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Reaction Kinetics of Gliadin−Glutenin Cross-Linking in Model Systems and in Bread Making

BERT LAGRAIN,* KRISTOF BRIJS, AND JAN A. DELCOUR

Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium

The gluten proteins gliadin and glutenin are important for wheat flour functionality in bread making, where, during baking, they polymerize through a heat-induced sulfhydryl−disulfide exchange mechanism. A model system was used to study the kinetics of this reaction. Thus, gluten was subjected to hydrothermal treatment with the rapid visco analyzer (RVA) with holding temperatures of 80, 90, and 95 °C. At these temperatures, ω-gliadin solubility did not change, but the solubilities of α- and γ-gliadin in 60% ethanol decreased according to first-order reaction kinetics. All reaction rate constants increased with temperature. The activation energies for the heat-induced exchange reaction were 110 and 147 kJ/mol for α- and γ-gliadin, respectively. Starch did not influence the reaction rates of the association of α- and γ-gliadin with glutenin. During gluten−starch model bread baking, glutenin oxidized first, and when the internal crumb temperature reached 100 °C, α- and γ-gliadin cross-linked to glutenin, again following first-order reaction kinetics. The experimental findings and similarities in temperature conditions and reaction kinetics suggest that the RVA system can be instrumental in understanding gluten behavior in concentrated food systems, such as bread making.

KEYWORDS: Wheat gluten; heat treatment; gliadin−glutenin interaction; sulfhydryl−disulfide interchange; kinetics; bread baking

INTRODUCTION

The storage proteins of wheat consist of monomeric gliadin and polymeric glutenin. They determine bread quality due to their unique ability to form cohesive, extensible, viscoelastic gluten. Gliadin consists of proteins containing α-, γ-, and ω-gliadins. In contrast to α- and γ-gliadins, which form three and four intramolecular disulfide (SS) bonds, respectively, ω-gliadins lack cysteine residues. Glutenin is a heterogeneous mixture of SS-linked polymers with a largely unknown polymer structure. A glutenin polymer consists of glutenin subunits (GS) of high molecular weight (HMW) or low molecular weight (LMW), which are connected through intermolecular SS bonds. The low molecular weight glutenin subunits (LMW-GS) are classified as B, C, and D types (*1*). Heat treatment of these proteins eventually leads to large gluten protein aggregates with further polymerization of glutenin and formation of gliadin−glutenin bonds through additional SS (cross-)linking in the process. There may be two types of reaction occurring during heating. The polymerization of glutenins at temperatures below 100 °C may involve oxidation of sulfhydryl (SH) groups as postulated by Weegels and co-workers (*2*) based on their finding of a decrease in free SH group levels. Also, the incorporation of gliadins into the glutenin structure, which occurs at higher temperatures, may result from SH−SS interchange, since no free SH groups are present in gliadin molecules (*3, 4*). Indeed, at temperatures exceeding 90 °C, free SH groups from glutenin can induce a covalent linkage with gliadin, as the free SH group carries out nucleophilic attack on the sulfur atom of an intramolecular SS bond (*3*). Although Kokini and co-workers (*5*) proposed that cross-links among gliadin molecules are formed above 70 °C in the absence of glutenin, others believe that gliadin cross-links only with glutenin (*4, 6*). The incorporation of gliadin monomers in the glutenin network leads to a large three-dimensional network structure (*7*).

Heat-induced SH−SS interchange reactions between gluten proteins were originally proposed by Schofield and co-workers (*8*). This interchange reaction also occurs in other proteins and has been demonstrated at 100 °C and pH 6.0 using a model system with trypsinogen, lysozyme, and glutathione. The reaction follows first-order kinetics, and the reagents have a half-life of about 10 min (*9*). Also, for whey proteins, reaction kinetics for the SH−SS exchange reaction have been described (*10*). However, to the best of our knowledge, the reaction kinetics for the heat-induced SH−SS interchange reaction between α- and γ-gliadin and glutenin have not been studied even if model systems have been used to study gluten polymerization reactions (*11*). As such, Lagrain and co-workers (*12*) applied a temperature profile and simultaneously measured rheological changes of a gluten−water suspension in a rapid visco analyzer (RVA). It was observed that below 95 °C, glutenin polymerizes mainly through oxidation (*2, 4, 12*).

* To whom correspondence should be addressed. Tel: + 32(0)16321634. Fax: + 32(0)16321997. E-mail: bert.lagrain@biw.kuleuven.be.

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Holding at 95 °C resulted in losses of solubility of both α- and γ-gliadins due to covalent linkage with glutenin (*12*). Recently, the occurrence of SH−SS exchange between gliadin and glutenin was also observed during heat treatment under high pressure (*13*).

In contrast to model systems that study gluten proteins as such in vitro, the study of reaction kinetics in bread making is very complex. First of all, besides gluten proteins, bread contains other constituents of which starch and water are the most predominant. Starch is, at least quantitatively, the main component in bread, and interactions between starch and gluten may influence their functionalities and bread quality parameters. The presence of gluten proteins impacts the swelling of starch granules during RVA analysis (*14*). However, to the best of our knowledge, it is not known whether the gelatinization of starch in the presence of gluten impacts gluten polymerization. During bread baking, starch granules swell and increasingly bind water. With only a limited amount of water available, the increased interaction of starch with water may well affect the other water-dependent changes during heating (*11*). Second, establishing reaction kinetics of gluten in bread making is very difficult because of temperature gradients in the system, and the continuously changing physical properties of the dough/bread product in terms of volume of the baking mass. Indeed, the physical, chemical, and structural transformations of starch and gluten proteins during baking are caused by a complex series of heat and mass transfer phenomena determined by an evaporation front at 100 °C (*15*). Finally, gluten itself undergoes a complex series of changes during bread making. Already during mixing and proofing, glutenin extractability first increases and then decreases during fermentation. Whether these extractability changes are due to de- and repolymerization (*16*), conformational rearrangements (*17*), or changes in dissolution properties by a changed effective surface area (*18*) is still subject to debate. In contrast to the changes during mixing and proofing, irreversible polymerization of gluten proteins occurs during bread baking, which contributes to the final bread structure and quality. A decrease in the level of extractable protein with time of baking has been observed (*19–21*). This can be ascribed to glutenin linking followed by glutenin−gliadin cross-linking, since the extractability of gliadins from bread is much lower than that from flour, and α- and γ-gliadins are affected more than ω-gliadins (*19, 22, 23*). During baking, gliadin and glutenin polymerize through heat-induced SH−SS interchange reactions (*19*).

Thus, to approach reaction kinetics of gluten proteins in bread baking, a simplification of the system is necessary. SH−SS interchange between glutenin and gliadin has been clearly established in different model systems (*3, 4, 13, 24*). The reaction, which results in gliadin extractability loss, is very important in bread making (*19*). Moreover, the kinetics of this type of reaction have been studied for different types of protein (*9, 10*) but not for gliadin subgroups. Against this background, the objective of this work was to study reaction kinetics of gliadin−glutenin cross-linking through a SH−SS exchange reaction. First, the solubilities of α-, γ-, and ω-gliadins in 60% ethanol were monitored in a model system following hydrothermal treatment of gluten and a gluten−starch mixture at different temperatures. To this end, gluten and a gluten starch mixture were subjected to RVA analysis with holding temperatures of 80, 90, and 95 °C. The extractabilities of both glutenin and gliadin at different stages of both processes were evaluated with size-exclusion (SE)-high-performance liquid chromatography (HPLC). Reversed-phase (RP)-HPLC was used to determine specific changes in α-, γ-, and ω-gliadin solubilities. On the basis of this information, reaction kinetics could be determined.

In a second part, these results were compared with the evolution of gliadin solubility during gluten−starch model bread making. This will allow comparison of the reaction kinetics in the model system with experimental data obtained during bread making.

MATERIALS AND METHODS

Materials. All chemicals and reagents were at least of analytical grade and from Sigma-Aldrich (Steinheim, Germany) unless otherwise specified. Commercial vital wheat gluten [Amygluten 110; moisture content (mc), 6.16%; crude protein content (N × 5.7), 78.9% on dry basis (db); and starch content, 10.4% on db] and wheat starch (Meritena 200; mc, 11.79%) were obtained by industrial gluten−starch separation of European spring wheat flour by Tate & Lyle Europe (Aalst, Belgium). A gluten−starch blend for RVA analysis was prepared with 50% gluten and 50% starch on a dry mass basis. A gluten−starch blend for model bread making was prepared with 16% gluten and 84% starch. The gluten and starch powders were passed through a 250 μm sieve.

Methods. *Controlled Heating and Cooling.* A RVA (RVA-4D, Newport Scientific, Sydney, Australia) was used to apply a temperature profile to 25.00 g of 20% (w/w) suspensions of gluten and gluten−starch blend in water (*24*). The RVA converted the current required to maintain constant mixing speed (160 rpm) of a paddle into an apparent viscosity value in Poise (P, 0.1 kg m^{-1} s^{-1}), the unit of dynamic viscosity. This apparent viscosity value was further referred to as RVA viscosity. At the start of the RVA analysis, suspensions were first homogenized by hand-shaking and then mixed in the RVA (900 rpm for 20 s). The temperature profile included a temperature increase from room temperature to 40 °C (in 1 min), a linear temperature increase (in 14 min) to 80, 90, and 95 °C for the gluten suspension and to 95 °C for the gluten−starch suspension, a holding step (40−60 min at 80, 90, and 95 °C), a cooling step (7 min) with a linear temperature decrease to 50 °C, and a final holding step at 50 °C (13 min). The pH during the test, that is pH 5.8, remained constant. The RVA was stopped at different points in the heating and holding phases of the profile, and the gluten or gluten−starch suspensions were frozen in liquid nitrogen, freeze-dried, and ground in a laboratory mill (IKA, Staufen, Germany). All RVA analyses were performed at least in triplicate.

Bread Making. Gluten−starch model bread was made according to the procedure of Finney (*25*) for 100 g of flour. Gluten−starch blend (16 g of gluten and 84 g of starch), sucrose (6.0 g), NaCl (1.5 g), compressed yeast (5.3 g) (Bruggeman, Ghent, Belgium), and water (59 mL) were mixed at 25 °C with a 100 g pin-mixer (National Manufacturing, Lincoln, NE) for 4.8 min, which was the optimal mixing time for the gluten−starch dough based on mixograph analysis. Dough was fermented at a temperature of 30 °C and relative humidity of 95%. During fermentation, it was punched (passed between rolls to be flattened) after 52, 77, and 90 min. Fully fermented dough was molded and proofed for 36 min in a baking pan [internal dimension (width × length × height), 8.0 cm × 14.5 cm × 5.5 cm] at 30 °C and 95% relative humidity and baked for 24 min at 225 °C. The pH of fermented dough was 5.6. Temperatures were measured by a K thermocouple with PID controller (model 2132, Eurotherm Controls, Leesburg, VA). The thermocouple was mounted on a frame set on the baking pan over the dough before introduction in the oven and located in the center of the baking pan 3.0 cm from the bottom. Samples from the center of the loaf were withdrawn after varying times of baking. To this end, a cubic part (2.5 cm × 2.5 cm × 2.5 cm) from the center of the dough/bread was cut between 1.75 and 4.25 cm from the bottom. Dough and partially and fully baked bread samples were immediately frozen in liquid nitrogen, freeze-dried, and ground in a laboratory mill (IKA).

SE-HPLC. SE-HPLC was conducted using a LC-2010 system (Shimadzu, Kyoto, Japan) with automatic injection. Hydrothermally treated gluten−starch blend and (partially baked) bread samples containing 1.0 mg of protein were shaken with 1.0 mL of a 0.05 M sodium phosphate buffer (pH 6.8) containing 2.0% sodium dodecyl sulfate (further referred to as SDS buffer) for 60 min at room

temperature. After centrifugation (5 min, 10000*g*), the supernatant (60 μL) was loaded on a Biosep-SEC-S4000 column (Phenomenex, Torrance, CA; separation range, 15000−500000). The elution solvent was (1:1, v/v) acetonitrile (ACN)/water containing 0.05% (v/v) trifluoroacetic acid (TFA). The flow rate was 1.0 mL/min at a temperature of 30 °C, and eluted protein was detected at 214 nm.

The elution profiles were divided into two fractions using the lowest absorbance reading between the two peaks as the cutoff point (*26*). The first fraction corresponded to the level of SDS extractable glutenin, and the second was assigned to the level of SDS extractable gliadin. The total SDS extractable protein, gliadin, and glutenin was calculated from the peak areas and expressed as percentage of the peak area of unheated gluten extracted with the above SDS buffer in the presence of 1.0% dithiothreitol (DTT).

SE-HPLC analysis was performed on duplicate samples. Each sample was extracted twice.

RP-HPLC. RP-HPLC was also conducted using a LC-2010 system (Shimadzu). Hydrothermally treated gluten (100 mg), gluten−starch blend (200.0 mg), and (partially baked) bread samples (500.0 mg) were shaken three times with 3.0 mL of 60% (v/v) ethanol (gliadin extract) for 10 min at room temperature and centrifuged (10 min, 8000*g*). The supernant was further used as gliadin extract, and the residues were shaken three times with 3.0 mL of 0.05 M Tris/HCl buffer (pH 7.5) containing 50% propan-1-ol, 2.0 M urea, and 1.0% (w/v) DTT for 20 min at 60 °C and centrifuged (10 min, 8000*g*). The supernatants obtained under reducing conditions were then kept under nitrogen and are further referred to as reduced glutenin extract. Aliquots (80 μL) of the gliadin and glutenin extracts were loaded on a Nucleosil 300-5 C8 column (Machery-Nagel, Düren, Germany). The elution system consisted of deionized water acidified with 0.1% (v/v) TFA (A) and ACN containing 0.1% TFA (v/v) (B). Proteins were eluted with a linear gradient from 24 to 56% B in 50 min and detected at 214 nm.

α-, γ-, and ω-Gliadins, B/C-LMW-GS, D-LMW-GS, and high molecular weight glutenin subunits (HMW-GS) were distinguished based on absorbance minima between specific peaks as outlined by Wieser, Antes, and Seilmeier (*27*). The absorbance area of the proteins was highly correlated to the concentration of protein (*27*). Hence, the relative proportion of each gliadin type was calculated as the ratio of the area of each type in the chromatogram to the total chromatogram area.

RP-HPLC analysis was performed on duplicate samples. Each sample was extracted twice.

Protein Content Determination. Protein contents (N × 5.7) of the gliadin extracts in 60% ethanol were determined after ethanol removal by evaporation using an adaptation of the AOAC Official Dumas Method to an automated Dumas protein analysis system (EAS variomax N/CN, Elt, Gouda, The Netherlands) (*28*). Protein contents from each gliadin extract were determined in duplicate.

Free SH Determination. Free SH groups were determined colorimetrically after reaction with 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) (*29*). Hydrothermally treated gluten−starch blends (1.0−2.0 mg of protein/mL) were dispersed in sample buffer (pH 6.5) containing 0.05 M sodium phosphate, 2.0% (v/v) SDS, 3.0 M urea, and 1.0 mM tetrasodium ethylenediamine tetra acetate and shaken for 60 min. DTNB reagent (0.1% w/v in sample buffer, 100 μL) was mixed with 1.0 mL of sample dispersion. After 45 min, the samples were centrifuged (3 min, 11000*g*), and the absorbance at 412 nm was determined. Absorbance values were converted to levels of free SH groups using a calibration curve constructed with reduced glutathione (*30*). Free SH determination was performed on duplicate samples. Each sample was extracted twice.

Data Analysis. The SH−SS exchange reaction can be written as

$$R_1SH + R_2(SS) \leftrightarrow R_1SSR_2SH \quad (1)$$

In this reaction, the 60% ethanol-insoluble glutenin (R_1SH) reacts via a free SH group with an intramolecular SS bond of gliadin $R_2(SS)$ soluble in 60% ethanol, leading to insoluble gliadin−glutenin (R_1SSR_2SH) entities (*3*). On the basis of the model system for heat-induced SH−SS exchange proposed by Volkin and Klibanov (*9*), we first investigated whether this reaction for gluten proteins follows first-order kinetics. This would be presented as follows:

$$-\frac{d[R_2(SS)]}{dt} = k[R_2(SS)] \quad (2)$$

and hence,

$$\ln[R_2(SS)]_t = -kt + [R_2(SS)]_0 \quad (3)$$

$[R_2(SS)]_0$ is the initial concentration (mM) of a type of gliadin. $[R_2(SS)]_t$ is the concentration (mM) at time t, and k is the first-order reaction rate constant (min^{-1}).

Concentrations of the gliadin types during hydrothermal treatment were calculated from the protein contents of the gliadin extracts and the relative proportions of each type of gliadin. The mean values and standard deviations were calculated using the Microsoft Excel package. Average molecular weights for α-gliadin (29260), γ-gliadin (30360), and ω-gliadin (45000) were used based on their average number of amino acids (*31, 32*). The rate constants for losses of gliadin solubility, their 95% confidence intervals, and the goodness of fit (R^2 value) were estimated on the basis of a linear regression analysis of the natural logarithm of the residual gliadin concentration as a function of treatment time. The temperature dependence of the reaction rate constants was determined using the Arrhenius model:

$$k = k_{ref} \exp\left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \quad (4)$$

E_a is the activation energy (J mol^{-1}) of the SH−SS exchange reaction, R is the universal gas constant (8.3143 J K^{-1} mol^{-1}), k_{ref} is the reaction rate constant at reference temperature T_{ref} (K), and k is the reaction rate constant at temperature T (K). Linearized Arrhenius plots were used to estimate the temperature dependency of the k values. The activation energy (E_a) was calculated by linear regression analysis by plotting the natural logarithm of k values as a function of the reciprocal of the absolute temperature.

RESULTS AND DISCUSSION

Reaction Kinetics of Gluten Proteins during RVA Analysis. Figure 1 shows the solubilities of the different types of gliadin in 60% ethanol during RVA analysis of a gluten−water suspension heated at a constant holding temperature of 80, 90, and 95 °C. The concentration of ω-gliadin remained constant during hydrothermal treatment even at 95 °C (**Figure 1a**). The association of α- and γ-gliadins to the insoluble glutenin as a function of time reduced the solubilities of both types already at 80 °C (**Figure 1b**,**c**). Treatment with DTT of the residual insoluble protein fraction restored the original gliadin solubilities (results not shown), confirming the involvement of SS cross-links in the aggregation. The natural logarithm of the residual α- and γ-gliadin concentrations as a function of treatment time resulted in a straight line. The experimental points indeed could be elegantly described using first-order kinetics assumptions (R^2 > 0.97) for α- and γ-gliadin solubility losses. **Table 1** lists the estimated first-order rate constants and half-lives at 80, 90, and 95 °C. The activation energy (E_a) of the association of gliadin to glutenin through a SH−SS exchange reaction could be calculated by plotting the natural logarithm of k values as a function of the reciprocal of the absolute temperature (**Figure 2**). For α-gliadin, a value of 110 kJ/mol was calculated, and for γ-gliadin, the E_a was 147 kJ/mol. While the difference in E_a values may seem strange at first sight, it is well of note that individual α-gliadin molecules contain only three intramolecular SS bonds, while individual γ-gliadin molecules contain four such moieties. γ-Gliadin possibly needed more energy to unfold and expose its sulfur groups than did α-gliadin. At temperatures exceeding 80 °C, γ-gliadin reacted with a higher rate than α-gliadin, probably due to the higher level of reactive sulfur

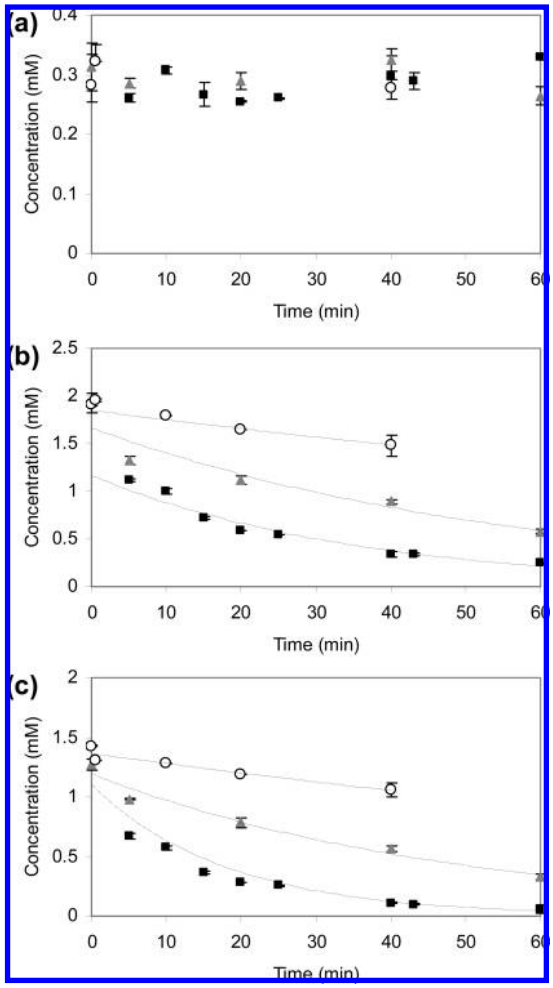

Figure 1. Solubility in 60% ethanol of (**a**) ω-gliadin, (**b**) α-gliadin, and (**c**) γ-gliadin during RVA analysis at holding temperatures of 80 (open circles), 90 (gray triangles), and 95 °C (black squares). Trendlines in parts **b** and **c** were calculated with a first-order exponential rate law.

groups. Regretfully, no comparable data on either first-order rate constants or activation energies of gliadin solubility losses are available.

Figure 3 shows the RVA viscosity profile of the gluten starch blend with a holding time of 40 min at 95 °C. The profile started at a low viscosity. At 60 °C, the viscosity strongly increased to a peak viscosity at 87 °C. After a strong viscosity decrease in the holding step (95 °C), the RVA viscosity remained constant. During cooling, the RVA viscosity increased again, and in the final holding step at 50 °C, a small increase in viscosity was observed.

The viscosity profile in **Figure 3** mainly shows how starch changed upon heating in water. The impact of the gluten component on the profile is small. The viscosity increase at 60 °C is the result of starch swelling substantially upon water uptake. This phenomenon is termed "pasting" (*33*). The subsequent viscosity decrease or "breakdown" indicates the extent of starch disintegration with rupture and breakdown of starch granules (*33*). The rapid increase in viscosity during cooling or "setback" was caused by a decrease of energy in the system that allowed more hydrogen bonding between starch chains and thus increased viscosity.

The heating step progressively reduced the level of SDS-extractable glutenin, while that of extractable gliadin remained constant. Holding at 95 °C decreased the levels of both extractable glutenin and gliadin. Glutenin became SDS unextractable during the first 10 min of holding at 95 °C (**Figure 3**). **Figure 4** shows the level of free SH groups during RVA analysis of the gluten−starch mixture. When the mixture was heated to 95 °C, the level of SH groups decreased and then remained constant. The decrease in free SH groups was due to polymerization reactions of the glutenin molecules, resulting in a complete loss of SDS extractability. However, this precipitation reaction through SS bonding slowed down with the progress of the polymerization reaction. This could be assigned to steric hindrance, resulting from the formation of a three-dimensional protein network from the linear native glutenin polymers (*34*). The strongly decreased probability of glutenin oxidation may well explain why the level of free SH groups remained constant at 2 μmol/g protein at 95 °C (**Figure 4**). At this temperature also, the SDS extractability of gliadin decreased (**Figure 3**). This indicates mainly gliadin−glutenin cross-linking through an SH−SS exchange mechanism. At 95 °C, the solubilities of α-gliadin and γ-gliadin in 60% ethanol decreased drastically, whereas that of ω-gliadin remained constant (results not shown). The α- and γ-gliadin solubilities in the gluten−starch blend obeyed first-order reaction kinetics. **Table 1** lists the estimated values for rate constant and half-life.

The results show that hydrothermal treatment in the RVA resulted first in an increased polymerization of glutenin through oxidation of its free SH groups. The ω-gliadins did not react with other proteins. They do not contain cysteine groups and, hence, are not able to form SS linkages. In contrast to ω-gliadins, α- and γ-gliadins form intramolecular SS bridges, and at higher temperatures, they react with glutenin through a SH−SS exchange mechanism following first-order kinetics. These results confirm earlier results on the kinetics of total gliadin solubility loss during heat treatment also following first-order reaction rates (*34, 35*). The rate constants of this reaction increased with temperature (**Table 1**). The activation energy for the reaction was higher for γ-gliadin (147 kJ/mol) than for α-gliadin (110 kJ/mol). Starch did not significantly ($P < 0.05$) influence the reaction rates of the association of α- and γ-gliadin to glutenin, suggesting the same level of gluten polymerization regardless whether or not starch is present. In the next paragraph, these results obtained with the RVA system are compared with those during gluten−starch model bread baking.

Reaction Kinetics of Gluten Proteins during Gluten−Starch Model Bread Baking. In contrast to the RVA model system, the temperature is not uniformly distributed during dough to bread transformation. Two separate regions can be distinguished. Once the crust is formed, its moisture is very low, and the temperature asymptotically tends to the oven temperature. In the crumb, moisture is constant, and the temperature asymptotically tends to 100 °C (*15*). In this study, samples were withdrawn from the center, and the temperature was measured in the center of the dough/bread.

Figure 5 shows the internal dough temperature during baking of gluten−starch bread as a function of time. The temperature in the center of the dough/bread increased from 30 to 95 °C in 13 min and then approached 100 °C for another 13 min. The crumb temperature in the center slowly decreased when bread was taken out of the oven and reached room temperature after about 2 h of cooling. During bread baking, the total level of SDS extractable protein decreased to 19% at the end of the process. When the internal dough temperature reached 60 °C, glutenin extractability had already decreased by 46%, while 90% of the gliadin fraction remained SDS extractable. After 12 min of baking and an internal crumb temperature of 90 °C, glutenin

Table 1. Rate Constants and Half-Lives as Calculated from α- and γ-Gliadin Solubilities in 60% Ethanol[a]

	α-gliadin			γ-gliadin		
sample	rate constant (min^{-1})	half-life (min)	R^2	rate constant (min^{-1})	half-life (min)	R^2
		RVA (gluten)				
80 °C	0.0067 (0.001)	103	0.99	0.0064 (0.002)	108.3	0.90
90 °C	0.014 (0.002)	50.7	0.98	0.021 (0.003)	33.5	0.97
95 °C	0.034 (0.003)	20.5	0.97	0.052 (0.004)	13.3	0.98
		RVA (gluten−starch)				
95 °C	0.035 (0.004)	19.7	0.97	0.055 (0.004)	12.6	0.98
		comparison (RVA−bread)				
100 °C (calculated from model)	0.046	15.1		0.090	7.7	
>95 °C (internal bread crumb)	0.11 (0.01)	6.4	0.98	0.13 (0.02)	5.3	0.98

[a] The values are calculated for gluten during RVA analysis with different holding temperatures (model system) and for a gluten−starch blend during RVA analysis at 95 °C. Rate constants and half-lives are also estimated for 100 °C in the model system and calculated from α- and γ-gliadin solubilities in 60% ethanol during bread baking at an internal crumb temperature exceeding 95 °C. Standard errors are given between parentheses.

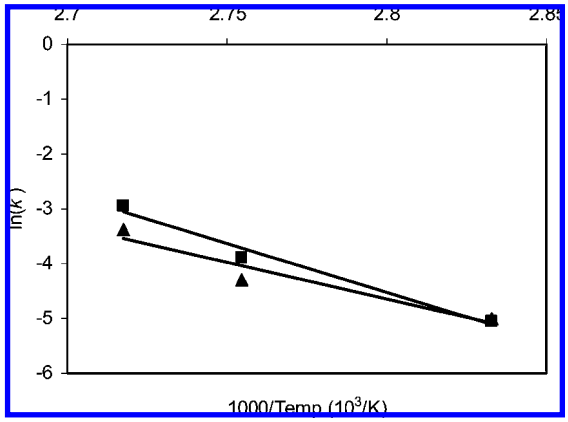

Figure 2. Effect of temperature (K) on the rate constants (k in min^{-1}) for solubility loss of α-gliadin (black triangles) and γ-gliadin (black squares) in 60% ethanol.

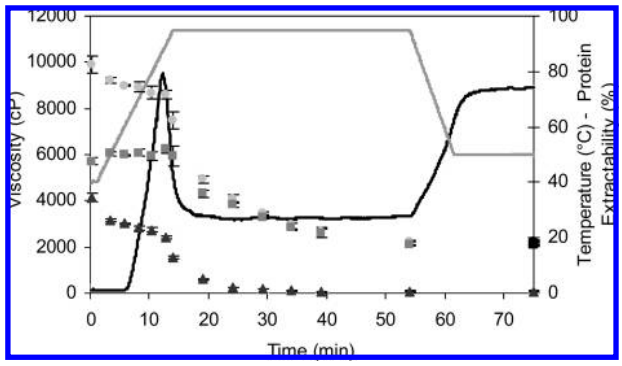

Figure 3. RVA profile (40 min of holding time at 95 °C) of a gluten−starch−water suspension (20% w/v) with indication of protein extraction yields with 2.0% SDS at different times. RVA viscosity (black line), temperature (gray line), total SDS extractable protein (gray circles), SDS extractable gliadin (gray squares), and SDS extractable glutenin (black triangles).

extractability had decreased by 80% and gliadin extractability by 27%. No SDS-extractable glutenin was present anymore at the end of baking, while that of extractable gliadin decreased by 70%. **Figure 6** shows the concentration of gliadin subgroups in 60% ethanol during gluten−starch model bread making with a central crumb temperature exceeding 95 °C. At this stage, both moisture content and crumb temperature remained relatively constant. The baking step only had a small impact on ω-gliadin solubility, but both α- and γ-gliadin solubilities decreased according to a first-order process. We used the data from the RVA system (**Table 1**) to estimate values for the rate

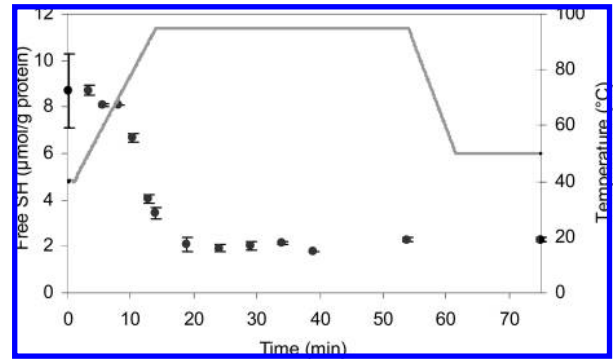

Figure 4. Changes in free SH content during RVA analysis of a gluten−starch blend.

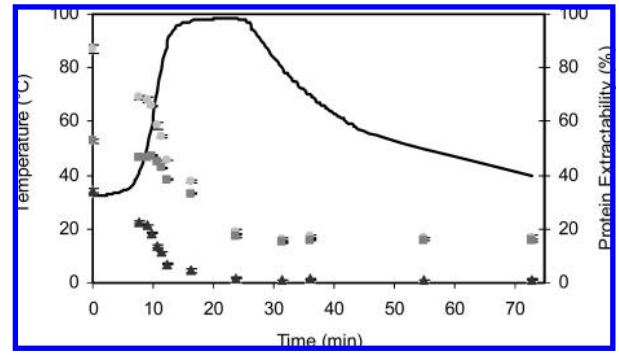

Figure 5. Internal crumb temperature (full line) of gluten−starch bread (100 g of flour) during and after baking with indication of extraction yields with 2.0% SDS at different times. Total SDS extractable protein (gray circles), SDS extractable gliadin (gray squares), and SDS extractable glutenin (black triangles).

constants and half-lives of α- and γ-gliadin reacting with glutenin at 100 °C and compared these with the experimental values in bread crumb at 100 °C. This approach worked better for γ-gliadin with an estimated rate constant of 0.090 min^{-1} and an experimental rate constant in bread crumb of 0.13 min^{-1}, than for α-gliadin with estimated and experimental rate constants of 0.046 and 0.11 min^{-1}, respectively (**Table 1**). The higher rate constants during bread baking suggest lower activation energies for α- and γ-gliadin solubility losses during the process. Both the reaction environment and shear conditions during bread baking differ from those during RVA analysis, and this may have affected the reactivity of the gluten proteins.

The fraction of gliadins that had lost solubility in 60% ethanol after heat treatment was solubilized after reduction of the insoluble polymeric protein fraction with DTT. Thus, gliadin

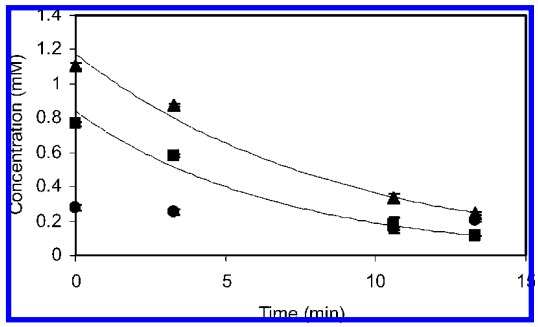

Figure 6. Solubility in 60% ethanol of ω-gliadin (black circles), α-gliadin (black triangles), and γ-gliadin (black squares) vs time during bread baking with an internal crumb temperature exceeding 95 °C. Trendlines were calculated with a first-order exponential rate law.

had been incorporated in the latter fraction (results not shown). This incorporation of gliadin in the insoluble glutenin fraction has also been demonstrated during wheat flour bread baking (*19*). **Figures 3** and **5** reveal some analogies. The temperature profile in the crumb during baking also consists of a heating step, a holding step at about 100 °C, and a cooling step. The changes in protein extractability also show similar trends. At temperatures below 90 °C, mostly glutenin polymerized. Above 90 °C, gliadin extractability decreased strongly. At a temperature of about 100 °C in the center of bread, α- and γ-gliadin cross-linked to glutenin through a SH−SS exchange mechanism. From the results, it could be postulated that the decreases in α- and γ-gliadin solubilities and the limited change in ω-gliadin solubility followed the same kinetics as observed during RVA analysis. Reduction of SS bonds with DTT rendered all proteins in bread extractable, and this confirmed that mainly SS covalent linkages between gluten proteins were formed during bread baking.

Although the conditions of dilute starch−gluten−water suspensions in the RVA are quite different from the concentrated starch−gluten−water systems found in baked products, the similarities in temperature conditions and reaction kinetics suggest that the RVA system can be instrumental in understanding gluten behavior in concentrated food systems, such as bread making.

ABBREVIATIONS USED

ACN, acetonitrile; db, dry basis; DTT, dithiothreitol; HMW-GS, high molecular weight glutenin subunits; HPLC, high-performance liquid chromatography; LMW-GS, low molecular weight glutenin subunits; MC, moisture content; RP, reversed-phase; RVA, rapid visco analyzer; SDS, sodium dodecyl sulfate; SE, size-exclusion; SH, sulfhydryl; SS, disulfide; TFA, trifluoroacetic acid.

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