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Foaming Properties of Wheat Gliadin

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ABSTRACT: We studied gliadin solubility, surface tension and foam behavior, and the presence of different gliadin types in gliadin aqueous solutions and foams as a function of pH. Gliadin has excellent foaming properties only at neutral and alkaline pH. Its solubility is minimal near neutral pH, while almost complete at acidic and alkaline pH. Surface tensions of gliadin solutions are minimal around neutral pH, higher at alkaline pH, and highest at acidic pH, which corresponds well with their respective foaming properties. Foams at acidic and alkaline pH values are enriched in γ-gliadin, while foams at pH 8.0 have a similar distribution of α- and γ-gliadins. Thus, γ-gliadin predominantly contributes to the foaming properties of gliadin. The poor foaming properties of gliadin at pH 2.0 improve in the presence of 0.25 and 1.0% NaCl. It follows that the presence of positively charged amino acid residues hinders the formation of stable foam at acidic pH.

KEYWORDS: Foam, gliadin, pH, salt, protein solubility

INTRODUCTION

Wheat kernel proteins are divided into gluten protein (generally about 80−85% of total wheat protein) and a heterogeneous group of non-gluten proteins (generally about 15−20% of total wheat protein). Gluten, the wheat storage protein, consists of comparable levels of gliadin and glutenin. It is important for the bread-making quality of wheat flour because it enables the formation of a viscoelastic dough. Dough elastic/strength properties are ascribed to the glutenin polymers, and gliadins are believed to act as plasticizers that weaken interactions between glutenin chains, thereby increasing dough viscosity. Gliadins have surface-active properties and, as a result, the potential to stabilize gas cells during bread making.[1−4]

On the basis of biochemical and genetic data, gliadins are classified into ω-, α-, and γ-gliadins.[5] Their primary structure consists of a hydrophilic central domain (CD) containing repetitive amino acid (AA) sequences particularly rich in Gln and Pro. The CD is enclosed by two terminal domains (TD) containing low levels of Gln and Pro and higher levels of hydrophobic AA than the CD. In addition, almost all ionizable AA, which are present in low levels, occur in the carboxyl-terminal domain (C-TD).[6] Gliadins of the α and γ types have a short amino-terminal domain (N-TD) consisting of only a few AA residues and a large CD and C-TD. The ω-gliadins, on the other hand, have a large CD, which covers about 90−96% of the protein sequence, while only short N-TD and C-TD are present.[6] The presence of hydrophilic and hydrophobic parts in α- and γ-gliadins indicates that they may have amphiphilic properties.[7]

The literature describes the surface-active properties of gluten proteins and, in particular, gliadin in different model systems. Mita et al.[8,9] studied the properties of gluten, gliadin, and glutenin foams in the presence of 3.0 M urea as a function of pH. Using reconstituted mixtures of gliadin and glutenin, they showed that gliadin plays a predominant role in gluten-foaming properties.[9] Furthermore, solutions of gliadins show lower surface tensions than those of glutenin.[9] Uthayakumaran et al.[10] found that γ-gliadin foams are of higher stability than ω-gliadin foams, while α-gliadin foams are less stable. To the best of our knowledge, no further research about the foaming properties of gliadin is reported thus far.

The degree at which foams can be created from liquids is proportional to the surface tension of these liquids. The lower the surface tension, the easier it is to create foams. Some proteins decrease the surface tension because of their ability to adsorb at the air−liquid interface. To study the surface-active behavior of gluten proteins, research also focused on the adsorption of gluten proteins at model interfaces. Keller et al.[11] and Banc et al.[7] both studied the adsorption of gluten proteins at an air−water interface. Gliadin shows a higher surface activity than glutenin,[11] and a film of purified γ-gliadin is more stable at the air−water interface than those of other gliadin types.[7] Gliadins adsorb readily onto a solid hydrophobic surface, and their three-dimensional orientation depends upon gliadin type and concentration at the surface.[1] The aggregation capacity of α-gliadins at the surface is higher than for other gliadin types. Sequential adsorption of purified gliadin types indicated that α-, β-, and γ-gliadins block adsorption of ω-gliadins.[1] Gliadins adsorb more at a solid hydrophobic surface at pH 5.5 than pH 4.0. The same conclusion was reached on the basis of surface tension measurements of liquids at pH 4.0 and 5.0.[2] However, the amount of gliadin adsorbed at a solid hydrophobic surface at pH 4.0 increases in the presence of 0.1 M NaCl.[12] Increasing adsorption of gliadins at a solid surface was explained as resulting from decreasing electrostatic repulsion between proteins at the interface.[2,12]

Although the adsorption of gliadins at surfaces in model systems has been described quite well, data on the relationship between the surface-active properties of gliadins on one hand and the foaming properties of gliadins on the other hand are scarce.

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Furthermore, it is not clear which gliadin types contribute to the foaming properties of gliadins. Because food systems differ in their pH, it is important to study the foaming properties of these gliadins as a function of pH.

The aim of our work was to elaborate on the foaming properties of gliadin. These were determined as a function of pH and linked to gliadin solubility, surface tensions, and the distribution of the different gliadin types. Furthermore, the effect of NaCl on the foaming properties of gliadin was examined.

MATERIALS AND METHODS

Materials. Commercial wheat gluten [moisture content, 6.7%; crude protein content, 80.22% on a dry basis (db) using 5.7 as the nitrogen/protein conversion factor for gluten proteins; starch content, 8.35% db] was from Cargill (Bergen op Zoom, The Netherlands). Denatured ethanol (97%, v/v) was from Brenntag (Mülheim/Ruhr, Germany). All other chemicals, solvents, and reagents were purchased from Sigma-Aldrich (Bornem, Belgium) and were of at least analytical grade, unless otherwise specified.

Methods. *Gliadin Extraction.* Gliadin was extracted in two steps from 100 g of wheat gluten with two quantities (1.0 L each) of 70% (v/v) ethanol. After shaking (20 min at 20 °C) and centrifugation (10000*g* for 10 min at 20 °C), the supernatants were pooled. Before the second extraction step, the cohesive glutenin residue was mechanically disrupted with a spatula. The ethanol in the supernatants was removed by rotary evaporation (50 °C), and the gliadin fraction was freeze-dried.

Aqueous Solubility of Gliadin. Prior to foam formation, gliadin (85% protein, db) was dispersed in deionized water [0.3% (w/v) protein]. The pH of the suspensions was adjusted to pH 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 using either 1.0 M HCl or 1.0 M NaOH. Gliadin suspensions at pH 2.0, 8.0, and 12.0 were also prepared in aqueous solutions of 0.05, 0.25, 1.00, and 2.00% (w/v) NaCl. Protein contents in supernatants (further referred to as gliadin solutions), obtained after centrifugation (10000*g* for 10 min at 20 °C) of the suspensions, were determined by the Dumas combustion method, an adaptation of the Association of Official Analytical Chemists (AOAC) official method 990.03,[13] to an automated Dumas protein analysis system (EAS, VarioMax N/CN, Elt, Gouda, The Netherlands), using 5.7 as the nitrogen/protein conversion factor for gluten proteins.

Foaming Properties. Foams were prepared on the basis of the whipping method described by Caessens et al.,[14] with small modifications. A volume of 100 mL of gliadin solution was placed in a graduated glass cylinder (internal diameter of 60 mm), the bottom of which was covered with a glass filter (thickness of 5 mm and diameter of 60 mm) and had a small tap to allow for the removal of the aqueous phase. The solution was whipped for 70 s using a rotating propeller (2000 rpm; outer diameter of 45.0 mm and thickness of 1.0 mm) at room temperature. The initial foam volume (FV) was that of foam measured at 2.0 min after the start of whipping. Foam volume loss was monitored for 60 min, and foam stability (FS, %) was defined as the percentage of foam volume remaining after 60 min relative to FV. After 60 min, the liquid under the foam was removed through the tap, while the residual foam on top of the glass filter was removed and recovered with 70% (v/v) aqueous ethanol solution. The gliadin solution, liquid, and foam were freeze-dried. The coefficient of variation for the determination of FV and FS was calculated on the basis of a 5-fold determination for a typical gliadin solution at pH 6.0 and did not exceed 10%.

Surface Tension Measurements of Gliadin Solutions. Surface tensions (N/m) of gliadin solutions were determined at room temperature using a torsion balance (model "OS" balance/tensiometer, Bidford on Avon, Alcester, Warwickshire, U.K.) equipped with a 40.0 mm circumference platinum (DuNuoy) ring. Recipients containing the gliadin solutions were cleaned with acetone and air-dried before use. The coefficients of variation for surface tension values were calculated on the basis of a 5-fold determination of each sample.

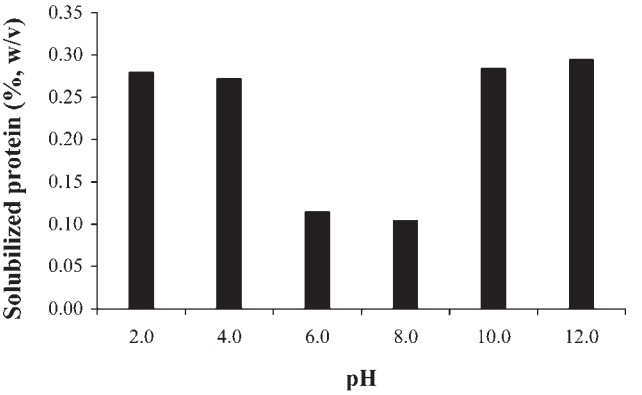

Figure 1. Protein solubility (%, w/v) of wheat gliadin dispersions (0.3%, w/v) as a function of pH.

Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC). The distribution of ω-, α-, and γ-gliadins at different pH within gliadin solutions and the resulting liquids and foams were determined by RP-HPLC. All freeze-dried gliadin samples (foam, liquid, and solution) were dissolved in 70% aqueous ethanol solution (5.0 mg of protein/mL) to ensure complete solubilization. Aliquots (40 μL) were injected on a Nucleosil C8 column (5 μm, pore size of 300 Å, 250 × 4.0 mm, Machery-Nagel, Düren, Germany) at 50 °C using a LC-2010 HPLC system (Shimadzu, Kyoto, Japan) with automated sample injection. The elution solvent consisted of deionized water (solvent A) and acetonitrile (solvent B), both containing 0.1% (v/v) trifluoroacetic acid (TFA). Gliadins were eluted with a linear gradient from 24 to 56% solvent B in 50 min at a flow rate of 1.0 mL/min and detected at 214 nm. The ω-, α-, and γ-gliadins were distinguished on the basis of absorbance minima between specific peaks as described by Wieser et al.[15] The percentage area of each gliadin type was expressed relative to the total area of the RP-HPLC chromatogram.

RESULTS AND DISCUSSION

Solubility of Gliadin as a Function of pH. The solubility profile of gliadin as a function of pH is shown in Figure 1. Wheat gliadin is poorly soluble near neutral pH values, which correspond to the isoelectric point (pI) of gliadin (ca. 7.8).[16] In contrast, at acidic and alkaline pH conditions, gliadin solubility exceeded 90%.

Foaming Properties of Gliadin as a Function of pH. Initially, an equal amount of protein (0.3%, w/v) was suspended in deionized water. On the basis of the pH versus solubility profile (Figure 1), the centrifuged gliadin solutions contained variable gliadin levels. Therefore, the foaming properties were also determined as function of pH at a constant protein level (0.10%, $w_{protein}/v$) present in the gliadin solutions. This concentration was taken as standard for all foaming tests and corresponds to the maximal gliadin concentration in the supernatant of a 0.3% ($w_{protein}/v$) gliadin suspension at pH 8.0, at which solubility was minimal.

Figure 2A shows the foaming properties of gliadin solutions as a function of pH, with the variable protein levels shown in Figure 1. At acidic pH, FV was slightly lower than at alkaline pH. FS was very low at pH 2.0 but increased with pH. At neutral and alkaline pH values, foams were quite stable (more than 60% of the foams still being present after 60 min). These results are in line with Mita et al.,[9] who also found the FS of gliadin foams, in the presence of 3.0 M urea, to be low at pH values below 5.0.

Figure 2B shows the foaming properties of gliadin solutions at a constant protein concentration (0.10%, $w_{protein}/v$). These were

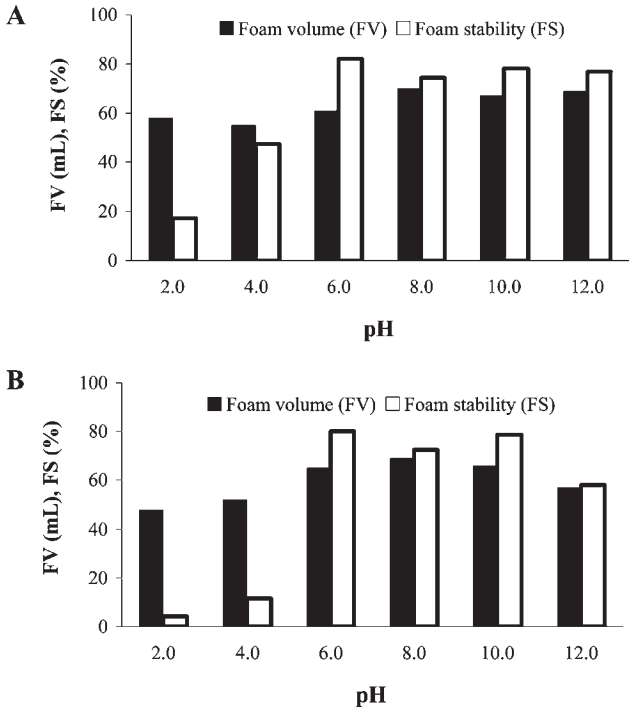

Figure 2. FV and FS measured 60 min after the start of whipping from (A) gliadin solutions with a protein concentration of 0.28, 0.27, 0.11, 0.10, 0.28, and 0.29% (w/v) at pH 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0, respectively, and (B) gliadin solutions at a constant protein concentration (0.10%, w/v).

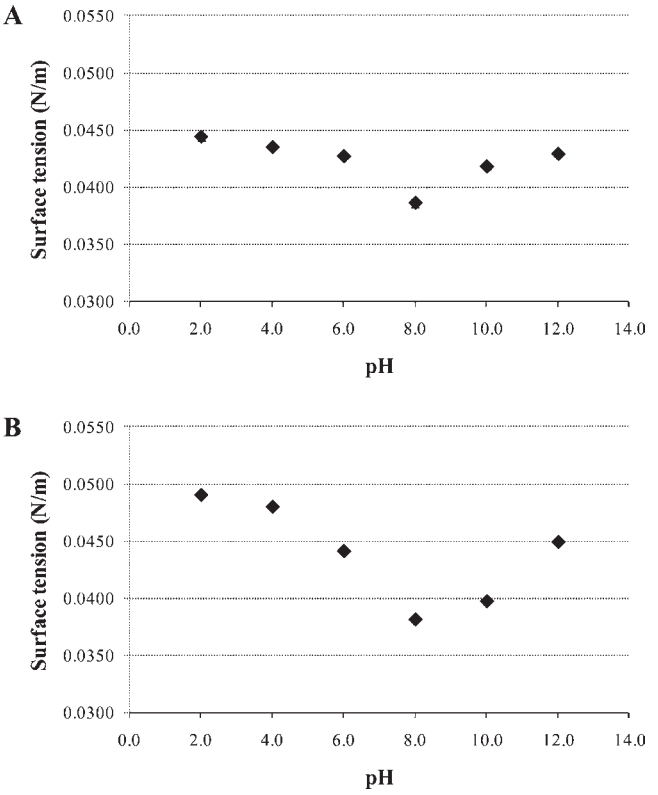

Figure 3. Surface tension of (A) initial gliadin solutions with a protein concentration of 0.28, 0.27, 0.11, 0.10, 0.28, and 0.29% (w/v) at pH 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0, respectively, and (B) gliadin solutions at a constant protein concentration (0.10%, w/v).

comparable to those obtained with gliadin solutions at varying protein concentrations (Figure 2A). At a constant protein level, FV at acidic pH was lower than neutral and alkaline pH (Figure 2B). FS was very low at pH 2.0 and 4.0, while at neutral and alkaline pH, foams were more stable. Slightly lower FV and FS were obtained at pH 12.0 than pH 6.0, 8.0, and 10.0. As a result, the differences in foaming properties of gliadin as a function of pH cannot be ascribed to variations in protein levels of the solutions.

We also determined the foaming properties of lyophilized egg white protein from fresh egg, solubilized in deionized water. FV of egg white protein was 75 mL, and FS was 80%. Thus, the foaming properties of egg white protein were similar to those of gliadin at neutral and alkaline pH conditions.

Surface Tension of Gliadin Solutions as a Function of pH. Air−liquid interfaces have a surface tension because of (van der Waals) interactions between molecules of the liquid at the surface. The degree at which foams can be created is proportional to this surface tension. The lower the surface tension, the easier it is to create foams. Substances with surface-active properties, such as some proteins, can adsorb at the air−liquid interface and decrease the surface tension, leading to an increased foaming ability.[17] Thus, to relate the surface-active properties of gliadins at different pH conditions to their foaming properties, the surface tensions of gliadin solutions with variable and constant protein levels (0.10%, w/v) were determined as a function of pH (panels A and B of Figure 3). A minimal surface tension was noted at pH 8.0, i.e., near the pI of gliadin.[16] Surface tensions increased toward more extreme pH conditions. At a constant protein concentration, they were lower at alkaline than acidic pH (Figure 3B). In the other setup, which implied variable protein concentrations, surface tensions were generally lower, except at pH 8.0, and differences between surface tensions at acidic and alkaline pH were smaller than at a constant protein concentration. The surface tension values are in line with the FV of gliadin solutions at different pH conditions (panels A and B of Figure 2).

According to the literature, because increasing electrostatic repulsion between proteins at air−water interfaces decreases protein adsorption at an interface, this increases surface tensions.[18,19] These increasing electrostatic repulsions go hand in hand with decreased foaming properties. At a constant protein concentration, the gliadin foaming properties at acidic pH are lower than at higher pH. Therefore, we suggest that electrostatic repulsion between gliadins at pH 2.0 and 4.0 is higher than that at higher pH values. This could explain the poorer foaming properties at acidic than higher pH. Moreover, it has been reported that lower levels of gliadins adsorb at lower pH (pH 4.0) than higher pH (pH 5.5) at model solid hydrophobic surfaces.[12] The high FS of gliadin solutions at neutral pH may be explained by their pI (near pH 7.8).[16] At such pH, gliadin has a low net charge and, thus, experiences only limited electrostatic repulsion. This results in efficient adsorption of gliadins at the interface.[12,19] Moreover, according to Mita et al.,[8] proteins form the most compact conformation near their pI, and therefore, under such conditions, large quantities can adsorb at the air−water interface. As a result of increasing adsorption of gliadins at the interface, the surface tension decreases. In addition to close packing of proteins at the interface, they can then also efficiently associate with each other and form a stable rigid protein layer at the interface.[17,19] Gliadins contain large levels of glutamine and nonpolar AA, which leads to strong hydrogen and hydrophobic interactions. It is believed that

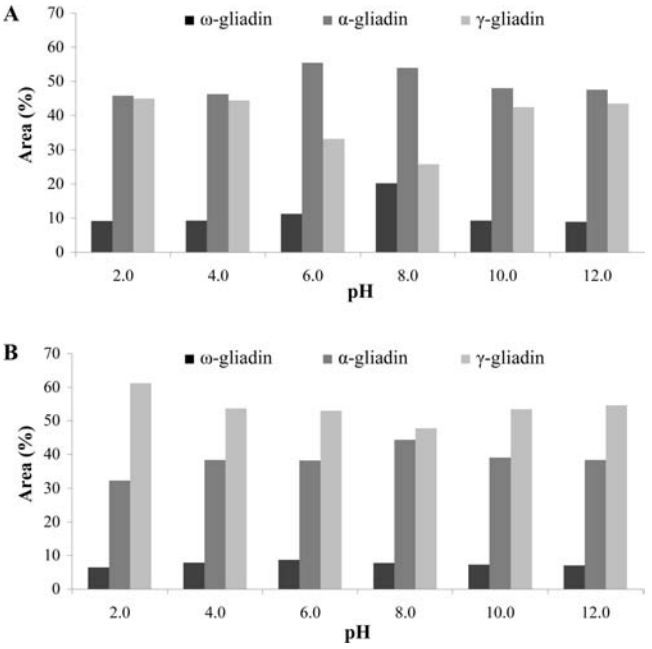

Figure 4. Distribution of gliadin types in (A) initial gliadin solution and (B) foams remaining 60 min after the start of whipping as a function of pH. The levels of different gliadin types (ω-, α-, and γ-gliadin) are expressed as the area percentage of each gliadin type relative to the total area of the RP-HPLC (C8) chromatogram.

these factors also contribute to the aggregation and, thus, the poor solubility of gluten proteins.[4]

Lower surface tensions at high than low pH may indicate less electrostatic repulsion between gliadins. However, because gliadin types differ in their hydrophobic−hydrophilic distribution along the AA chain,[6,20,21] the foaming properties of gliadin solutions at different pH values could also be determined by a varying contribution of the different gliadin types.

Distribution of Gliadin Types within Gliadin Solutions as a Function of pH. The ω-gliadins are the most hydrophilic ones, whereas the γ-gliadins represent the most hydrophobic ones.[15] RP-HPLC showed that gliadin, extracted from commercial gluten with 70% (w/v) ethanol solution, consists of comparable levels of α- and γ-gliadin (45% each) and 10% ω-gliadin. A similar distribution of gliadin types was observed in gliadin solutions at pH 2.0, 4.0, 10.0, and 12.0 (Figure 4A). This was expected, because almost all proteins are soluble at these pH values (Figure 1).

However, gliadins are poorly soluble in water at pH 6.0 and 8.0. Figure 4A shows that, at such pH values, gliadin solutions contained mostly α-gliadins. γ-Gliadins and even more so ω-gliadins were present in lower levels. The relative levels of dissolved γ-gliadins were lower at pH 6.0 and 8.0 than other pH values. This can be explained by the fact that γ-gliadins are the most hydrophobic gliadins and, thus, precipitate most at pH conditions near the pI of gliadins.[15]

Because the distributions of gliadin types within gliadin solutions at acidic and alkaline pH were very similar, we conclude that differences in foaming properties at alkaline or acidic pH are not due to differences in composition of the gliadin solutions. Therefore, it is expected that the pH-dependent foaming properties of gliadins are rather explained by the presence of charges on the protein and/or the specific adsorption of gliadin types at the liquid−air interface during foam whipping.

Distribution of Gliadin Types within Gliadin Foams as a Function of pH. To examine the importance of specific gliadin types in the foaming of gliadin at different pH values, their distribution within the foams remaining 60 min after the start of whipping was determined. The amount of proteins in the foams at pH 2.0 and 4.0 was less than 10% of the proteins in the gliadin solution, while at pH 6.0, 8.0, 10.0, and 12.0, between 15 and 20% of the proteins were present in the foams.

Figure 4B shows that, except for pH 8.0, foams were enriched in γ-gliadins. α-Gliadins were also present in relatively high levels. The residual foam from the gliadin solution at pH 2.0 contained higher levels of γ-gliadin than those at the other pH values. The relative contribution of γ-gliadin in foam at pH 8.0 was decreased, while that of α-gliadin was slightly increased (Figure 4B); therefore, eventually, similar levels of α- and γ-gliadins were present. The levels of ω-gliadin in the different foams were low (Figure 4B), even at pH 8.0, where the relative level of ω-gliadin in the corresponding gliadin solution was higher (Figure 4A). The distribution of gliadin types within the liquid phase at 60.0 min after the start of whipping was very similar to that of the corresponding gliadin solutions (results not shown). This seems evident because only less than 20% of the gliadins were present in the foam.

Our results indicate that, except at pH 8.0, mainly γ-gliadins are present in foams at 60 min after the start of whipping and that they are thus important for the foaming properties of gliadin. Although equal levels of α- and γ-gliadins were present in the foam at pH 8.0, γ-gliadins still seem to be very important because their relative level in foam was much higher than that within the solution. Thus, γ-gliadins predominantly adsorb at the air−water interface. To the best of our knowledge, the importance of γ-gliadin for the foaming capacity of gliadin has never been reported. Furthermore, the relative level of α-gliadin in foam was higher at pH 8.0 than that at the other pH values, which indicates that α-gliadins also play an important role in foaming near their pI. This can be explained as follows. On the basis of the primary structures of α- and γ-gliadins, the presence of a hydrophilic CD and a more hydrophobic C-TD, which contains most of the ionizable AA,[6] indicates that they have amphiphilic properties.[7] However, because of an additional polyglutamine sequence in the C-TD of α-gliadins, α-gliadins are less hydrophobic than γ-gliadins.[6,20,21] As a result, α-gliadins are less amphiphilic than γ-gliadins. Therefore, we believe that, at pH values different from the pI of gliadins, the combination of charges and the additional polyglutamine sequence in the C-TD of α-gliadins may result in a less pronounced amphiphilic character of α-gliadins than γ-gliadins. We, thus, expect relatively lower levels of α-gliadins than γ-gliadins in foams. However, at the pI, charges in proteins cancel each other out, and as a result, electrostatic interaction can lead to increased protein−protein interactions at the liquid−air interface, which possibly explains equal levels of α- and γ-gliadins in foams.

Our results show that the distributions of gliadins within foams at acidic and alkaline pH are similar. On the basis of this observation, we believe that the poor foaming properties of gliadin solutions at acidic pH are the result of net charges on the protein chains. Both Elofsson et al.[12] and Wannerberger et al.[3] showed electrostatic repulsive forces to dominate adsorption and interactions between gliadins at model solid surfaces. Our experiments confirm their suggestion that these forces should be considered in the context of the stability of air bubbles. Because acidic pH values have a negative effect on the surface-active properties

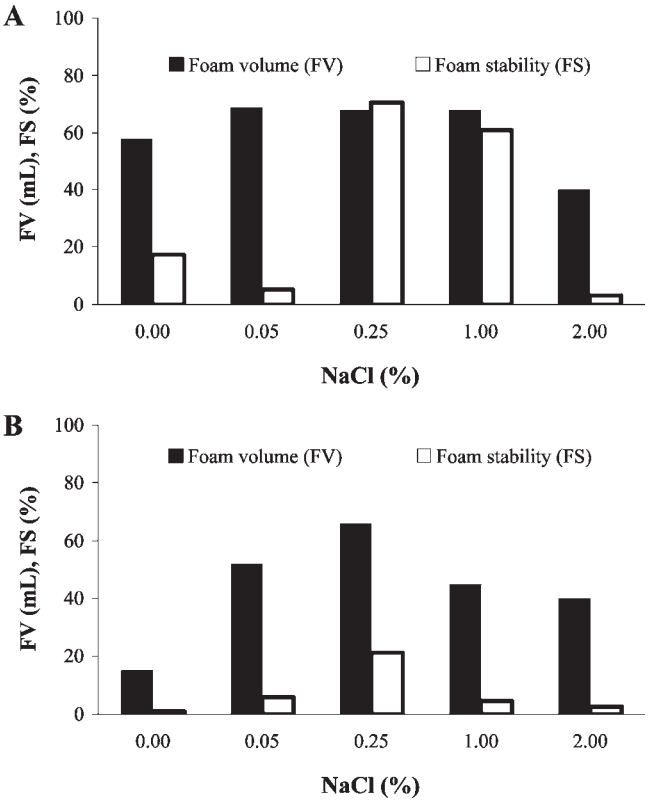

Figure 5. FV and FS (measured 60 min after the start of whipping) of gliadin at pH 2.0 in the presence of 0.00, 0.05, 0.25, 1.00, and 2.00% (w/v) NaCl from (A) gliadin solutions with a protein concentration of 0.28, 0.30, 0.22, 0.05, and 0.02% (w/v) protein and (B) gliadin solutions at a constant protein concentration (0.02%, w/v).

of gliadins, one could imagine that this influences their functionality during processing of wheat-based aerated food systems at acidic pH.

Elofsson et al.[12] found that, when the ionic strength is increased at pH 4.0, the levels of gliadins at the interface increase. As a result, the addition of salt, a common constituent in many food systems, may well improve the poor foaming properties at acidic pH. Therefore, we studied the foaming properties at pH 2.0, 8.0, and 12.0 at different NaCl concentrations.

Effect of NaCl on the Foaming Properties of Gliadin. The addition of 0.05, 0.25, 1.00, or 2.00% NaCl (w/v) affected neither the FV nor the FS of gliadin foams at pH 8.0 and 12.0. In contrast, at pH 2.0, both the addition of 0.25 and 1.00% NaCl drastically improved FS, whereas FV only slightly improved (Figure 5A). The addition of 0.05 or 2.00% NaCl did not increase FS. The protein concentrations at pH 2.0 in the presence of 0.05, 0.25, 1.00, and 2.00% NaCl were 0.30, 0.22, 0.05, and 0.02% (w/v), respectively, showing that the gradual addition of salt precipitates some protein. At pH 2.0, the lower added NaCl concentrations decreased the surface tension of gliadin solutions (Figure 6A) but resulted in only slightly increased FV (Figure 5A). Surface tensions seem little related to FS. At pH 12.0, surface tensions gradually decreased up to 2.0% NaCl addition, while at pH 8.0, surface tensions did not change. However, salt addition caused important changes in foaming properties neither at pH 8.0 nor at pH 12.0 (results not shown).

The poor FS of a gliadin solution at pH 2.0 containing no added NaCl might be related to reduced levels of protein−protein interactions because of electrostatic repulsion at the

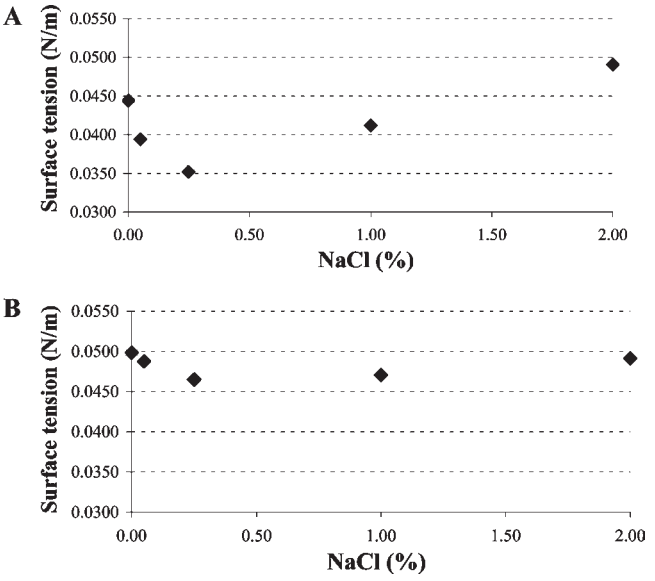

Figure 6. Surface tension of gliadin solutions at pH 2.0 at 0.00, 0.05, 0.25, 1.00, and 2.00% (w/v) NaCl with a (A) protein concentration of 0.28, 0.30, 0.22, 0.05, and 0.02% (w/v) and (B) constant protein concentration of 0.02% (w/v).

interface,[18] while the poor FS at pH 2.0 and 2.00% NaCl may well be attributed to the strongly decreased protein solubility, making less proteins available to stabilize foam bubbles. Therefore, the foaming properties and surface tensions were also determined at a constant protein concentration (0.02%, w/v). In this case, an optimal FV was obtained at 0.25% NaCl, followed by 0.05, 1.00, and 2.00%, respectively (Figure 5B). The FS readings showed a similar profile as the FV values. At a constant protein concentration, surface tension values were higher but showed a profile as a function of the NaCl concentration similar to that at variable protein concentrations (panels A and B of Figure 6). At a constant protein concentration, surface tensions were much in line with FV readings (Figure 5B and Figure 6B).

RP-HPLC analysis of the gliadin solutions at pH 2.0 showed that the addition of NaCl caused relatively more precipitation of γ-gliadins than the other gliadin types (Figure 7A). This can be explained by their higher hydrophobicity than that of ω- and α-gliadins. The relative level of ω-gliadin in solution strongly increased at 1.00 and 2.00% NaCl. Figure 7B indicates that mostly γ-gliadins were present in foams at pH 2.0, in both the absence or presence of added NaCl. No residual foam was left at pH 2.0 after 1.0 h at 0.05 and 2.00% NaCl. The level of ω-gliadin in foams at pH 2.0 and 1.00% NaCl was much lower than that in solution. This indicates that ω-gliadin hardly contributes to the formation and stabilization of gliadin foams.

Our results indicate that chloride ions mask positive charges on the protein chain at acidic pH, leading to decreased surface tensions and improved foaming properties. This is in line with the literature showing that the addition of salt at pH levels above or below the pI of proteins results in increased protein adsorption at interfacial surfaces because of charge masking.[18,19] However, under our experimental conditions, we did not observe improved foaming properties for gliadins when adding NaCl above their pI.

In conclusion, we showed that the foaming properties of gliadin strongly depend upon the pH. This is attributed to the presence of ionizable AA. Moreover, γ-gliadin contributes most to the foaming properties of gliadin, irrespective of the pH. In this

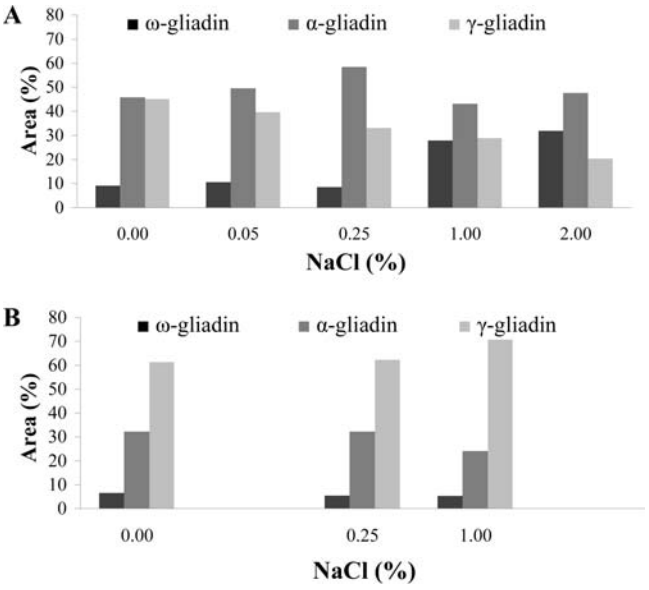

Figure 7. Distribution of gliadin types (ω-, α-, and γ-gliadin) in (A) initial gliadin solution and (B) foams remaining 60 min after the start of whipping at pH 2.0 in the presence of 0.00, 0.05, 0.25, 1.00, and 2.00% (w/v) NaCl. The levels of different gliadin types are expressed as the area percentage of each gliadin type relative to the total area of the RP-HPLC (C8) chromatogram.

respect, the present results contribute to an understanding of the gliadin foaming behavior and the underlying mechanisms.

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ABBREVIATIONS USED

AA, amino acid(s); CD, central domain(s); db, dry basis; FS, foam stability; FV, initial foam volume; pI, isoelectric point; RP-HPLC, reversed-phase high-performance liquid chromatography; C- and N-TD, C- and N-terminal domain(s); TFA, trifluoroacetic acid

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