

Influence of Degree of Protein Aggregation on Mass Transport Through Wheat Gluten Membranes and Their Digestibility—An In Vitro Study

Sandra Domenek,¹ Lothar Brendel,² Marie-Hélène Morel,¹ and Stéphane Guilbert^{1,3}

ABSTRACT

Cereal Chem. 81(3):423–428

The influence of the network structure of wheat gluten on the barrier properties against enzymes was investigated in vitro. The changes in the network structure were introduced by different temperature treatments. The modifications were assessed with solubility studies of wheat gluten proteins in sodium dodecyl sulfate (SDS). The physical barrier properties of wheat gluten membranes were investigated with transport studies examining the transfer of a model protein with no enzymatic activity (BSA) through gluten membranes. The protein network was an effective barrier for BSA, although lightly cross-linked films were mechanically instable. Membrane breaks occurred in function of the cross-linking density (per-

centage of SDS-insoluble proteins) after only 24 hr for lightly cross-linked films ($\approx 30\%$ SDS-insoluble proteins), while highly cross-linked membranes ($\approx 80\%$ SDS-insoluble protein) were tight up to more than 33 days. The digestion experiments of the gluten films with pepsin showed that the hydrolysis of wheat gluten films with $>72\%$ of SDS-insoluble protein was significantly retarded. In conclusion, technological treatments to increase the cross-linking density of gluten have the potential to slow the digestion of cereal-based foodstuff and to reduce the degradation rate of composite biomaterials.

Structure stability and enzyme barrier properties of composite wheat gluten-based biomaterials are of great interest for both food and nonfood applications. In cereal-based food, products with low glycemic response have received increasing interest because the decrease of the blood glucose response of foodstuff has beneficial metabolic effects (Bornet et al 1997; Jenkins et al 2000). For example, it is known that due to the continuous but highly structured wheat gluten matrix in pasta, the release of the dispersed carbohydrate phase during digestion is retarded (Colonna et al 1990), which leads to a low but sustained postprandial glucose response (Granfeldt and Björck 1991). This mechanism allows a low insulinemic index (Bornet et al 1997), which is particularly beneficial for the dietary management of diabetes and other metabolic diseases (Bornet et al 1997; Jenkins et al 2000). Variations of the gluten thermosetting parameters of the fabrication process control the starch accessibility and enzymatic degradation in cereal-based foods (Holm and Björck 1988; Colonna et al 1990; Björck et al 1994; Fardet et al 1999). In the field of nonfood composite materials based on wheat gluten, the problem is very similar. Gluten bioplastics are promising biodegradable materials (Cuq et al 1998; Domenek et al 2004). Combination with fillers or fibers is often proposed to reduce the cost or to improve the mechanical properties of the material, as well as to control the material's degradability. In this work, we focus on the role of the protein network on the accessibility for digestive enzymes. More specifically, we investigate the protein network's physical barrier properties and its biochemical susceptibility to digestion by a protease. To examine the former aspect, we conducted transport experiments where the transfer of a model molecule through a gluten membrane was measured. For the investigation of the latter, gluten samples were subjected to proteolytic digestion. In both cases, the findings were related to the gluten structure with the quantification of the fraction of gluten proteins, which are insoluble in sodium dodecyl sulfate (SDS) buffer. This quantity reflects the protein network's cross-link density (Redl et al 1999a,b; Domenek et al 2002; Redl et al 2003). It was varied by applying different temperature treatments

to samples (Weegels et al 1994a,b; Weegels and Hamer 1998; Domenek et al 2002), which actually appear in the industrial fabrication process of food or nonfood wheat-based materials (Autran et al 1989; Abecassis et al 1994; Icard-Vernière and Feillet 1999).

MATERIALS AND METHODS

Vital wheat gluten was provided by Amylum (Aalst, Belgium). Protein, starch, lipid, and ash quantities were 76.5, 11.8, 5.0, and 0.8% of dry mass, respectively. Moisture content was 7.2% (wet mass basis). Chemicals were purchased from Sigma (St. Louis, MO).

Gluten Sample Preparation and Analysis

Wheat gluten was equilibrated for four days at 100% rh and 20°C, thereafter the average water content was $19.40 \pm 0.11\%$ (wet mass basis). The wet gluten powder (3 g) was hot-molded under a heating press between two Teflon sheets (Techmo, Nazelles-Negrin, France) for 10 min at 150 bar with press temperatures of 70, 80, 90, 100, and 120°C. The resulting films were immediately cooled to 0°C and stored at -28°C in a commercial freezer. The film thickness measured with a hand-held micrometer (Braive Instruments, Checy, France) was 0.25 ± 0.03 mm irrespective of pressing temperature (average of five measurements per film). The different films were labeled with their respective press temperature followed by lower case characters to distinguish repeat experiments.

Size-Exclusion HPLC

The samples (0.8 ± 0.005 g) were ground in a laboratory ball mill (Prolabo, France) under liquid nitrogen for 1 min. The frozen powder was blended with 4 ± 0.05 g of unmodified wheat starch (Sigma) and ground for an additional 2 min. The resulting gluten/starch blend was subjected to size exclusion-HPLC analysis. The gluten samples were analyzed according to Domenek et al (2002). The ground gluten and starch blend (160 mg) was agitated for 80 min at 60°C in 20 mL of 0.1 mol/L of sodium phosphate buffer (pH 6.9) containing 1% SDS and subsequently centrifuged (Beckmann Avanti J30-I, 37,000 \times g, 30 min). The supernatant contained the SDS-soluble protein fraction (F_s). The SDS-insoluble protein fraction (F_i) was extracted with 5 mL of SDS-phosphate buffer containing 20 mmol/L of dithioerythriol (DTE) for 60 min at 60°C and tip-sonicated to bring the gluten proteins into solution. After centrifugation (37,000 \times g, 30 min), the supernatant was mixed 1:1 with SDS-phosphate buffer containing 40 mmol/L of iodoacetamide (IAM). IAM alkylated the thiol groups which prevented their subsequent oxidation. Both extracts (F_s and F_i , 20 μ L) were submitted to SE-HPLC fractionation.

¹ Laboratory of Cereal Technology and Agropolymers, ENSA.M-INRA, 2 place Viala, 34060 Montpellier Cedex 1, France.

² Present address: Theoretische Physik, Universität Duisburg-Essen, 47048 Duisburg, Germany.

³ Corresponding author. Phone: +33 (0)4 99 61 28 31. Fax: +33 (0)4 67 52 20 94. E-mail: guilbert@ensam.inra.fr

SE-HPLC analysis was conducted on a TSK-G 4000 SW XL (TosoHaas) size-exclusion analytical column (7.5 × 300 mm) preceded by a TSK 3000-SW XL (TosoHaas) guard column (7.5 × 75 mm). The columns were eluted at room temperature with a 0.1 mol/L of sodium phosphate buffer (pH 6.9) containing 0.1% SDS. The flow rate was 0.7 mL/min and proteins were detected at 214 nm. The column was calibrated with protein standards according to Dachkevitch and Aufran (1989).

Digestion Experiments

To prepare the gluten samples for the proteolytic digestions, the frozen samples (4 g) were ground in the laboratory ball mill under liquid nitrogen for 2 min. The samples were stored over P₂O₅ at room temperature. For the enzymatic hydrolysis, the gluten powder (10 mg) was preincubated for 20 min at 37°C in 1 mL of phosphate-citrate buffer (0.153 mol/L, pH 5.5) including 160 μL of

HCl (0.1 mol/L). The sample was adjusted to pH 2 with 3M HCl. Pancreatic pepsin (2% in 0.1 mol/L of HCl) was added to achieve an enzyme to substrate (E/S) ratio of 6. The hydrolysis was conducted for 20 min at 37°C. The reaction was stopped by the addition of trichloroacetic acid (TCA 97.7% [Acros, France], final concentration 12%) and subsequent heating at 75°C for 10 min. The samples were centrifuged for 45 min at 20,817 × g and 10°C. The extent of hydrolysis was calculated from the measurement of free amino acids released, detected with the ninhydrin reaction (Prochazkova et al 1999). The supernatant was diluted three times with TCA (12%). An aliquot of the supernatant (40, 60, or 80 μL) was adjusted to 200 μL with TCA (12%) and to 1 mL with water and submitted to the ninhydrin reaction. The absorbance of the reaction solution was read at 570 nm (Ultraspec 2000, Pharmacia Biotech, Cambridge, UK). The slope and the intercept of the linear regression curve of the measured absorbance against the volume of the digestion solution (initial supernatant volume of 40, 60, or 80 μL) was calculated. The slope was used to compute the percentage of liberated amino acids as compared with the total number of peptide bonds in the sample, using the molar extinction coefficient of ninhydrin-α-amino groups obtained from calibration curves prepared with leucine ($\epsilon_{\text{Leucine}} = 22,785 \text{ L/mol/cm}$)

$$\frac{aa_{\text{liberated}}}{aa_{\text{total}}} = \frac{\text{slope} ([V_{\text{reaction}} \times V_{\text{digestion}} \times \text{dilution}] / [m_{\text{sample}} \times \epsilon_{\text{leucine}}]) M_r (\text{leucine}) \times 100}{1} \quad (1)$$

where $aa_{\text{liberated}}$ is the quantity of liberated amino acids, aa_{total} the total quantity of peptide bonds, V the reaction volume of the reaction solution, and the digestion solution; m_{sample} is the initial sample mass; and M_r is the molar weight of leucine. Each digestion experiment was repeated 10 times. Variance analysis was performed using Statgraphics software (Manugistics, Rockville, MD).

BSA Transport Measurement

The measuring apparatus is shown in Fig. 1. It consists of two glass compartments with different volumes. Subscript 1 will denote quantities referring to the donor compartment ($V_1 = 50 \text{ mL}$) and subscript 2 will denote quantities referring to the receiver compartment ($V_2 = 750 \text{ mL}$). The receiver compartment was thermoregulated with a double-wall jacket at 20°C linked to a cryostat. The gluten membranes were fixed to the donor compartment, which in turn was immersed in the receiver solution. The diameter of the effective aperture was 35 mm. Both compartments were stirred independently; donor compartment with an impeller stirrer (Ika RW 11 basic) and receiver compartment with a magnetic stirrer.

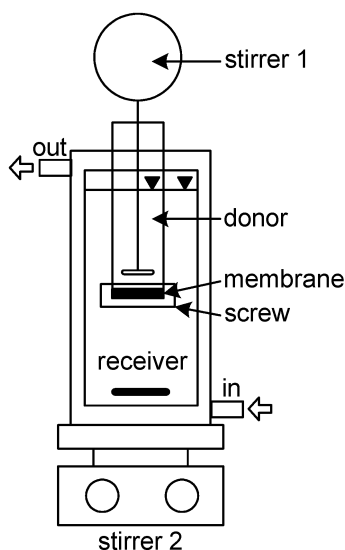


Fig. 1. Scheme of diffusion cell. Receiver compartment ($V_2 = 750 \text{ mL}$) is thermoregulated with a double-wall jacket. Donor compartment ($V_1 = 50 \text{ mL}$) is immersed in the receiver compartment. Both compartments are stirred independently with an impeller stirrer (stirrer 1) and a magnetic stirrer (stirrer 2). The membrane is fixed between two Teflon seals and tightened with a screw.

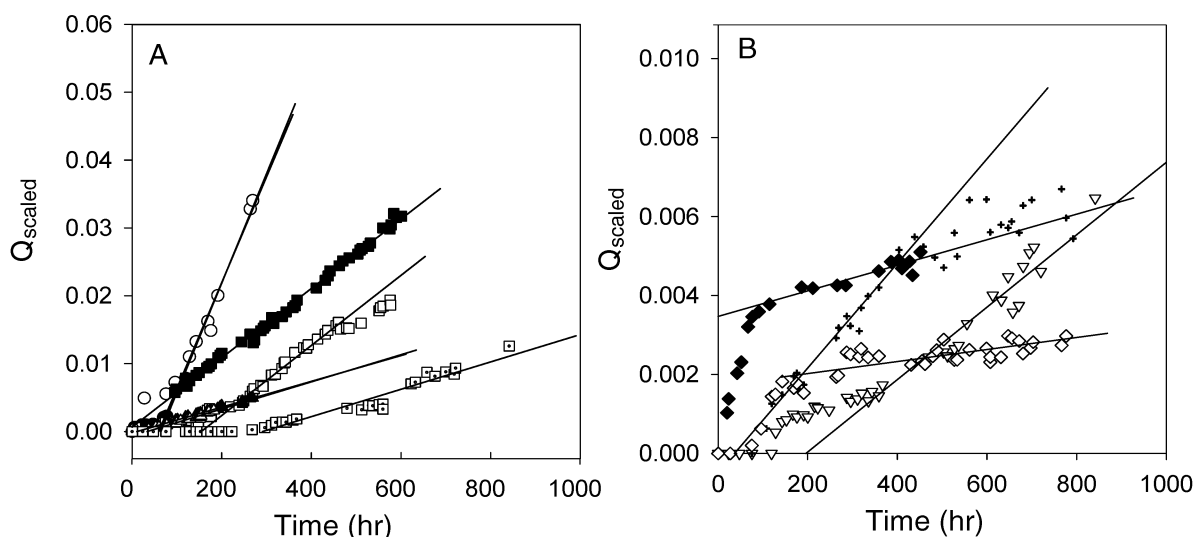


Fig. 2. Scaled BSA concentration in receiver compartment. Curve labels contain preparation temperature, lower case letters distinguish experiments performed with gluten membranes pressed at the same temperatures: 70°C (○), 80°C-a (□), 80°C-b (■), 80°C-c (▲), 80°C-d (△), 80°C-e (◻), 80°C-f (▽), 100°C (+), 120°C-a (◆), 120°C-b (◇).

The phosphate buffer (5 mmol/L of sodium phosphate buffer, 3 g/L of NaN_3 , pH 6.8–6.9) was equilibrated in the measuring apparatus overnight at 20°C. The membrane was separately equilibrated in the phosphate buffer overnight at room temperature (22.5°C). The thickness of the swollen films was measured with a hand-held micrometer (Braive Instruments, 5 repeats). Then membrane disks were cut from the swollen film and fixed between the two compartments. The donor compartment was filled with buffer solution (45 mL), and the transport experiment was started by the addition of 5 mL of concentrated bovine serum albumin (BSA) solution (1 mmol/L), which gave an initial concentration difference of 100 $\mu\text{mol/L}$. Two control samples were taken immediately to examine the exact start concentrations and to check the membrane tightness. The sample volume was replaced with the phosphate buffer to maintain the total volume constant. Control experiments without BSA were conducted on gluten membranes treated at 80°C.

BSA Quantification with Reversed-Phase HPLC

The BSA concentration in the receiver compartment was monitored by RP-HPLC using a Waters system (LC Module Iplus, equipped with a Waters autosampler and a Waters in-line degasser) controlled by Millennium software (Waters). A Waters Delta Pak C 18 column (3.9 × 300 mm, 15 mm particle size, 300 Å pore size) was employed, preceded by a Waters Nova Pak C 18 guard pack. The column was maintained at 35°C. The flow rate was 0.5 mL/min. RP-HPLC solvent A was an aqueous solution of 0.1% TFA (HPLC grade) and solvent B was a solution of 0.08% TFA in acetonitril (HPLC grade). The solvents were filtered through a 0.45-mm Milipore filter. The column was equilibrated with 35% solvent B and eluted with a gradient that increased to 90% solvent B at 22 min, where it was held for 4 min before being returned to 35% B for 22 min to reequilibrate the column. The detector wavelength was set to 214 nm.

RESULTS AND DISCUSSION

Transport Experiments

The results of BSA transport experiments are shown in Fig. 2, where the increase of the BSA quantity is plotted versus time. To ease readability, the plot is subdivided into two parts that have different scales on the ordinate. The BSA quantity is scaled with respect to initial concentration in the donor compartment

$$Q_n = Q_2 \cdot V_1 / Q_1 \cdot V_2 \quad (2)$$

for easier comparison. Following the scaling procedure, the maximum concentration in the receiver compartment is

$$V_1 / (V_1 + V_2) = 1/16 \quad (3)$$

A feature common to the curves belonging to samples with a SDS-insoluble protein fraction $F_i \leq 41\%$ (all curves in Fig. 2A

and 80°C-f in Fig. 2B) is the presence of two regimes, after an initial lag-phase, the behavior of which differs for each sample, the increase of concentration is well approximated by a constant relationship. In principle, these features are characteristic for a diffusive transport where the lag-phase corresponds to the time to reach a quasi-stationary linear concentration gradient within the membrane (Crank 1975; Amsden 1998). However, the interpretation of diffusive transport cannot be applied to the samples with $F_i \geq 41\%$ (Fig. 2B), where the BSA concentration increase results virtually in a saturation for later times, although the concentration is still small compared with the equilibrium value. Additionally, while the rates of the BSA increase in the receiver compartment are rather reproducible for samples with similar F_i , the lag-phase is not. This can be seen in Fig. 2A, where the lag-phase is not observable for $F_i = 35\%$ (80°C-a); whereas in the duplicate experiment it is observed at 150 hr (80°C-b); and in the repeat experiment (80°C-e) no transport at all is observed for 240 hr.

Close examination of the BSA-concentration curves revealed that the transport was not of diffusive type. It can be shown (see Appendix) that for diffusive transport, the product of lag-time and the slope of the linear part of the concentration curve cannot exceed 0.115. This constraint is violated for almost all experiments. To further examine the velocity of the transport phenomenon, we interpreted (notwithstanding the argument given above) the reproducible concentration evolution of BSA in the receiver compartment as if it were of diffusive origin. Doing this, we can compute an apparent diffusion coefficient D' by evaluating the slope of the linear part (see Appendix). The resulting values, which are shown in Table I together with F_i , are of same order of magnitude as D_0 , the diffusion coefficient for BSA in water, which amounts to $6.77 \cdot 10^{-11} \text{ m}^2/\text{sec}$. This would imply either a partition coefficient (K) being much larger than unity (see Appendix) or a diffusive transport phenomenon essentially unaffected by the presence of the membrane. Neither interpretation is plausible when compared with the results of Fardet et al (1998), who found $K = 0.45$ and a decrease of the diffusion constant of 50% in a very lightly cross-linked gluten network.

Consequently, the quite fast BSA transport indicated the presence of larger openings in the membrane due to the apparition of membrane damages. The observation of significant lag-phases excludes their preexistence. For example, for $F_i > 41\%$, the experiment duration of 800 hr was insufficient to observe damage for all samples (data not shown because of persistently zero concentration). The observed apparent diffusion coefficient D' being higher or in the same order of the BSA diffusivity in water evidences the existence of a convective transport, which could be due to the stirring, leading to such a virtually stationary hydrodynamic state. Moreover, there were cases where a transport occurred only temporarily (Fig. 2B, curves 100°C, 120°C-a, and 120°C-b), which might be explained by the effect of clogging, which in turn suggests channel-like damages.

TABLE I
Barrier Properties of Heat-Treated Wheat Gluten Films in Function of Degree of Protein Aggregation (F_i)

Preparation Temperature ^a	F_i (%) ^b	Membrane Thickness (mm)	Slope Transport Rate ^c	D' ^d
Film 70°C	28 ± 1	1.10 ± 0.10	6.33	8.69
Film 80°C-a	35 ± 2	0.53 ± 0.04	14.3	9.50
Film 80°C-b	35 ± 2	0.53 ± 0.02	14.2	9.34
Film 80°C-c	37 ± 2	0.52 ± 0.02	5.56	3.61
Film 80°C-d	37 ± 2	0.50 ± 0.03	5.28	3.29
Film 80°C-e	37 ± 2	0.52 ± 0.05	5.61	3.64
Film 80°C-f	41 ± 2	0.49 ± 0.05	2.56	1.56
Film 100°C	68 ± 3	0.29 ± 0.01	3.86	1.34
Film 120°C-a	86 ± 3	0.22 ± 0.03	0.898	0.25
Film 120°C-b	87 ± 3	0.28 ± 0.06	0.422	0.15

^a Lower case letters distinguish experiments.

^b F_i degree of protein aggregation.

^c $10^{-9} \times \text{sec}$.

^d Apparent diffusion constant ($10^{-11} \times \text{m}^2 \times \text{sec}$).

An interesting feature of the velocity of BSA transport through the membranes can be seen in Table I. Although the F_i changed slightly between the different experimental runs, the BSA transport rate of parallel experiments was remarkably reproducible. The change of F_i seems to be related to a change in the gluten powder, which showed an increased cross-linking degree with storage time. Due to the long run times of the transport experiments (>1 month) the analysis of sample series extended over approximately one year. The 80°C films served as the control experiment all along the working time. The films 80°C-a and 80°C-b were prepared and analyzed at the beginning; 80°C-c, 80°C-d, and 80°C-e were prepared and analyzed at half-time course; and 80°C-f was the last experiment. The reproducibility of the transport, although not of diffusive type, might indicate the presence of a characteristic damage size due to an intrinsic length scale, which in turn is

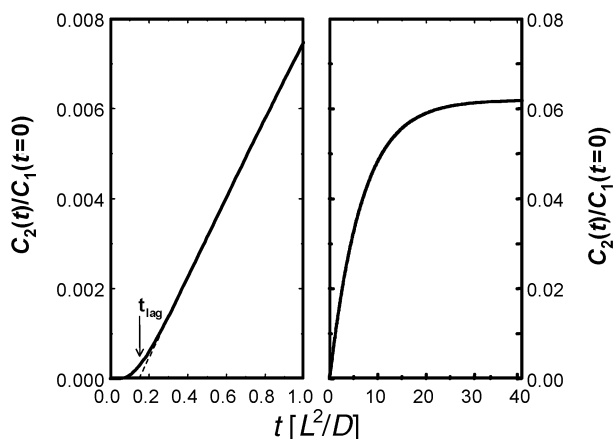


Fig. 3. Temporal evolution of the concentration at the interface between membrane and receiving chamber for early (left) and later times (right). Tangent (dashed line) fitted to the linear part defines the time t_{lag} (as intersection with the abscissa) and together with its slope s is used to determine the unknowns D and β (and thus K). Solution was obtained numerically for parameters $\beta = 0.1$ and $\nu = 15$.

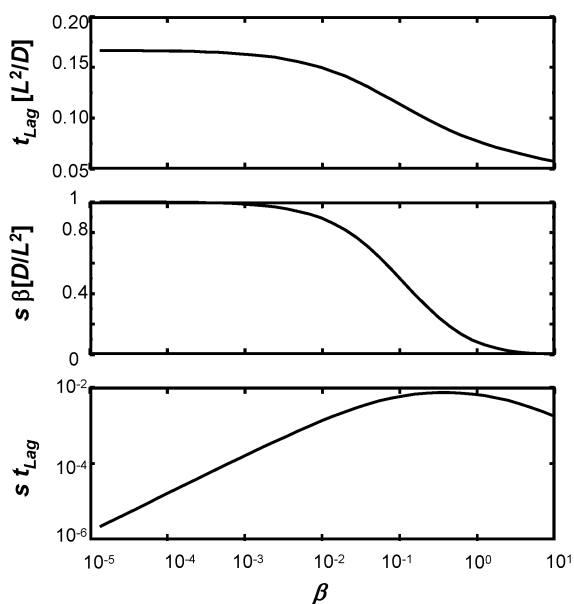


Fig. 4. Tangent fitted to the linear part of $C_2(t)/C_1(t = 0)$ (see Fig. 3) defines time lag t_{lag} (top) as intersection with the abscissa and possesses the slope s (middle). In the limit of small β , $t_{lag}D/L^2$ approaches $1/6$ while sL^2/D becomes β . Exact factors decrease for larger β , which corresponds to $\nu = 15$. The (dimensionless) product $s t_{lag}$ (bottom) depends solely on β and can thus be used to identify it (provided the product does not exceed ≈ 0.0072 , in which case the model does not apply).

given by the degree of cross-linking. Furthermore, this indicates that the resistance of the gluten network to the membrane damage plausibly increased for higher cross-link densities, but the statistics provided by the data do not allow for a precise quantitative evaluation of the damage probability.

The physical evolution of the wheat gluten membrane during long-time immersion was additionally evidenced by the finding that the F_i of membranes that were subjected to the diffusion experiment for more than 30 days was significantly reduced. The F_i of membrane 80°C-e (41%) diminished to 33% after the experiment, and the F_i of the membranes used in the control experiment from $F_i = 38$ diminished 37% to 28 and 23% respectively. The decrease of the F_i was mainly linked to an increase in the solubility of large protein aggregates. We suggest that the cleavage of the network chains might be assigned in part to the action of proteases present in wheat gluten. Due to the well-known thermal stability of proteases, they might not have been fully inactivated during the initial film pressing. The inactivation would be more important for higher temperature treatments. As a second effect, during the long hydration time, noncovalent bonds between the proteins in the gluten network might open, resulting in additional solubilization of proteins.

Digestion Experiments

The results of the enzymatic hydrolysis of the wheat gluten samples with pepsin are given in Table II. The variance analysis performed on the data set classed the samples into two statistically different groups at the 95% confidence level. The enzymatic degradation of the gluten membranes was slightly retarded by increasing cross-linking degrees. The hindrance became significant at cross-linking degrees higher than $\approx 72\%$. Pepsin is an aspartic protease with a broad specificity. Hence, a slowdown of the enzymatic reaction due to conformational changes may be unlikely. Because pepsin is an endoprotease (cleaving within protein chains), we hypothesize that more cuts might be needed to liberate peptides from the highly cross-linked polymer network. Garcia-Rodenas et al (1994) investigated the enzymatic digestion of cast wheat gluten-casein films. They found no significant difference between film and native material. Because the casting technique does not involve severe heat-treatments of the film (Garcia-Rodenas 1994; Gontard et al 1996) and will thus cause no high F_i values (Domenek et al 2004), this finding underlines the present result that modifications in the digestion rates were only obtained in the high F_i range.

CONCLUSIONS

The investigation of the influence of the protein aggregation on the digestibility of gluten materials was made with a double approach. First, the physical accessibility of the protein network was studied with transport experiments using a model protein. Second, the enzymatic digestibility of the protein network was assessed with essays in vitro. For possible further studies with

TABLE II
Hydrolysis (%) of Gluten Film Samples Heat-Treated at Different Temperatures^a

Sample	F_i ^b (%)	Hydrolysis ^c (%)	σ (%) ^d
Gluten	16	7.03a	0.23
Film 70°C	31	7.11a	0.24
Film 80°C	42	6.69a	0.24
Film 90°C	52	6.43a,b	0.24
Film 100°C	72	6.53a	0.27
Film 120°C	90	5.77b	0.23

^a Multiple range test for hydrolysis in function of the treatment temperature.

^b F_i degree of protein aggregation.

^c Values followed by a and b are statistically different at $P < 95\%$.

^d Standard deviation (10 repeats).

experiments using gluten-based composite materials, where technological modifications that influence F_i are applied, we judge three factors responsible for a possible dependence of the degradation or digestion on F_i .

1. The diffusion of large molecules through the protein matrix itself does not play a role because the matrix can be considered as impermeable for enzymes.

2. Mechanical damage is responsible for the transport of the model protein. However, the mechanical stability of the membrane can be significantly improved by the cross-link density.

3. Cross-linking degrees above F_i values of 72% significantly slow the enzymatic degradation of the protein matrix.

Technological modifications of the wheat gluten protein network have, therefore, the potential to decrease the degradation rate of foodstuff or composite materials, provided that the filler is surrounded by the protein matrix. To fabricate new cereal-based foodstuffs with reduced glycemic index, an optimum has to be looked for where the continuous gluten matrix that envelops the starch granules (regarded as filler) keeps both its barrier properties due to high cross-linking degrees and its elasticity to prevent breaks in the matrix during starch granule swelling. In biodegradable composite materials, we might achieve both higher mechanical stability and enhanced barrier properties of the gluten matrix due to increased cross-linking degrees, which are in turn attainable by controlled heat-treatments of the material.

LITERATURE CITED

- Abecassis, J., Abbou, R., Chaurand, M., Morel, M.-H., and Vernoux, P. 1994. Influence of extrusion conditions on extrusion speed, temperature, and pressure in the extruder on pasta quality. *Cereal Chem.* 71:247-253.
- Amsden, B. 1998. Solute diffusion in hydrogels. An examination of the retardation effect. *Polym. Gels Networks* 6:13-43.
- Autran, J., Ait-Mouh, O., and Feillet, P. 1989. Thermal modification of gluten as related to end-use properties. Pages 563-593 in: *Wheat is Unique: Structure, Composition, Processing, End-Use Properties and Products*. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Björck, I., Granfeldt, Y., Liljeberg, H., Tovar, J., and Asp, N. 1994. Food properties affecting the digestion and absorption of carbohydrates. *Am. J. Clin. Nutr.* 59:S699-S705.
- Bornet, F., Billaux, M., and Messing, B. 1997. Glycaemic index concept and metabolic diseases. *Int. J. Biol. Macromol.* 21:207-219.
- Colonna, P., Barry, J.-L., Cloarec, D., Bornet, F., Gouilloud, S., and Galmiche, J.-P. 1990. Enzymic susceptibility of starch from pasta. *J. Cereal Sci.* 11:59-70.
- Crank, J. 1975. *The Mathematics of Diffusion*, 2nd Ed. Oxford Science Publication Clarendon Press: Oxford.
- Cuq, B., Gontard, N., and Guilbert, S. 1998. Proteins as agricultural polymers for packaging production. *Cereal Chem.* 75:1-9.
- Dachkevitch, T., and Autran, J. 1989. Prediction of baking quality of bread wheat in breeding programs by size-exclusion high performance liquid chromatography. *Cereal Chem.* 66:448-456.
- Domenek, S., Morel, M.-H., Bonicel, J., and Guilbert, S. 2002. Polymerization kinetics of wheat gluten upon thermosetting: A mechanistic model. *J. Agric. Food Chem.* 50:5947-5954.
- Domenek, S., Feuilletoy, P., Gartraud, J., Morel, M.-H., and Guilbert, S. 2004. Biodegradability of wheat gluten based biomaterials. *Chemosphere* 54:551-559.
- Fardet, A., Hoebler, C., Djelveh, G., and Barry, J. 1998. Restricted bovine serum albumin diffusion through protein network of pasta. *J. Agric. Food Chem.* 46:4635-4641.
- Fardet, A., Abecassis, J., Hoebler, C., Baldwin, P., Buléon, A., Bérot, S., and Barry, J.-L. 1999. Influence of technological modifications of protein network from pasta on in vitro starch degradation. *J. Cereal Sci.* 30:133-145.
- Garcia-Rodenas, C., Cuq, J., and Aymard, C. 1994. Comparison of in vitro proteolysis of casein and gluten as edible films or as untreated proteins. *Food Chem.* 51:275-280.
- Gontard, N., Thibault, R., Cuq, B., and Guilbert, S. 1996. Influence of relative humidity and film composition on oxygen and carbon dioxide permeabilities of edible films. *J. Agric. Food Chem.* 44:1064-1069.
- Granfeldt, Y., and Björck, I. 1991. Glycemic response to starch in pasta: A study of mechanisms of limited enzyme availability. *J. Cereal Sci.* 14:47-61.
- Holm, J., and Björck, I. 1988. Effects of thermal processing of wheat on starch. II. Enzymic availability. *J. Cereal Sci.* 8:261-268.
- Icard-Vernière, C., and Feillet, P. 1999. Effects of mixing conditions on pasta dough development and biochemical changes. *Cereal Chem.* 76:558-565.
- Jenkins, D., Axelsen, M., Kendall, C., Augustin, L., Vuksan, V., and Smith, U. 2000. Dietary fiber, lente carbohydrates and the insulin-resistant diseases. *Brit. J. Nutr.* 83:S157-S163.
- Prochazkova, S., Varum, K., and Ostgaard, K. 1999. Quantitative determination of chitosans by ninhydrin. *Carbohydr. Res.* 320:82-92.
- Redl, A., Morel, M.-H., Bonicel, J., Guilbert, S., and Vergnes, B. 1999a. Rheological properties of gluten plasticized with glycerol: Dependence on temperature, glycerol content and mixing conditions. *Rheol. Acta* 38:311-320.
- Redl, A., Morel, M.-H., Bonicel, J., Vergnes, B., and Guilbert, S. 1999b. Extrusion of wheat gluten plasticized by glycerol: Influence of process conditions on flow behavior, rheological properties and molecular size distribution. *Cereal Chem.* 76:361-370.
- Redl, A., Morel, M.-H., and Guilbert, S. 2003. Heat and shear mediated polymerization of plasticized wheat gluten protein upon mixing. *J. Cereal Sci.* 38:105-114.
- Weegels, P., and Hamer, R. 1998. Temperature-induced changes of wheat products. Pages 95-130 in: *Interactions: The Keys to Cereal Quality*. R. Hamer and R. Hoseney, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Weegels, P., Verhoek, J., deGroot, A., and Hamer, R. 1994a. Effects on gluten of heating at different moisture contents. I. Changes in functional properties. *J. Cereal Sci.* 19:31-38.
- Weegels, P., Verhoek, J., deGroot, A., and Hamer, R. 1994b. Effects on gluten of heating at different moisture contents. II. Changes in physico-chemical properties and secondary structure. *J. Cereal Sci.* 19:39-47.

[Received April 28, 2003. Accepted November 17, 2003.]

APPENDIX

List of Abbreviations

F_i , percentage of SDS-insoluble gluten protein fraction
 C_1 resp. C_2 , BSA concentration in the donor respectively receiver compartment
 Q_1 resp. Q_2 , BSA quantity in the donor respectively receiver compartment
 Q_n , scaled BSA quantity
 V_1 resp. V_2 , buffer volume in the donor respectively receiver compartment
 L , membrane thickness
 K , partition coefficient
 A , membrane area
 D , diffusion coefficient of BSA
 D' , apparent diffusion coefficient of BSA
 D_0 , diffusion coefficient of BSA in water (20°C)

Theoretical Considerations

For the calculation of the apparent diffusion coefficient of BSA within the gluten membrane, we define $C(x)$ being the concentration profile within the membrane ($x \in [0...L]$ counted downstream) and K being the partition coefficient, defined as the ratio of the concentration at the membrane's surfaces and the bulk concentration in the corresponding chamber

$$C(x=0) = KC_1 = K \frac{Q_1}{V_1} \text{ and } C(x=L) = KC_2 = K \frac{Q_2}{V_2} \quad (1)$$

The parameters $Q_1(t=0)$, V_1 , V_2 , L and A are known; D and K are sought. To simplify the expression for the boundary conditions we use the scaled concentration

$$u(x) = C(x) \frac{V_1 + V_2}{Q} \quad (2)$$

where $Q \equiv Q_1(t=0)$ is the total amount of material [because $Q_2(t=0) = 0$]. This does not affect the relationship

$$\frac{C_2(t)}{C_1(t=0)} = \frac{C(x=L,t)}{C(x=0,t=0)} = \frac{u(x=L,t)}{u(x=0,t=0)} \quad (3)$$

useful for the comparison to the experimental data. Furthermore, we choose L as unit of length and L^2/D as unit of time, which yields the dimensionless diffusion equation

$$\frac{\partial u}{\partial t} = \frac{\partial^2 u}{\partial x^2} \quad (4)$$

together with the boundary conditions

$$u(x=0,t) = 1 + v \left(1 - \beta \int_0^t \partial_x u(x=0,t') dt' \right) \quad (5)$$

$$u(x=1,t) = \beta \int_0^t \partial_x u(x=1,t') dt' \quad (6)$$

with

$$v \equiv \frac{V_2}{V_1} \quad (7)$$

being the relative volume of the receiving chamber and

$$\beta \equiv K \frac{LA}{V_2} \quad (8)$$

the membrane's relative effective volume. Constant concentration in the donor chamber ($v=0$) is a well known problem and can be solved analytically (Crank 1975), yielding the somewhat unhandy expression for the concentration at the receiver interface

$$u(x=1) = 2\beta \sum_{n=0}^{\infty} \frac{\alpha_n^2 + \beta^2}{\alpha_n^2 + \beta^2 + \beta} \frac{\cos \alpha_n}{\alpha_n^2} (1 - \exp(-\alpha_n^2 t)) \quad (9)$$

where the coefficients α_n are the positive roots of

$$\alpha_n \tan \alpha_n = \beta \quad (10)$$

To discuss $v > 0$ (more specifically, $v = 15$ with our experimental set-up), we revert to numerically obtained solutions. Typically, for a membrane volume that is negligible compared with the chambers and a partition coefficient of order unity, the parameter β is rather small. In this case, it has the obvious meaning of separating two time scales in the problem: short time (order of unity) it takes to build up a quasi-linear concentration profile within the membrane, and long time (of order $1/\beta$) it takes to reach equal concentration in both chambers. The transition between the two regimes is marked by a concentration rising linearly in time and fitting a tangent there as shown in Fig. 3, can be used to determine the unknowns D and β (and thus K): tangent's intersection with the abscissa, denoted t_{Lag} as definition of the short time scale, amounts to $L^2/(6D)$ in the limit of very small β while slope s equals $\beta D/L^2$. For larger β , the factors $1/6$ and β change and can be read in Fig. 4. To identify the value of β for a given tangent in the first place, one uses the (dimensionless) product of the slope and the time lag $s \times t_{\text{Lag}}$, assuming β is not unreasonably large enough to have a unique relationship.