With these assumptions, the effect of pH on the reaction rate constants k_E and k_F could be determined. The relation between the rate constants and the pH was linear for cystine elimination (correlation coefficient of 0.96) (**Figure 6**), but no clear correlation was found for LAN formation. Hence, the change of k_E with pH is more pronounced than for k_F. The higher pH dependency of the elimination reaction has also been demonstrated for other proteins, since the hydroxyl anion catalyzes the β-elimination reaction of cystine (*10*, *19*, *23*). Although the elimination reaction is slowed down by a pH decrease, it is not inhibited at a pH below 7.0. Hence, it possibly occurs in several cereal-based foods that undergo heat treatment. In contrast to the elimination reaction, the rate of LAN formation is nearly pH independent at the pH values studied. The double bond of DHA has strong affinity toward SH groups and readily reacts with protein SH groups to form LAN (*24*). However, in this case, the consumption of free SH in other reactions may explain the rather small temperature and pH dependency of the LAN formation.

Practical Implications of β-Elimination of Cystine and of Related LAN Formation. To determine the practical relevance of β-elimination of cystine, the proposed kinetic model was applied in conditions mimicking real biotechnological processes involving wheat gluten, such as bread baking. Bread is a very complex system. Apart from its major component starch, it contains gliadin, glutenin, and other proteins, as well as other constituents such as nonstarch polysaccharides and lipids. However, the presented model may well give an indication of a possible occurrence and relevance of β-elimination reactions and LAN formation. Typical conditions during bread baking are a pH between 5 and 6 and an internal crumb temperature close to 100 °C (*25*). A typical internal crumb temperature versus time curve (*25*) resembles best the temperature profile as measured in the sample at 110 °C. When comparing the two temperature profiles, and taking into account the slower temperature increase in bread crumb, it can be estimated that the end of baking approximately corresponds to 20 min heating in the model system. With the kinetic model for gliadin at pH 6.0 and 110 °C

(103 °C sample temperature) it can be estimated that after 20 min heating under these conditions up to 0.4% of all cystine is destroyed and nearly quantitatively converted to DHA. 0.04% of all cystine is converted to free SH and 0.01% to LAN. These values are far below the detection limits of the methods used in this study. While during bread making it seems that β-elimination reactions are outnumbered by the reactions leading to SS bonds and probably do not contribute much to the final gluten network, the situation is different in systems of higher pH. Many soft wheat products (cakes, cookies, pretzels) that are chemically leavened end up with a pH of 7 and higher (*26*). A notable example is pretzel making, where the doughs are passed through a bath of 1.0% NaOH (i.e., pH 13.4) at temperatures above 90 °C (*27*). When taking the same temperature estimates as for bread making but now at pH 8.0, up to 3% of all cystine is destroyed as calculated from the gliadin model system. This results in a 2.5% conversion of cystine to DHA and free SH and a 0.4% conversion to LAN. In this case the level of free SH resulting from β-elimination (about 0.3 μmol/g protein) is about 20 times lower than the level of free SH in unheated gluten (7 μmol/g protein) (*6*). The newly generated free SH groups may serve as catalysts for SH−SS-exchange reactions leading to faster gluten polymerization. Moreover, the level of LAN generated in these conditions approaches the levels reported for dityrosine cross-links in flour dough (0.6 linkages per 100 SS bonds) (*28*). A similar reasoning, but now for pH 11 (the highest pH value for which the above model is based on experimental data) and which might be relevant for what happens during the pretzel dough lye treatment at even higher pH, would predict that 2% of all cystine is destroyed already after 1 min reaction time at a sample temperature of 120 °C. It can be estimated that, after 5 min in these conditions, 10% of all cystine would be destroyed, resulting in the formation of nearly 2 μmol of LAN/g of protein. These values can even be much higher on the pretzel surface as it is subjected to a pH of more than 13 and temperatures higher than 150 °C. This indicates that, besides dityrosine, LAN can be present as a nonreducible cross-link in some cereal-based food applications. Indeed, at higher pH values, which often are applied in the production of gluten-based bioplastics, the role of β-elimination of cystine becomes increasingly important. This is illustrated by literature describing more protein cross-linking and stronger gluten films cast at alkaline pH (*13*, *29*) and further supported by work from our group for gluten proteins *in toto* treated at pH 8.0 and 13.0 where even higher levels of cystine destruction and LAN formation were measured than for gliadin alone (*11*).

ABBREVIATIONS USED

dm, on dry matter; DHA, dehydroalanine; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); EDTA, tetrasodium ethylenediamine tetraacetate disodium salt; HPAEC-IPAD, high-performance anion-exchange chromatography with pulsed amperometric detection; LAN, lanthionine; NADH, reduced ; SDS, sodium dodecyl sulfate; SE, size exclusion; SH, sulfhydryl; SS, disulfide.

ACKNOWLEDGMENT

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