

Adsorption Studies of Interaction Between Water-Extractable Nonstarch Polysaccharides and Prolamins in Cereals

U. Elofsson,¹ A.-C. Eliasson,^{1,2} M. Wahlgren,¹ A.-M. A. Loosveld,³ C. M. Courtin,³ and J.A. Delcour³

ABSTRACT

Cereal Chem. 77(5):679–684

Adsorption of cereal storage protein and nonstarch polysaccharides onto methylated silica surfaces, as measured by ellipsometry, was used to study possible interactions between those components. All fractions, rye secalin, wheat gliadin, rye arabinoxylan, and wheat arabinogalactan peptide, were surface-active to some degree. Sequential adsorption of rye arabin-

oxylan and wheat arabinogalactan peptide on top of gliadin and secalin, respectively, indicated that an interaction occurred. As ionic strength and pH influenced both the adsorption and the interaction of the components studied, these phenomena are believed to be of electrostatic nature.

Wheat flour is unique in its ability to produce leavened products. Such ability is related to properties of the wheat flour dough such as its gas-holding capacity (Baker and Mize 1941) and viscoelastic behavior (Bloksma 1990). Of the other cereals, only rye shows some breadmaking performance. Rye is grown almost exclusively in the northern part of Europe and is used in many specialty products such as sourdough and crisp breads (Lampinen 1995). In Sweden especially, wheat and rye flours are mixed for breadmaking purposes.

It is not totally clear why rye flour has (much) poorer breadmaking performance than wheat flour (He and Hoseney 1991). Gas-holding capacity and viscoelastic behavior of dough, largely responsible for a good breadmaking performance, are mainly conveyed on wheat flour doughs by wheat flour storage proteins. They are able to form a large continuous gluten network during dough formation. Although the rye storage proteins have been studied to much lesser extent, the formation of large aggregates occurs not only with wheat protein but also with certain rye proteins (Field et al 1983). It has further been shown that certain rye protein fractions have a behavior at the air-water interface that is very similar to that of wheat proteins (Wannerberger et al 1997). In spite of comparable protein properties, rye flour doughs have a much lower gas-holding capacity than their wheat counterparts. Reasons for this could be the lower protein content and less functional protein in rye flours and the lack of aggregation by rye proteins because of interactions with nonstarch polysaccharides (NSP). Such interactions have been suggested in several studies (Amadò and Neukom 1985, Hamer and Lichtendonk 1987, Weegels et al 1991). Based on the observation that the separation of gluten and starch is facilitated in the presence of hemicellulases, Weegels and coworkers (1991) argued that NSP may prevent the aggregation of gluten proteins. If such an effect were present in wheat, it would be expected to be of even greater importance in rye, where the protein content usually is lower and the NSP content higher.

As rye proteins apparently do not form large aggregates in dough, other factors may play a role in conveying gas-holding capacity to rye doughs. This suggests that NSP fractions such as arabinoxylan and β -glucan, through their high molecular weight and viscosity-inducing properties, may partially make up for the absence of a gluten network. However, the literature concerning NSP functional effects in breadmaking is confusing, probably as a result of differences in chemical composition of NSP fractions used in breadmaking procedures and evaluation and definition of bread quality parameters.

The present investigation was undertaken to study whether interactions between water-extractable arabinoxylan or a second cereal NSP, arabinogalactan peptide (Fincher and Stone 1974, Loosveld et al 1997), and wheat gliadin or rye secalin exist. Interactions were studied on a molecular level by adsorption of proteins on methylated silica, and subsequent interactions with arabinoxylan or arabinogalactan peptide in solution. Adsorption was monitored with ellipsometry, a technique that has been used by several authors to study the interfacial behavior of proteins (Wahlgren et al 1993, Wannerberger et al 1996).

MATERIALS AND METHODS

Chemicals

Dextran (31391, Fluka, Buchs, Switzerland), a protein free polysaccharide, was of *Leuconostoc* origin, and had a molecular weight of \approx 110 kDa. Bovine serum albumin (BSA) series NR A-4503 Lot 99F0014 was obtained from Sigma (St. Louis, MO). Sodium acetate and acetic acid (Merck, Darmstadt, Germany) were of analytical grade (100%). 1-Butanol and 1-propanol were of analytical grade (99.0% and 99.5% respectively) and purchased from Lab-Scan (Dublin, Ireland). All other reagents were of at least analytical grade unless otherwise specified.

Isolation and Purification of Water-Extractable Arabinoxylan and Arabinogalactan Peptide from Flour

Arabinoxylan from a European rye cultivar (Halo) was purified and characterized by Delcour et al (1999). In the procedure, rye whole meal is extracted with water; the starch in the extract is hydrolyzed with thermamyl; and a precipitate is formed on addition of ethanol to the resulting solution. Part of the crude arabinoxylan (R-PrAX) obtained was then further deproteinized by clay-adsorption treatment and referred to as (R-AX).

The arabinogalactan peptide was isolated from the European wheat cultivar Skirlou according to the method by Loosveld et al (1997). This method consisted of extracting inactivated flour (Fincher and Stone 1974) with water; precipitation of protein material by a heating step; hydrolyzing starch with thermamyl and amyloglucosidase; dialysis; and precipitation of wheat water-extractable arabinogalactan peptide by a number of ethanol precipitation steps. The arabinogalactan peptide was characterized as described by Loosveld et al (1998) and is referred to as (W-AGP). Sample compositions are shown in Table I.

Preparation of Protein Samples

Gliadin was extracted from flour of the wheat cultivar Dragon by 70% (v/v) ethanol as described by Wannerberger et al (1996), yielding a material with 97.8% (dry basis) protein content (N \times 5.7, according to the Kjeldahl method) and was kindly provided by Lars Wannerberger, Lund University, Sweden. In one adsorption experiment, gliadin from the wheat cultivar Sport was used.

¹ University of Lund, Department of Food Technology, Box 124, S-221 00, Lund, Sweden.

² Corresponding author. Phone: +46 46 222 9674. Fax: +46 46 222 9517. E-mail: Ann-Charlotte.Eliasson@livsteki.lth.se

³ Laboratory of Food Chemistry, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, B-3001 Heverlee, Belgium.

The alcohol-soluble secalins were extracted from a commercial rye flour (Nord Mills AB, Malmö, Sweden) and purified according to the procedure used by Shewry et al (1983) with some modifications. Quantitative extraction was performed at room temperature by stirring 200 g of flour with 2.8 L of water-saturated 1-butanol for 3 × 60 min. Each suspension was centrifuged (MSE Mistral 4L centrifuge, London, England) in a screw-capped polypropylene centrifuge tube at 1,700 × g (18°C, 20 min). The lipid containing supernatants were discarded. The residues were stirred with 2.4 L of 0.5M NaCl (2 × 60 min) to extract the water-extractable proteins and centrifuged as described above. Final extractions were with 2.8 L of 50% (v/v) 1-propanol (3 × 60 min). The supernatants were first rotary-evaporated at room temperature until ≈40% of 1-propanol had been removed, and then dialyzed for 48 hr against several exchanges of distilled water. The retentates were lyophilized with a Hetosicc freeze dryer (Heto Birkeröd, Denmark). The Kjeldahl protein content (N × 5.7) in the secalin fractions was determined by a Kjeltec Auto 1030 analyzer (Tecator, Sweden) as 85% (dry basis).

SDS-PAGE of the protein fractions (data not shown) showed that the gliadin as well as the secalin fractions both contained several bands in the molecular weight region 94–14.4 kDa. Under

nonreducing conditions, the secalin fraction gave streaking when the electrophoresis was run.

Preparation of Methylated Silica Surfaces

Silica surfaces, with an oxide layer of 300–350 Å obtained by thermal oxidation, were kindly provided by Stefan Welin-Klintström, University of Linköping, Sweden.

The silica surfaces were first cleaned at 80°C for 5 min with NH₃, H₂O₂, and H₂O (1:1:5, v/v) and then with HCl, H₂O₂, and H₂O (1:1:5, v/v). They were rinsed with twice-distilled water after each step, and finally twice with ethanol followed by three times with trichloroethylene. The surfaces were methylated by 0.1% of dichlorodimethylsilane in trichloroethylene for 1 hr, after which they were rinsed three times with trichloroethylene and twice with ethanol. The methylated surfaces were stored in ethanol until used.

All the glassware was cleaned with 1:1 concentrated sulfuric and nitric acid before use.

Ellipsometry

The principle of this optical method is based on the changes in ellipticity of plane polarized light on the reflection at the interface (Azzam and Bashara 1977). A thin-film ellipsometer (type 43603-200E, Rudolph Research, Fairfield, NJ), equipped with a 5mW He-Ne laser (Melles Griot, Carlsbad, CA) was used. The mode was null ellipsometry, and the equipment was automated according to the principles of Cuypers (1976). From the changes in the ellipsometric angles (Δ and Ψ), the thickness and the reflective index of a protein layer were calculated according to McCrackin et al (1963), with the assumption of a homogeneous film at the surface. The calculations were performed using a three-phase model (surface, adsorbate, and ambient) utilizing the measured pseudorefractive index for the silicon with the oxide layer. The concentration of the substance in the film (Γ , mg/m²), was calculated from the values of the thickness and the refractive index. Because the adsorbed mass can be determined more accurately at low surface coverage than the film thickness and the refractive index (Cuypers et al 1983), Γ is the value presented in the current study. The values used for the ratio between molar weight and molar refractivity and for the partial specific volume were 4.1 g/mL and 0.75 mL/g, respectively.

Adsorption Measurements

The adsorption in 0.01M acetate buffer solution at pH 4.0 and 5.5, with or without 0.1M NaCl, was measured at 25°C with an agitation of 325 rpm. The pseudorefractive index of the bare surface was first determined in buffer solution with the methylated silica

TABLE I
Monosaccharide Composition; Arabinogalactan, Arabinoxylan, β -Glucan, and Protein Content; Arabinose-to-Xylose Ratio; Arabinose-to-Galactose Ratio; and Xylose Substitution Pattern of R-AX, R-PrAX, and W-AGP

	R-PrAX	R-AX	W-AGP
Dry substance (% ds)	93.5	89.5	93.0 ^a
Arabinose (% ds)	24.8	41.3	37.1
Xylose (% ds)	38.8	66.9	3.0
Mannose (% ds)	1.0	0.6	1.5
Galactose (% ds)	2.9	2.9	51.6
Glucose (% ds)	10.4	6.0	2.3
Arabinoxylan (% ds)	54.2	93.4	4.0
Arabinose/xylose	0.59	0.59	...
Unsubstituted xylose (% of xylose)	57.5	56.1	...
3-Monosubstituted xylose (% of xylose)	26.2	29.1	...
2,3-Disubstituted xylose (% of xylose)	16.3	14.8	...
Arabinogalactan (% ds)	4.4	4.5	77.6
Arabinose/galactose	0.69
β -Glucan (% ds)	4.7	3.0	2.7
Protein (% ds)	21.7	0.0	11.1

^a Values from estimated moisture content of 7%.

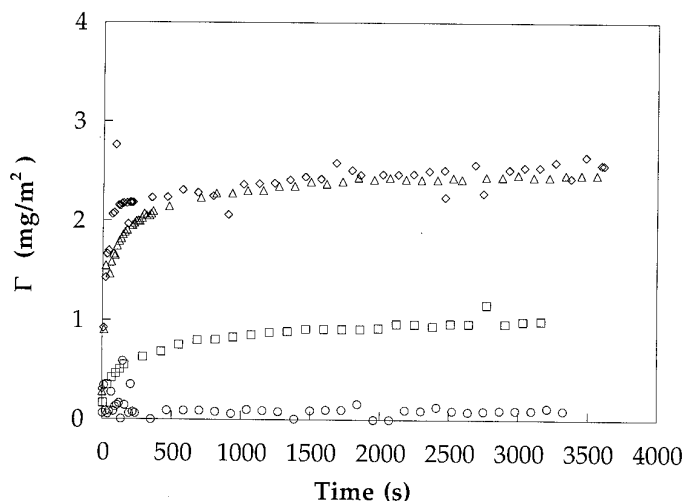


Fig. 1. Adsorbed amount (Γ) of nonstarch polysaccharides on hydrophobic silica surfaces vs. time. Measurements of 0.01M acetate buffer at 25°C, 325 rpm, 3.4 μ g/mL protein concentration, 1.7 mg/mL polysaccharide concentration. R-PrAX (Δ), R-AX (\square), W-AGP (\diamond), dextran (\circ).

surface in the cuvette. The measurements started with addition of 40 μL of protein stock solution to the cuvette, giving a final concentration of $\approx 3.4 \mu\text{g/mL}$. Adsorption was measured for 60 min. The cuvette was then rinsed with buffer solution (5 min, 20 mL/min). Desorption was measured for another 10 min before the next sample solution was added for the sequential studies. For polysaccharide adsorption, 0.5 mL of the stock solution was added to a volume of 4.5 mL, giving a final concentration of 1.7 mg/mL in the cuvette. The rinsing procedure and the desorption measurement were then repeated as above. The maximum variation in these measurements was 0.6 mg/m^2 , which corresponds to $\approx 13\%$.

RESULTS

Adsorption of NSP-Fractions to Methylated Silica Surfaces

The adsorptions of R-PrAX, R-AX, and W-AGP in 0.01M acetate buffer at pH 5.5 are shown in Fig. 1. The fractions with high protein content (R-PrAX and W-AGP) (Table I) adsorbed to relatively high and similar plateau levels of $\approx 2.5 \text{ mg/m}^2$ after 60 min of adsorption. In contrast, the adsorbed plateau levels of the

protein-free R-AX were considerably lower after 60 min of adsorption ($\approx 1.0 \text{ mg/m}^2$). The initial adsorption for W-AGP was very fast, and the adsorbed level was close to the plateau value within 5 min, whereas the adsorption of both the arabinoxylan fractions was significantly slower. The adsorption under the same conditions of dextran is also shown in Fig. 1 for comparison. Under these conditions, dextran did not adsorb at all. No effect on the adsorption of either R-PrAX or R-AX was found when the pH was lowered to pH 4.0 (data not shown).

Adsorption of Cereal Storage Proteins and NSP-Fractions

Adsorption of secalin or gliadin followed by each of the NSP fractions was performed in 0.01M acetate buffer at pH 4.0 and 5.5 on methylated silica surfaces.

As seen in Fig. 2, under the conditions used, the adsorption behavior of both proteins was very similar. The adsorbed levels after 60 min were ≈ 2.0 and 4.0 mg/m^2 at pH 4.0 and 5.5, respectively. At higher ionic strength (0.01M acetate buffer with 0.1M NaCl), the plateau value for the adsorption of secalin increased to 5.0 mg/m^2 at both pH levels (Figs. 3 and 4). For gliadin, the

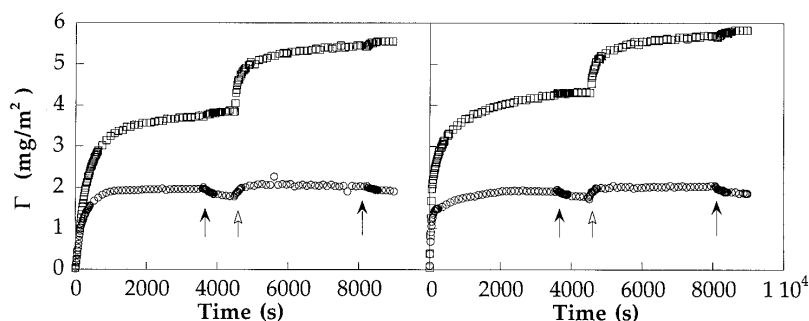


Fig. 2. Adsorbed amount of secalin (left) and gliadin (right) on hydrophobic silica surfaces vs. time, followed by adsorption of crude arabinoxylan (R-PrAX) at pH 4.0 (\circ) and pH 5.5 (\square). Measurements of 0.01M acetate buffer at 25°C, 325 rpm, 3.4 $\mu\text{g/mL}$ protein concentration, 1.7 mg/mL polysaccharide concentration. Adsorption was measured for 60 min, followed by rinsing for 5 min. Desorption was measured for 10 min before the second sample solution was added. Solid arrows indicate change to rinsing buffer; open arrows indicate change to the second sample solution.

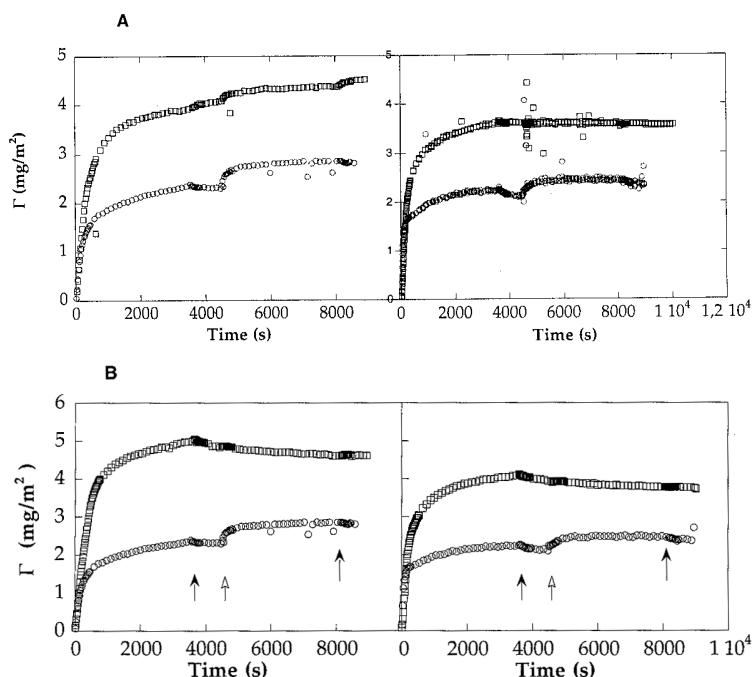


Fig. 3. Adsorbed amount of secalin (left) and gliadin (right) onto hydrophobic silica surfaces vs. time, followed by adsorption of R-AX. Measurements of 0.01M acetate buffer at 25°C, 325 rpm, 3.4 $\mu\text{g/mL}$ protein concentration, 1.7 mg/mL polysaccharide concentration. Adsorption was measured for 60 min, followed by rinsing for 5 min; measurements of desorption made during 10 min before the second sample solution was added. Solid arrows indicate change to rinsing buffer; open arrow indicates change to the second sample solution. Gliadin sample in this experiment was from the wheat cultivar Sport. **A**, R-AX at pH 4.0 (\circ) and pH 5.5 (\square); **B**, R-AX at pH 4.0 without (\circ) and with (\square) 0.1M NaCl.

higher ionic strength resulted in an adsorption increase from 2.0 to 4.0 mg/m² only at pH 4.0, whereas at pH 5.5, no significant difference was found.

The addition of R-PrAX, R-AX, and W-AGP to a preadsorbed protein layer (secalin or gliadin) is illustrated in Figs. 2–5. Generally, the polysaccharide fractions adsorbed in a manner similar to that of a preadsorbed layer of protein, regardless of whether the protein was secalin or gliadin. The sequential adsorption was performed at two different pH levels (pH 4.0 and pH 5.5). At pH 4.0, a small increase (<0.5 mg/m²) in the adsorbed level was observed after sequential addition of the R-PrAX and R-AX fractions (Figs. 2 and 3), while an increase of as much as 1.4 mg/m² was found for W-AGP (Fig. 5). In contrast, at pH 5.5, R-PrAX showed the highest adsorption to protein layers (Fig. 2), followed by W-AGP (Fig. 4). The R-AX fraction (Fig. 3A) did not adsorb at all (gliadin) or to a very low extent (secalin) to protein layers under these circumstances (at pH 5.5). At increased ionic strength, no adsorption of either W-AGP or R-AX fractions occurred on top of the adsorbed proteins for both pH 4.0 (Fig. 3B and 5) and pH 5.5 (Fig. 4). No, or very limited, desorption occurred upon dilution of either the proteins or the polysaccharide fractions under these conditions (Figs. 2–5).

The results obtained for the sequential adsorption of the cereal proteins and the polysaccharide fractions can be compared with those obtained for the sequential adsorption of dextran and bovine serum albumin (BSA), a globular protein. At pH 5.5, dextran did not adsorb to a bare methylated silica surface (Fig. 1), and it did not adsorb to a cereal protein (secalin) layer in sequential adsorption measurements (results not shown). On the other hand, the sequential adsorption of BSA to a preadsorbed secalin layer was of the same order as the adsorption of R-PrAX (Fig. 2).

DISCUSSION

The levels of the R-PrAX, R-AX, W-AGP, and dextran adsorbed to methylated silica surfaces as a function of time in 0.01M

acetate buffer at pH 5.5 are shown in Fig. 1. Generally, nonprotein containing carbohydrates are not surface active, which, as seen in Fig. 1, was confirmed for dextran. It is therefore interesting that a rather high amount (1.0 mg/m²) of the R-AX fraction, which did not contain any protein (Table I), still adsorbed to a methylated silica surface. In agreement with the present findings, arabinoxylan has previously been found to reduce the surface tension of water (Izodarczyk et al 1991, Sarker et al 1998).

Although R-PrAX and W-AGP both contain proteins, it must be assumed that their protein fractions differ substantially. As an arabinogalactan peptide in general contains ≈7–8% of protein covalently embedded in its structure (Fincher and Stone 1974), almost all protein in the W-AGP fraction is an integral part of the arabinogalactan peptide itself. Arabinoxylan, on the contrary, does not contain covalently bound protein. This means that the protein present in the R-PrAX fraction is a contaminant.

While the adsorption kinetics were similar, the R-PrAX adsorption plateau value was 2.5 mg/m², more than twice that of R-AX. This increase is clearly to be ascribed to the residual protein presence in the R-PrAX sample. W-AGP adsorbed with a considerable faster kinetic to the silica surface than the arabinoxylan fractions, but to the same plateau value (2.5 mg/m²) as the R-PrAX. One should not assume that because the adsorbed amount is the same for R-PrAX and W-AGP, that the layer compositions are similar. Due to the covalent linkage between peptide and carbohydrate, the adsorbed layer could, for example, contain more carbohydrates for adsorption from W-AGP than from R-PrAX. The difference in kinetics also indicates that different types of chemical entities are adsorbing in the two cases.

The adsorbed amounts of both secalins and gliadins increased with pH (Fig. 2). This behavior may be caused by a decreased lateral electrostatic repulsion between the proteins at the interface as the pH approaches the isoelectrical interval (Norde 1986), which for prolamins in general often is between pH 5.0 and 9.0 and for gliadins is between pH 6.5 and 8.1 (Shewry and Milfin 1985, Lindahl

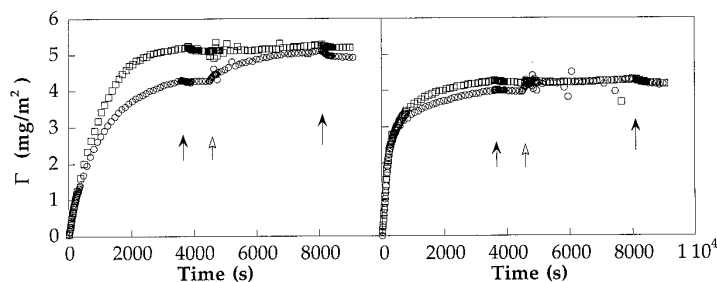


Fig. 4. Adsorbed amount of secalin (left) and gliadin (right) onto hydrophobic silica surfaces vs. time, followed by adsorption of W-AGP at pH 5.5 without (○) and with (□) 0.1M NaCl. Measurements of 0.01M acetate buffer at 25°C, 325 rpm, 3.4 μg/mL protein concentration, 1.7 mg/mL polysaccharide concentration. Adsorption was measured for 60 min, followed by rinsing for 5 min; measurements of desorption made during 10 min before the second sample solution was added. Solid arrows indicate change to rinsing buffer; open arrow indicates change to the second sample solution.

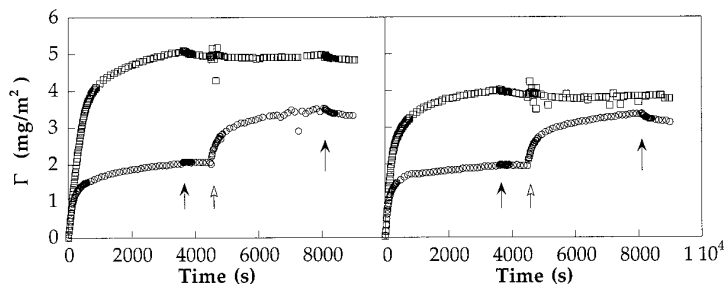


Fig. 5. Adsorbed amount of secalin (left) and gliadin (right) onto hydrophobic silica surfaces vs. time, followed by adsorption of W-AGP at pH 4.0 without (○) and with (□) 0.1M NaCl. Measurements of 0.01M acetate buffer at 25°C, 325 rpm, 3.4 μg/mL protein concentration, 1.7 mg/mL polysaccharide concentration. Adsorption was measured for 60 min, followed by rinsing for 5 min; measurements of desorption made during 10 min before the second sample solution was added. Solid arrows indicate change to rinsing buffer; open arrow indicates change to the second sample solution.

1990). A similar trend has been found at the air-water interface as measured with the surface balance technique (Wannerberger et al 1997). A further confirmation of the relevance of electrostatic phenomena for the interactions was the higher adsorbed amounts found at pH 4.0 when 0.1M NaCl was added to the 0.01M acetate buffer (Fig. 3B). For gliadins, the plateau value at pH 4.0 at the higher ionic strength was similar to the values at pH 5.5, both with and without salt. This is in contrast to the secalins, where the presence of salt led to an increase in the adsorbed amount both at pH 4.0 and at pH 5.5 (Figs. 3B and 4). A difference in the isoelectric point of the proteins in the adsorbed layers may explain this observation. The adsorbed gliadins may contain a smaller fraction of proteins that are charged at pH 5.5. The adsorption then becomes less sensitive to the influence of ion concentration and electrostatic screening. The difference in the plateau value between secalin and gliadin at high ionic strength, pH 5.5 could thus be due to a difference in close packing of the proteins at the surface caused by a difference in lateral electrostatic repulsion. However, the discrepancy may also suggest a higher degree of globular structure in the adsorbed secalins.

Sequential addition of different surface active fractions can give rise to several adsorption phenomena. Preferential adsorption of one of the fractions can result either in blocking from further adsorption or in exchange reactions, depending on the order in which the fractions are added. Such behaviors have been found for several proteins and protein fractions (Arnebrant and Nylander 1986, Wannerberger et al 1996, Elofsson et al 1997). Adsorption onto pre-adsorbed layers has also been observed, as in the sequential adsorption of β -lactoglobulin followed by lactoferrin (Wahlgren et al 1993). The arabinoxylan fractions R-PrAX and R-AX behaved similarly in the sequential adsorption to gliadins and secalins at pH 4.0 (Figs. 2 and 3). The increase in adsorbed amount was ≈ 0.25 mg/m², while for the W-AGP fraction, the corresponding increase was 1.4 mg/m² on both secalins and gliadins (Fig. 5). The final adsorbed amount after sequential addition of R-AX and R-Pr-AX is $\approx 2-3$ mg/m². Note that the increase is similar for both these fractions, especially as the adsorption of these fractions to clean silica surfaces varies considerably. The similarity in adsorption behavior of R-PrAX and R-AX at pH 4.0 suggests that the increase resulted from adsorption of arabinoxylans and was not, as could have been expected, affected by the protein content in the R-Pr-AX. Although, it can not be completely ruled out that in the case of R-Pr-AX, there is an exchange between protein and carbohydrates from solution and proteins initially adsorbed to the interface as the final adsorbed levels of R-PrAX and protein after sequential addition of R-PrAX were similar to those for adsorption of this fraction alone to methylated silica surfaces. The substantially higher adsorbed amount observed for W-AGP after sequential addition can thus be related to the covalently bound peptides in the W-AGP fraction (Fig. 5). An even higher increase in adsorbed amount (1.7 mg/m²) were found at pH 5.5 when R-PrAX was sequentially adsorbed to cereal storage proteins (Fig. 2). In contrast, R-AX did not adsorb at all to the gliadin covered surfaces at this pH level (Fig. 3A) and W-AGP only to a small degree, as shown in Fig. 5. The results imply that at the higher pH there is a significant contribution to the adsorption after sequential addition from unbound proteins in the R-PrAX fraction and from the peptide part of W-AGP (Table I). The effect of pH on the adsorption of unbound proteins may be of electrostatic origin in line with the significant adsorption of BSA to secalin under the same conditions. BSA (pI 5.2) is slightly negatively charged at pH 5.5, whereas the net charge of the cereal proteins adsorbed at the surface may well be of the opposite sign (Shewry and Milfin 1985, Lindahl 1990). Similar results were found by Wannerberger et al (1996), who studied the sequential adsorption of gliadins and a water-soluble cereal protein fraction that may be considered comparable to the proteins in the R-PrAX fractions in this study.

An increase in ionic strength fully blocked the adsorption of all the polysaccharide fractions to either of the prolamins, at both pH 4.0 and 5.5 (Figs. 3-5). This confirms the importance of elec-

trostatic interactions in the sequential adsorption at pH 5.5 as suggested above. Furthermore, it also implies that electrostatic interactions are involved in the adsorption of the polysaccharide fractions to preadsorbed protein layers at pH 4.0.

Although the air-water interface differ in several aspects from a methylated silica surface, such as mobility for molecules, surface energy, etc., the interactions observed in this study might very well also influence the behavior of the components at this interface.

CONCLUSIONS

All NSP-fractions studied here, rye arabinoxylan with and without a protein fraction (R-PrAX and R-AX, respectively), and a wheat arabinogalactan peptide (W-AGP), are surface-active as shown by adsorption to a methylated silica surface in aqueous 0.01M acetic acid buffered solution of pH 4.0-5.5. Cereal storage proteins from rye and wheat, secalins and gliadins, displayed similar adsorption behaviors under the same conditions. However, higher adsorbed amounts were found for secalins when the ionic strength was increased. Sequential adsorption of NSP-fractions implied an interaction between cereal storage proteins and both rye arabinoxylans as well as the wheat arabinogalactan peptide. This interaction was independent of the protein content in the polysaccharide fraction and seemed to be of electrostatic origin.

ACKNOWLEDGMENTS

We acknowledge the experimental support from Ly Van Hung. Financial support was obtained from the Swedish Research Council for Engineering Sciences and from the EU (Fair Project CT97-3069).

LITERATURE CITED

- Amadó, R., and Neukom, H. 1985. Minor constituents of wheat flour: The pentosans. Pages 241-251 in: *New Approaches to Research on Cereal Carbohydrates*. R. D. Hill and L. Munck, eds. Elsevier Science: Amsterdam.
- Arnebrant, T., and Nylander, T. 1986. Sequential and competitive adsorption of β -lactoglobulin and k-casein on metal surfaces. *J. Colloid Interface Sci.* 111:529-533.
- Azzam, R. M. A., and Bashara, N. M. 1977. *Ellipsometry and polarized light*. North-Holland: Amsterdam.
- Baker, J. C., and Mize, M. D. 1941. The origin of the gas cell in bread dough. *Cereal Chem.* 18:19-34.
- Bloksma, A. H. 1990. Dough structure, dough rheology, and baking quality. *Cereal Foods World* 35:237-244.
- Cuypers, P. A. 1976. *Dynamic ellipsometry: Biochemical and biomedical applications*. Thesis. Rijksuniversiteit: Limburg, Holland.
- Cuypers, P. A., Corsel, J. W., Janssen, M. P., Kop, J. M. M., Hermens, W. T., and Hemker, H. C. 1983. The adsorption of prothrombin to phosphatidylserine multilayers quantitated by ellipsometry. *J. Biol. Chem.* 258:2426.
- Delcour, J. A., Rouseu, N., and Vanhaesendonck, I. P. 1999. Pilot-scale isolation of water extractable arabinoxylans from rye. *Cereal Chem.* 76:1-2.
- Elofsson, U. M., Paulsson, M. A., and Arnebrant, T. 1997. Adsorption of β -lactoglobulin A and B in relation to self-association: Effect of concentration and pH. *Langmuir* 13:1695-1700.
- Field, J. M., Shewry, P. R., and Milfin, B. J. 1983. Aggregation states of alcohol-soluble storage proteins of barley, rye, wheat and maize. *J. Sci. Food Agric.* 34:362-369.
- Fincher, G. B., and Stone, B. A. 1974. A water soluble arabinogalactan-peptide from wheat endosperm. *Aust. J. Biol. Sci.* 27:117-132.
- Hamer, R. J., and Lichtendonk, W. J. 1987. Structure-function studies on gluten proteins. Reassembly of glutenin proteins after mixing. Pages 227-237 in: *Proc. International Workshop on Gluten Proteins*, 3rd. R. Lasztity and F. Bekés, eds. World Scientific: Singapore.
- He, H., and Hoseney, R. C. 1991. Gas retention of different cereal flours. *Cereal Chem.* 68:334-336.
- Izodarczyk, M., Biliaderis, C. G., and Bushuk, W. 1991. Physical properties of water-soluble pentosans from different wheat varieties. *Cereal Chem.* 68:145-150.

- Lampinen, R. 1995. Newcoming of rye? Pages 9-13 in: International Rye Symposium: Technology and Products. K. Poutanen and K. Autio, eds. VTT Technical Research Centre: Espoo, Finland.
- Lindahl, L. 1990. Rheological properties in wheat flour systems. Molecular and interfacial basis. Thesis. Lund University: Lund, Sweden.
- Loosveld, A., Grobet, P. J., and Delcour, J. A. 1997. Contents and structural features of water extractable arabinogalactan in wheat flour fractions. *J. Agric. Food Chem.* 45:1998-2002.
- Loosveld, A., Maes, C., van Casteren, W. H. M., Schols, H. A., Grobet, P. J. and Delcour, J. A. 1998. Structural variation and levels of water-extractable arabinogalactan-peptide in European wheat flours. *Cereal Chem.* 75:815-819.
- McCrackin, F. L., Pasaglia, E., Stromberg, R. R., and Steinberg, H. L. 1963. Measurement of the thickness and refractive index of very thin films and optical properties of surfaces by ellipsometry. *J. Res. Natl. Bur. Stand.-A* 67:363-377.
- Norde, W. 1986. Adsorption of proteins from solution at the solid-liquid interface. *Adv. Colloid Interface Sci.* 25:267-340.
- Sarker, D. K., Wilde, P. J., and Clark, D. C. 1998. Enhancement of protein foam stability by formation of wheat arabinoxylan-protein crosslinks. *Cereal. Chem.* 75:493-499.
- Shewry, P. R. and Mifflin, B. J. 1985. Seed storage proteins of economically important cereals. Pages 1-83 in: *Advances in Cereal Science and Technology*. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Shewry, P. R., Parmar, S., and Mifflin, B. J. 1983. Extraction, separation, and polymorphism of the prolamin storage proteins (secalins) of rye. *Cereal Chem.* 60:1-6.
- Wahlgren, M. C., Arnebrant, T., and Paulsson, M. A. 1993. The adsorption from solutions of β -lactoglobulin mixed with lactoferrin or lysozyme onto silica and methylated silica surfaces. *J. Colloid Interface Sci.* 158:46-53.
- Wannerberger, L., Eliasson, A.-C., and Sindberg, A. 1997. Interfacial behaviour of secalin and rye flour-milling streams in comparison with gliadin. *J. Cereal Sci.* 25:243-252.
- Wannerberger, L., Wahlgren, M., and Eliasson, A.-C. 1996. Adsorption of protein fractions from wheat onto methylated silica surfaces. *Cereal Chem.* 73:499-505.
- Weegels, P. L., Marseille, J. P., and Voorpostel, A. M. B. 1991. Enzymes as a processing aid in the separation of wheat into starch and gluten. Pages 199-203 in: *Gluten Proteins 1990*. W. Bushuk and R. Tkachuk, eds. Am. Assoc. Cereal Chem.: St Paul, MN.

[Received December 20, 1999. Accepted June 26, 2000.]