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Gluten Protein Composition in Several Fractions Obtained by Shear Induced Separation of Wheat Flour

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Recently, it was found that applying curvilinear shear flow in a cone−cone shearing device to wheat flour dough induces separation, resulting in a gluten-enriched fraction in the apex of the cone and gluten-depleted fraction at the outer part. This article describes whether fractionation of the various proteineous components occurs during and after separation of Soissons wheat flour. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and size-exclusion high performance liquid chromatography (SE-HPLC) were found to be suitable techniques for this. It is concluded that all protein fractions migrate to the center of the cone as a result of which the composition of the gluten-enriched fraction remains rather similar to that in the original flour. However, the larger glutenin polymer fraction migrated faster, as a result of which the concentration of large polymers was increased with a factor 2.4 compared to that of Soissons flour. The concentration of monomers in the gluten-enriched fraction was decreased to 70% of the original concentration in the original wheat flour.

KEYWORDS: Wheat flour; fractionation; gluten; separation; SE-HPLC; SDS−PAGE

INTRODUCTION

In recent publications, a new separation process for wheat flour into starch and gluten was proposed (*1*, *2*). In this process, a relatively dry dough (approximately 47% water is added on flour weight) is exposed to a curvilinear simple shear flow obtained in a cone−cone shearing device. The simple shear flow leads to aggregation of the gluten protein. When the aggregates are large enough, they migrate upon the curvilinear component of the flow toward the center of the flow field, i.e., the protein accumulates at the apex of the shearing device. This process seems to have the potential to be more environmentally friendly because it hardly requires any water and does not involve any washing steps such as those used during the current dough and batter separation processes (*3*), provided that sufficient purity can be obtained.

The traditional dough separation process consists of a washing step, which results in a loss of protein. The protein yield of flour fractionation for batter and dough-batter processes is approximately 70−90%. The remaining part of the proteins is lost during processing (*3*). Consequently, the composition of the vital gluten obtained after the process is different from the composition originally present in wheat flour. Wheat flour proteins consists of several types of proteins; glutenin, gliadin, albumin, and globulin (*4*). Studying gluten protein compositions by size-exclusion high performance liquid chromatography (SE-HPLC) results in four fractions: the high molecular weight (HMW) glutenin polymer, low molecular weight (LMW) glutenin polymer, HMW monomers (gliadins), and LMW monomers (albumins and globulines).

The new process does not make use of a washing step yet, and therefore, it is likely that the process produces gluten fractions with different chemical composition and functional properties than the wheat gluten fractions obtained with the current industrial separation processes. A certain extent of fractionation of proteins is likely to occur in the new process as well because Peighambardoust et al. (*1*) found that the glutenin macro polymer (GMP) content did not scale linearly with the protein content under all circumstances. It is therefore important to obtain more quantitative information about the exact protein composition after shearing. GMP is a highly aggregated polymer mixture that consists of mainly the HMW glutenin polymer, but it also contains the LMW glutenin polymer and HMW monomeric protein (*5*). SE-HPLC is therefore a more appropriate tool to study changes in chemical composition.

Therefore, the aim of this article is to describe the protein composition in the gluten-enriched and gluten-depleted layers of the shearing device as a function of shearing time. The variation in wheat flour composition of the layers will be visualized by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE), still based on the method of Laemmli (*6*). In addition, SE-HPLC is used to provide more quantitative information about the size distribution of the various protein fractions. Already, since the 1980s researchers have used this technique to analyze wheat proteins in relation to gluten quality (*7*−*11*). The protein composition is

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compared with the starting material and kneaded dough. Kneaded dough is measured to show that the methods provide results that are in agreement with previous research on gluten protein composition.

MATERIALS AND METHODS

Experiments were performed using wheat flour (Meneba, Rotterdam, The Netherlands) from a single wheat cultivar (Soissons). Protein content, Farinograph water absorption, stability time, peak time, and tolerance index of the flour were determined to be 11.2% (on dry basis), 53.2% (on 14.5% moisture flour basis, i.e., 53.7% on 14% moisture), 14 min, 23.5 min, and 30 BU, respectively. Water absorption is determined by the addition of tap water to 300 g of Soissons flour (moisture content 14.5%) to which 6 g of NaCl (Merck, Darmstadt, Germany) was added (i.e., 2.0 w% on flour basis).

Sample Preparation Shearing Device. Sheared dough samples were obtained using an in-house developed shearing device, which is based on a cone−plate rheometer. It consists of a stationary and rotating cone, in between which the sample material was exposed to a well-defined shear. The hypotenuse of the shearing device is 8.5 cm. The shearing device is described in more detail by Peighambardoust et al. and Manski et al. (*1*, *12*). The shearing device was connected to a Brabender Do-corder 330 unit (Brabender OHG, Duisburg, Germany). Temperature and torque values were measured online by an interface and controlling unit. The temperature in the system was controlled by a circulating water flow that was temperature controlled at 15 °C. The dough composition for all shear experiments was 70.4 g of Soissons flour, 33.2 g of water, and 1.40 g of NaCl. A temperature of 15 °C was chosen as this gave a proper amount of separation (*2*).

Soissons flour, NaCl, and water were manually mixed in a beaker glass by a spatula. The water was added in three fractions during mixing. A sample was made by adding 5% less than the water absorption determined by Farinograph. To prevent dehydration, the mixture was immediately transferred into the shearing device after mixing. The samples were rested for 15 min at 15 °C in a closed shearing device.

Samples were initially sheared at 5 rpm (6 s^{-1}) for 4 min to avoid wall slipping. Then, the rotation rate was increased in 1 min to 15 rpm (18 s^{-1}). The total shearing time for the dough was 8 or 60 min. Shearing for 8 min gave a mixture, in which the aggregation of gluten protein had taken place, and the first gluten migration had started. After 60 min, a substantial amount of gluten migration had taken place. After processing, the material was cooled to approximately 5 °C inside the shearing device in stationary position. Then, the material was divided into 5 layers of equal length along the hypotenuse, 1.7 cm each. Layers were encoded from top to bottom. The samples were immediately frozen in liquid nitrogen.

Dough Mixing. Kneaded Soissons dough was prepared by mixing water, flour, and NaCl in a 300 g Farinograph mixer. The dough, same composition as that in the shear experiments, was kneaded for 8 or 60 min at 63 rpm. The kneading process took place at 15 °C. After processing, the kneaded dough was frozen in liquid nitrogen.

Freeze-Drying. All frozen samples were freeze-dried overnight to a moisture content of 3.5% or lower. Afterward, the materials were powered using an IKA Mill (IKA type A11, Staufen, Germany) and sieved through a sieve of 0.355 mm.

Protein Content. The protein contents of the different freeze-dried samples were determined by DUMAS using a NA 2100 Nitrogen and Protein Analyzer (ThermoQuest-CE Instruments, Rodeno, Italy). The conversion factor for gluten protein, 5.7, was used to calculate the protein content. Methionine was used as the standard.

SDS−PAGE. The total protein compositions of kneaded and sheared dough (gluten-enriched and gluten-depleted fractions) were compared with the starting material, Soissons flour. The amounts added to the gel were adjusted in order to dose approximately 2 mg of protein in each tube. This 2 mg of protein was suspended in 1 mL of 0.5% (w/v) SDS−0.05 M sodium phosphate buffer (pH 6.9) solution containing 0.05 M NEM (*N*-ethylmaleimide, Sigma Aldrich, Germany). Afterward, the suspensions were heated for 5 min at 40 °C and stirred overnight (∼20 h) followed by sonication of the material in an ultrasonic disintegrator for 30 s (5 μm; fitted with a 3 mm exponential microtip) to dissolve as much protein as possible. Finally, samples were centrifuged (20,000*g*) at room temperature for 3 min.

Native SDS−PAGE analysis was performed using a Bio-Rad Mini-Protean 3 cell (BioRad Laboratories, Herculas, California, US). Samples were prepared by mixing 100 μL of sample solution with 200 μL of sample buffer. Samples were separated on a 10% Tris-HCl ReadyGel (Bio-Rad Laboratories, Hercules (CA), USA) A broad range marker, of which 6 μL was injected, was used as the standard. The marker (Prestained SDS Page standards, high range, BioRad Laboratories, Hercules, (CA), USA) contained myosin (202400 Da), β-galactosidase (116580 Da), bovine serum albumin (98080 Da), and ovalbumin (47110 Da). From the samples (sheared and kneaded), 15 μL was injected into the wells. From each material, two separate samples were produced using the procedure described above. All samples were injected on the SDS−PAGE gel once. The gel was run at a constant voltage of 100 V for 1 h.

After running the gel, it was washed three times with water and shaken cautiously for 5 min. The gel was then colored by Bio Safe Coomassie Stain (Bio-Rad Laboratories, Hercules (CA), USA) for 1 h while shaking. Finally, the gel was rinsed with water after which pictures were made of the gel.

Sample Preparation for SE-HPLC Analysis. All materials were analyzed by studying the soluble, nonsoluble and total protein fractions. Proteins were extracted from the samples using a two-step extraction procedure according to Gupta et al. (*7*). Extractions were performed in duplicate. Sample amounts were adjusted to obtain 2 mg of protein per mL of solvent solution. The protein was suspended in 1 mL of 0.5% (w/v) SDS−0.05 M sodium phosphate buffer (pH 6.9) solution containing 0.05 M NEM. The samples were heated at 40 °C for 5 min and subsequently stirred at room temperature for 2 h. Afterward, samples were centrifuged at 10,000*g* and 15 °C for 30 min. The supernatant was collected, filtered through a 0.45 μm filter, and stored for further analysis.

To obtain the nonsoluble protein fraction, the pellet was resuspended in 1 mL of 0.5% (w/v) SDS−0.05 M sodium phosphate buffer (pH 6.9) containing 0.05 M NEM. The samples were stirred overnight (∼20 h) at room temperature, followed by sonication of the material in an ultrasonic disintegrator (Soniprep150) for 30 s (5 μm; fitted with a 3 mm exponential microtip). The samples were centrifuged at 10,000*g* for 30 min. Supernatant was collected and filtered through a 0.45 μm filter and stored for further analysis.

To obtain the total protein fraction, 2 mg of protein from each sample was suspended in 1 mL of 0.5% (w/v) SDS−0.05 M sodium phosphate buffer (pH 6.9) containing 0.05 M NEM. Then, it was heated for 5 min at 40 °C and stirred for 20 h at 200 rpm at room temperature, followed by the sonication of the material in an ultrasonic disintegrator for 30 s (5 μm; fitted with a 3 mm exponential microtip). The samples were centrifuged at 10,000*g* for 30 min. The supernatant was collected and filtered through a 0.45 μm filter prior to further analysis.

SE-HPLC. Soluble, nonsoluble, and total protein fractions were analyzed by SE-HPLC. Duplicate injections were performed to obtain information about the protein size distribution in the various samples. Since duplicate samples were analyzed for duplicate weighing, 4 analyses were done for each sample. Analysis was performed on a BioSep-SEC-S4000 (Phenomenex, Torrance (CA) USA) size-exclusion column (330 × 7.8 mm). The eluent consisted of 50% (v/v) acetonitrile and 50% (v/v) Milli-Q water. The eluent contained 0.1% (v/v) trifluoroacetic acid. The injection volume was 10 μL. Flow rate was set to 0.5 mL/min, and detection was done at a wavelength of 210 nm. The temperature in the sample tray was kept at room temperature. After analysis, the chromatograms were divided into four different parts, each retention-time range representing a distinct type of protein.

RESULTS

Effect of Shearing on Protein Content. The degree of starch-gluten separation is dependent on processing time. **Table 1** shows the protein content in the various layers in the shearing device, the starting material, and the kneaded samples.

Table 1 shows that shearing separated the dough into protein-enriched and protein-depleted fractions. Continued shearing led to a further separation. Layer 5, representing the apex of the cone, contained the protein-enriched fraction, whereas layer 1 represents the depleted fraction. Since wheat protein contains various protein types, we will study the effect of shearing on the protein

composition of these fractions in more detail. The materials shown in **Table 1** were therefore analyzed by SDS−PAGE and SE-HPLC.

SDS−PAGE. Figure 1 shows clear differences between the various samples. After shearing, the gluten-depleted layer contained less high molecular weight proteins than the gluten-enriched layer (lanes 6−7). A change in the amount of HMW proteins can be observed for the gluten-enriched fractions after 60 min of shearing, though the effect is subtle when the protein composition is compared to Soissons flour. Differences were also visible at the entrance of the gel. Less material of layer 1, sheared for 60 min, is blocked at the entrance of the lane.

Table 1. Measured Protein Concentration (% w/w) for Various Process Conditions[a]

treatment	layer	rotation rate	processing time	average protein %	standard deviation
Soissons flour		unprocessed		11.2	0.15
shearing	1	15 rpm	8 min	7.0	0.18
	2			11.4	0.32
	3			11.7	0.02
	4			14.2	1.04
	5			21.2	0.98
shearing	1	15 rpm	60 min	5.4	0.35
	2			10.2	1.31
	3			12.4	0.52
	4			15.8	0.66
	5			37.5	0.82
kneading		63 rpm	8 min	11.4	0.50
kneading		63 rpm	60 min	11.0	0.11

[a] Layer 1 represents the rim of the cone, and layer 5 is the apex.

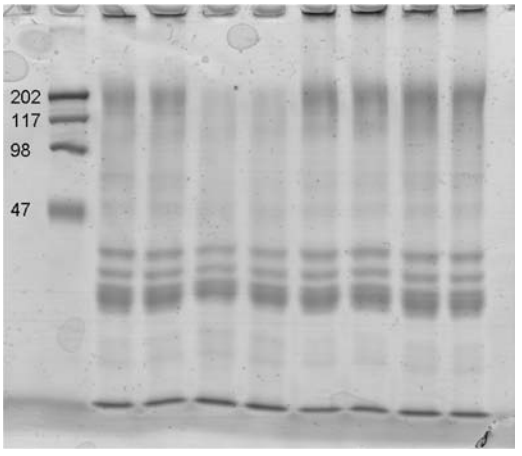

Figure 1. SDS−PAGE gel of Soissons flour for different process conditions. The lanes indicate (1) marker, (2−3) Soissons flour (raw material), (4−5) shearing for 60 min layer 1, (6−7) shearing for 60 min layer 5, and (8−9) kneading for 60 min.

SE-HPLC. A typical chromatogram obtained is depicted in **Figure 2**. The first part contains the large, HMW polymers. The second part contains a range of smaller LMW glutenin polymers (small polymers). Together, these fractions present the polymeric proteins of gluten. The third part of the chromatogram consists of HMW monomers and gliadins (large monomers). The fourth part contains the albumins and globulins, i.e., the small monomers (*10*, *11*).

The areas of each part of the chromatogram were measured for samples tested. The results are presented in Supporting Information. From the tables in Supporting Information, ratios between the large and small polymeric fractions were expressed as ratio of the large monomer fraction. Also, the ratio between the large small monomeric fraction is calculated. This is done for the total protein fractions, soluble fractions, as well as the nonsoluble fractions. Results of the SE-HPLC experiments are presented in **Table 2**. We focus on the ratios to include a kind of internal reference because in the case of kneading, it is expected that the monomeric fraction is not influenced.

Kneading. The effect of kneading on molecular composition was studied using SDS−PAGE and SE-HPLC. The results are shown in **Figure 1** and **Table 2**. Kneading for 8 or 60 min led to an increased ratio of polymeric to large monomers, compared to the composition in Soissons flour. It did not give differences in the ratio of large to small monomers. The large polymeric fraction was probably increased due to the degradation of protein that could not be extracted by SDS. The breakdown of this fraction upon processing may then give rise to an increase in polymeric fraction, while the other fractions are more or less constant. Continuation of the kneading process to 60 min resulted in further breakdown of these high molecular weight aggregates, as a result of which this ratio between polymeric materials and monomers decreased.

For the soluble fraction, as given in **Table 2**, kneading only gives significant effects in the ratio of polymeric to large monomers. This value increased upon kneading, with a maximum at 8 min and a subsequent decrease after 60 min of kneading. The results for the nonsoluble protein fraction are presented in **Table 2**. After sonication, all protein fractions are detected in the samples, although they are in small amounts. Kneading leads to a decreased ratio of polymeric to HMW monomeric protein, but

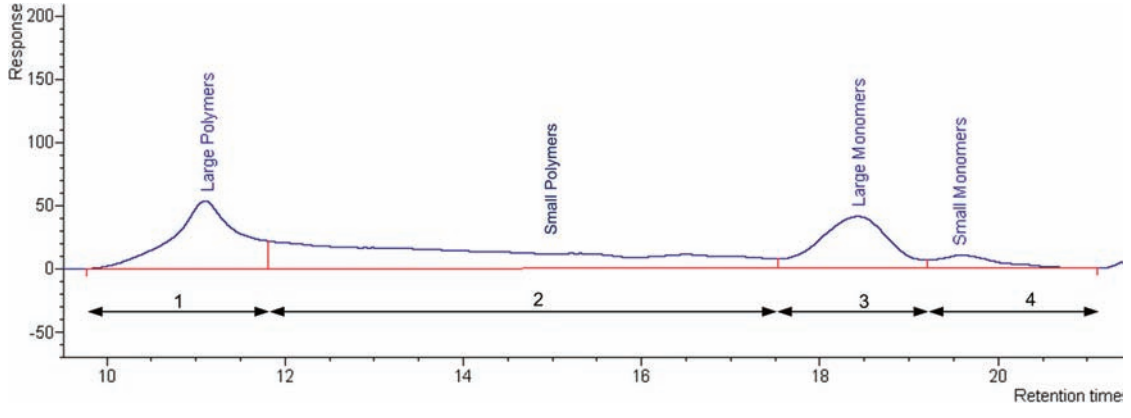

Figure 2. Typical chromatogram of gluten protein obtained by SE-HPLC. The ranges represent (1) large (HMW) polymeric proteins, (2) small (LMW) polymeric proteins, (3) large (HMW) monomeric protein, and (4) small (LMW) monomeric protein.

Table 2. SE-HPLC Ratios

			fraction (1 + 2)/3			fraction 3/4		
treatment (protein %)	time (min)	layer	average	standard deviation	significant difference	average	standard deviation	significant difference
			(a) For the Total Protein Fraction					
Soissons (11.2%)[a]			0.83	0.06	a	2.92	0.05	e
shearing (7.0%)	8	1	0.81	0.10	a	2.12	0.07	f
shearing (21.2%)	8	5	1.43	0.02	b	3.55	0.02	g
shearing (5.4%)[b]	60	1	0.57	0.04	a	1.49	0.02	h
shearing (37.5%)	60	5	1.38	0.03	b	3.71	0.04	i
kneading	8		1.21	0.03	c	2.87	0.02	e
kneading	60		1.11	0.03	d	2.92	0.04	e
			(b) For the Soluble Protein Fraction					
Soissons (11.2%)[a]			0.76	0.08	a	3.29	0.04	e
shearing (7.0%)	8	1	0.88	0.09	a	2.30	0.03	f
shearing (21.2%)	8	5	0.96	0.07	b	3.72	0.03	g
shearing (5.4%)[b]	60	1	0.63	0.06	a	2.00	0.04	h
shearing (37.5%)	60	5	0.98	0.06	b	3.90	0.02	i
kneading	8		1.52	0.03	c	3.20	0.04	e
kneading	60		1.32	0.05	d	3.22	0.06	e
			(c) For the Nonsoluble Protein Fraction					
Soissons (11.2%)[a]			5.02	0.10	a	3.26	0.24	e
shearing (7.0%)	8	1	2.22	0.04	a	4.99	0.16	f
shearing (21.2%)	8	5	3.92	0.04	b	4.01	0.10	g
shearing (5.4%)[b]	60	1	2.52	0.04	a	5.17	0.17	h
shearing (37.5%)	60	5	3.56	0.17	b	4.37	0.21	i
kneading	8		2.58	0.11	c	3.66	0.12	e
kneading	60		3.95	0.17	d	2.82	0.21	e

[a] Starting material. [b] Average and standard deviation were calculated over 2 measurements (instead of 4).

at prolonged kneading time, this ratio increased again. The HMW to LMW monomeric ratios were not significantly different, considering the high standard deviation. The protein profile for the raw material Soissons wheat flour, as well as the profile obtained for the kneaded dough, is in agreement with previous studies (*10*, *11*).

Shearing. Table 2 shows the SE-HPLC results concerning the total protein fraction after shearing. For both processing times, the ratios of the calculated fractions for the gluten-enriched layers and the gluten-depleted layers differed from the starting material. Shearing for 8 min led to an increase in the polymeric to HMW monomeric fraction immediately. The depleted fraction, however, was not influenced yet. Increasing the shearing time to 60 min, decreased the ratio between polymeric to HMW monomeric protein in the depleted fraction compared to that in Soissons flour.

The HMW to LMW monomeric fractions were influenced immediately. Already after 8 min of shearing, an effect could be found in the depleted and enriched layer, which became more pronounced after 60 min of processing. The HMW to LMW monomeric ratio increased for the protein-enriched layer and decreased for the protein-depleted layer. It can therefore be concluded that upon gluten migration, we observed a preference for the larger polymeric and HMW monomeric fractions to migrate, leading to enrichment in polymeric protein for the protein-enriched layer. Nevertheless, all gluten fractions were found in the gluten-enriched and depleted layers. This implies that all protein fractions migrate to a certain extent.

Table 2 indicates also the significant differences between the samples. Most remarkable difference for the polymeric to large monomeric fraction is the clear difference between the gluten-enriched layers versus the other samples. The monomeric fractions, however, varied extremely between the samples.

Table 2 describes the same ratios for the soluble protein fraction. As can be seen, shearing led to an increased ratio of polymeric to HMW monomeric fractions in the gluten-enriched layer, which did not further increase when shearing time was increased to 60 min. But, the gluten-depleted layer behaved in a less clear manner because the ratio increased after 8 min and then decreased at 60 min.

Also, here the effect of shear on the ratio of the HMW to LMW monomeric fractions is clear; the ratio increased rapidly in relation to the kneaded samples. This confirms that especially the larger-molecular weight fractions migrate and that the LMW monomeric fractions migrate less. The ratio of the HMW to LMW monomeric fractions decreased in the gluten-depleted layers. This ratio further decreased with prolonged processing time. As expected, this ratio consistently increased in the gluten-enriched layer.

Table 2 shows the results of the nonsoluble fractions. Sonication was used to dissolve this fraction (partly). The ratio of polymeric to HMW monomeric fractions is the highest for the starting material (Soissons flour); shearing generally decreases this ratio. After shearing, the gluten-enriched layer shows higher ratios than the gluten-depleted layer. The ratio of the HMW to LMW monomeric fractions decreased from the gluten-depleted layers to the gluten-enriched layers to Soissons flour. This indicates that the HMW monomeric components seem to migrate more to the center than the LMW ones. No distinct differences can be observed for the shearing times. The standard deviations were quite high (due to the low concentrations of the dissolved material) making it difficult to obtain significant differences.

DISCUSSION

This article further explores a new separation principle based on the use of shear-induced migration. The application of a curvilinear shear field provides a new principle to separate wheat

flour into gluten and starch. In a previous paper, we hypothesized that this separation consists of two steps (*1*, *2*). First, the aggregation of the gluten and second the migration of the gluten along a curved shear field. In addition, Peighambardoust et al. (*1*) obtained results from which it could be derived that during separation also a certain extent of gluten protein fractionation occurred. To challenge this hypothesis and increase our understanding of the underlying mechanism, the effect of shear on gluten composition was studied in more detail.

The molecular composition of the enriched fractions were analyzed in more detail and compared to the unprocessed material (Soissons flour). Besides, a comparison was made with kneaded dough. As the type of flow is different for these types of equipment, a difference in protein composition was expected.

Differences in protein composition can be observed between the starting Soissons flour, the sheared, and the kneaded flour. The quantitative SDS−PAGE gel shows that the gluten-depleted fraction contained less high molecular weight protein. This difference cannot be caused by the difference in the protein amount, as a correction is made for this (see Materials and Methods section). The SE-HPLC results confirmed these results.

The influence of kneading and shearing on gluten composition was different. During shearing, the larger polymers are immediately separated toward the enriched layer, while the monomeric fraction needs more time as can be concluded from **Table 2**. The kneading process influences the ratios between the fractions drastically, but continued kneading did not change the ratio between the polymeric and high monomeric fraction anymore. Both processing techniques influenced the protein composition to a certain extent. The results with the soluble and nonsoluble fractions confirmed the effects described above.

The question that remains is to describe the extent of gluten fractionation. It is clear that in case the protein composition changed significantly, the chromatograms measured by SE-HPLC should be completely different for the proteins extracted from the different layers. However, the shape of the chromatograms was rather comparable, indicating that the fractionation is limited. Therefore, it is not the case that during shearing a certain group of proteins is completely removed from a definite layer. In other words, the gluten-enriched layer is not completely depleted from small monomers, and layer 1 still contains polymers. In addition, the change in ratios between the various fractions (**Table 2**) is less than the change in total protein content (**Table 1**).

Supporting Information provides quantitative information about fraction 4 during the total protein analysis during SE-HPLC. It shows that the percentage of the small monomers on the total dissolved material is different for the various samples. Soissons flour and the kneaded samples have a comparable fraction. However, the area fraction captured by fraction 4 changed for the sheared samples between layer 1 and 5 of the shearing device. The values of the sheared samples are different, which is caused by the fact that those samples were extracted from, respectively, gluten-enriched and gluten-depleted layers and the fact that it is measured during time. The gluten-depleted fractions contained more small monomers.

Another way of presenting the data obtained during the SE-HPLC analysis of the total protein samples is given in **Table 3**. **Table 3** describes the distribution of protein for the analyzed samples in an absolute manner. In this case, the assumption is made that all protein can be recovered on the SE-HPLC column. The values given in the table indicate the amount of protein for 100 g of dry dough materials. Changes for the distribution can be observed. Therefore, for Soissons flour itself, most of the protein is present in fractions 2 and 3. It can be seen that the content of all fractions have increased in layer 5, compared to that in Soissons.

Table 3. Protein Distribution in 100 grams of Material for the Various Samples

		Protein distribution (in g) per fraction for 100 gram dried material			
sample	protein (g)	fraction 1	fraction 2	fraction 3	fraction 4
Soissons	11.2	0.75	3.54	5.15	1.76
layer 1, 8 min	7.0	0.54	1.95	3.07	1.45
layer 1, 60 min	5.4	0.33	1.05	2.40	1.62
layer 5, 8 min	21.2	3.24	7.91	7.89	2.16
layer 5, 60 min	37.5	5.93	13.65	14.03	3.90
kneading 8 min	11.2	0.99	4.32	4.37	1.52
kneading 60 min	11.2	1.06	4.02	4.56	1.56

Standardized Protein Composition Distribution of the Various Samples

sample	fraction 1	fraction 2	fraction 3	fraction 4
Soissons	1.00	1.00	1.00	1.00
layer 1, 8 min	1.15	0.88	0.95	1.32
layer 1, 60 min	0.93	0.61	0.97	1.91
layer 5, 8 min	2.28	1.18	0.81	0.65
layer 5, 60 min	2.36	1.15	0.81	0.66
Kneading 8 min	1.32	1.22	0.85	0.87
Kneading 60 min	1.41	1.13	0.88	0.89

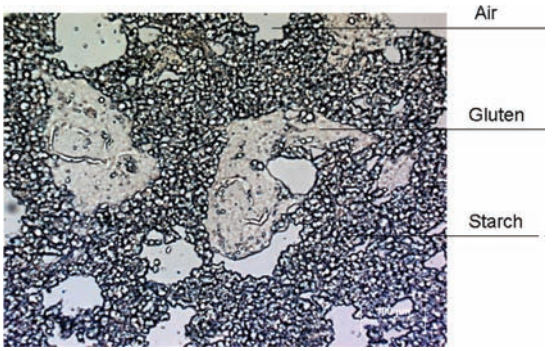

Figure 3. Light microscopy picture of the gluten-depleted layer. Process conditions were 60 min of shearing at 15 rpm at 15 °C.

This implies that all fractions migrate, though the migration of fraction 1 was faster than the migration of the other fractions (i.e., the increase in layer 1 was larger).

In **Table 3**, we have standardized the distribution of protein in the samples. In this case, we have taken into account the final protein concentration of each sample and the protein distribution over the various fractions as obtained for Soissons flour. Therefore, the values given in **Table 3** are divided by the areas for Soissons and multiplied by the change in protein concentration (protein content Soissons/protein content sample). This gave the remarkable result that the fifth layer after shearing for 8 or 60 min is almost identical. In other words, upon prolonged processing the protein concentration increases, while the compositions remains constant. This could indicate that the material ready for migration (i.e., the aggregated protein) determined the distribution of protein in the gluten-enriched layer. The gluten-depleted fraction showed a decrease in polymeric protein and an enrichment of the monomeric protein after 60 min of processing.

If we compare the explanation given above with a microscopic picture of a gluten-depleted layer, we observe that the protein material is still present in clusters, even though a major part of the protein is already migrated. **Figure 3** depicts a microscopic overview (Axiovert inverted DIC Microscope including digital camera) of layer 1 after 60 min of shearing. Thin coupes of 10 μm each are made by a cryotome. The air bubbles were formed when a drip of dimethylformamide was added.

The existence of the gluten aggregates could be caused by the presence of the remaining polymeric protein. The aggregates can still be present in the first layer because aggregate formation needs some time. The fact that large protein fractions migrate fast implies that changes in composition over the various layers also occur quickly (**Table 2**). The aggregates formed at the end of the shearing process will therefore be different in protein composition. The difference in composition will lead to differences in rheological properties of the aggregates as well. The gluten-depleted layers contain a relatively high percentage of large monomers. The monomeric fraction contains the gliadin protein that acts as a plasticizer, making the aggregate softer and less elastic. This could cause the gluten aggregates to become too weak to be pulled toward the center of the cone. They will probably break instead.

The reasoning above is also in line with **Table 3** in which we showed that the composition of the fifth layer remains the same. Before migration can take place, an aggregate with a defined composition and related rheological properties has to be created. The above-described hypothesis could also explain why separation is promoted by certain process conditions as reported earlier. For example, an increased temperature can induce additional cross-links which may influence the rheological properties of the aggregates.

The aim of the research was to determine if shear-induced migration resulted in fractionation of the various protein fractions in dough. As described, this fractionation can be observed; however, all components migrate to a certain extent, as a result of which complete fractionation is not observed. The main conclusion is that in the separation process, all components migrate but not at an equal rate. Low-molecular weight monomeric proteins tend to migrate more slowly than higher-molecular weight components. This will lead to a certain extent of fractionation. We hypothesize that the concentration of the high molecular weight gluten is related to the principle of migration, probably through the mechanism that this concentration strongly determines the rheological properties of the gluten aggregates.

Supporting Information Available: Percentage relative area of total, soluble, and nonsoluble protein analyzed by SE-HPLC. This material is available free of charge via the Internet at http://pubs.acs.org.

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