Concentrated separation of wheat flour into starch and gluten

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Introduction

Introduction

A large part of the world population depends on wheat as it is a major staple food. Wheat is grown all around the world in a wide range of climates, has a high yield per hectare and is very suited for long term storage. It is not only widespread, but it is also a versatile crop. There is a wide diversity of food products produced from the wheat kernel, and it is likely that this diversity will increase even further. (Delcour *et al.* 2010)
For wheat kernels, the most important components are the starch fraction, the gluten fraction and the germ. The starch represents the major part of the kernel (being 70-80 % of the dry weight) and is in fact the energy storage for the future plant. The gluten protein is the second-largest component (being approximately 12 % of the dry weight), being a mixture of mostly storage proteins. The amount of gluten, so protein, differs for every wheat flour type. (Goesaert *et al.* 2005; Delcour *et al.* 2010) Wheat is currently predominantly used for human consumption and (animal) feed, but a growth in the use of wheat components for the production of biomaterials and biofuels is foreseen in the next decades. The availability of fossil energy sources is slowly but surely dwindling, and the use of biological sources will become more important as a result of that. (Balat 2007) Starch and gluten fractions are intimately associated in the kernel and cannot be separated easily by, for example, a dry separation process such as wind sifting. Separation of starch and gluten from wheat is more difficult than the separation of some legumes, potatoes and corn. The current industrial process to separate gluten from the starch relies on the use of a large amount of water, which has to be removed after the separation. Besides, this water washes away part of the raw material into the waste water, so that it can not be used for human consumption. This thesis is aimed at finding a new way of separation that would not require a strong dilution with water, such that a more sustainable separation process could be developed for wheat flour into its main constituents, requiting less energy, water and utilising more of the raw flour.

Wheat production

Wheat is produced for food and feed purposes, and increasingly for the production of biomaterials and -fuels. (Balat 2007) The quality and quantity of wheat produced depends on the climate and soil conditions, and the availability of fertilizer and irrigation. Most wheat is grown in Canada, the USA, Germany and France. The yield per hectare of Dutch wheat is higher than in most countries. For example, comparing Canada and the USA with European countries indicated 3 times more yield per hectare for the European countries. The yield in Canada and the USA is approximately 2-3 tonnes/ha while this is 7 -9 tonnes/ha in Europe (Wrigley *et al.* 2009). The Dutch climate is not optimal for high

quality wheat production and therefore Dutch wheat is mainly used in the feed industry. In The Netherlands, wheat with the quality for bakery applications is therefore mainly imported from foreign countries with a less moderate climate.

<u>Starch</u>

The major part of a wheat kernel is composed of starch. Starch consists of amylose and amylopectin. Amylose is a linear polymer composed of α-D-glucose units linked by α-1,4 bonds. Amylopectin is composed as well of α-D-glucose units linked by α-1,4 bonds, but is highly branched due to glycosidic α-1,6 bonds. (Delcour *et al.* 2010) The starch is present in semi-crystalline granules having a bimodal size distribution of 6 and 22 μm. Starches play an important role in many food applications; for example as thickener, filler, matrix component or nutritional component providing energy.

Starch granules have strong interaction with water. When heated above their gelatinisation temperature (usually around 60 ˚C), they swell much more. The gelatinisation process is accompanied by partial or complete melting of the semi-crystalline starch and leakage of amylose to the surrounding liquid. The granules remain discernable, but swell to many times their original volume. At even higher temperatures, the granules will decompose and the starch will dissolve molecularly.

During baking bread, the starch in the dough gelatinises and takes up water (Goesaert *et al.* 2005). This provides the firmness to the bread in the baking process, while retrogradation (re-crystallisation) of the amylase lends additional strengthening after cooling of the bread.

Starch is a valuable raw material in its isolated form, because it is used as starting material for the production of glucose, maltodextrins and other saccharides via hydrolysis. (Delcour *et al.* 2010) Soft drinks are usually sweetened with glucose or fructose syrup, produced through starch hydrolysis (possibly followed by an enzymatic isomerisation step to convert part of the glucose to fructose). Starch is also used as texturizer, carrier or thickener. The sheer size of the market volume of the products derived from starch makes efficient isolation of the starch an important issue.

<u>Gluten</u>

Wheat gluten protein is a valuable product as well. In flour-based products, gluten tends to self-associate and form a strong, but flexible network, which gives well-developed dough its characteristic properties and consistency (before heating, which will induce the starch to take over this role at least partly). The gluten matrix is also important for retaining the carbon dioxide that is produced during the proofing of the dough.

Gluten consists of a complex mixture of many different proteins. The major fractions in gluten are the glutenins and the gliadins. Glutenins contribute to the dough elasticity, so

the resistance to extension. The gliadins have little or no resistance to extension and appear to be responsible for the viscous properties of (developed) gluten products. Gliadin is extremely sticky while glutenins are rubbery and resilient. Albumins and globulines, the water soluble and low molecular weight proteins, are thought to be of minor importance for the functional properties of dough. (Delcour *et al.* 2010) Glutenin is the polymeric fraction bound by inter and intra molecular bonds. Gliadin is the monomeric protein linked by intramolecular bonds. (Muller *et al.* 1995; Wieser 2007)

Gluten is a valuable additive to foods, especially in the bakery industry. In Europe, it is common to add gluten to the flour to increase the protein concentration and to improve the final dough properties needed to allow good proofing of bread. Also in other products, gluten is used more often, for example as binder and texturizing agent in meat products, such as sausages and hamburgers, and in the feed industry as calf milk replacer or as pet food. (Delcour *et al.* 2010)

Gluten quality

The quality of wheat gluten is usually related to its vitality. This vitality expresses the capability of the gluten matrix to extend without rupturing. In other words, it describes to what degree the gluten matrix can be deformed while retaining its original functionality. The quality of gluten also includes the degree of tolerance to processing. (Day *et al.* 2006; Delcour *et al.* 2010) High quality wheat flour can withstand severe process conditions. The potential quality of wheat gluten is determined by the wheat cultivar (and the growth conditions). However, the quality might be compromised during the separation processes of the wheat flour into its constituents. For example, intensive mixing or kneading during separation and purification may lead to lower quality, just as elevated temperatures, during drying.

Current separation processes

One crucial step in both the production of foods and non-food products is the efficient separation of the raw material into its major constituents. Therefore the process separates much of the raw material into the highly valuable fractions and will not use much additional resources to achieve this. Inefficient processes may lead to water shortages and waste water treatment issues, use much energy (e.g., to dehydrate the products), while an ineffective process will lose part of the feedstock and also cause waste issues. Our fossil resources will become scarcer and more expensive in the coming decades, and it is likely that bio-based raw materials will serve as feedstock for many other products, such as synthetic materials and sources of energy. At the same time, it is crucial that the supply of foods is not threatened in this development. We therefore need more effective and efficient processes.

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┌──────────────┐
│  Biopolymer  │                              Milk, dough
│   mixture    │
└──────┬───────┘
       │
       │        *Separation process driven by:*
       │        ● *Density*
       │        ● *Particle size*
       │        ● *Solubility*
       │
       ▼
┌──────────────┐                           Starch & Gluten
│              │                          Fat & Skimmed milk
│ Constituents │
│              │
└──────────────┘
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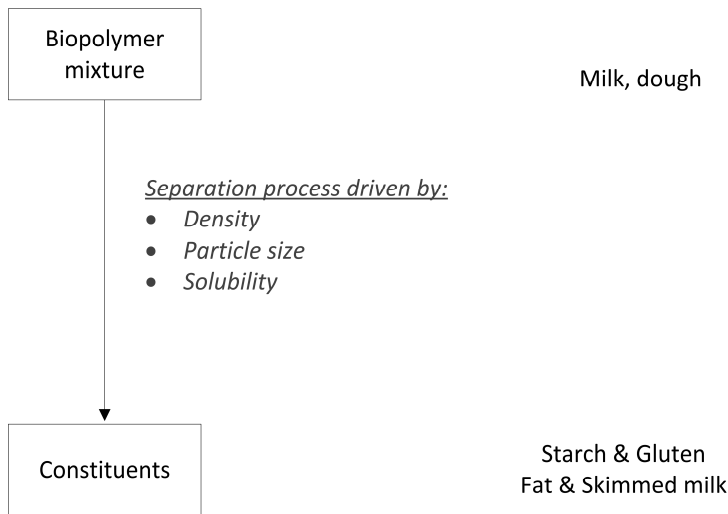
Figure 1.1: Separation of biopolymer blends

Most of the current separation processes for biopolymeric materials (such as wheat flour) make use of differences in density, particle size or solubility (Figure 1.1), and the currently used process for separation of gluten and starch is not different. The commonly used industrial separation technique for wheat flour separation is a wet processing step. As a first step, wheat flour is produced, by coarse disintegration, allowing the separation of the germ and husks, followed by fine(r) milling. Although variations exist, one first mills the kernel, and then prepares a dough or batter by adding water while mixing, and then washes the dough or batter to remove the starch. Figure 1.2 represents the current industrially used separation technique in simplified form. Generally speaking wheat flour separation is based on producing a dough or a batter which is washed/rinsed by water. It is also possible to use a combination of steps, a dough-batter separation process. (Van der Borght *et al.* 2005; Day *et al.* 2006; Delcour *et al.* 2010)

The Martin process is a traditional method to recover the gluten fraction from wheat flour. A dough is made by mixing flour and water to a moisture content of 40-60%. The dough is then rested so that the dough can become fully hydrated, and a strong dough is formed. A large amount of water is then added while mixing to remove the starch fraction and water extractable components. The final protein concentration in the gluten end product can go up to 75% due to extra washing steps. (Van der Borght *et al.* 2005; Day *et al.* 2006; Delcour *et al.* 2010)

A variation of the Martin process is the dough-batter process. By this method, a stiff dough is first formed as well, but this is then subsequently dispersed in a large amount of water while mixing. (Van der Borght *et al.* 2005; Day *et al.* 2006; Delcour *et al.* 2010)
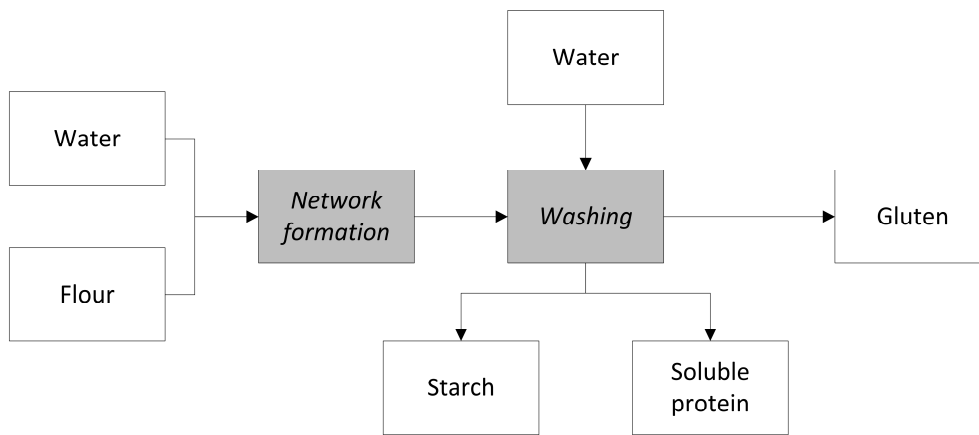
Figure 1.2: Schematic overview of the current separation process of wheat flour into starch and

An alternative separation method is the batter process; therefore, wheat flour and water are mixed into a dough/batter. By further addition of water, the batter is separated in a gluten-rich curd that is low in starch. The starch remains suspended in the water. This process is mostly performed between 40-55 °C. The gluten curd can be recovered by sieves, while the starch and water pass. (Van der Borght *et al.* 2005; Day *et al.* 2006; Delcour *et al.* 2010)

The starch slurry, that still contains some gluten protein in small aggregates, will be separated by centrifugation, by tabling or in hydrocyclones or decanters. These separations make use of the density differences of starch and gluten. Sieving is used to separate the starch from large gluten structures. Centrifugation of small volumes of slurry results in a dense starch fraction at the bottom, the so called high quality starch or A-starch. The top part consists of the gluten traces and the low quality starch or B-starch. The last type consists of smaller starch granules and could contain some unextractable arabinoxylans, low amounts of protein, ash and cell wall material from the wheat. Hydrocyclones can separate the components by centrifugal force. Starch leaves the system in the apex of the hydrocyclone, while all the other material leaves at the top. Tabling is a method when a suspension flows over an inclined table, where starch is given the time to settle while the gluten, water and soluble material flow off. At the end the table is washed with water to remove the B-starch. A decanter can also be used to separate the starch slurry. (Van der Borght *et al.* 2005; Day *et al.* 2006; Delcour *et al.* 2010)

Evidently, the current starch-gluten separation process consumes large amounts of water and energy. Another disadvantage of the process is the loss of soluble gluten protein in the waste stream, which creates a waste treatment issue, but at the same time represents a loss of valuable raw material.

There have been several attempts to develop more efficient separation processes, but those were found to be unsuitable for large-scale industrial application. (Delcour *et al.* 2010) Most of these processes were still based on the use of an excess of water. These wet separation processes involve, e.g. steeping of the wheat kernels, milling of the wet wheat and separation of bran, germ, protein and starch. After hydration of the kernels by water, the steeped wheat is generally grinded. In some cases, the wheat was fermented or the bran was removed by sieving so that a starch/protein suspension is obtained, which is then separated by washing, sieving and/or centrifugation to obtain gluten and starch. In case the suspension was centrifuged, all proteins could be recovered. The heavy processing of the wheat kernels devitalizes the gluten protein, while the wet processing of wheat kernels leads to agglomeration of gluten protein by bran, which makes the separation more difficult. (Van der Borght *et al.* 2005)

<u>From simple shear flow to shear-induced separation</u>

Almost a decade ago, work started on using well-defined shear flow as a new process parameter. (Van der Goot *et al.* 2008) Initial studies focused on improved understanding of the phenomena occurring during a thermo-mechnical treatment. (Van den Einde 2004; Peighambardoust 2006) studied the behaviour of dough under simple shear flow and found remarkable process tolerance of the dough to the shear flow. This study was extended by Peressini *et al.* (2008) who found that dough aggregates into gluten and starch patches when exposed to shear flow in a Couettte-device. Manski (2007) showed the formation of hierarchically structured fibrous protein materials under well-defined flow. In all of the observations described above well-defined shear flow was essential; the same behaviour could not be observed in regular mixers.

Next to structure formation, Peighambardoust *et al.* (2008) found that gluten tends to concentrate in the apex of a conical shearing device. Gluten enrichment to at least 40% protein was observed. This observation was then used to propose a new principle for separation of gluten and starch from wheat gluten. The new process was called "shear-induced separation", and was based on the application of shear flow on flour and water (and some salt). Due to shearing, a dough was formed, after which the gluten aggregated and started to migrate and accumulated in the centre of the conical device. The rest of the material in the device became depleted of gluten protein (and hence was rich in starch). It was hypothesized that the separation mechanism consists of two steps, namely concentration of gluten protein to visible aggregates and subsequent migration of these aggregates towards the centre. (Peighambardoust *et al.* 2008)

The separation took place in a concentrated system of approximately 55-60% solids, and was neither expected, nor could be predicted from the behaviour of more diluted dough or batter systems. The separation does not require elevated temperatures, and it does

not need a high mechanical energy input. It therefore has potential to be developed into a mild and efficient separation process for gluten and starch, while the quality of gluten protein may well be retained due to the mild conditions. The process could lead to water and energy savings, as the process takes place in a concentrated dough matrix. Therefore the shear-induced separation process can contribute to more sustainable processing. The principle of separation by shear seems to combine mildness (maximising value) with no production of waste water, strong reduction or elimination of waste streams and low energy input.

As mentioned earlier, wheat is one of the most important staple foods for humans across the world. In 2050, it is expected that the world population has grown to more than 9 billion people. At the same time, large regions around the world are rapidly becoming more affluent. Both trends put strain on our capability to produce sufficient foods and feed, and on our environment, as more waste is generated, and more land is used for food and feed production. This trend is accelerated by the fact that our fossil resources are becoming scarcer, and subsequently more expensive. As a result, many countries are already using crops that were originally only used for food and feed, for the production of biomaterials and fuels. We may expect that this trend will continue, even when a second-generation technology for biorefining (using non-digestible parts of the crop) becomes available.

The combined effect is that bioresources will become more valuable, while the availability of water and fuels will become less. Due to this trend, it is more important to develop optimized processes for the fractionation of these bioresources that make the maximum use of the raw material, but with a minimum of water and energy, while avoiding the production of waste with low or negative value. The trend of sustainable production causes optimal use of raw materials and creates processes having less impact on the environment.

<u>Towards a new process for the separation of wheat flour</u>

The path from an observation in an in-house developed laboratory-scale shearing device to full-scale production facilities consists of many different steps. This thesis takes the first steps towards a true process by trying to further understand the mechanisms behind the separation process. The overall objective of this thesis is to identify the principles that underlie the shear-induced separation mechanism of starch and gluten as was previously identified. Part of this is the identification of the fundamental driving forces that are responsible for the separation, and the optimal process conditions and matrix composition. This can then be translated to an optimal geometry, plus the quality of the components that may ultimately be expected to be feasible. Finally, the identification of the principles will allow the development of guidelines for the design of

larger separation equipment, and will offer a perspective on more general applicability outside the field of gluten-starch separation.

Outline of the thesis

The aim of this thesis is to take the next step towards industrial application of the shear-induced separation process of wheat flour into its constituents. Main focus is on improving understanding of the mechanisms driving the separation. The research described in this thesis covers three topics, being: (I) investigating the separation principles; (II) determining the gluten quality after separation (III) improving the shearing device. Figure 1.3 schematically represents the outline of this thesis.

Chapter 2 describes the effects of the process conditions on the separation process. Settings such as process temperature, rotation rate and process time were varied. The chapter shows that the separation follows a two step mechanism (i.e. aggregation and migration) and that those steps are favoured by different process conditions.

The separation process was studied with dough that includes NaCl. As NaCl is an undesirable addition for industrial application due to negative health effects and negative environmental impact, it was investigated whether salt addition could be lowered or even omitted. Thus, **Chapter 3** describes the influence of NaCl addition to the system and its effect on the amount of separation. Separation in the shearing device was possible without NaCl addition, although the dough became less process tolerant. The process parameters therefore should be chosen more carefully. Remarkably, the process seems to be faster without salt.

The influence of shear processing on the molecular composition is studied in **Chapter 4**. The gluten composition at various locations in the shearing device was studied by SE-HPLC and SDS PAGE. All fractions of the gluten protein took part in the separation process, and migrated in the shearing device. Larger molecules seemed to migrate slightly faster.

Chapter 5 describes the kneading and baking properties of gluten obtained via shear-induced separation compared to commercial gluten. Baking mini-breads containing shear-induced gluten resulted in breads with comparable quality as baked with the addition of commercial vital wheat gluten. The kneading tests even suggested an enhanced effect compared to commercial gluten.

Chapter 6 and Chapter 7 describe a new shearing device, which is developed to study the effect of shear rate and the device geometry on the separation process. Remarkable differences were found. It turned out that not only the shear rate determines the final situation; the volume/size of the material area plays a major role as well. To explain all

observations, the mechanism behind the separation was extended to a three step separation process.

The thesis ends with a general discussion in **Chapter 8** about the consequence of the current work, a future outlook and further possibilities of the shear-induced separation process.
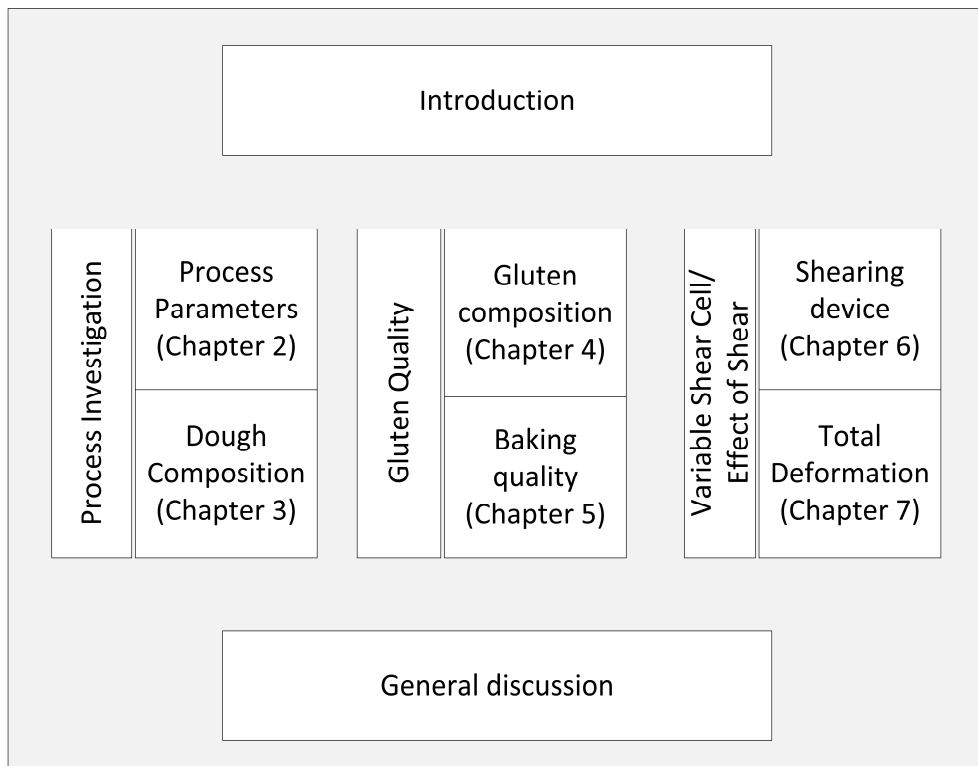

Figure 1.3: Schematic overview of the thesis entitled "Concentrated separation of wheat flour into starch and gluten"

References

Balat, M. (2007). An overview of biofuels and policies in the European Union. *Energy Sources Part B-Economics Planning and Policy* 2(2): 167-181.

Day, L., Augustin, M.A., Batey, I.L. and Wrigley, C.W. (2006). Wheat-gluten uses and industry needs. *Trends in Food Science & Technology* 17(2): 82-90.

Delcour, J.A. and Hoseney, R.C. (2010). Principles of cereal science and technology. St. Paul, Minnesota, AACC International

Goesaert, H., Brijs, K., Veraverbeke, W.S., Courtin, C.M., Gebruers, K. and Delcour, J.A. (2005). Wheat flour constituents: how they impact bread quality, and how to impact their functionality. *Trends in Food Science & Technology* 16(1-3): 12-30.

Manski, J.M. (2007). Flow-induced structuring of dense protein dispersions, PhD thesis, Food Process Engineering, Wageningen University, Wageningen

Muller, S. and Wieser, H. (1995). The location of disulfide bonds in alpha-type gliadins. *Journal of Cereal Science* 22(1): 21-27.

Peighambardoust, S.H. (2006). Development of dough under shear flow, PhD thesis, Food and Bioprocess Engineering, Wageningen University, Wageningen

Peighambardoust, S.H., Hamer, R.J., Boom, R.M. and Van der Goot, A.J. (2008). Migration of gluten under shear flow as a novel mechanism for separating wheat flour into gluten and starch. *Journal of Cereal Science* 48(2): 327-338.

Peressini, D., Peighambardoust, S.H., Hamer, R.J., Sensidoni, A. and Van der Goot, A.J. (2008). Effect of shear rate on microstructure and rheological properties of sheared wheat doughs. *Journal of Cereal Science* 48(2): 426-438.

Van den Einde, R.M. (2004). Molecular modification of starch during thermomechanical treatment, PhD thesis Food and Bioprocess Engineering, Wageningen University, Wageningen

Van der Borght, A., Goesaert, H., Veraverbeke, W.S. and Delcour, J.A. (2005). Fractionation of wheat and wheat flour into starch and gluten: overview of the main processes and the factors involved. *Journal of Cereal Science* 41(3): 221-237.

Van der Goot, A.J., Peighambardoust, S.H., Akkermans, C. and Van Oosten-Manski, J.M. (2008). Creating novel structures in food materials: The role of well-defined shear flow. *Food Biophysics* 3(2): 120-125.

Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology* 24(2): 115-119.

Wrigley, C.W. and Gooding, M.J. (2009). Wheat in the grain chain: managing production to optimize grain quality. *Cereal Foods World* 54(2): 58-63.

Influence of process conditions on the separation behaviour of starch-gluten systems

Abstract

Separation of wheat flour into its constituents starch and gluten was studied using a cone-cone shearing device, with emphasis on the effect of rotation rate, processing time, temperature and water content. This study confirms the two step mechanism previously proposed for the gluten migration: aggregation of gluten protein into gluten domains that subsequently migrate to the apex of the cone. The results show that optimal process conditions for gluten migration are different from the process conditions for gluten aggregation. While gluten agglomeration (step 1) benefits from high temperature, low rotation rate and high water content, gluten migration (step 2) is positively influenced by a high dough viscosity and higher rotation rate.

Introduction

Separation of biomaterials into their constituents is an important industrial activity. Most of the traditional separation techniques are based on differences in solubility and density and are mostly not very efficient in energy and solvent usage. Novel separation processes should therefore combine energy efficiency and mild processing to obtain high quality products. Besides, the use of solvents, often water in the case of food products, should be reduced.

The most important industrial processes for separating wheat flour into starch and gluten are based on kneading and washing (Van der Borght *et al.* 2005). These processes involve at least one wet separation step. Recently, a new separation principle was introduced for wheat flour (Peighambardoust *et al.* 2008). It was found that the differences in rheological behaviour of the constituents can be used as a promising new route for separation of biomaterials. By exposing wheat flour dough to a curvi-linear shear field, a gluten enriched fraction and a gluten-depleted fraction were obtained under relatively dry conditions. The separation occurred through a two step mechanism. Initially, gluten are clustered into aggregates (step 1), which then migrate to the apex of the cone (step 2). This new process could potentially lead to large water and energy savings. In addition, improved product/gluten characteristics could be expected, because the process is based on simple shear processing, which process made wheat dough rather process tolerant. (Peighambardoust *et al.* 2005; Peighambardoust *et al.* 2007)

Process conditions play an important role in all separation processes (Larsson *et al.* 1996; Robertson *et al.* 2003; Frederix *et al.* 2004; Kuktaite *et al.* 2005; Van der Borght *et al.* 2005). Peighambardoust *et al.* (2008) studied the effect of processing time on the separation process. They found that the separation required a minimum processing time, but too long processing resulted in redispersion of the protein fraction throughout the whole mixture. However, the role of the other process parameters in the new separation process is not clear yet.

From previous work, it can be concluded that the type of flow applied to wheat flour dough influences the formation of gluten aggregates (i.e. step 1). Steady simple shear flow promotes the gluten network formation while transient shear flow leads to elongation and break up of the gluten network (Peighambardoust *et al.* 2007). In addition, the use of a Couette device, in which step 2 could not take place, showed the existence of a relation between the shear rate and the size of the gluten aggregates. A lower shear rate had a positive influence on the size of the gluten aggregates. A higher shear rate resulted in break up of those gluten aggregates (Peressini *et al.* 2008).

Therefore, the aim of this chapter was to investigate the effect of the process conditions

being shear rate, processing time, processing temperature and water content on the shear-induced separation of starch and gluten from wheat flour in a curvi-linear shear field.

Materials and methods

Experiments were performed with Soissons wheat flour (Meneba, Rotterdam, The Netherlands) from a single wheat cultivar. Proteins content, Farinograph water absorption, stability time, peak time and tolerance index of flour were 11.2% (on dry basis), 53.2% (on 14.5% moisture flour basis), 14 min, 23.5 min and 0.3 BU, respectively. Water absorption is determined by the addition of tap water to 300 g Soissons flour (moisture content 14.5%) to which 6 g NaCl was added (i.e. 2.0% on flour basis).

Shearing device

Separation of starch and gluten was studied in a laboratory scale shearing device, which configuration is based on a cone-plate rheometer. This shearing device was developed in -house (Wageningen University, The Netherlands) and consists of a stationary and a rotating cone. The shearing device was earlier described in detail by Peighambardoust *et al.* (2008) and Manski *et al.* (2007). The shearing device was connected to a Brabender Do-corder 330 unit (Brabender OHG, Duisburg, Germany) with an interface and controlling unit for on-line measuring of temperature and torque values. The temperature of the Shear Cell was controlled using a circulating water flow. The experimental scheme is outlined in Table 2.1. A rotation rate of 10 rpm corresponds to a simple shear rate of 24 s^{-1}. All samples were initially sheared at 5 rpm for 4 min to avoid wall slip and to obtain a homogenous starting material. Then, the rate was increased in 1 min to the set rotation rate. In case of constant deformation, all experiments started with 4 min shearing at 5 rpm after which the shear rate is gradually increased to the set rotation rate in 1 min. The settings used were: 165 min at 5 rpm, 83 min at 10 rpm, 41 min at 20 rpm, 14 min at 60 rpm. After processing, the material was cooled to approximately 5 °C inside the stationary shearing device.

Preparation of dough

Equal amounts of wheat dough (105 g) were used for all experiments. Dough was prepared by combining wheat flour, NaCl and tap water in a beaker glass and mixed using a spatula. Water was added in 3 fractions to the mixture . Formulations of the used flours are summarized in Table 2.2. The mixture was distributed evenly over the surface of the bottom cone of the shearing device. The upper cone was placed on top of the

Table 2.1:
Overview of the different process parameters used during the experiments [a]

	W (%)	t (min)[b]	v (s^{-1})	T (°C)	s (s^{-1} min)
Standard[c]	48.2	55	36	15	1980
Water content	53.2	55	36	15	1980
	58.2	55	36	15	1980
Process time	48.2	5	36	15	180
	48.2	10	36	15	360
	48.2	25	36	15	900
Rotation rate	48.2	55	12	15	660
	48.2	55	72	15	3960
	48.2	55	144	15	7920
Temperature	48.2	55	36	10	1980
	48.2	55	36	25	1980
	48.2	55	36	40	1980
Constant deformation	48.2	14	144	15	2016
	48.2	41	48	15	1968
	48.2	83	24	15	1992
	48.2	165	12	15	1980
Combined optimal process	53.2	55	12	40	660

[a] Where W = water absorption, t = processing time, v = shear rate, T = temperature and s = total deformation

[b] Start-up time excluded from process time. Start-up time not included in the deformation

Table 2.2:

Composition of the dough used during the different shear runs.

For water absorption (%)	Soissons flour (g)	Tap water (g)	NaCl (g)
48.2	70.4	33.2	1.40
53.2 [a]	68.1	35.5	1.35
58.2	66.0	37.7	1.31

[a] Farinograph water absorption

sample. A vertical compression force of 300 kgf (2940 N) was put onto the material, which was kept constant during all experiments. The material was rested for 15 min prior to processing at the process temperature to allow relaxation of the material.

<u>Treatment of samples</u>

The hypotenuse of the shearing device is 8.5 cm. The material is divided in 5 equal layers of 1.7 cm each, after shearing and encoded.

The material was immediately frozen using liquid nitrogen. Samples were collected and freeze-dried at least for 48 h to an average moisture content of 3.5%. After freeze drying the samples were powdered with an IKA Basic mill (type A11) (IKA, Staufen, Germany) and sieved using a pore size of 0.355 mm.

<u>Protein content</u>

The protein content of the freeze-dried sample material and freeze-dried Soissons flour was determined by Dumas using a NA 2100 Nitrogen and Protein Analyser (ThermoQuest -CE Instruments, Rodeno, Italy). The conversion factor for gluten protein is 5.7. Methionine was used as standard during analysis. Duplicate measurements are performed; variations of maximally 1% were observed. All protein contents are expressed based on dry matter.

Results

<u>Reproducibility</u>

To check the reproducibility, one experiment was carried out three times. The process conditions used were 15 rpm, 15 °C and 60 min using water absorption of 48.2%. The compositions of the different sections including error bars are shown in Figure 2.1. The protein concentrations of layer 1 to layer 5 measured in experiment 1 were: 6.8%, 7.5%,
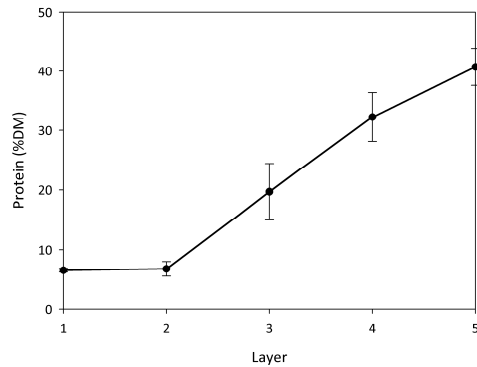
Figure 2.1: Protein concentration in all layers after processing at standard conditions: temperature 15 ˚C, rotation rate 15 rpm, time 60 minutes. Water absorption is 48.2%.

14.8%, 27.1% and 37.1%. The protein concentrations of layer 1 to layer 5 measured in experiment 2 were: 6.2%, 5.2%, 25.1%, 35.2% and 43.2%. The concentrations of layer 1 to layer 5 measured in experiment 3 were: 6.6%, 7.6%, 19.2%, 34.5% and 41.8%. Main variations were observed in the protein concentration of the intermediate layers.

Influence of processing time

The processing time had a pronounced influence on the separation processes. Large gluten aggregates were observed in the whole material when the dough was sheared for 10 min only. The sample sheared for 15 min showed clear aggregates in the upper layers of the shearing device, and darker yellow bands in region 4 and 5 suggesting larger-scale migration of gluten. The samples sheared for longer periods consisted of a gluten-depleted upper part and gluten-rich lower part.

Figure 2.2a shows the different torque curves obtained for the shear experiments at different rotation times. Even though the differences between the curves are relatively
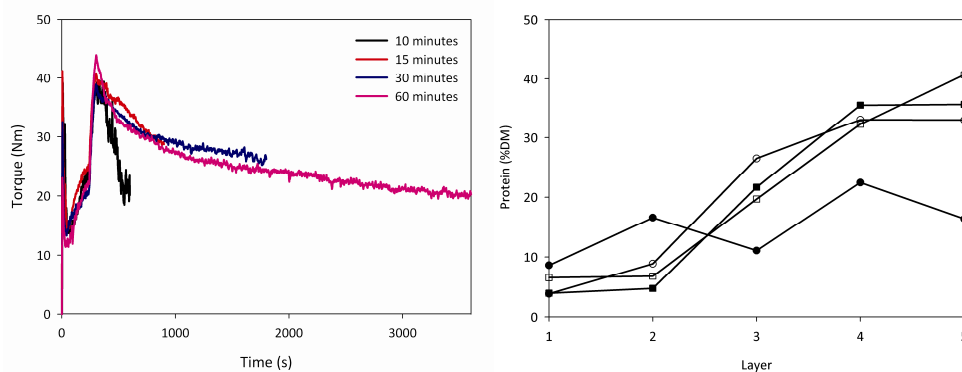

Figure 2.2: a) Online measured torque curves for varying processing time. b) Protein concentration in all layers after processing. Experiments were executed at 15 ˚C at 15 rpm. Water absorption is 48.2%. Processing time ●/-- 10 minutes, ○/--15 minutes, ▨/-- 30 minutes □/-- 60 minutes.

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minor, the sample sheared for only 10 min shows a faster decrease in torque than the others.

The reason for the latter behaviour is not clear, but could be related to variations in the manually controlled vertical pressure. Figure 2.2b shows the protein concentration for all layers of the samples as function of processing time. The material that was sheared for only 10 min showed a fluctuating protein concentration profile. Obviously, the separation of protein started quickly, leading to regions that were already depleted, while other regions were enriched with protein. The samples sheared for longer periods showed a consistent increase in protein concentration over the different layers. Local separation, i.e., the formation of aggregates, was observed in all samples. However, the amount of aggregates was different; shorter shearing times resulted in more aggregates. A shearing time of 15–60 min yielded similar separation, however, differences were observed in the intermediate layers mainly. The enriched gluten fractions contained 33.0% (15 min), 35.6% (30 min) and 40.7% (60 min) protein. A shearing time of 30 min causes the strongest depletion of protein in the first and second layer (protein content 3.9% and 4.7%). This suggests an optimum in processing time, confirming results obtained by Peighambardoust *et al.* (2008). They stated that prolonged shearing had a negative effect on the visco-elastic properties of the gluten, leading to a reduction in driving force for separation.

<u>Influence temperature</u>

Samples sheared at 10 °C and 15 °C showed gluten aggregates in the whole material, while the material sheared at 25 °C and 40 °C did not contain clearly visible aggregates. The torque curves are depicted in Figure 2.3a. A low processing temperature
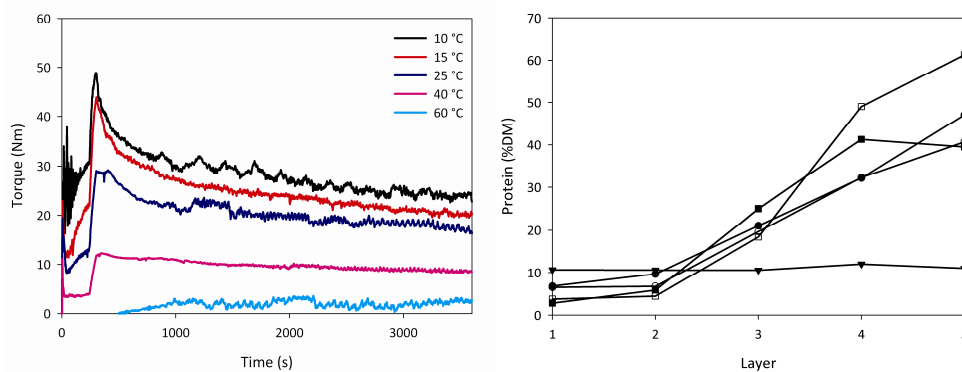

Figure 2.3: a) Online measured torque curves for varying processing temperatures. The lowest temperature corresponds with the highest torque value. b) Protein concentration in all layers after processing. Temperature ●/-- 10 °C, ○/--15 °C, ▨/-- 25 °C, □/-- 40 °C and ▼/-- 60 °C. Experiments were executed at 15 rpm for 60 minutes. Water absorption is 48.2%.

corresponds with a higher torque curve. A higher temperature makes the material less viscous, which leads to lower torque values. In addition, a higher processing temperature resulted in a smaller difference between the lowest and highest measured torque values. All torque curves showed a decrease in torque value upon time, indicating some dough weakening. Figure 2.3b shows the protein concentration in all samples. Shearing the material at 40 °C gave the highest protein concentration in the lower region of the shearing device; a protein concentration of 61.3% was measured. The samples sheared at 25 °C and 40 °C showed the lowest protein concentration in the starch rich layers. In layer 1 a protein concentration of 2.8% was measured for shearing at 25 °C and 3.8% for 40 °C. An explanation for this could be that the hydrophobic interactions become more important (Oakenfull *et al.* 1977) at higher temperature. This effect favours the formation of gluten aggregates, leading to a positive effect on the first step (aggregation) in the separation process.

Influence of the rotation rate

Visual inspection of the samples after shearing showed remarkable differences when varying the rotation rate. The sample processed at 5 rpm showed a clear division between a peripheral white starch rich region and central yellowish gluten-rich region. Materials sheared at 5 and 15 rpm were more elastic in the gluten rich regions and were more powdery in the starch layers. The samples sheared at 30 and 60 rpm were smoother than the samples sheared at 5 and 15 rpm; the sample sheared at 60 rpm even felt rubbery. However, this sample became also quite warm due to viscous dissipation, which will have changed the properties of gluten protein and starch. Separation behaviour will therefore be different. It is possible that the increased temperature might have a negative influence on the separation process. Because gluten starts to loose its visco-elasticity quickly when the product temperature exceeds 50 °C (Attenburrow *et al.* 1990). In addition, starch starts to gelatinise which will have its (negative) influence on the separation process.

Figure 2.4a shows the torque curves during shearing. When a low rotation rate (5 rpm) was applied during the whole experiment, a gradual increase in torque was recorded till 1000 s, after which the torque slowly decreased again. A peak in torque was observed in all other experiments at the moment that the rotation rate in the second part of the experiment was set to the final rotation rate (which was higher than the rotation rate in the starting period). The height of the torque peak depends on the final rotation rate. After the peak dough behaved remarkably shear thinning; the torque value hardly increased upon increased rotation rate.

Figure 2.4b shows the change in protein concentration in each layer over the height of the shearing device. The highest protein concentration was measured in the material

Figure 2.4: a) Online measured torque curves for varying rotation rates. b) Protein concentration in all layers after processing. Rotation rate ●/-- 5 rpm, ○/-- 15 rpm, ▨/-- 30rpm and □/-- 60 rpm. Experiments were executed at 15 °C for 60 minutes. Water absorption is 48.2%.

sheared at 5 rpm (59.6% in layer 5, and 4.4% in layer 1). The material sheared at 60 rpm did not show a significant change in protein concentration in the 4 inner layers and showed only a slightly higher concentration in the outer layer 5 (20%).

Influence of water content

Samples with higher water absorption values were stickier. This stickiness complicated the division of the samples containing water absorption of 53.2% and 58.2% into 5 well-defined layers, which may have influenced the accuracy. Figure 2.5a shows the online measured torque curves. The torque curve indicates that high water content leads to less viscous dough, which is in accordance with expectations. The curve of the sample with a
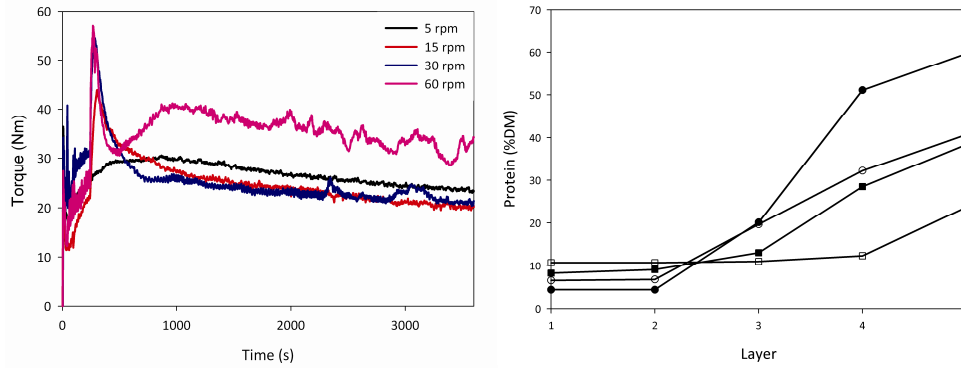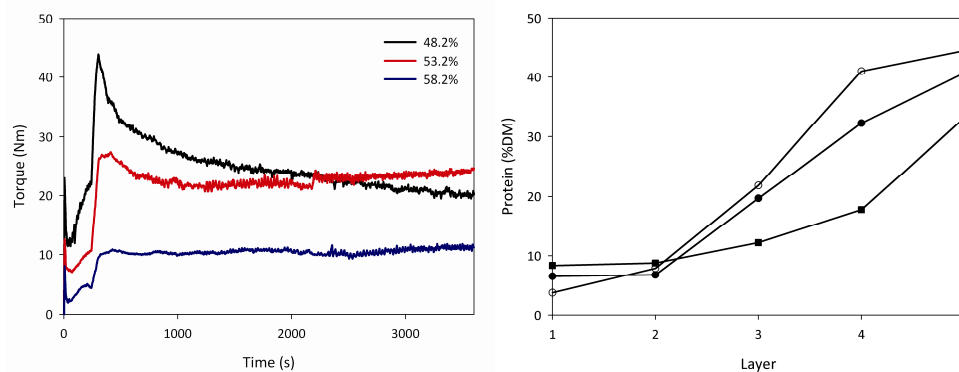

Figure 2.5: a) Online measured torque curves for varying water absorption values. b) Protein concentration in all layers after processing. Experiments were executed at 15 rpm, 15˚C for 60 minutes. Water absorption values ●/-- 48.2%, ○/--53.2% and ■/-- 58.2%.

water absorption value of 58.2% is rather constant in time. For water absorption of 48.2% the torque value decreased during the experiment, indicating a lower viscosity, which suggests dough weakening. The curves for water absorption of 53.2% and 58.2% are more constant during time. In case of 53.2% water absorption, the torque curve even increased upon time.

Figure 2.5b shows the protein concentration in the layers sheared with varying water absorption. The highest water absorption gave the least effective separation. The best separation was obtained using the Farinograph water absorption, 53.2%, but the differences in protein contents are small compared to the system with water absorption of 48.2%.

Constant deformation

We also performed experiments with a constant overall deformation (integral of shear rate over time); to find out whether the same deformation gave the same results or whether other effects play a role as well. All shear runs had the same start-up conditions, 4 min shearing at 5 rpm after which in 1 min the shear rate is gradually increased to the set rotation rate. The settings were: 165 min at 5 rpm, 83 min at 10 rpm, 41 min at 20 rpm, 14 min at 60 rpm. The corresponding torque curves are shown in Figure 2.6a and b shows that separation was obtained in all samples. A constant deformation obtained at intermediate rotation rates (10–20 rpm) gave a more or less comparable degree of separation, but the samples sheared at a high and a low shear rate behaved significantly different. The sample sheared at 60 rpm showed the least noticeable separation. This is probably due to heating as a result of viscous dissipation. The material became
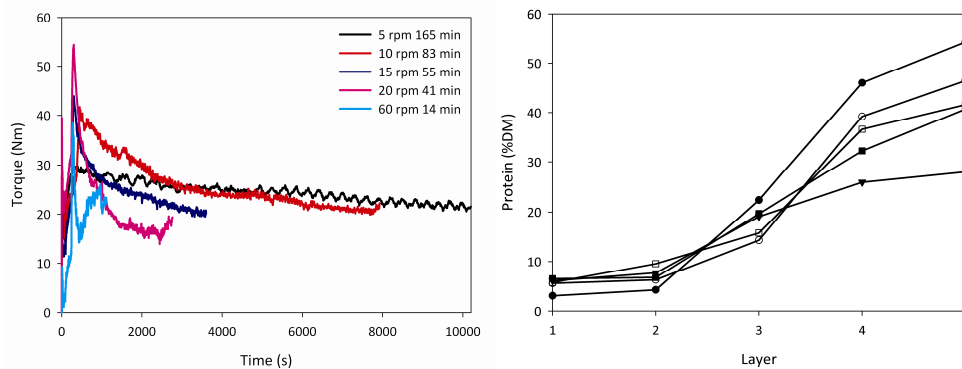

Figure 2.6: a) Online measured torque curves at constant deformation. b) Protein concentration in all layers after processing. Experiments were executed at 15 ˚C and a water absorption of 48.2%. Rotation rate/processing time combinations ●/-- 5 rpm 165 minutes, ○/-- 10 rpm 83 minutes, ▨/-- 15 rpm 55 minutes, □/-- 20 rpm 41 minutes and ▼/-- 60 rpm 14 minutes.

noticeably rubbery in the upper layers. A low shear rate (5 rpm) gave the best separation (54.3% in layer 5). The results confirmed that a low absolute rotation rate is beneficial to obtain a good separation.

<u>Combined optimal conditions</u>

Finally, an experiment was performed in which all best process conditions from the previous sections were combined in a single experiment. Wheat flour dough was sheared at 5 rpm for 60 min at a temperature of 40 °C. The water absorption used was 53.2%, so the Farinograph water absorption value. Figure 2.7 shows the torque curve related to this experiment. Due to the applied temperature and water content, the torque value was low and more or less constant. The system did not result in any appreciable separation: the measured protein concentration in all layers was around the starting concentration (layer 1 10.7%, layer 5 10.3% protein). But, many large protein aggregates were visible. This experiment suggests that the interaction between the various process parameters is probably highly non-linear.
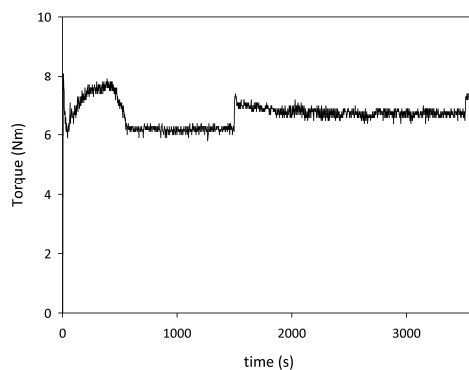
Figure 2.7: Online measured torque curve for combined optimal conditions. The experiment was executed at 5 rpm, 40 ˚C for 60 minutes. Water absorption value dough: 53.2%.

Discussion

The aim of the chapter was to describe the effect of variation in process parameters on the shear-induced separation of gluten and starch from wheat flour. Therefore, the parameters were systematically varied. In a final experiment, all optimal process conditions were combined, but this did not result in the most optimal separation process. Furthermore, constant deformation also led to different results when the rotation rate was varied. To explain the results, we refer to the two stage mechanism earlier proposed by Peighambardoust *et al.* (2008). The first step consists of the local formation of gluten aggregates, while in the second stage the gluten aggregates migrate to the apex of the shearing device.

The formation of aggregates is enhanced by the application of a low shear rate. The final size of the local gluten aggregates decreases with increasing shear rate, due to increasing rupture of gluten domains by the shear forces (Peressini *et al.* 2008). Temperature also influences the formation of aggregates, explaining a positive effect on the first step. The viscosity of the dough decreases at elevated temperatures, leading to lower shear forces and less gluten aggregate rupture. Also water addition increases the rate of aggregation, because water addition increases the volume fraction of gluten phase compared to the starch particles, favouring gluten aggregation. It is expected that the differences in aggregate properties will have an influence on the second step of the separation mechanism. Shape and size of the aggregates will play a role in the migration behaviour. In addition, the resistance that the gluten aggregates experience during migration is influenced by the process conditions at which they are formed. Visual observations suggest that a low rotation rate favours the formation of strong and compact aggregates that can resist higher shear forces before breaking.

Unfortunately, little theoretical information is available about the migration of macromolecules in a curvi-linear shear field. Dill (1979) described the migration and separation of DNA molecules in a shearing device consisting of two concentric cones. These DNA molecules can be approximated as random coils that behave as a Hookean spring when stretched in the curvi-linear shear field. The curvature of the flow lines result in a force component that acts radially inwards, resulting in a migration of the DNA molecules towards the centre of the cell. The migration velocity in case of DNA was hypothesized to be proportional to the square of the rotation rate (Dill 1979; Agarwal *et al.* 1994). Dill further states that the migration is proportional to the aggregate size (even to the power 5), the matrix viscosity, and the applied shear rate. Although it is clear that the dough system differs significantly from the DNA system, there are similarities, namely that gluten aggregates are also elastic to a large extent and that they will extend in a shear field. Since our system is curved as well, one might expect the same type of behaviour by the stretched gluten aggregates, provided that they are strong enough to allow strong deformation without breaking. Thus, the nature of the driving force for both migration processes might be rather similar.

We schematically summarized the effects described above in Figure 2.8. Figure 2.8a summarizes the effect of shear stress on the maximum size that a gluten aggregate can obtain. A lower shear stress (induced by higher water content, lower shear rate, or higher temperature) leads to larger aggregates. Figure 2.8b then summarizes the effect of shear stress on the migration for various aggregate sizes: larger gluten aggregates migrate faster to the apex of the cone. In other words, a higher stress leads to a larger driving force. Figure 2.8c combines the two previous graphs. From Figure 2.8a, the maximum aggregate size can be read, which is then used to derive the driving force for
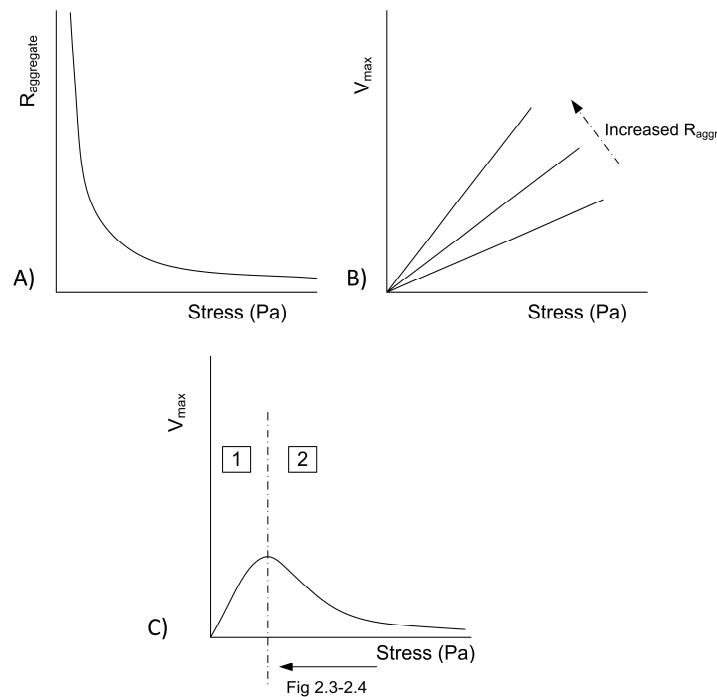
Figure 2.8: Schematic representation of the separation process. a) Influence of shear stress on the gluten aggregate formation (Step 1). b) Influence of shear stress on the aggregate migration (Step 2) c) The influence of shear stress on the total amount of migrated gluten protein material.

migration. Now an optimum in migrated gluten protein can be obtained. When shear stresses are too high, gluten aggregates are not formed anymore or existing aggregates will break up.

Figure 2.8 can be used to explain why the combination of the optimal process conditions does not have a linearly cumulative effect on the separation behaviour. Except for the water content, all changes in process conditions favoured the formation of aggregates. But combining those optimal process conditions led to a low dough viscosity and thus a low force onto the aggregates. As a result, the aggregates were hardly stretched, hardly any driving force for migration was created and no separation was obtained. Therefore it can be said that the chosen 'optimal' conditions (Figure 2.6) were mainly based on the mechanism of the first step (formation of gluten aggregates). The chosen conditions were unfavourable for the second step (migration). Indeed, aggregates were found to be present, which have not been migrated to the apex. Figure 2.8c suggests that the chosen conditions were probably too much towards region 1.

Figure 2.8 can also be used to understand the outcomes of the constant deformation experiments. Low shear rates were found to be favourable for the separation of wheat flour. Larger gluten aggregates can be formed at this shear rate. Visually, the aggregates

created at lower rotation rates seemed to be more compact than those formed at higher shear rates. Their greater strength combined with the lower shear rates means that they can become larger. At a certain stage, these aggregates are then so large, that they will be stretched significantly leading to gluten migration. At intermediate processing conditions, the growing aggregates will be stretched earlier, when they are still smaller. Not all gluten was yet aggregated, and therefore the separation is less complete. The effect becomes smaller at higher shear rates, since the dough is rather shear thinning. At very high shear rates, viscous dissipation became important leading to a significant increase in product temperature leading to additional effects such as starch gelatinisation and loss of gluten elasticity.

Figure 2.8 summarizes the knowledge on the separation process described in this chapter. We used general principles on gluten aggregation and migration of elastic materials in a curved shear filed. Therefore we expect that Figure 2.8 is not only applicable to the flour studied here, but can also be applied to describe the separation of other flours. However, differences then could be in the slope of the lines in Figure 2.8a and b to indicate different flour strengths and aggregation capabilities. From Figure 2.8 it can be concluded that step 1 and 2 of the separation mechanism are influenced differently the majority of the process parameters. In some conditions, the effects even counteract each other. Therefore, we conclude that it will be difficult or impossible to find the optimal settings for the separation in this device. Nevertheless, the fact that protein enrichment up to 60%, and protein depletion to about 2% was already obtained without knowing the most optimal processing conditions, indicates the promising opportunities of the new separation principle outlined.

Conclusion

Wheat flour (dough) can be separated into starch depleted and gluten enriched fractions using a conical shearing device, over a broad range of process conditions. A protein enriched fraction of 61.3% was obtained (40 °C) and a protein depleted fraction of 2.8% protein (25 °C) was obtained without optimising the separation process.

This study confirms the theory that the migration of gluten follows a two step mechanism. In the first step, gluten is formed into aggregates that grow steadily larger while shearing. In the second step, these aggregates migrate towards the apex of the cone. The two steps of the separation process are promoted by different process conditions, but the effects of process conditions on the two steps can be counteractive. Gluten agglomeration is promoted by high temperature, low rotation rate and high water content. Migration is favoured by a higher rotation rate and a higher dough viscosity (or low water content).

Acknowledgements

References

Agarwal, U.S., Dutta, A. and Mashelkar, R.A. (1994). Migration of macromolecules under flow: the physical origin and engineering implications. *Chemical Engineering Science* 49 (11): 1693-1717.

Attenburrow, G., Barnes, D.J., Davies, A.P. and Ingman, S.J. (1990). Rheological properties of wheat gluten. *Journal of Cereal Science* 12(1): 1-14.

Dill, K.A. (1979). Theory for the separation of very large DNA molecules by radial migration. *Biophysical Chemistry* 10(3-4): 327-334.

Frederix, S.A., Courtin, C.M. and Delcour, J.A. (2004). Influence of process parameters on yield and composition of gluten fractions obtained in a laboratory scale dough batter procedure. *Journal of Cereal Science* 39(1): 29-36.

Kuktaite, R., Larsson, H., Marttila, S. and Johansson, E. (2005). Effect of mixing time on gluten recovered by ultracentrifugation studied by microscopy and rheological measurements. *Cereal Chemistry* 82(4): 375-384.

Larsson, H. and Eliasson, A.C. (1996). Phase separation of wheat flour dough studied by ultracentrifugation and stress relaxation .2. Influence of mixing time, ascorbic acid, and lipids. *Cereal Chemistry* 73(1): 25-31.

Manski, J.M., van der Goot, A.J. and Boom, R.M. (2007). Formation of fibrous materials from dense calcium caseinate dispersions. *Biomacromolecules* 8(4): 1271-1279.

Oakenfull, D. and Fenwick, D.E. (1977). Thermodynamics and mechanism of hydrophobic internaction. *Australian Journal of Chemistry* 30(4): 741-752.

Peighambardoust, S.H., Hamer, R.J., Boom, R.M. and Van der Goot, A.J. (2008). Migration of gluten under shear flow as a novel mechanism for separating wheat flour into gluten and starch. *Journal of Cereal Science* 48(2): 327-338.

Peighambardoust, S.H., van Brenk, S., van der Goot, A.J., Hamer, R.J. and Boom, R.M. (2007). Dough processing in a Couette-type device with varying eccentricity: Effect on glutenin macro-polymer properties and dough micro-structure. *Journal of Cereal Science* 45(1): 34-48.

Peighambardoust, S.H., van der Goot, A.J., Hamer, R.J. and Boom, R.M. (2005). Effect of simple shear on the physical properties of glutenin macro polymer (GMP). *Journal of Cereal Science* 42(1): 59-68.

Peressini, D., Peighambardoust, S.H., Hamer, R.J., Sensidoni, A. and Van der Goot, A.J. (2008). Effect of shear rate on microstructure and rheological properties of sheared wheat doughs. *Journal of Cereal Science* 48(2): 426-438.

Robertson, G.H. and Cao, T.K. (2003). Effect of processing on functional properties of wheat gluten prepared by cold-ethanol displacement of starch. *Cereal Chemistry* 80(2): 212-217.

Van der Borght, A., Goesaert, H., Veraverbeke, W.S. and Delcour, J.A. (2005). Fractionation of wheat and wheat flour into starch and gluten: overview of the main processes and the factors involved. *Journal of Cereal Science* 41(3): 221-237.

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Influence of sodium chloride on shear flow induced starch-gluten separation from Soissons wheat dough

Abstract

Wheat dough can be separated into a starch-rich and a gluten-rich fraction by subjecting the dough to curvilinear shear flow. This chapter presents the effect of salt (NaCl) addition on the shear-induced separation process. The separation (defined as the changes in protein concentration in the various layers, compared to the starting material) was promoted by NaCl addition up to a concentration of 4 w%. Dough without NaCl showed limited separation, but this effect could be partly compensated by a decreased processing time. Rheology measurements did not show clear differences in G' and tan δ value, for dough with different NaCl concentration. But, shear stress and normal force did vary for various NaCl concentrations when applying a constant shear rate.

Nevertheless, the large differences in separation behaviour are probably more related to the influence of salt on gluten aggregation and properties resulting thereof, than the differences in dough rheological properties.

Introduction

Separation of wheat dough into its constituents, starch and gluten, is an important industrial process. The current industrial separation processes, based on kneading and washing (Van der Borght *et al.* 2005), are energy and water intensive and lead to a significant protein loss. Therefore, a need exists for an alternative separation method that is more efficient in terms of protein recovery, water and energy consumption.

Peighambardoust *et al.* (2008) described an alternative separation procedure. Subjecting wheat flour dough to curvilinear shear flow (which is obtained by simple shear flow in a curved geometry), using a conical shearing device, led to the creation of a central region (referred as apex) that was rich in gluten, and a peripheral band that was rich in starch. Those results were obtained with a recipe, originally mentioned for bread applications, therefore inclusion the salt sodium chloride. But, this salt is strongly undesired for application on industrial scale. Disposal of waste water will be expensive and time consuming. In addition, salt reduction is also important from a nutritional point of view. However, it is well-known that NaCl has a strong, and often positive, influence on the dough properties (Miller and Hoseney 2008). It is therefore probable that salt will be of influence on the separation behaviour as well. Since this shear-induced separation is based on rheological properties, we will connect the rheological properties of the sheared material to the separation behaviour as function of the salt content.

Salt plays an important role in baked dough products and related processes. It is generally used at levels of about 1–2% (based on flour weight). Salt acts as a flavour enhancer and as stabilizer for the yeast fermentation rate (Miller and Hoseney 2008). It takes longer mixing before a certain dough strength is obtained with NaCl than without NaCl, but dough containing NaCl can resist mixing for longer time periods (Butow *et al.* 2002; Miller and Hoseney 2008). Farinograph studies confirmed that the addition of NaCl increased the stability time and dough stability (Tanaka *et al.* 1967). The extensibility of dough is increased by addition of NaCl (Butow *et al.* 2002). Several studies using the Farinograph showed that the water absorption decreased upon salt addition, but this is not observed in a mixograph (Miller *et al.* 2008). Furthermore, NaCl solubilises part of the wheat proteins (Osborne 1924). Finally, Ukai *et al.* (2008) showed that the association (i.e. aggregation) behaviour of wheat protein is favoured upon salt addition, which was reflected by an increase in intermolecular β-sheet structure content.

Larsson (2002) investigated the relation between rheological parameters and NaCl concentration for dough mixed for 5 min in a Farinograph. The water content, 46% on total basis, was kept constant in all experiments. A moderate, but significant, increase in the elastic and viscous moduli was observed when increasing the NaCl concentration

from 0 w% to 2 w% (based on flour weight). Also Hoseney (1994) described a strengthening effect of dough. Preston (1989) observed this dough strengthening effect at low salt concentrations, using Farinograph, Extensiograph, and Alveograph methods. Generally, the strengthening effects were related to the electrostatic shielding of the charged amino acids on the gluten protein surface, which induces stronger inter-protein interactions. Contrary to the findings above, Salvador *et al.* (2006) found a slight reduction in the visco-elastic modulus. Melander and Horváth (1977) described the latter effect as an increase in the ordering of the water structure inside a dough. They concluded that the slight reduction of the visco-elastic modulus upon salt addition was caused by a decrease of the inter-protein hydrophobic interactions. The differences of the effects of salt on the dough rheological properties could be caused by the different starting materials, (e.g. the moisture content and flour type), mixer types and process conditions. So, overall, it has to be concluded that no unambiguous agreement in literature exists.

As outlined above, the exact influence of salt on the properties of dough is not harmonized with each other. Besides, the effects of salt on the functional properties of dough are only studied in mixing processes, the impact of shearing on functional properties of the dough may well be different (Peighambardoust *et al.* 2006b; Peressini *et al.* 2008). The shear-induced separation of wheat flour may well provide additional insight on the influence of NaCl on that. Therefore, wheat flour dough with salt concentrations of 0, 0.5, 1, 2, 4, and 7 w% (based on flour weight), were processed in a shearing device and analysed on the distribution of protein. Rheological differences between the different dough formulations were determined through rheological analyses; amplitude, frequency, and constant shear rate sweeps.

Materials and methods

Materials

Experiments were carried out with Soissons flour from a single wheat cultivar (Meneba, Rotterdam, The Netherlands). Protein content, moisture content, Farinograph water absorption, stability time, peak time, and tolerance index of flour were 11.2% (on dry basis), 14.5%, 53.2% (which means 53.5% on 14% moisture), 14 min, 23.5 min and 30 BU, respectively. Water absorption was determined by the addition of tap water to 300 g Soissons flour (moisture content 14.5%) to which 6 g NaCl (Merck, Germany) was added (i.e. 2.0% on flour wet basis).

Farinograph processing

Farinograph characteristics were determined for several NaCl concentrations based on flour weight, according to AACC method 54-21. The characterisation took place using 63 rpm and 30 °C. NaCl was added additionally to the flour in a 300 g Farinograph. The water absorption values are given in Table 3.1. The NaCl concentrations are expressed in percentage NaCl on flour wet basis (14.5% moisture).

Table 3.1:
Measured Farinograph water absorption and calculated amounts for processing of Soissons flour, tap water and NaCl in the shearing device for each NaCl concentration.

NaCl concentration (w%) [a]	Farinograph water absorption (%)	Soissons flour (g) [b]	Tap water (g) [b]	NaCl (g) [b]	Molarity (M)
0	54.2	70.79	34.21	0	0.000
0.5	54	70.64	34	0.35	0.176
1	53.7	70.55	33.75	0.7	0.355
2	53.2	70.32	33.29	1.4	0.720
4	52.9	69.53	32.71	2.76	1.444
7	52.7	68.27	31.98	4.75	2.542

[a] Flour weight basis (so including the natural moisture content)
[b] Calculated amounts of the compounds were used in the same ratio for the rheological analyses. Soissons flour was set in that case to 50.00g

Shearing device

Processing of the starting material was performed using a laboratory scale shearing device, which is based on a cone-plate rheometer. The in-house developed shearing device (Wageningen University, The Netherlands) consists of a stationary and a rotating cone. In the narrow gap between the cones, the sample material was exposed to shear flow. The exact configuration of the shearing device was described previously by Peighambardoust *et al.* (2008) and Manski *et al.* (2007).

The shearing device was connected to a Brabender Do-Corder 330 unit (Brabender OHG, Duisburg, Germany) with an interface and controlling unit for on-line measuring of the temperature and torque readings. The temperature of the shearing device was controlled through a circulating water flow through the system. The circulating water temperature was set to 15 °C during the experiment.

Preparation of dough

The preparation of the dough was described earlier (Van der Zalm *et al.* 2009b). The amounts used for each experiment are given in Table 3.1. The ingredients were manually mixed in a beaker glass using a spatula. Meanwhile water was added in three fractions to the mixture. The amount of water added was 5% less than the water absorption determined by Farinograph. The ingredients were mixed and immediately transferred to the shearing device to prevent dehydration of the mixture. Part of the shearing trials included a resting time before processing. In that case the samples were rested for 15 min at 15 °C in a closed shearing device. All samples were initially sheared at 12 s^{-1} (5 rpm) for 4 min to avoid slippage and to obtain a homogenous starting material. The shear rate was then increased to 36 s^{-1} (15 rpm) over 1 min. The total duration of the shearing process was 60 min. After processing, the material was cooled to approximately 5 °C in stationary position. A vertical compression force of 300 kgf (2940 N) was put onto the material and kept constant during the experiments.

Freeze drying

The hypotenuse of the shearing device is 8.5 cm. The material was divided in 5 equal layers of 1.7 cm each, and encoded. The various fractions were immediately frozen using liquid nitrogen. The samples were then freeze dried to an average moisture content of 3.5%. The freeze dried material was powdered with an IKA Mill (IKA type A11, Staufen, Germany) and sieved using a pore size of 0.355 mm.

Protein content

The protein content of the freeze dried samples and Soissons flour, were determined using Dumas with a NA 2100 Nitrogen and Protein Analyser (ThermoQuest-CE Instruments, Rodeno, Italy). A conversion factor of 5.7 was used for gluten protein. Methionine was used as standard during analysis. Duplicate experiments were performed. The maximum variation in protein concentration was 3.3% for duplicate measurements in case of a high protein concentration. Most samples showed an experimental variation of approximately 0.5% in protein content in repeated protein measurement.

Rheological characterisation

Rheological measurements, including the determination of normal forces at constant shear rate, were performed with a stress controlled Paar MCR 301 rheometer (Anton Paar, Graz, Austria). Dough was kneaded at 63 rpm and 15 °C in a 50 g Farinograph for 3 min, using 50 g Soissons flour and on top of that tap water and NaCl. Triplicate rheological measurements were performed for the 2% NaCl sample to check

reproducibility. All other samples were measured once to determine the rheological properties of the material. We choose to use a resting period of 15 min before all rheological characterisations. This is used more often for dough rheology. (e.g. Edwards *et al.* 2001; Larsson 2002; Angioloni *et al.* 2005; Salvador *et al.* 2006).

Oscillatory strain amplitude sweeps were carried out using a serrated plate–plate configuration (diameter 25 mm, gap 1 mm). Measurements were performed over a strain range of 0.001– 400% in logarithmic steps at a frequency of 1 Hz. Temperature was kept constant at 15 °C.

Oscillatory frequency sweeps were made using a constant strain of 0.01% at a temperature of 15 °C. Serrated plate–plate geometry was used during experiments (diameter 25 mm, gap 1 mm). The used strain value was within the linear visco-elastic region as determined in the amplitude sweeps. The frequency was increased logarithmically between 0.1 Hz and 100 Hz.

Normal forces were measured for all NaCl concentrations at a constant shear rate of 0.1 s^{-1}. Also these experiments were conducted using serrated plate–plate geometry (diameter 25 mm, gap 1 mm). Temperature during analysis was 15 °C.

Results

Water absorption by the Farinograph method

The NaCl concentration influenced the water absorption value of Soissons flour dough. The torque curves, resulting from the determination of the water absorption by using the Farinograph method, showed large differences as can be seen in Figure 3.1. The addition of NaCl to Soissons flour dough decreased the required water content, which is in line with earlier studies (Among others Tanaka *et al.* 1967). The strongest effect can be seen for high salt concentrations. For low salt concentration, the Farinograph curves
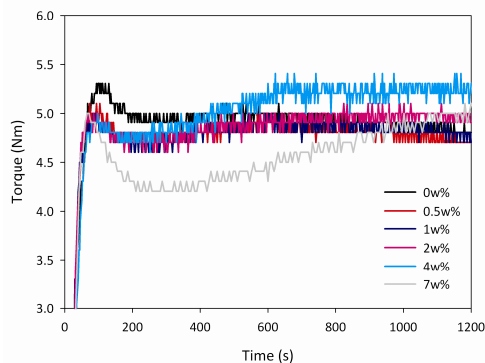

Figure 3.1: Torque curves of the Farinograph water absorption determination of dough with varying NaCl concentration.
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were rather similar. The hydration peak precedes a decrease in torque value, which is then subsequently followed by a moderate rise in torque. The stability time increased slightly for higher salt content. The 4 w% NaCl dough behaved initially similar as the other types of dough, but its torque values remained higher. The dough with 7 w% NaCl showed a much stronger decrease in torque, and only recovered to normal torque values after approximately 800 s.

Torque curves shear runs

Figures 3.2a and 3.2b show the torque curves of the shear runs for varying NaCl concentration, with and without a resting period of 15 min. In general, the torque values were slightly lower for the samples with a resting period, due to relaxation of the material during resting. The dough without NaCl gave a much lower torque value than the other dough samples after resting. The dough made with 7 w% NaCl did not show the peak that was observed with the other doughs, after the increase of the rotation rate at 240 s.
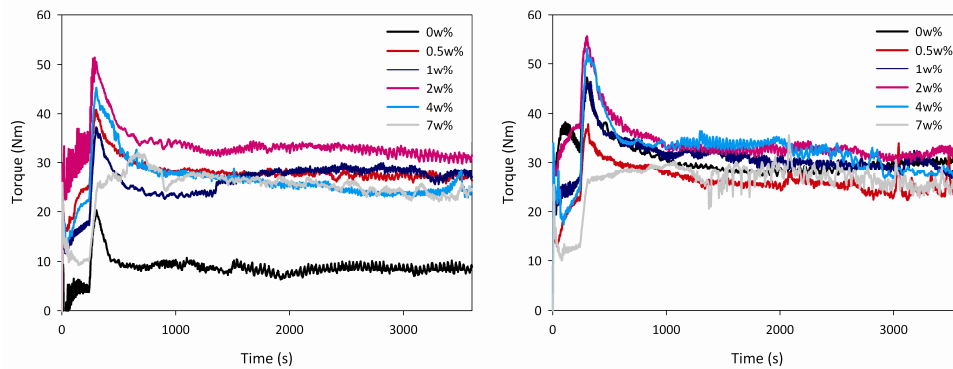
Figure 3.2: a) Torque curves of the shear runs performed with a resting time of 15 minutes for varying NaCl concentration in Soissons dough. b) Torque curves of the shear runs performed without resting period for varying NaCl concentration in Soissons dough. In both cases the process conditions were 60 minutes, 15 rpm, 15 °C.

Protein content

Table 3.2 shows the protein content of the sheared samples with varying NaCl concentrations, in the different layers of the shearing device. A gluten enriched fraction at the apex of the cone was obtained for all NaCl concentrations, with and without a resting time. The highest protein concentration in the apex was measured for the dough with 4 w% NaCl. In both cases, with and without resting, the concentration was around

Table 3.2: Measured protein content (% w/w) for each layer after shearing at different NaCl concentrations. Layer 1 represents the rim of the cone, layer 5 is the centre.

NaCl concentration (%)	Layer	Without resting time		With resting time	
		Protein %	*SD*	*Protein %*	*SD*
0	1	10.7	0.58	9.1	0.24
	2	11.2	0.37	11.9	0.08
	3	11.4	0.31	13.2	0.07
	4	11.3	0.12	13.0	0.11
	5	16.8	0.65	13.4	0.00
0.5	1	9.2	0.04	6.1	0.06
	2	9.2	0.28	10.0	0.69
	3	14.6	0.24	21.6	0.38
	4	21.8	0.11	19.5	0.00
	5	30.9	0.14	21.1	0.61
1	1	9.9	0.32	6.4	0.20
	2	3.5	0.25	10.6	0.28
	3	15.7	1.30	22.0	0.05
	4	16.8	0.10	26.2	0.13
	5	33.0	0.25	26.8	0.92
2	1	7.0	0.05	6.6	0.09
	2	8.1	0.36	7.6	0.32
	3	22.4	0.17	19.2	0.56
	4	30.2	0.64	34.5	0.02
	5	44.5	2.44	41.9	0.43
4	1	6.1	0.27	4.5	0.07
	2	10.3	0.32	5.4	0.29
	3	22.6	0.66	20.7	0.33
	4	48.1	0.93	45.7	0.43
	5	55.3	1.13	54.9	0.27
7	1	8.6	0.12	10.7	0.25
	2	10.0	0.07	11.5	0.33
	3	8.7	0.04	11.5	0.52
	4	15.9	0.32	7.9	0.34
	5	19.6	0.47	10.6	0.58

55% (w/w) protein. However, when the NaCl concentration was increased to 7 w%, hardly any separation was observed irrespective of resting. Dough without NaCl gave only limited separation under those process conditions. Without resting time, the protein content in the second layer was lower than in the first and third layer. It seems that the second layer is depleted from protein. This behaviour was also observed in previous research and was there interpreted as indication of the first stages of migration of gluten protein to the apex of the cone (Van der Zalm *et al.* 2009a; 2009b).

Oscillatory shear experiments

Figure 3.3a illustrates the results of the oscillatory shear experiments for varying NaCl concentrations. No differences could be observed between the samples made in the range of 0–4 w% NaCl. The 7 w% NaCl sample gave a somewhat higher elastic modulus (G'), with the same strain dependency. Figure 3.3b shows the phase angle (tan δ) values for increasing strain. Also the phase angle values did not clearly vary in the range of 0–4 w% NaCl.

Frequency sweeps

Figure 3.3c shows the dependency of the elastic modulus on the angular frequency for doughs with varying NaCl concentrations. The elastic modulus first increased slightly (up to 2 w% NaCl), then decreased (4 w% NaCl), and then increased again (7 w% NaCl). There seems to be an effect on G' values, though there was no correlation between the NaCl concentration and G' values. Nevertheless, the 7 w% NaCl dough had higher G' values. The tan δ values showed minor dependence, as can be seen in Figure 3.3d. The doughs up to 4 w% NaCl showed overlapping tan δ values. Only the 7 w% NaCl dough had a higher tan δ value over the whole angular frequency range.

Constant shear rate sweep

During the separation experiments, a constant shear rate was applied. To mimic the shear runs, we tried to use the same shear conditions during the rheological analysis as created during the separation experiments in the shearing device. This was unsuccessful, due to sample breakage in the rheological experiments. Therefore, it was decided to apply a lower shear rate, namely 0.1 s^{-1}. Figure 3.4a illustrates the influence of a constant shear rate during time on the shear stresses created in the system. During the first minute, the stresses in the dough system with a low NaCl concentration (0–2 w%) built up high stresses and subsequently underwent a maximum after 90 s, due to sample breakage. The dough samples with 4 and 7 w% NaCl built up stress at a much lower rate and did not (yet) show a clear maximum in stress. Salt addition thus reduced the accumulation of stress. Simple shear flow led to the build-up of normal forces as well,
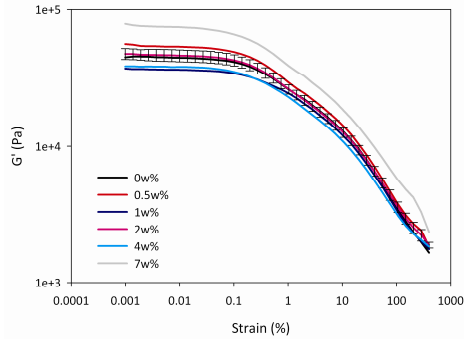
Figure 3.3: a) Elastic modulus (Pa) versus strain (%) for Soissons flour dough with varying NaCl concentration. The dough was kneaded for 3 minutes at 63 rpm in a 50 gram farinograph bowl.

Figure 3.3: b) Tan delta (phase angle) versus strain (%) for Soissons dough with varying NaCl concentration. The dough was kneaded for 3 minutes at 63 rpm in a 50 gram farinograph bowl.

Figure 3.3: c) Elastic modulus versus frequency. Frequency dependency of Soissons dough for varying NaCl concentration. The dough was kneaded for 3 minutes at 63 rpm in a 50 gram farinograph bowl.

Figure 3.3: d) tan delta versus frequency. Frequency dependency of Soissons dough for varying NaCl concentration. The dough was kneaded for 3 minutes at 63 rpm in a 50 gram farinograph bowl.

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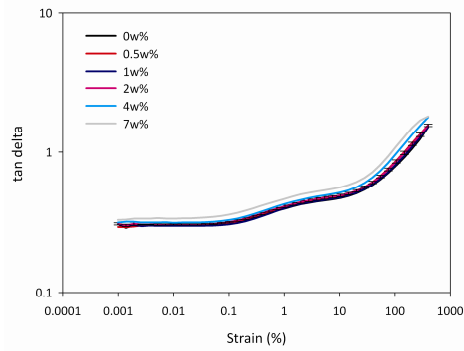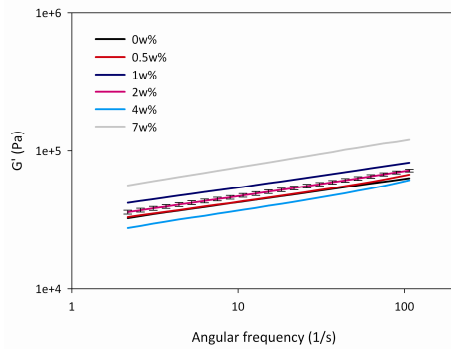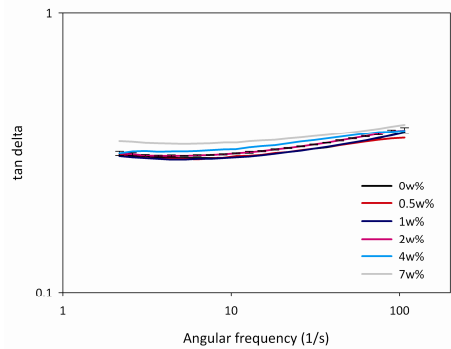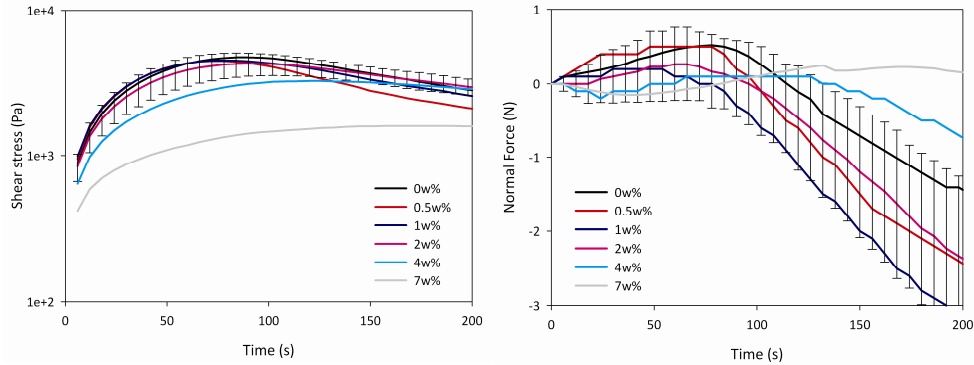Figure 3.4: a) Shear stress during time at constant shear rate of 0.1 s^{-1} for varying NaCl concentrations. b) Normal force during time at constant shear rate of 0.1 s^{-1} for varying NaCl concentrations.
The dough was kneaded for 3 minutes at 63 rpm in a 50 gram Farinograph bowl.

which can be observed already at this low shear rate (Figure 3.4b). Though the experiments should be interpreted with care, it seems that moderate salt concentrations (0.5–2 w%) resulted in the highest normal forces, and a subsequent decrease due to breakage.

Discussion

The aim of this chapter is to study the influence of salt on the separation of starch and gluten from wheat flour under curvilinear shear flow. The previous section showed that the separation behaviour of wheat dough is strongly dependent on the NaCl concentration. Up to a concentration of 4 w% NaCl, separation is promoted. Increasing the concentration to 7 w% NaCl negatively influenced the separation process.

Rheological characterisation

Unfortunately, it was not possible to characterise the rheological properties of the dough as it was placed in the shearing device. The properties of hand-mixed dough (i.e. the real starting material) could not be measured, since those samples did not form a cohesive mass. Shearing a dough for a few minutes led to gluten aggregates and the onset of separation. Alternatively, we could have chosen to measure zero-developed dough as presented by Campos *et al.* (1996) and Lee *et al.* (2001). They prepared dough samples by mixing ice-particles and flour, followed by ice melting. However, Peighambardoust *et al*. (2006a) showed that the mixing behaviour was of zero-developed dough and dough prepared by flour–water mixing were not the same.

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Therefore, we decided to measure the properties of dough prepared by wheat flour–water samples that were shortly kneaded at the temperature (15 °C) used during the separation experiments. By kneading the samples for only 3 min, a comparable response was found in the Farinograph water absorption test. The stage of dough development might however be different, because at 3 min, the torque value of the 0 w% NaCl dough is already decreasing while that of the other samples is still increasing. According to Kim *et al.* (2008) the stage of dough development might influence the visco-elastic properties of dough. Phan-Thien *et al.* (1997) measured shear stresses created for a constant shear rate ranging from 0.000665 s^{-1} to 10.5 s^{-1} as function of time. They also measured normal stress differences. Stress–strain curves at a constant shear rate of 0.1 s^{-1} were obtained by Kim *et al.* (2008). It should be noted however that both research groups measured the dough rheological responses in dough that contained considerably more water. Because the separation effect is lost at higher water content (Van der Zalm *et al.* 2009b), dilution was not an option.

Even though the rheological measurements gave rather high experimental variation, one can divide the different types of dough into three classes: no salt, moderate salt, and high salt concentration. It seems that a higher salt concentration results in a lower yield strain and reduced normal forces. This difference may explain why a high salt concentration leads to a reduced separation.

The rheological measurements emphasize the importance of normal forces. Once more, higher salt concentrations can lead to smaller build-up of normal forces and little or no decline during time. It should be remarked that the values of the normal forces are very high, since the measurements were performed using a very low shear rate. It can thus be expected that a higher shear rate, such as applied during experiments performed in the shearing device, will lead to higher normal forces. This might account for the migration (and separation) phenomena observed. Even though the shear rate applied in the rheological test (0.1 s^{-1}) is much lower than applied in the shearing device (36 s^{-1}), an impression can be obtained how the material would behave in the shearing device. The 360 times higher shear rate should lead to an extremely large normal force. The shearing device was designed in such a way that the material was not able to leave the device, which will cause even higher stresses in the system. The normal force could be related to the formation of larger gluten aggregates, which deform upon shear flow. These aggregates grow in size and ultimately are forced towards the centre of the curvilinear flow field. This effect resembles the Weissenberg effect described by Sperling (2001). The forces onto the aggregates present in the system, caused by the curvature in shear field, induce the larger aggregates to migrate to the apex of the cone.

Resting period

A resting period of 15 min seems to be negative for separation. This might be related to relaxation phenomena and repolymerisation of the structure. This may lead to breakage of the gluten aggregates and subsequent to poorer separation.

New insights in shear-induced separation

The rheological measurements can be used to explain the dependence of the separation behaviour on NaCl concentration. NaCl influences the network formation in dough systems (Preston 1989; Hoseney 1994; Larsson 2002). At low salt concentrations (0, 0.5, 1 w% NaCl), the gluten interactions are relatively weak. Thus, aggregates are formed quickly, but will also lead to easy breakage. The driving force for subsequent migration of gluten to the apex of the cone remains small. A higher salt concentration (2–4 w% NaCl) induces stronger interactions between gluten aggregates, hence stronger and larger aggregates and better and faster migration. At very high salt concentration (7 w% NaCl), the interactions become so strong, that they cannot easily deform in the shear flow field anymore. Breakage occurs too quickly and the formed gluten aggregates redisperse again, leading to low migration.

Peighambardoust *et al.* (2008) observed that when dough, containing 2 w% NaCl, was sheared for more than 120 min, redistribution of gluten was observed in the shearing device. This could mean that also shearing weakens dough during time, which could be defined as overshearing. To check whether overshearing had taken place when shearing dough without salt, additional shearing experiments were performed with shorter processing times, i.e. 10 and 30 min. Both experiments gave a higher concentration of gluten protein in the apex of the cone. The concentrations measured in layer 1–5 after shearing for 10 min: 8.7%, 11.1%, 16.2%, 17.0%, and 26.4%. Shearing for 30 min gave 11.1%, 11.3%, 13.5%, 12.8%, and 17.8%. Compared to the protein concentrations measured after shearing for 60 min (Table 3.2), the protein concentrations measured after shearing for 10 or 30 min were higher in the layers 4 and 5. Obviously, in the first minutes separation was obtained, and further processing eventually led to a redistribution of the gluten.

In previous research we described results obtained after shearing a wheat flour mixture containing 2 w% NaCl for 10 min. The process included a resting period of 15 min. A protein content around 20% was obtained in the upper layer (Van der Zalm *et al.* 2009b). If we compare this to the results with a dough without NaCl sheared for 10 or 30 min, a remarkable observation can be done. Shearing for 10 min without NaCl showed a comparable or maybe even better amount of separation. Thus, we see fast, but limited separation for dough without salt. This is in line with the fact that dough without salt can be developed faster, but is less process tolerant (Butow *et al.* 2002). This could lead to

faster overmixing and overshearing of the material.

Shear-induced separation of wheat flour seems to be possible for dough without salt, as long as one takes the low process tolerance into account. Addition of NaCl results in a stronger dough that is more resistant to applied shear forces. To separate wheat flour without salt, shorter processing times are necessary and the separation process should be defined more accurately. Decreasing the rotation rate and increasing the process temperature of the separation process could promote the formation of aggregates, leading to higher gluten yields at the apex of the cone (Van der Zalm *et al.* 2009b).

Conclusion

The NaCl concentration in dough has a strong influence on the separation behaviour of dough into starch and gluten. Up to a NaCl concentration of 4 w%, separation is promoted. No salt or a very high salt concentration did not result in significant separation using standard conditions. Nevertheless, shearing dough without NaCl for shorter times like 10 and 30 min, a significant amount of separation could be observed. So, dough containing no or a low NaCl concentration may well be separated, but the process conditions are much more critical.

The effects of salt on the rheological behaviour of dough are not sufficient to explain the differences in separation behaviour. However, the time-dependent accumulation of shear stress during constant deformation, and the simultaneous development of normal force, confirms that salt increases the interactions between gluten aggregates. Without salt, the interaction is weak as a result of which the shear force can easily break the aggregates leading to poor separation. Higher salt concentrations improve the interaction, leading to stronger aggregates, which grow subsequently larger that they migrate towards the centre of the cone. Excessive salt makes the gluten interaction too strong, leading to aggregate breakage upon prolonged deformation, and hence reduced separation.

Acknowledgements

References

Angioloni, A. and Dalla Rosa, M. (2005). Dough thermo-mechanical properties: influence of sodium chloride, mixing time and equipment. *Journal of Cereal Science* 41(3): 327-331.

Butow, B.J., Gras, P.W., Haraszi, R. and Bekes, F. (2002). Effects of Different Salts on Mixing and Extension Parameters on a Diverse Group of Wheat Cultivars Using 2-g Mixograph and Extensigraph Methods. *Cereal Chemistry* 79(6): 826-833.

Campos, D.T., Steffe, J.F. and Ng, P.K.W. (1996). Mixing wheat flour and ice to form undeveloped dough. *Cereal Chemistry* 73(1): 105-107.

Edwards, N.M., Peressini, D., Dexter, J.E. and Mulvaney, S.J. (2001). Viscoelastic properties of durum wheat and common wheat dough of different strengths. *Rheologica Acta* 40: 142-153.

Hoseney, R.C. (1994). Bread Baking. *Cereal Food World* 39: 180-183.

Kim, Y.R., Cornillon, P., Campanella, O.H., Stroshine, R.L., Lee, S. and Shim, J.Y. (2008). Small and large deformation rheology for hard wheat flour dough as influenced by mixing and resting. *Journal of Food Science* 73(1): E1-E8.

Larsson, H. (2002). Effect of pH and Sodium Chloride on Wheat Flour Dough Properties: Ultracentrifugation and Rheological Measurements. *Cereal Chemistry* 79(4): 544-545.

Lee, L., Ng, P.K.W., Whallon, J.H. and Steffe, J.F. (2001). Relationship between rheological properties and microstructural characteristics of nondeveloped, partially developed, and developed doughs. *Cereal Chemistry* 78(4): 447-452.

Manski, J.M., van der Goot, A.J. and Boom, R.M. (2007). Formation of Fibrous Materials from Dense Calcium Caseinate Dispersions. *Biomacromolecules* 8(4): 1271-1279.

Melander, W. and Horváth, C. (1977). Salt effects on hydrophobic interactions in precipitation and chromatography of proteins: An interpretation of the lyotropic series. *Archives of Biochemistry and Biophysics* 183(1): 200-215.

Miller, R.A. and Hoseney, R.C. (2008). Role of Salt in Baking. *Cereal Food World* 53(1): 4-6.

Osborne, T.B. (1924). The vegetable proteins. London.

Peighambardoust, S.H., van der Goot, A.J., Boom, R.M. and Hamer, R.J. (2006a). Mixing behaviour of a zero-developed dough compared to a flour-water mixture. *Journal of Cereal Science* 44(1): 12-20.

Peighambardoust, S.H., van der Goot, A.J., van Vliet, T., Hamer, R.J. and Boom, R.M. (2006b). Microstructure formation and rheological behaviour of dough under simple shear flow. *Journal of Cereal Science* 43(2): 183-197.

Peressini, D., Peighambardoust, S.H., Hamer, R.J., Sensidoni, A. and Van der Goot, A.J. (2008). Effect of shear rate on microstructure and rheological properties of sheared wheat doughs. *Journal of Cereal Science* 48(2): 426-438.

Phan-Thien, N., SafariArdi, M. and MoralesPatino, A. (1997). Oscillatory and simple shear flows of a flour-water dough: A constitutive model. *Rheologica Acta* 36(1): 38-48.

Preston, K.R. (1989). Effects of Neutral Salts of the Lyotropic Series on the Physical Dough Properties of a Canadian Red Spring Wheat Flour. *Cereal Chemistry* 66(3): 144-148.

Salvador, A., Sanz, T. and Fiszman, S.M. (2006). Dynamic rheological characteristics of wheat flour-water doughs. Effect of adding NaCl, sucrose and yeast. *Food Hydrocolloids* 20(6): 780-786.

Sperling, L.H. (2001). Introduction to physical polymer science. New York, John Wiley & Sons.

Tanaka, K., Furukawa, K. and Matsumoto, H. (1967). The effect of acid and salt on the farinogram and extensigram of dough. *Cereal Chemistry* 44(6): 675-680.

Ukai, T., Matsumura, Y. and Urade, R. (2008). Disaggregation and reaggregation of gluten proteins by sodium chloride. *Journal of Agricultural and Food Chemistry* 56(3): 1122-1130.

Van der Borght, A., Goesaert, H., Veraverbeke, W.S. and Delcour, J.A. (2005). Fractionation of wheat and wheat flour into starch and gluten: overview of the main processes and the factors involved. *Journal of Cereal Science* 41(3): 221-237.

Van der Zalm, E.E.J., Sawalha, A., Van der Goot, A.J. and Boom, R.M. (2009a). Separation of wheat flour into starch and gluten - Mechanisms and differences between flour cultivars. Proceedings International Symposium on Food Rheology and Structure, Zürich, 344-347

Van der Zalm, E.E.J., Van der Goot, A.J. and Boom, R.M. (2009b). Influence of process conditions on the separation behaviour of starch-gluten systems. *Journal of Food Engineering* 95(4): 572-578.

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Gluten protein composition in several fractions obtained by shear-induced separation of wheat flour

Abstract

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.

Introduction

In recent publications, a new separation process for wheat flour into starch and gluten was proposed (Peighambardoust *et al.* 2008; Van der Zalm *et al.* 2009). 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 (Van der Borght *et al.* 2005), 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 (Van der Borght *et al.* 2005). 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 (Osborne 1907). 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.* (2008) 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 (Weegels *et al.* 1996). 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 (1970). 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 (Singh *et al.* 1990; Batey *et al.* 1991; Gupta *et al.* 1993; Johansson *et al.* 2001; Kuktaite *et al.* 2004). The protein composition is 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).

<u>Sample Preparation Shearing Device</u>

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.* (2008) and Manski *et al.* (2007). The shearing device was connected to a Brabender Docorder 330 unit (BrabenderOHG, 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 (Van der Zalm *et al.* 2009).

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 0.355mm.

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-0.05M 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 (20,000g) 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 SafeCoomassie 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, non soluble and total protein fractions. Proteins were extracted from the samples using a two-step extraction procedure according to Gupta *et al.* (1993). 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 0.05M 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 10,000g 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 10,000g 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 10,000g 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 (330x7.8mm). 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 4.1 shows the protein content in the various layers in the shearing device, the starting material, and the kneaded samples. Table 4.1 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 4.1 were therefore analyzed by SDS-PAGE and SE-HPLC.

SDS-PAGE

Figure 4.1 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

Table 4.1:
Measured protein concentration (% w/w) for various process conditions. Layer 1 represents the rim of the cone, layer 5 is the apex.

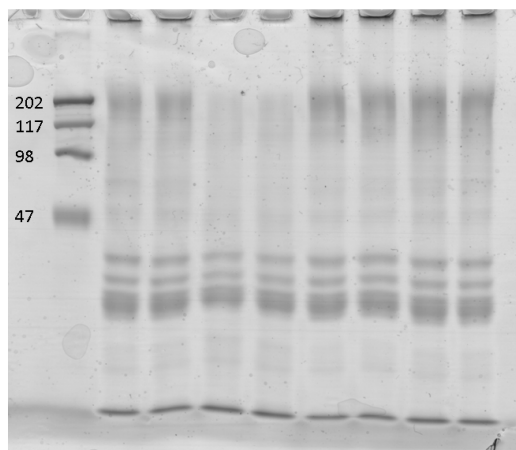| Treatment	Layer	Rotation rate	Processing time	Average Protein %	Standard deviation
Soissons flour		unprocessed		11.2	0.15
Shearing	1	15 rpm	8 min	7.0	0.18
	2			11.4	0.32
	3			11.7	0.02
	4			14.2	1.04
	5			21.2	0.98
Shearing	1	15 rpm	60 min	5.4	0.35
	2			10.2	1.31
	3			12.4	0.52
	4			15.8	0.66
	5			37.5	0.82
Kneading		63 rpm	8 min	11.4	0.50
Kneading		63 rpm	60 min	11.0	0.11

Figure 4.1: SDS PAGE gel of Soissons flour for different process conditions. The lanes indicate: (1) Marker, (2-3) Soissons flour (raw material), (4-5) shearing 60 min layer 1, (6-7) shearing 60 min layer 5, (8-9) kneading 60 min.

the entrance of the gel. Less material of layer 1, sheared for 60 min, is blocked at the entrance of the lane.

SE-HPLC

A typical chromatogram obtained is depicted in Figure 4.2. 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 (Kuktaite *et al.* 2004, Johansson *et al.* 2001)

The areas of each part of the chromatogram were measured for samples tested. The results are presented in the Appendix. From the tables in the Appendix, 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 4.2. 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 4.1 and Table 4.2a. 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
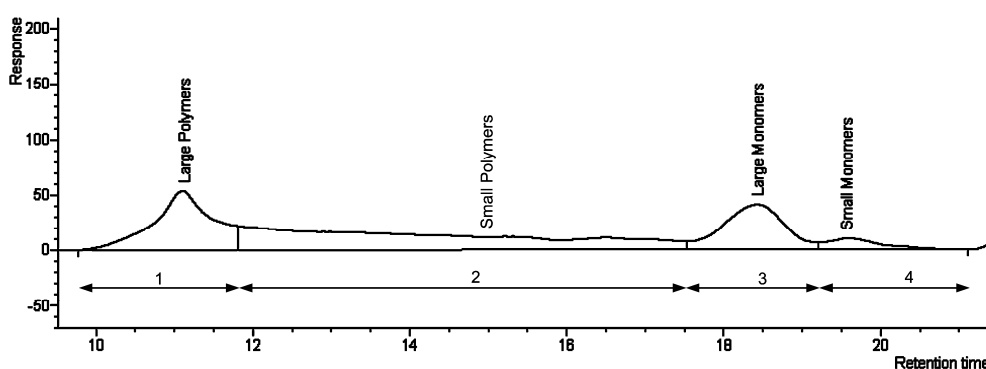

Figure 4.2: Typical chromatogram of gluten protein obtained by SE-HPLC. The ranges represent (1) Large (HMW) polymeric proteins (2) Small (LMW) polymeric proteins (3) Large (HMW) monomeric protein (4) Small (LMW) monomeric protein

Table 4.2:
SE– HPLC Ratios

Treatment (protein %)	Time (min)	Lay-er	Fraction (1+2)/3			Fraction 3/4		
			average	stdev	significant difference	average	stdev	significant difference
Table 4.2a: SE-HPLC ratios for total protein fraction								
Soissons (11.2%) *	-		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%) **	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
Table 4.2b: SE-HPLC ratios for the soluble protein fraction								
Soissons (11.2%) *	-		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%) **	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
Table 4.2c: SE-HPLC ratios for nonsoluble protein fraction								
Soissons (11.2%) *	-		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%) **	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

* Starting material
** Average and standard deviation were calculated over 2 measurements (instead of 4)

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 4.2b, 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 non soluble protein fraction are presented in Table 4.2c. 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 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 (Johansson *et al.* 2001; Kuktaite *et al.* 2004).

Shearing

Table 4.2a 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 4.2a indicates also the significant differences between the samples. Most remarkable difference for the polymeric to large monomeric fraction is the clear difference between the glutenenriched layers versus the other samples. The monomeric fractions, however, varied extremely between the samples.

Table 4.2b 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 4.2c shows the results of the non soluble 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 centre 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 (Peighambardoust *et al.* 2008; Van der Zalm *et al.* 2009). First, the aggregation of the gluten and second the migration of the gluten along a curved shear field. In addition, Peighambardoust *et al.* (2008) 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 4.2a. 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 4.2) is less than the change in total protein content (Table 4.1).

The Appendix 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 4.3. Table 4.3a 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

Table 4.3a:
Protein distribution in 100 grams of material for the various samples.

Sample	Protein (g)	Protein distribution (in g) per fraction for 100 gram dried material			
		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

Table 4.3b:
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

5, compared to that in Soissons. 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 4.3b, 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 4.3a 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
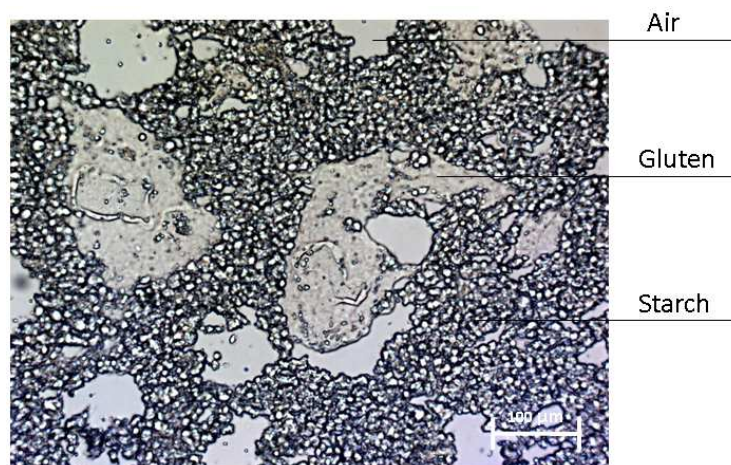
Figure 4.3: Light microscopy picture of the gluten-depleted layer. Process conditions were 60 minutes shearing at 15 rpm at 15˚C.

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 4.3 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 4.2a). 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 4.3b 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.

References

Batey, I.L., Gupta, R.B. and Macritchie, F. (1991). Use of size-exclusion high-performance liquid-chromatography in the study of wheat-flour proteins - an improved chromatographic procedure. *Cereal Chemistry* 68(2): 207-209.

Gupta, R.B., Khan, K. and Macritchie, F. (1993). Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. *Journal of Cereal Science* 18(1): 23-41.

Johansson, E., Prieto-Linde, M.L. and Jonsson, J.O. (2001). Effects of wheat cultivar and nitrogen application on storage protein composition and breadmaking quality. *Cereal Chemistry* 78(1): 19-25.

Kuktaite, R., Larsson, H. and Johansson, E. (2004). Variation in protein composition of wheat flour and its relationship to dough mixing behaviour. *Journal of Cereal Science* 40(1): 31-39.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.

Manski, J.M., van der Goot, A.J. and Boom, R.M. (2007). Formation of fibrous materials from dense calcium caseinate dispersions. *Biomacromolecules* 8(4): 1271-1279.

Osborne, T.B. (1907). The proteins of the wheat kernel, Carnegie Institution of Washington.

Peighambardoust, S.H., Hamer, R.J., Boom, R.M. and Van der Goot, A.J. (2008). Migration of gluten under shear flow as a novel mechanism for separating wheat flour into gluten and starch. *Journal of Cereal Science* 48(2): 327-338.

Singh, N.K., Donovan, R. and Macritchie, F. (1990). Use of sonication and size-exclusion high-performance liquid-chromatography in the study of wheat-flour proteins. II. Relative quantity of glutenin as a measure of breadmaking quality. *Cereal Chemistry* 67 (2): 161-170.

Van der Borght, A., Goesaert, H., Veraverbeke, W.S. and Delcour, J.A. (2005). Fractionation of wheat and wheat flour into starch and gluten: overview of the main processes and the factors involved. *Journal of Cereal Science* 41(3): 221-237.

Van der Zalm, E.E.J., Van der Goot, A.J. and Boom, R.M. (2009). Influence of process conditions on the separation behaviour of starch-gluten systems. *Journal of Food Engineering* 95(4): 572-578.

Weegels, P.L., vandePijpekamp, A.M., Graveland, A., Hamer, R.J. and Schofield, J.D. (1996). Depolymerisation and re-polymerisation of wheat glutenin during dough processing .1. Relationships between glutenin macropolymer content and quality parameters. *Journal of Cereal Science* 23(2): 103-111.

Appendix: Percentage relative area of total, soluble, and nonsoluble protein analyzed by SE-HPLC.

Appendix A: Percentage relative area of total protein analysed by SE-HPLC

Treatment (protein %)	Time (min)	Layer	Total area (a.u.)	SD total area	Average Layer 1	Stdev	Average layer 2	Stdev	Average Layer 3	Average stdev	Average Layer 4	stdev
Soissons (11.2%)	-		25816.7	368.8	6.6	0.6	31.7	1.0	46.0	1.1	15.7	0.4
Shearing (7.0%)	8	1	20377.6	927.3	7.7	0.2	27.8	2.3	43.9	1.3	20.7	0.9
Shearing (21.2%)	8	5	35449.9	865.5	15.3	0.1	37.3	0.3	36.9	0.4	10.4	0.1
Shearing (5.4%)	60	1	15998.7	194.8	6.1	0.1	19.4	0.6	44.5	0.6	30.0	0.1
Shearing (37.5%)	60	5	35784.8	499.5	15.8	0.5	36.3	0.7	37.7	0.4	10.2	0.3
Kneading	8		30773.3	584.5	8.8	0.5	38.6	0.4	39.0	0.4	13.6	0.2
Kneading	60		28998.8	495.7	9.5	0.3	35.9	0.6	40.7	0.5	14.0	0.4

Appendix B: Percentage relative area of soluble protein analysed by SE-HPLC

Treatment (protein %)	Time (min)	Layer	Total area (a.u.)	SD total area	Average Layer 1	stdev	Average layer 2	stdev	Average Layer 3	Average stdev	Average Layer 4	stdev
Soissons (11.2%)	-		26169.2	1099.1	6.8	0.8	30.1	1.0	48.4	1.4	14.7	0.1
Shearing (7.0%)	8	1	19120.2	507.2	8.0	1.4	30.1	1.4	43.1	0.7	18.8	0.4
Shearing (21.2%)	8	5	27052.4	464.0	11.3	1.2	31.9	1.2	44.8	0.4	12.0	0.2
Shearing (5.4%)	60	1	16288.5	259.9	5.6	0.6	24.0	0.7	47.0	0.9	23.5	0.4
Shearing (37.5%)	60	5	25994.6	1220.2	10.4	1.2	33.3	1.2	44.8	0.4	11.5	0.1
Kneading	8		36116.4	1415.9	12.4	0.5	41.3	0.6	35.3	0.4	11.0	0.3
Kneading	60		33548.5	812.4	16.2	1.0	34.1	0.7	38.0	0.8	11.8	0.5

Appendix C: Percentage relative area of total protein analysed by SE-HPLC

Treatment (protein %)	Time (min)	Layer	Total area	SD total area	Average Layer 1	stdev	Average layer 2	stdev	Average Layer 3	stdev	Average Layer 4	stdev
Soissons (11.2%)	-		10602.3	756.1	27.6	2.7	51.7	2.0	15.8	0.7	4.8	1.0
Shearing (7.0%)	8	1	16755.8	699.0	19.8	1.4	45.1	0.4	29.3	0.3	5.9	0.9
Shearing (21.2%)	8	5	10151.3	842.3	29.5	0.4	46.3	0.5	19.33245	0.5	4.8	0.3
Shearing (5.4%)	60	1	21666.1	127.9	22.1	0.8	45.8	0.6	26.9	0.6	5.2	0.8
Shearing (37.5%)	60	5	10118.6	1073.6	31.2	2.6	43.1	1.7	20.9	2.3	4.8	0.5
Kneading	8		2646.5	251.2	22.5	2.2	44.5	3.2	25.9	0.9	7.1	0.6
Kneading	60		4788.8	834.7	34.9	4.6	39.6	6.1	18.8	0.6	6.7	1.2

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Quality of shear fractionated wheat gluten
–
comparison to commercial vital wheat gluten

Abstract

The functional properties of gluten obtained with a shear-induced separation process, recently proposed by Peighambardoust *et al.* (2008), are compared with a commercially available vital wheat gluten. Two tests were performed. First, a relatively strong wheat flour, Soissons, was enriched with gluten protein. The resulting dough was then evaluated on its kneading performance. Second, a weak flour, Kolibri, was enriched to evaluate the baking properties. The wheat flour enriched with gluten protein obtained via the shear-induced separation process (SCG) showed comparable to improved gluten functionality relative to commercial available vital wheat gluten protein (CVWG). The differences in functionality cannot be directly related to the composition as analyzed with SE-HPLC, because the composition of the gluten materials was rather comparable. The differences in functionality may therefore be related to the different drying techniques used or to the inherent mildness of the shear-induced separation technique.

Introduction

Traditional separation processes for wheat flour fractionation, such as the dough-batter processes, are based on kneading and washing of dough with excess water (Van der Borght *et al.* 2005). Disadvantages of this process are the high consumption of energy and water, and the loss of protein during the separation process into the waste water. In addition, kneading and drying steps might negatively influence the vitality, and thereby the functional properties of the gluten product.

Some years ago, a new separation mechanism for wheat flour was proposed (Peighambardoust *et al.* 2008; Van der Zalm *et al.* 2009). The separation mechanism is based on shearing wheat flour dough in a conical device. As a result the flour was separated in a gluten-enriched and a gluten-depleted fraction. The total moisture content of the dough during this process was approximately 45% w/w. The fact that no excess water is used, implies that more protein remains in the product than with the conventional dough-batter process. The inclusion of the water soluble protein in the gluten fraction may impact the functional properties of the gluten fraction, but it is not known in what extent.

The gluten proteins determine many properties of a flour and therefore the baking quality of the final dough. It is known that traditional processing negatively influences the functional properties of wheat dough, reflected by a decrease in GMP content. Previously, it was found that shearing, did not lead to a decrease of amount the glutenin macro polymer (GMP) even over longer treatment times. This longer shear treatment could also be expressed as higher tolerance for specific mechanical energy. (Peighambardoust *et al.* 2005; Peighambardoust *et al.* 2006; Peighambardoust *et al.* 2008) Peighambardoust *et al.* (2010) compared the effect of various processing techniques (kneading and shearing) on the functional properties of dough. Dough aeration during processing, gas holding capacity during proving, and baking properties were monitored for dough obtained by kneading or shearing processes. It could be concluded that the way of processing influenced these properties.

Uthayakumaran *et al.* (1999) investigated the effect of the total protein content and the glutenin-to-gliadin ratio on dough extensibility and baking properties. Increasing the protein content in the dough while keeping the glutenin/gliadin ratio constant, resulted in an increase of the mixing time, the mixograph peak resistance, the extensibility and the maximum resistance to extension. It also resulted in an increased loaf volume of the baked bread. The same effects could be obtained by increasing the glutenin/gliadin ratio at constant protein content. Goesaert *et al.* (2005) reviewed the effect of wheat flour constituents on functionality and bread baking quality. The non-gluten protein albumin

and globulin should have a minor role in bread baking, while the gluten protein glutenin and gliadin do play a role. Gliadin influences the dough viscosity while glutenins influence the dough elasticity. Gluten protein quality and quantity thus play an important role for the final dough functionality.

During this research, we evaluated the functionality of the gluten fraction obtained after shearing. This gluten was compared with commercial vital wheat gluten in respect to their chemical composition, kneading and baking properties. It can be expected that the separation processes (dough-batter process and shear-induced separation process) result in different gluten protein composition. In this chapter, we therefore describe the influence of the gluten fractions obtained by both separation techniques with respect to the kneading and baking behavior. During the kneading tests, Soissons flour will be enriched with gluten protein, while for the baking tests weak biscuit flour (Kolibri) will be enriched by additional gluten protein.

Materials and methods

Experiments were performed with two types of flour. Soissons flour from a single wheat cultivar (Meneba, Rotterdam, The Netherlands) was used to obtain the gluten batch (i.e. shear cell gluten, abbreviated as SCG) and it was used during the kneading tests. Kolibri flour (Meneba, The Netherlands), a weaker flour, is used for the baking experiments. The enrichment of the Soissons and Kolibri doughs was performed with commercial vital wheat gluten (CVWG) (Roquette, Barentz BV, Hoofddorp, The Netherlands) and with the gluten obtained in house by shear-induced separation. The commercial gluten had a protein content of 80.4% (on dry matter basis, using DUMAS conversion factor N = 5.7).

Soissons and Kolibri flour were characterized by the Farinograph method. Farinograph water absorption (FWA) was determined by the addition of tap water to 300 g flour (moisture content 14.5%) to which 6 g NaCl was added (i.e. 2.0% on flour basis). AACC Approved Method 54-21 was followed. Protein content, Farinograph water absorption, stability time, peak time and tolerance index of Soissons flour were determined to be 12.0% (on dry matter basis), 53.7% on 14% moisture basis, 14 minutes, 23.5 minutes and 30 BU respectively. For Kolibri flour, the characteristics were determined as follows: 11.3% protein on dry matter basis, Farinograph water absorption 58.2% on 14% moisture basis. The stability time, peak time and tolerance index were 1.5 minutes, 1.5 minutes and 40 BU respectively.

Shearing device

The gluten fractions were obtained by exposing wheat flour dough to well-defined shear flow in a shearing device, which configuration is based on a cone-plate geometry. The gluten fraction was obtained in an up-scaled version of the shearing device which was used in previous starch-gluten separation studies (Van der Zalm *et al.* 2009; 2010a). The dimensions of this shearing device are described in more detail by Habeych *et al.* (2008). The heating system was modified. During the research of Habeych *et al.* (2008) the heating was performed electrically, to obtain high temperatures. In this version the heating is performed by a water chamber which is connected to the bottom cone. Peighambardoust *et al.* (2004, 2005, 2006) (performed dough analyses in a previous version of the shearing device. That device had comparable dimensions.

The in-house developed shearing device is mounted on a Brabender Do-Corder 330 driver unit (Brabender, Duisburg, Germany) which was connected to an interface and controlling unit for online torque and temperature registration. The device consists of a static upper cone and a rotating bottom cone. Temperature of the upper- and lower cone was controlled by circulating water through the system. The material was processed for 60 minutes at 12 s^{-1} (5 rpm) and a temperature of 15 °C. The pressure onto the hydraulic cylinder, so the lower cone as well, was 2.5 bar (25 N/cm^2). Prior to processing, the material was allowed to rest for 15 minutes. After processing, the material was cooled till approximately 5 °C in stationary position.

Production of sheared gluten

A batch of shear separated gluten was obtained by mixing gluten from multiple separation runs. The dough for the separation runs was made separately for each run. This dough was obtained by manually mixing Soissons flour, NaCl and tap water. Water was added in three fractions to the flour/NaCl mixture. Material was spread equally in the lower cone before closing the device. The amount of dough processed in the shearing device was 295 gram: 197.8 g Soissons flour, 93.3 g tap water and 3.93 g NaCl. After shearing, a gluten-enriched and a gluten-depleted fraction were obtained. The material inside the shearing device was divided in 4 fractions and encoded. Layer 1 had a size of 2.5 cm, layer 2 of 2.5 cm, layer 3 of 2.5 cm and layer 4 of 5.0 cm along the hypotenuse of the shearing device. Layer 4 is the layer in the center of the cone and contained the gluten-enriched material. The material was immediately frozen using liquid nitrogen and stored until freeze drying. Samples were freeze dried at least for 48h to a maximal moisture content of 3.5%. Later on, samples were powdered with an IKA Basic mill (IKA, type A11, Staufen, Germany) and sieved using a pore size of 0.355 mm.

The gluten-enriched material (i.e. layer 4) from 22 individual shearing experiments was collected and mixed to obtain one shear separated gluten protein batch of 345 g.

Protein content

The protein contents of the freeze dried flours, gluten and samples were determined by Dumas using a FlashEA 1112 series Nitrogen Analyzer (Thermo Fisher Scientific Inc, Waltham, MA, USA). Methionine was used as standard. The conversion factor for protein was 5.7. Duplicate measurements were performed for each sample; variations of maximally 1% were observed. All protein contents are expressed based on dry matter.

Kneading experiments

Kneading tests were performed to investigate the influence of the two types of gluten, CVWG and SCG. A series of kneading experiments were carried out with Soissons wheat flour having different levels of enrichment of protein. This led to three different samples: Soissons (reference; without addition), Soissons with CVWG and Soissons with SCG.

Kneading experiments were performed in a 300 grams Farinograph. Process settings were: rotational rate 63 rpm, temperature 30 °C and mixing time 60 minutes. During all measurements, the FWA was kept constant at 53.2%, which means that the FWA was not corrected for the additional gluten. This correction could not be carried out as we could not obtain sufficient gluten material through shear processing. The kneading experiments were performed with Soissons flour, which has 11.2% protein based on dry matter, and at 2 enrichment levels of about 2 and 4% added gluten.

Baking experiments

Baking experiments were performed to visualize the differences between both gluten sources. The baking experiments were performed with Kolibri flour, a weak biscuit flour. Three combinations were compared: Kolibri (reference; without addition), Kolibri with CVWG and Kolibri with SCG. Baking tests were performed at 11.3% (Kolibri flour), ~13.0% and ~15.0% protein. To obtain these concentrations gluten was added in various amounts. Salt, yeast and sucrose were added to this. All samples were prepared in duplicate.

Kneading of the dough for the baking experiments was performed in a 50 gram Farinograph. In all cases, the mixer was filled with 80 gram of dough, i.e. flour, water and NaCl. Care was taken to ensure that salt and yeast were not directly into contact with each other at the beginning of the experiment to prevent inactivation of yeast. The dough was kneaded at 63 rpm at 30 °C for 3 minutes. A correction was made for the FWA for the various protein concentrations. Therefore, we have determined the FWA for Kolibri flour that was enriched with CVWG. The measured FWA values were: Kolibri 11.3% protein, FWA 57%; Kolibri + commercial gluten 13% protein, FWA 58.4%; Kolibri + commercial gluten 15% protein FWA 59.3%. These FWA values were also used for Kolibri

enriched with SCG. The dough was used for the measurement of the CO_2 production rate during proving (5 g), the dynamic dough density determination (10 g) and for the baking experiments (2 times 25 g each).

Proving dough volume

Carbon dioxide (CO_2) production during proving was measured according to the method described by Peighambardoust *et al.* (2010). Dough (5 g) was placed inside an air-tight 250 ml Erlenmeyer flask. The CO_2 production inside could be measured by connecting a tube with a 100 ml inverted graduated cylinder. The cylinder was placed inside a 500 ml beaker glass filled with oxalic acid solution at pH 3 to prevent CO_2 dissolution. The cylinder and flask were partly immersed in a water bath, and kept constant at 35 °C. CO_2 production was recorded approximately every 5 minutes for 3 hours. Duplicate experiments were performed for each type of dough, which included a new kneading process for each experiment.

Dynamic dough density during proving

According to the method described by Campbell *et al.* (2001) and Fang and Campbell (2000) the dynamic dough density was used to study the gas production and the retention during proving. A Sartorius dough density determination kit (YDK 01 LP, Sartorius AG, Goettingen, Germany) was used. Measurements were performed on an analytical balance that was connected to a laptop. Dough samples of approximately 10 g were placed in silicone oil with a density of 0.95 g/cm^3 (Momentive, Albany, NY, USA) as performed previously by Ktenioudaki *et al.* (2009). The temperature was maintained at 35 °C using a jacketed beaker glass.

The density of a 10 g sample of dough was first measured in air in the top cup, after which the sample was immersed in silicone oil in the bottom cup. Floating of the sample was prevented by two wires mounted on the bottom cup. The weight was recorded every 10 seconds until the sample started to gain weight due to oil absorption. The final weight was determined as well.

The static dough density was determined by the same setup; this dough did not contain yeast however. The weight was measured during time, to check if this was kept constant. The dough density was calculated with the following equation:

$$\rho = \frac{m_{air}}{m_{air} - m_{liquid}} \rho_{liquid}$$
Equation 5.1

in Equation 5.1 ρ is the dough density, m_{air} is the weight of the dough in air, m_{liquid} is the weight of the dough in the liquid and ρ_{liquid} is the density of the liquid.

Bread baking

The dough used for the baking experiment was gently rounded and placed inside a lightly greased baking tin (dimensions: top 5.4 cm^2, bottom 4.4 cm^2, height 33 mm) that was divided into three equal parts. Only the compartments at the outer side were used and those were filled with 25 g dough.
Proving was performed inside a climate chamber EKE 15.80.31 (Weiss Enet Industrietechniek B.V., Tiel, The Netherlands). The proving consisted of two steps; the 1st proving step lasted 40 minutes and the 2nd proving step 60 minutes. The temperature of the climate chamber was 35 °C and the relative humidity was set to 85% to suppress moisture loss. In between the two proving steps, the dough was sheeted and folded.
The baking itself was carried out with a bread baking machine (Princess, Silver Breadmaker® 1935, Breda, The Netherlands). The system was pre-heated for 15 minutes; breads were baked at 170 ˚C for 25 minutes. The internal structure and the shape of the bread was studied by slicing from each bread a slice with a thickness 3 mm.

Bread volume determination

The baked breads were analyzed on their weight, dimensions and volume. Loafs were weighed on an electronic lab balance after cooling down for 30 minutes. The largest distance for each side, were determined. Bread volume was determined with the rapeseed displacement method. For each measurement, a jar was filled with rapeseed. Breads were then gently placed in the jar. The abundant volume of the rapeseed, being equal to the volume of the bread, was collected en measured in a 100 ml graduated cylinder.

Sample preparation for Size Exclusion – HPLC analysis

The protein composition of the CVWG and the SCG was compared for their total protein fraction. Proteins were extracted from the samples using an extraction procedure according to Gupta *et al.* (1993). The extraction process was performed in duplicate. Sample amounts were corrected so that each sample contained 2 mg protein per mL solvent solution. The protein was suspended in 1 mL 0.5% (w/v) SDS-0.05M sodium phosphate buffer (pH 6.9) solution. The samples were heated at 40 ˚C for 5 minutes and subsequently stirred at room temperature for 20 hours to assure dissolution, followed by the sonication of the material in an ultrasonic disintegrator for 30 seconds (amplitude 5 µm, fitted with a 3 mm exponential microtip). The samples were centrifuged at 10,000g for 30 minutes. The supernatant was collected and filtered through a 0.45 µm filter prior

to Size Exclusion-HPLC (SE-HPLC) analysis.

SE - HPLC

The total protein fraction was 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 the duplicate weighing, 4 analyses were done for each sample. Analysis was performed on a BioSep-SEC-S4000 (Phenomenex, Torrance, CA, USA) size-exclusion column (330x7.8mm). The eluent consisted of 50 % (v/v) acetonitrile and 50 % (v/v) MilliQ 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 10 ˚C. After analysis, the chromatograms were divided in four different parts, each retention-time range representing a distinct type of protein.

Results

SE-HPLC

The protein compositions of the CVWG and SCG as determined with SE-HPLC were found to be rather similar. Figure 5.1 shows the chromatograms for the two samples. The chromatograms are divided in 4 fractions, named large polymers (1), small polymers (2), large monomers (3) and small monomers (4).

Both materials contain all 4 protein fractions. However, the fourth (low molecular weight) fraction was less prominent in the CVWG. A more extreme difference was expected, given the washing step in the commercial separation process. By analyzing the chromatogram, we obtained the areas of the various fractions. Table 5.1 summarizes these results.

There are some differences between the CVWG and the SCG, but they are minor. The SCG contains more small monomers (which includes the water soluble material) than CVWG.
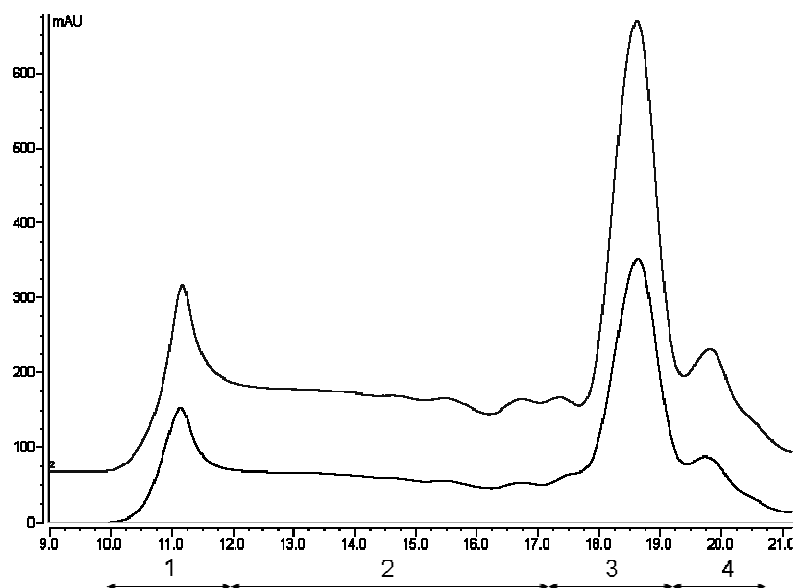
Figure 5.1: SE-HPLC chromatograms for two types of gluten. The lower line represents commercial gluten, the upper line the gluten obtained by shear-induced separation.

Table 5.1:
Ratios of the various ratios from SE-HPLC

	Total amount of polymers relative to amount of large monomers [(1+2)/3]	Amount of large monomers compared to amount of small monomers [3/4]
Commercial vital wheat gluten (CVWG)	1.23	4.39
Sheared gluten (SCG)	1.23	3.57
Soissons	1.09	3.12
Kolibri	1.19	2.94

Kneading experiments

The influence of gluten addition on the kneading behavior of the dough is investigated using CVWG and SCG. Figure 5.2 shows the result. During the kneading process, the water content was kept constant in all experiments, which could otherwise have influenced the development of a gluten network. As this was the case for CVWG and the SCG, one can compare the runs. The CVWG gave less resistance to mixing than the SCG indicating a lower cohesiveness. The same experiment was performed with Soissons flour enriched with 4% gluten, which yielded the same trend. The resistance measured for the Soissons plus 4% added CVWG gave torque values in the same range as Soissons flour plus 2% added SCG. Addition of 4% sheared gluten resulted in a maximal torque value of 8.3 Nm. Remarkably, the stability and change of the torque value during time stays rather equal during all experiments.
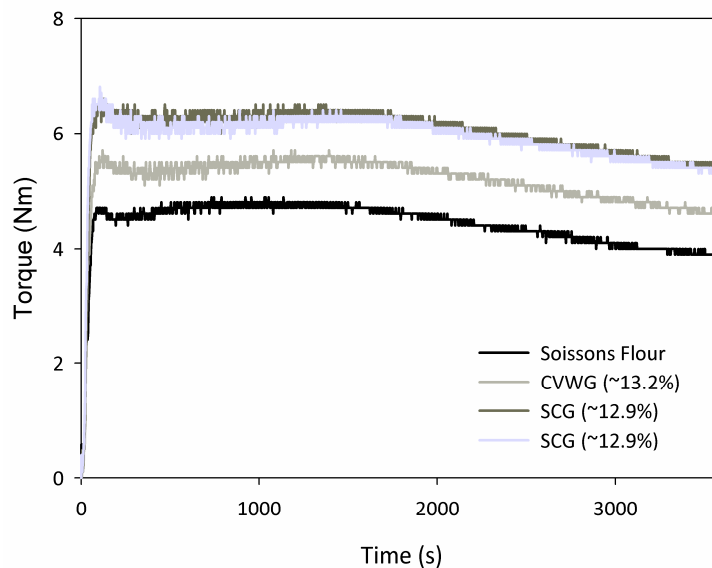

Figure 5.2: Farinograph curve for Soissons flour, Soissons flour enriched with 2% commercial vital wheat gluten (CVWG) and Soissons flour enriched with 2% shear-induced wheat gluten (SCG). Kneading took place at 63 rpm. Water content was in all situations the FWA of the standard flour, so 53.2%.
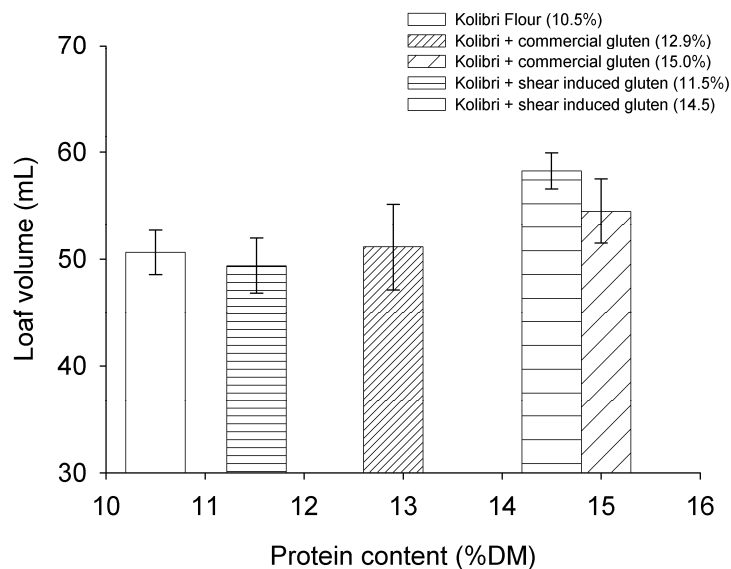
Figure 5.3: Loaf volume of baked breads. Bread was made by addition of CVWG or gluten obtained by shear-induced separation

Baking experiments

Figure 5.3 shows the volumes of the baked breads. Also the standard deviation of the volume measurements is depicted in the graph. The internal crumb structure and the shape of the various breads are visualized in Figure 5.4.

Enrichment of the biscuit flour Kolibri with small amounts of CVWG did not result in an increased bread volume immediately, and the crumb structure became more fine. The breads enriched with CVWG up to approximately 15% protein exhibited increased volumes and a finer crumb structure. Enriching the bread with SCG increased the bread volumes more strongly, while the crumb structure remained similar.

Yeast activity

The CO_2 production during time turned out to be constant for all dough compositions. The approximate CO_2 production is 10 ml per hour for 5 g dough. No differences were observed for the various dough types. The different way of processing, which could have led to variations in gluten protein therefore did not influence the CO_2 production in dough. This indicates that during all proving steps the CO_2 production was not influenced by different flours and protein types. This result is supported by the dough density measurements: all samples showed comparable dough densities. All samples started with a density of 1.2 g/cm^3, while after 60 minutes this was decreased towards 0.4 g/cm^3.
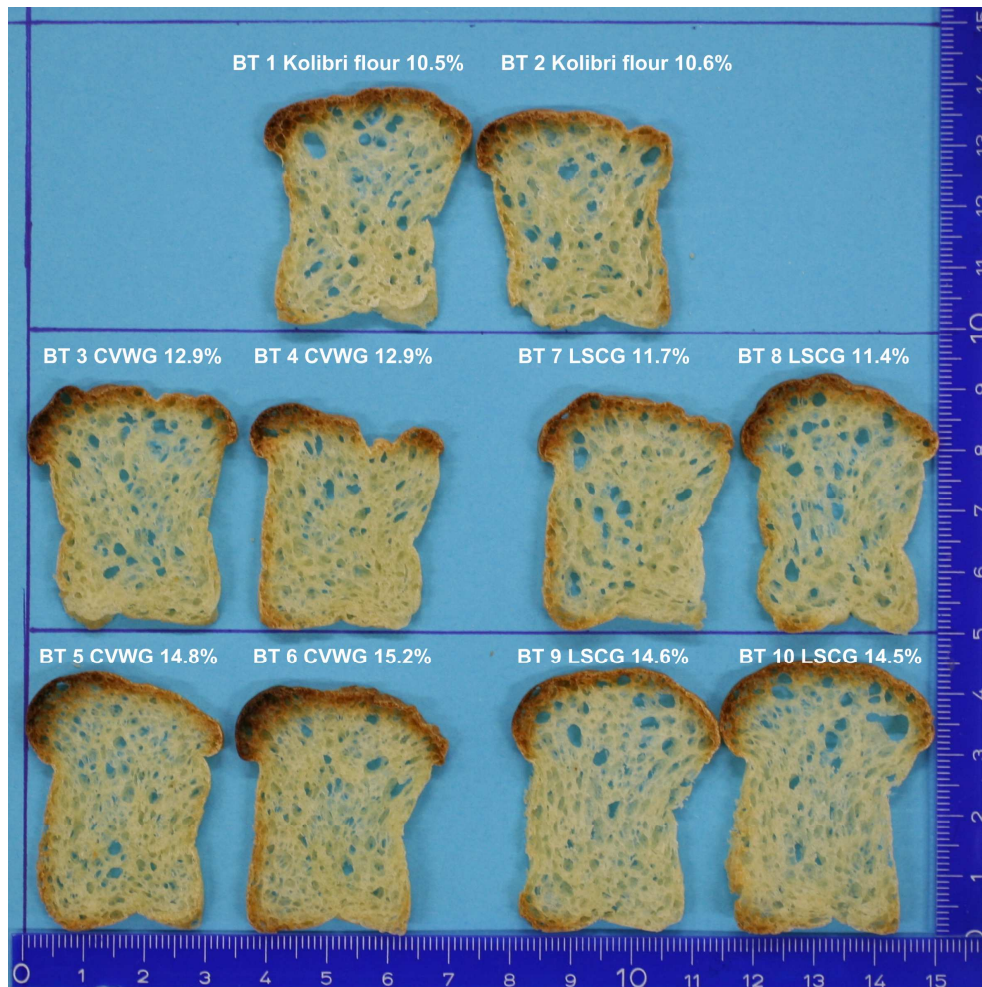
Figure 5.4: Crumb structure and shape of the various breads. From each bread baking test (BT), one slice is analyzed. The percentage indicates the protein concentration measured in the dough used for the bread baking. CVWG = Commercial Vital Wheat Gluten, SCG = gluten obtained via the shear-induced separation process

Discussion

The aim of this chapter is to investigate the effect of separation techniques on the functional properties of wheat gluten protein. The quality of gluten obtained with shear-induced fractionation (SCG) was compared to that of CVWG, by adding both types of gluten to a weak flour. Besides, both gluten compositions were analyzed with SE-HPLC; the kneading behavior, CO_2 production and proving behavior were assessed, and small bread loafs were baked and analyzed. Differences were expected due to differences in the separation process.

The recovery of gluten protein during the (non-optimized) shear-induced separation process is much lower than in the current industrial processes. In addition, the protein percentage of commercial gluten is approximately 80% (on dry matter basis) while the gluten obtained by shearing is around 30% protein (on dry matter basis). Also the recovery was lower. During the shear processing only 20% of the total available protein in the shearing device is collected in the enriched layers. Nevertheless, we think that this fraction is representative for the protein fraction obtained in case we could have a higher recovery. Previously, we showed that the gluten composition obtained in the gluten-enriched fraction was independent on gluten concentration and recovery (Van der Zalm *et al.* 2010b).

The baking tests yielded differences in loaf volumes and crumb structures. At this stage, it is not evident how this effect is related to the gluten composition. The protein compositions of CVWG and SCG were more or less similar. It seems that soluble monomers are not completely lost in the current industrial separation process, which implies that the shear-induced separation technique is not unique in that respect, though it contains a slightly higher fraction of monomers. It is possible that minor changes in the protein composition have a major effect on functionality already. It is also possible that differences in ability to (re-)form disulphide bridges are not detected by SE-HPLC.

The kneading tests showed an increase of the dough strength and dough resistance for wheat flour enriched by gluten obtained by shear-induced separation. The Farinograph curve for dough enriched with SCG and CVWG are however rather comparable. The main effect observed is the higher toque-value after the addition of gluten obtained by shear-induced separation. This suggests a larger water holding capacity of the material, indicating the presence of larger amount of slightly cross-linked proteins. This fraction could indeed not be detected by SE-HPLC. Two possible causes were identified for this

observation. A difference is that the drying process in the production process of CVWG is different from that used for SCG. Commercially available gluten is dried by thermal drying, while SCG was freeze dried. The vitality of gluten (i.e. the ability to (re-)form disulfide bridges) is influenced by the thermal treatment applied. The vitality of the CVWG will have been influenced much stronger by thermic drying, than the vitality of the SCG by freeze drying.

Another possibility is that the differences in functionality could be explained by the different processing technique itself. During the shear-induced separation much, less water is used during the processing, which may have a lower impact on the starch and gluten compared to the excess water used in the commercial process. This could influence the relative vitality of the gluten products. Furthermore, it was shown that shearing did not negatively influence the GMP-content in dough. Also the strain hardening properties were not negatively influenced (Peighambardoust *et al.* 2006). The latter is important, because strain hardening behavior is an important aspect for dough stabilization.

The CO_2 production during proving was the same for CVWG and SCG, therefore both gluten sources did not influence or limit the CO_2 production by yeast. Wheat dough enriched with SCG resulted in a larger bread volume, but the crumb structure was coarser compared to dough enriched with CVWG. The loaf volume depends on dough expansion and the ability of the matrix to stretch before it ruptures and the limit of expansion. (Sroan *et al.* 2009) In addition, bubble stabilization and disproportionation might play a role (Mills *et al.* 2003). As the loaf volume and the crumb structure were different for both gluten sources, while the CO_2 production was similar, the protein composition, and the lack of water for gluten development may be factors influencing the difference between the two systems. Nevertheless, the appearance of the baked bread of dough enriched with SCG had a good shape.

The differences in loaf volume and structure cannot be caused by differences in NaCl concentration as great care was taken that NaCl was present in the added SCG. Therefore we may assume that NaCl was equally distributed over the shearing device. Besides, the amount of NaCl present in the sheared gluten was subtracted from the NaCl added to the dough.

Conclusion

The processing method to obtain wheat gluten protein has a clear influence on the functional properties of dough. Addition of SCG showed at least the same fortification effect on kneading and baking behavior compared with CVWG. SCG yielded a stronger consistency of the dough, while baking with SCG gave a larger bread volume, although

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the crumb structure remained similar. For both tests, it should be taken into account that Farinograph water absorption and drying conditions were not identical for both methods.

The differences could not be explained by the SE-HPLC measurements, which showed only minor differences between the shear fractionated and commercial gluten. We expect that the better performance of the shear fractionated gluten is either due to the milder drying procedure applied, or due to the milder and more concentrated conditions during the fractionation process itself.

Acknowledgements

References

Campbell, G.M., Herrero-Sanchez, R., Payo-Rodriguez, R. and Merchan, M.L. (2001). Measurement of dynamic dough density and effect of surfactants and flour type on aeration during mixing and gas retention during proofing. *Cereal Chemistry* 78(3): 272-277.

Fang, C. and Campbell, G.M. (2000). Effect of measurement method and moisture content on wheat kernel density measurement. *Food and Bioproducts Processing* 78(4): 179-186.

Goesaert, H., Brijs, K., Veraverbeke, W.S., Courtin, C.M., Gebruers, K. and Delcour, J.A. (2005). Wheat flour constituents: how they impact bread quality, and how to impact their functionality. *Trends in Food Science & Technology* 16(1-3): 12-30.

Gupta, R.B., Khan, K. and Macritchie, F. (1993). Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. *Journal of Cereal Science* 18(1): 23-41.

Habeych, E., Dekkers, B., van der Goot, A.J. and Boom, R. (2008). Starch-zein blends formed by shear flow. *Chemical Engineering Science* 63(21): 5229-5238.

Ktenioudaki, A., Butler, F., Gonzales-Barron, U., Mc Carthy, U. and Gallagher, E. (2009). Monitoring the dynamic density of wheat dough during fermentation. *Journal of Food Engineering* 95(2): 332-338.

Mills, E.N.C., Wilde, P.J., Salt, L.J. and Skeggs, P. (2003). Bubble formation and

stabilization in bread dough. *Food and Bioproducts Processing* 81(3): 189-193.

Peighambardoust, S.H., Fallah, E., Hamer, R.J. and van der Goot, A.J. (2010). Aeration of bread dough influenced by different way of processing. *Journal of Cereal Science* 51(1): 89-95.

Peighambardoust, S.H., Hamer, R.J., Boom, R.M. and Van der Goot, A.J. (2008). Migration of gluten under shear flow as a novel mechanism for separating wheat flour into gluten and starch. *Journal of Cereal Science* 48(2): 327-338.

Peighambardoust, S.H., van der Goot, A.J., Hamer, R.J. and Boom, R.M. (2004). A new method to study simple shear processing of wheat gluten-starch mixtures. *Cereal Chemistry* 81(6): 714-721.

Peighambardoust, S.H., van der Goot, A.J., Hamer, R.J. and Boom, R.M. (2005). Effect of simple shear on the physical properties of glutenin macro polymer (GMP). *Journal of Cereal Science* 42(1): 59-68.

Peighambardoust, S.H., van der Goot, A.J., van Vliet, T., Hamer, R.J. and Boom, R.M. (2006). Microstructure formation and rheological behaviour of dough under simple shear flow. *Journal of Cereal Science* 43(2): 183-197.

Sroan, B.S., Bean, S.R. and MacRitchie, F. (2009). Mechanism of gas cell stabilization in bread making. I. The primary gluten-starch matrix. *Journal of Cereal Science* 49(1): 32-40.

Uthayakumaran, S., Gras, P.W., Stoddard, F.L. and Bekes, F. (1999). Effect of varying protein content and glutenin-to-gliadin ratio on the functional properties of wheat dough. *Cereal Chemistry* 76(3): 389-394.

Van der Borght, A., Goesaert, H., Veraverbeke, W.S. and Delcour, J.A. (2005). Fractionation of wheat and wheat flour into starch and gluten: overview of the main processes and the factors involved. *Journal of Cereal Science* 41(3): 221-237.

Van der Zalm, E.E.J., Van der Goot, A.J. and Boom, R.M. (2009). Influence of process conditions on the separation behaviour of starch-gluten systems. *Journal of Food Engineering* 95(4): 572-578.

Van der Zalm, E.E.J., Van der Goot, A.J. and Boom, R.M. (2010a). Influence of sodium chloride on shear flow induced starch-gluten separation from Soissons wheat dough. *Journal of Food Engineering* 99(3): 366-372.

Van der Zalm, E.E.J., Grabowska, K.J., Strubel, M., Van der Goot, A.J., Hamer R.J. and Boom, R.M. (2010b). Gluten Protein Composition in Several Fractions Obtained by Shear Induced Separation of Wheat Flour, *Journal of Agricultural and Food Chemistry* 58(19): 10487–10492

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Starch-gluten separation by shearing:
influence of the device geometry

Abstract

Separation of wheat flour into starch and gluten is possible by shearing in a conical device. This chapter describes the effect of the device geometry on the separation. The gap distance between the two cones and the cone angle were both varied. Modifications in the geometry of the shearing device lead to alterations in the shear rate profile applied to the dough. The geometry influenced both the aggregate formation and the following migration of the aggregates to the centre of the cone. This study confirms that the primary aggregation is mostly influenced by the shear rate, while the migration of the aggregates is influenced by the shear stress. However, the constraining of the dough by the walls of the cones also influences the gluten migration. Gluten clusters were found in all cases; however their migration to the centre only starts when they become similar in size compared to the space between upper and lower cone. This space is determined by the gap size and cone angle. Obviously, restriction of the growth of the gluten aggregates is a prerequisite for gluten migration. It is therefore clear that not only the shear rate but also the exact configuration of the device is important for separation. This insight may lead to significant optimisation of the process of separation by shearing.

Introduction

The separation of wheat flour into its main constituents starch and gluten is an important industrial process. The current processes are based on kneading and washing of dough, and thus are very intensive in water use. Subsequent removal of that water involves a lot of energy, while the waste water contains water soluble proteins from the flour as well. (Van der Borght *et al.* 2005) This gives rise to a waste water issue and loss of protein. Recently, a separation method for wheat flour in a conical shearing device was introduced (Peighambardoust *et al.* 2008; Van der Zalm *et al.* 2009b). The device used consists of two cones of which one cone rotates. The dough is placed in between the cones, and undergoes simple shear deformation as a result. Until now, the influence of temperature, rotation rate and processing time was investigated, but the influence of the exact geometry of this device is not clarified yet. This is the scope of this chapter.

The current hypothesis for the underlying mechanism for starch gluten separation during shear processing states that the separation consists of two steps. First, the gluten protein forms aggregates at mesoscopic scale upon continuous deformation. Second, when the aggregates are large enough, they migrate to the apex of the cone. (Peighambardoust *et al.* 2008; Van der Zalm *et al.* 2009b) This behaviour is observed for a variety of flour types, salt concentrations and a broad range of process conditions (Van der Zalm *et al.* 2009a; Van der Zalm *et al.* 2009b; 2010).

Previous devices were designed such that the shear rate was assumed to be equal over the height. (Peighambardoust *et al.* 2004; Manski *et al.* 2007; Habeych *et al.* 2008) In that case, the gap distance of the shearing device at the centre of the cones should be 0 mm. Nevertheless, we were not able to measure the exact gap distance between the upper and lower cone in previous devices. From experiments, we know however that there is usually some material in the centre of the cone, which indicate that the gap at that spot will not be exactly 0 mm. This implies that the shear rate would not be completely uniform in this device. That explains why Peighambardoust *et al.* (2008) speculated that the resulting shear rate gradient could have an influence on the separation behaviour. It should be taken into account that dough is processed in the shearing device, a material which flows difficultly.

We have developed a new shearing device to investigate the effects of gap distance at the centre of the cone and cone angles in more detail. Several upper cones were made with various cone angles. By applying those different cones, the shear rate profile can be changed in the devices. This chapter reports the influence of the exact geometry (overall space size and increment of the gap from centre to rim of the device) on the mechanism

and overall performance of the separation process and describes the new scientific insights resulting from the new experiments.

Materials and Methods

Soissons wheat flour (Meneba, Rotterdam, The Netherlands) from a single wheat cultivar was used to perform the experiments. The flour used had a protein content of 11.2% (w/w) and a moisture content of 14.5% (w/w). Farinograph water absorption of the Soissons flour, determined according to AACC-method 54-21, was 53.2% based on 14.5% moisture in flour, so 53.5% based on 14% moisture. Water absorption of the flour is determined in combination with 2w% NaCl (Merck, Germany) which is added on top of the sample. The formed dough had a stability time of 14 minutes, a peak time of 23.5 minutes and a tolerance index of 30 BU.

<u>Shearing device</u>

A new shearing device was developed in-house and used for all the experiments presented here. The device, based on the concept of a rheometer, applies a well-defined deformation to the material in between the two cones. The current device is an improved version of the devices made and described earlier (Peighambardoust *et al.* 2004; Manski *et al.* 2007; Peighambardoust *et al.* 2008). The devices were developed to study the influence of simple shear deformation on breakage and structure development in a number of biopolymer systems (Van den Einde *et al.* 2004; Van der Goot *et al.* 2008). In this respect, it differs from current structuring devices like extruders and kneaders, which apply a complex flow pattern, so multiple types of forces, to a material (Jongen *et al.* 2003). A schematic drawing of the shearing device is given in Figure 6.1. The shearing device is connected to a Brabender Do-corder 330 unit (Brabender OHG, Duisburg, Germany).

In earlier experiments, the closure of the system during operation was found to be crucial. The closing system used here is an improved design compared to previous versions of our shearing devices. The shearing device is closed hydraulically. A flexible PTFE (Teflon) ring closed the space by pressing onto the lower cone. Figure 6.2 describes the closing in more detail. The PTFE ring is perpendicular positioned to the cone. Grooves are present in the upper and lower cones to prevent slippage. The temperature is regulated by a circulating water flow. At two places, the temperature can be recorded. Temperature and torque values were measured online by an interface and controlling unit.

The shearing device consists of two cones; a static upper cone and a rotating lower cone. The diameter of the upper cone is 0.13 m, the diameter of the lower cone 0.138 m and

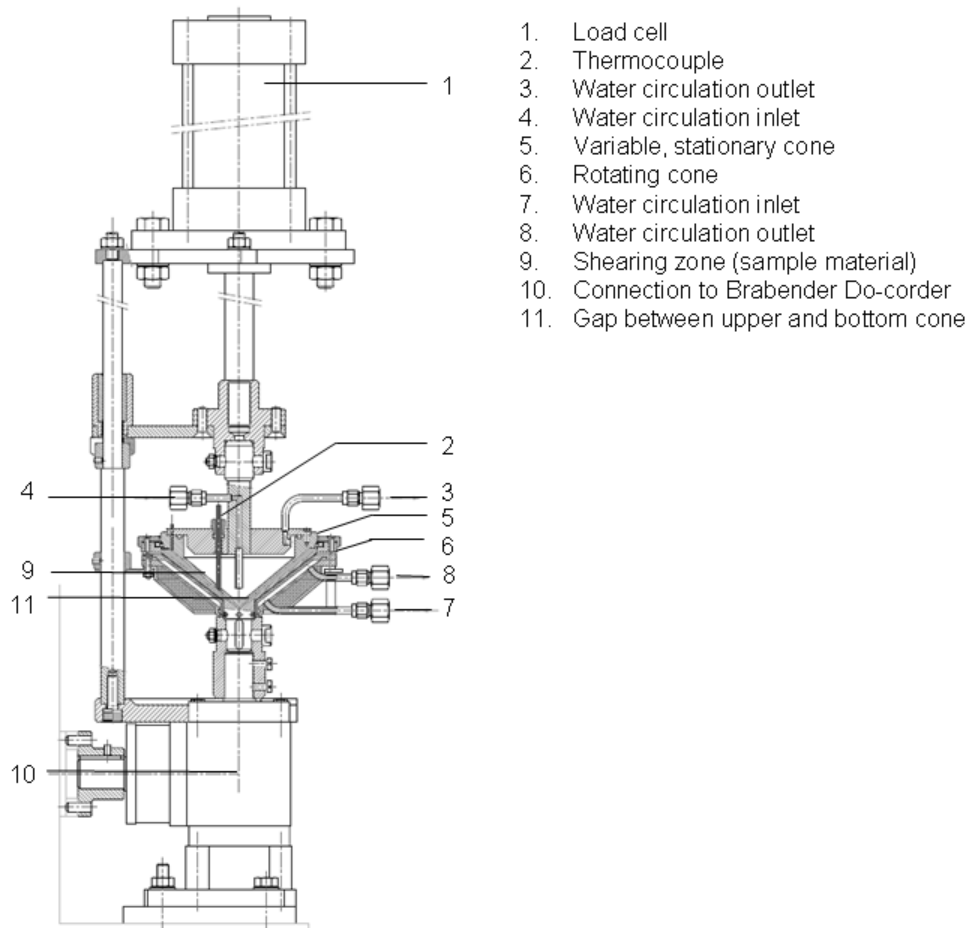| 1. | Load cell |
2.	Thermocouple
3.	Water circulation outlet
4.	Water circulation inlet
5.	Variable, stationary cone
6.	Rotating cone
7.	Water circulation inlet
8.	Water circulation outlet
9.	Shearing zone (sample material)
10.	Connection to Brabender Do-corder
11.	Gap between upper and bottom cone

Figure 6.1: overview of the shearing device

the hypotenuse of the lower cone 0.094 m. The space between the cones is filled by the dough. The angle of the gap was varied from 2.5˚, 5.0˚ to 7.5˚ by replacing the upper cone. It is also possible to work with parallel plates, which implies an angle of 0˚ between the cones. The overall space could also be varied by changing the gap distances by spacers in between the cones. Gap distances investigated were 3, 2, 1, and 0 mm. This is the gap distance in the tip of the shearing device.

Preparation of dough

As the volume between the two cones changes when using different cones and gap sizes, various amounts of wheat dough had to be used for the experiments. The volumes of the shearing zones are given in Table 6.1; the formulations of the dough are given in the
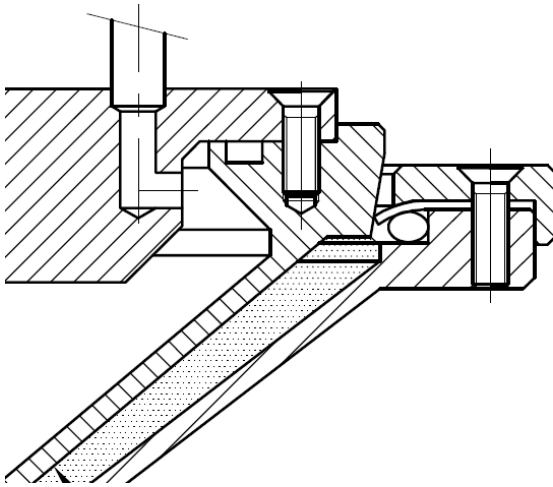
Figure 6.2: Closure system for the shearing device

Appendix. The mass ratios between flour: water: NaCl is in all cases 50.3 : 23.8 : 1. Dough was prepared by manually mixing the ingredients by a spatula. Water was added in 3 fractions during the mixing.

The dough mixture was distributed evenly in the bottom cone of the shearing device. The system was then closed, and a vertical compression force of approximately 2 bar (20 N/ cm^2) was imposed. This pressure was kept constant during all experiments. Prior to shearing, the material was rested at 15 °C for 15 minutes to allow relaxation.

Shearing process

All samples were sheared for 60 minutes. The process consists of 4 minutes shearing at 5 rpm to avoid wall slip. Within 1 minute the rotation rate was increased to 15 rpm. After the shearing process, the material in the shearing device was cooled in stationary position to approximately 5 °C.

Table 6.1:
Volume of shearing zone for each shearing device combination (ml)

Cone angle	Parallel	2.5°	5°	7.5°
Gap distance				
0 mm	0	67	80	117
1 mm	25	80	97	127
2 mm	38	85	112	140
3 mm	52	88	127	157

<u>Treatment of samples</u>

After shearing, the material inside the shearing device was divided into 5 different sections (layers) along the hypotenuse of the device. After opening the shear device, the material was taken form the cell and immediately frozen using liquid nitrogen and stored until freeze drying. Samples were freeze dried for at least 48 h to a maximum moisture content of 3.5%. Subsequently, the samples were powdered with an IKA Basic mill (type A11) (IKA, Staufen, Germany) and sieved using a pore size of 0.355 mm.

<u>Protein content</u>

The protein content (conversion factor 5.7) of the material from the various layers was determined by Dumas using a NA 2100 Nitrogen and Protein Analyser (ThermoQuest-CE Instruments, Rodeno, Italy). Methionine was used as standard during analysis. Duplicate measurements were made for all samples; variations of maximally 1% were observed. The protein content is expressed based on dry matter.

Results

This section describes the various structures that we have observed during the experiments and the final protein concentration in the layers.

An array of different cone angles and gap sizes was investigated; Figure 6.3 outlines the materials obtained after shearing by showing top view pictures. The visible (yellowish) dark regions are clusters of gluten protein. In some experiments, these clusters were large, as for example can be seen in the lower right corner (7.5°, 3 mm). One can also see the concentration of the gluten protein in the centre of the device. The definition in this case for aggregates is an accumulation of gluten protein, the definition for a cluster is grown aggregates, so accumulation of various aggregates. From Figure 6.3, it can be concluded that larger gaps and larger cone angles both lead to the formation of large clusters, which are still more or less spherical. At smaller gap distances and smaller cone angles, the aggregates seem to be smaller, while at the same time the driving force for migration towards the centre is stronger.

<u>Shearing at 2.5° angle</u>

The average protein concentrations measured for each layer after shear experiments in the 2.5° angle cone at different gap distances, are given in Figure 6.4a. Visually, the material after shearing looked the same for the experiments performed at a gap of 0, 1 and 2 mm. In those experiments, a comparable degree of separation was obtained. The material sheared at a distance of 3 mm however contained more aggregated material and larger clusters of gluten protein. Taking into account that the starting concentration
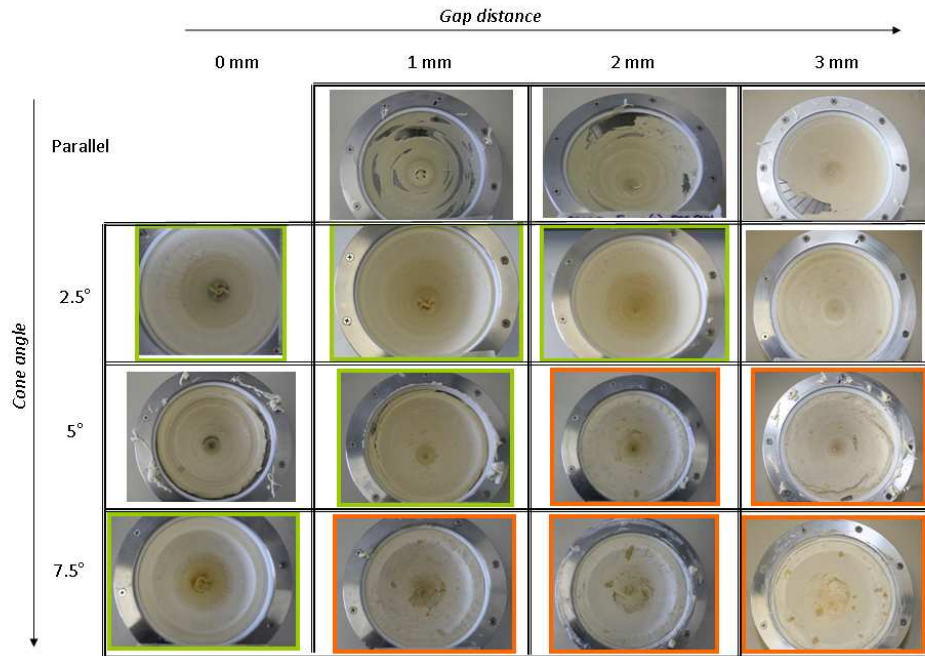
Figure 6.3: Overview of top view pictures of all shear runs performed. Shear runs are performed at 15 rpm at 15˚C and sheared for 60 minutes. The outer diameter of the stainless steel device is 17.5 cm. Green squares indicate separated systems; while red indicate gluten cluster formation.

consists of approximately 11% protein (dry matter basis), hardly any overall migration of protein had taken place with a gap size of 3 mm.

Shearing at 5.0° angle

The protein concentrations measured after shearing at the four different gap distances in the 5.0° cone are given in Figure 6.4b. The two smallest gaps (1 and 0 mm) gave a darker area in the tip of the cone, which indicates of stronger migration. However, this area was not large (see Figure 6.3). Figure 6.4b confirms this, as there is a large difference in protein concentration only between the 4th and the 5th layer of this shearing run. Again, gluten clusters could be observed in the material processed in the experiment with the largest gap. It is remarkable that the protein concentration in layer 4 is low for most conditions and the enrichment in gluten protein is only visible in the 5th layer. A fluctuating protein concentration is noticeable for the experiment performed at a gap distance of 0 mm.
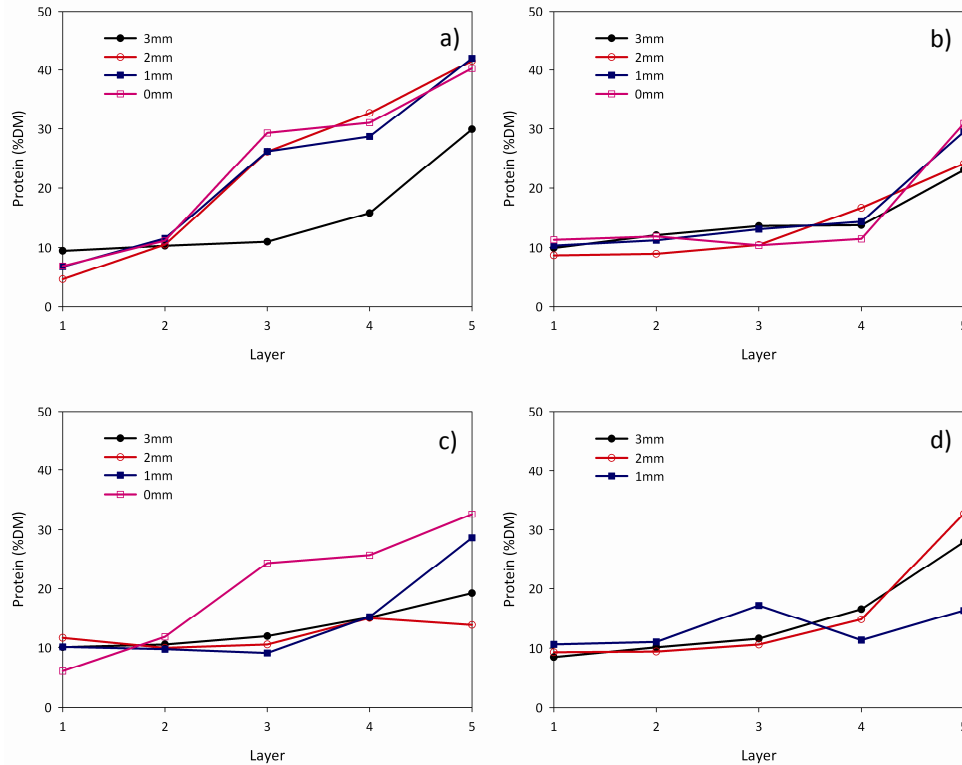
Figure 6.4: Protein concentration in the various
layers after shearing dough a) in the 2.5° angle cones. b) in the 5° angle cones c) in the 7.5° angle
cones d) in between parallel cones
All experiments were performed at 15 rpm, 15˚C for 60 minutes. Water absorption was 48.2%.

Shearing at 7.5° angle

Figure 6.4c summarizes the shear experiments performed in the 7.5° cone shearing
device. Also here, larger gap sizes (2 and 3 mm) showed hardly any separation, but the
experiment with 1 mm gap size shows a certain extent of separation. The experiment at
0 mm gives by far the best separation in this configuration. Visually, the results obtained
do not look very different from a standard shear run performed at a cone angle of 2.5˚
and a gap distance of 1 to 2 mm; which implies that layer 4 and 5 were filled with a
gluten-enriched material, while the other layers were gluten-depleted.

Shearing with parallel plates

In the conical shearing device configuration, it is also possible to work with parallel plates
(i.e., 0˚ cone angle). The results of these shear runs are shown in Figure 6.4d. Performing
these experiments was rather difficult, because material was lost due to leakage at the

rim of the device. The leakage indicates that the dough exhibits enormous forces in this configuration. This confirms our previous findings on normal forces. The material showed hardly any visual separation after shearing and it did not contain clusters or aggregates. Actually, it resembled a normal dough.

Discussion

The aim of this study was to investigate the effect of the shearing device geometry on the shear-induced separation process of starch and gluten. The novel shearing device therefore possessed two additional process parameters. The first parameter was the angle between the cones, which could be changed from parallel plates (0°), to 2.5°, 5.0° and 7.5° respectively. The second parameter is the distance between the cones, which was varied from 0 to 3 mm in steps of 1 mm. In some cases, controlling the gap distance was difficult, as the normal force exerted by the dough during shearing is large. Especially with a gap distance of 0 mm, the hydraulic closing system could not completely counterbalance that force. Although the gap decreased during resting and shearing, the gap of 0 mm was never reached. The gap for the experiments performed at the other angles could be controlled; for an angle of the 2.5° cone till 0.4 mm, for the 5° cone till 0.1 mm, and for the 7.5° cone till 0.2 mm respectively at the end of the processing time. One should note that wheat starch consists of two types of granules, with a diameter of approximately 6 and 22 μm (i.e., 0.006 to 0.022 mm) respectively. Already a few of these granules in between the closing rings may cause such a gap. Besides dough is relatively stiff, so hard to deform, which makes the closing of the system more difficult.

The application of different geometries led to completely different structures in the dough. In some experiments, large gluten clusters were obtained; other settings led to gluten migration. The smallest angle (2.5°) showed most migration, so the material was separated well. These process conditions were chosen based on already existing knowledge (Van der Zalm *et al.* 2009b). Based on the amount of material added in our previous study, we can estimate that the gap distance in the former configuration of the shearing device probably was probably 1-2 mm and a fixed cone angle of 2.5°. Figure 6.4, shows clearly that these conditions gave the best separation.

As the rotation rates are the same for all combinations of the configurations, the material in the shearing device is exposed to a different shear rate profile. The local shear rate γ applied to the samples can be calculating using Equation 6.1:
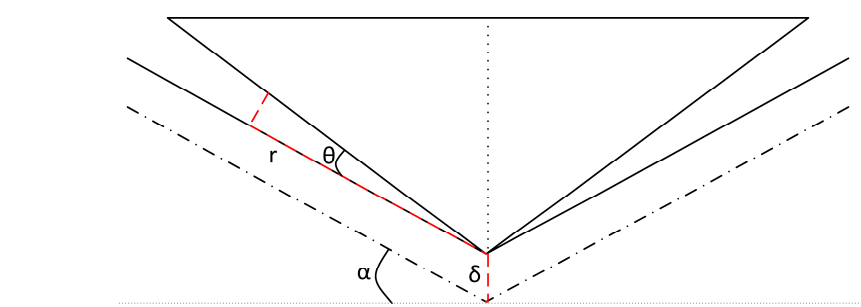
Figure 6.5: Schematic drawing of the shearing device.

$$\dot{\gamma} = \frac{2\pi \cdot \left(\dfrac{N}{60} \right) \cdot r}{\delta + r \cdot \tan(\theta)}$$

Equation 6.1

In this equation, N is the rotational speed (rpm), r the position on the hypotenuse of the shearing device (mm), δ the gap distance (mm) at the centre of the device, and θ the angle between the cones. (see Figure 6.5)

In case of a gap distance being 0 mm, a constant shear rate is obtained at all positions of the shearing device. For larger gap sizes, the shear rate differs at each position in the shearing device, and becomes larger towards the rim of the device. Table 6.2 shows the development of the shear rate along the hypotenuse of the shearing device. Table 6.2 also allows comparison of shear experiments in which the shear rate is locally comparable. For example, a shear rate of 6.5-7 s^{-1} can be obtained at r = 30 mm using a cone angle of 7.5°, a gap distance of 3 mm. In that experiment, large gluten clusters were obtained, also at that specific position. A comparable shear rate was also obtained in the experiment using the following settings: r= 10 mm, cone angle 2.5°, gap distance 2 mm. In that experiment, no gluten clusters were observed, but gluten migration took place. This leads us to the conclusion that the shear rate only is not sufficient to explain the differences in structure development during shearing. Obviously other factors play a role as well.

The same conclusion can be drawn by comparing this study with results obtained earlier. There, we showed that gluten separation during 60 minutes of shearing was promoted by a low rotation rate, so a low shear rate. (Van der Zalm *et al.* 2009b) Therefore it could be expected that changes in geometry in such a way that the shear rate was decreased, resulted in more separation as well. However, that was clearly not observed. Increasing the gap distance led to the formation of gluten clusters instead of gluten migration.

After the shearing process, the structures obtained with differing gaps and cone angles

Table 6.2:
Calculated shear rate at several positions in the shearing device. Shear rate is calculated at a rotation rate of 15 rpm.

Gap (mm)	theta (°)	tan theta (in rad)	Shear rate		
			r = 10 mm	r = 30 mm	r = 60 mm
3	0	0.000	5.24	15.71	31.42
	2.5	0.044	4.57	10.93	16.77
	5	0.087	4.05	8.38	11.42
	7.5	0.132	3.64	6.78	8.65
2	0	0.000	7.85	23.56	47.12
	2.5	0.044	6.45	14.24	20.40
	5	0.087	5.46	10.19	13.00
	7.5	0.132	4.74	7.92	9.52
1	0	0.000	15.71	47.12	94.25
	2.5	0.044	10.93	20.40	26.04
	5	0.087	8.38	13.00	15.08
	7.5	0.132	6.78	9.52	10.59
0	0	0.000	-	-	-
	2.5	0.044	35.98	35.98	35.98
	5	0.087	17.95	17.95	17.95
	7.5	0.132	11.93	11.93	11.93

are very diverse. Separated material could clearly be observed in a number of experiments. Also smaller aggregates with a diameter up to 2 mm could be observed. The experiments performed at large gaps and/or in combination with a large angle between the two cones, showed much larger gluten clusters. Obviously, when the space is large, the clusters are not restricted to grow and consequently are not deformed in the flow. As a result, they grow larger steadily. We suspect that these clusters remain more or less spherical for much longer time and would only start to deform when they become similar in size to the (local) distance between the two cones. Consequently, lack of confinement for the gluten clusters seems to reduce the driving force towards the cone. It illustrates that a prerequisite for migration is the deformation of the gluten clusters.

The diameter of the protein clusters depends also on the shear rate applied. A lower shear rate leads to larger aggregates (Van der Zalm *et al.* 2009b). These results confirm the behaviour hypothesized earlier: the clusters slowly grow with the total deformation (shear rate multiplied by time). With the same shearing time, a larger shear rate corresponds to a larger total deformation.

The experiments performed in the shearing device make use of a well defined shear flow, but it is not possible to observe the separation process in-line. Therefore, we carried out small-scale experiments to obtain some (qualitative) visualisation of the process. Remarkably, the strand formation described above and use of linear shear in combination with dough, has not been described yet in literature, as far as we are aware of. Dough sheeting seems to be related to the use of linear shear, but in sheeting one applies extensional flow. (Venkatesh Murthy *et al.* 2008) Extension generally leads to break-up of structures, instead of the formation of larger structures. Windhab *et al.* (2005) describe for instance the effect of various types of flow on emulsions. Elongation has an enormous effect on the breakup of these particles. (Windhab *et al.* 2005) Peighambardoust *et al.* (2007) concluded that the microstructure of dough was changed due to shearing in an eccentric Couette cell. Shear processing influences the break up of gluten aggregates (Peighambardoust *et al.* 2007). Therefore, a small amount of manually kneaded wheat flour dough was placed in between two Perspex plates, and a shear force is applied by hand to the material. After a short period of manually shearing, gluten cluster formation was observed. At continued shearing, the clusters form a roll of gluten protein perpendicular to the shear direction. (Figure 6.6) Although it is hard to control the process, the gluten cluster formation could be readily reproduced on a qualitative basis. Kieffer et al. (1999) observed with Soissons wheat dough also some demixing of the dough in a high-pressure capillary viscosimetry. During the process the dough is exposed to uniaxial deformation, so that gluten and starch is separated from each other. (Kieffer *et al.* 1999)

This simple experiment shows that the shearing results in the formation of gluten aggregates which grow with continued shearing. When the clusters become comparable in size to the gap size between the two plates, they became confined and formed rolls that were perpendicular oriented towards the shear direction. Eventually, those rolls covered the complete width of the plates. With respect to the first step (aggregate and cluster formation), the deformation (i.e simple shear) applied was comparable in case of the parallel plates and the shearing device. Also the resulting structure was rather similar. First, the gluten protein forms aggregates and/or clusters under the influence of the shear flow. When they become larger, they are squeezed in between the upper and lower walls, and form gluten strands perpendicular to the shear direction. This behaviour
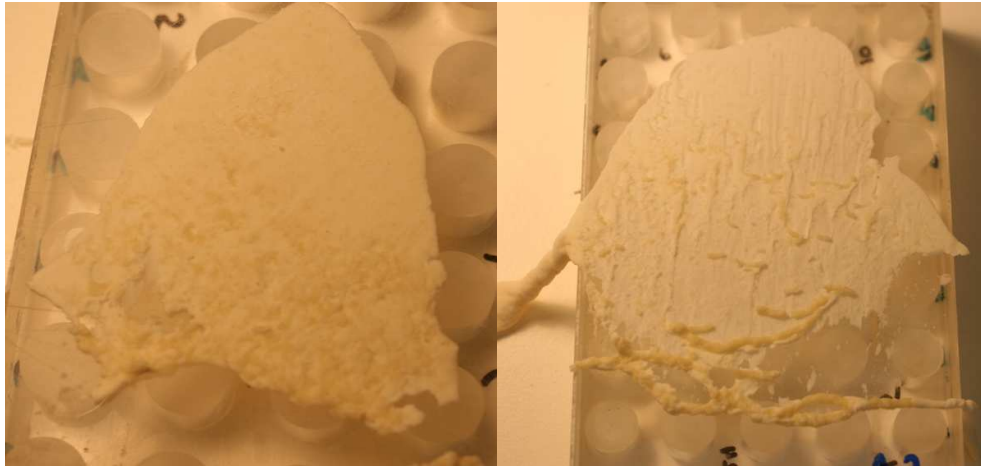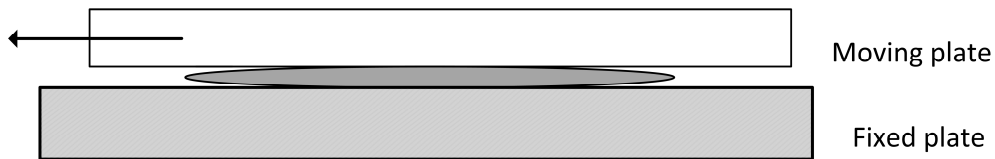
Figure 6.6: Separation of wheat flour into starch and gluten when shear is applied to it between two parallel plates a) experimental setup, the upper plate is 20 cm long, in between there is a thin layer of dough; b) maximal covered distance for shearing was approximately 5 times 20 cm, so 1m; c) maximal covered distance for shearing was 2m

was also observed in the conical shearing device. This means that the hypothesis earlier described about the separation mechanism is confirmed. These strands were not observed during the experiments given in Figure 6.3. To observe the formation of strands perpendicular to the shear direction is difficult because it is an intermediate step between aggregate formation and gluten migration. Figure 6.7 described the separation mechanism of wheat flour via both techniques schematically.

The second step, the migration, was not observed with the two plates. This indicates that the migration is most likely related to the curvilinear nature of the flow field in the conical device. The strands, perpendicular to the shear flow, are oriented towards the centre of the device and thus exposed to an inhomogeneous field, which results in their migration towards the centre. As this curvilinearity is not present between the two plates, no migration could be seen.

We suspect that during the separation process in the shearing device, the aggregates formed will be initially elongated in the direction of the shear flow, followed by migration of it to the tip of the device. This intermediate step is not visible in the experiments performed with the Perspex plates. No formation of gluten strands parallel
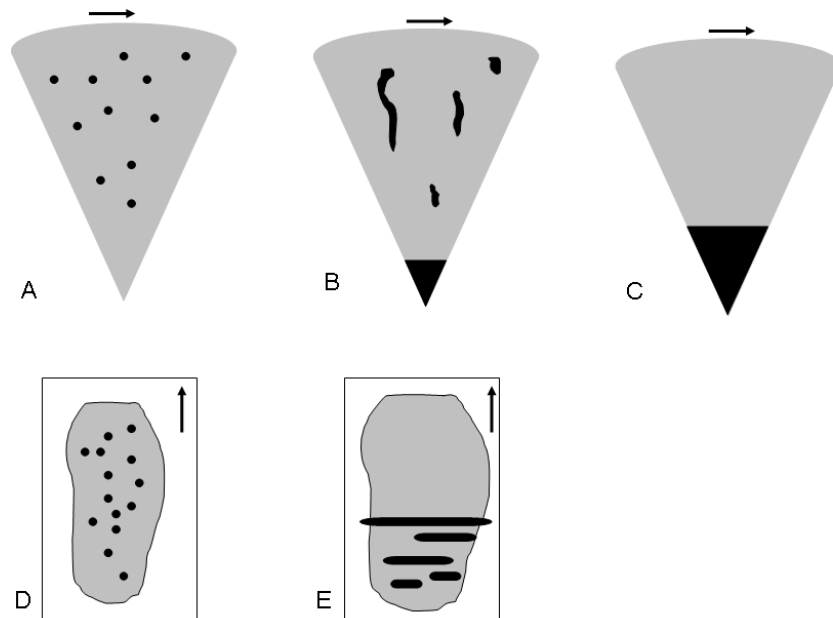
Figure 6.7: Separation of wheat flour into starch and gluten. Grey is the dough matrix, black the gluten rich areas. Step A represents the formation of aggregates in the shearing device, Step B the formation of gluten strands in the shearing device, Step C is the final situation of the separation process in the shearing device, Step D reflects the formation of aggregates after shearing with Perspex plates and Step E the formation of gluten strands. In both systems the gluten strands are formed perpendicular to the shear direction.

to the flow direction could be observed. This could be caused by differences in the strength of the applied shear force, which could be different in both systems. So, it could be that the aggregates immediately form gluten strands perpendicular to the shear direction, and that the formation of strands parallel to the shear direction is not necessary. Therefore this intermediate step is not depicted in Figure 6.7.

Conclusion

This study shows that the geometry of the shearing device is important for the shear-induced separation of wheat flour dough. The shear rate profile is different in all configurations as the rotation rate was kept constant. Although it seems that the shear rate is an important design parameter for the separation processes, other factors related to the exact device geometry play a role as well.

Previously we proposed a two-step mechanism. First, the gluten forms aggregates, which grow while shearing. When the aggregates become comparable in size to the (local) distance between the two cones, the aggregates deform, and the second step, migration, follows. This hypothesis was confirmed via a two-plate method, which showed the formation of gluten aggregates, and their subsequent extension into rolls perpendicular to the shear direction. Thus, the first step is due to the shearing itself. The second step is dependent on the curvilinear element in the cone device, as it was not observed with the plates.

The new insights presented in this chapter may provide guidelines towards a further development of an effective device for shear-induced separation of gluten and starch from wheat dough.

Acknowledgements

References

Habeych, E., Dekkers, B., van der Goot, A.J. and Boom, R. (2008). Starch-zein blends formed by shear flow. *Chemical Engineering Science* 63(21): 5229-5238.

Jongen, T.R.G., Bruschke, M.V. and Dekker, J.G. (2003). Analysis of dough kneaders using numerical flow simulations. *Cereal Chemistry* 80(4): 383-389.

Kieffer, R. and Stein, N. (1999). Demixing in wheat doughs - Its Influence on dough and gluten rheology. *Cereal Chemistry* 76(5): 688-693.

Manski, J.M., van der Goot, A.J. and Boom, R.M. (2007). Formation of Fibrous Materials from Dense Calcium Caseinate Dispersions. *Biomacromolecules* 8(4): 1271-1279.

Peighambardoust, S.H., Hamer, R.J., Boom, R.M. and Van der Goot, A.J. (2008). Migration of gluten under shear flow as a novel mechanism for separating wheat flour into gluten and starch. *Journal of Cereal Science* 48(2): 327-338.

Peighambardoust, S.H., van Brenk, S., van der Goot, A.J., Hamer, R.J. and Boom, R.M. (2007). Dough processing in a Couette-type device with varying eccentricity: Effect on glutenin macro-polymer properties and dough micro-structure. *Journal of Cereal Science* 45(1): 34-48.

Peighambardoust, S.H., van der Goot, A.J., Hamer, R.J. and Boom, R.M. (2004). A New Method to Study Simple Shear Processing of Wheat Gluten-Starch Mixtures. *Cereal*

Chemistry 81(6): 714-721.

Van den Einde, R.M., Akkermans, C., Van der Goot, A.J. and Boom, R.M. (2004). Molecular breakdown of corn starch by thermal and mechanical effects. *Carbohydrate Polymers* 56(4): 415-422.

Van der Borght, A., Goesaert, H., Veraverbeke, W.S. and Delcour, J.A. (2005). Fractionation of wheat and wheat flour into starch and gluten: overview of the main processes and the factors involved. *Journal of Cereal Science* 41(3): 221-237.

Van der Goot, A.J., Peighambardoust, S.H., Akkermans, C. and Van Oosten-Manski, J.M. (2008). Creating novel structures in food materials: The role of well-defined shear flow. *Food Biophysics* 3(2): 120-125.

Van der Zalm, E.E.J., Sawalha, A., Van der Goot, A.J. and Boom, R.M. (2009a). Separation of wheat flour into starch and gluten - Mechanisms and differences between flour cultivars. Proceedings International Symposium on Food Rheology and Structure, Zürich, 344-347

Van der Zalm, E.E.J., Van der Goot, A.J. and Boom, R.M. (2009b). Influence of process conditions on the separation behaviour of starch-gluten systems. *Journal of Food Engineering* 95(4): 572-578.

Van der Zalm, E.E.J., Van der Goot, A.J. and Boom, R.M. (2010). Influence of sodium chloride on shear flow induced starch-gluten separation from Soissons wheat dough. *Journal of Food Engineering* 99(3): 366-372.

Venkatesh Murthy, K. and Raghavarao, K. (2008). Analysis of dough sheeting and heat transfer during baking of unleavened flat bread (Chapathi). *International Journal of Food Engineering* 4(3): Article 1.

Windhab, E.J., Dressler, M., Feigl, K., Fischer, P. and Megias-Alguacil, D. (2005). Emulsion processing - from single-drop deformation to design of complex processes and products. *Chemical Engineering Science* 60(8-9): 2101-2113.

<u>Appendix Chapter 6</u>

Specification dough for shearing experiments

	Cone type	Parallel	2.5˚ angle	5˚ angle	7.5˚ angle
gap (mm)					
0	Flour (g)	-	58.33	69.65	101.86
	Water (g)	-	27.61	32.97	48.22
	NaCl (g)	-	1.16	1.38	2.03
1	Flour (g)	21.76	69.65	84.45	110.56
	Water (g)	10.3	32.97	39.98	52.34
	NaCl (g)	0.43	1.38	1.68	2.20
2	Flour (g)	33.08	73.99	97.5	121.88
	Water (g)	15.66	35.03	46.16	57.70
	NaCl (g)	0.66	1.47	1.94	2.42
3	Flour (g)	45.27	76.61	110.56	136.68
	Water (g)	21.43	36.27	52.34	64.70
	NaCl (g)	0.9	1.52	2.2	2.72

The following chapter will be submitted for publication

Constraining the gluten aggregate formation
is a prerequisite for shear-induced
separation

Abstract

Wheat flour was separated into a gluten-enriched and a gluten-depleted (i.e. starch-rich) fraction, with a cone-in-cone shearing device, with variable angle between the two cones. It was found that shearing for a specific time at the same rotational speed led to much more inward migration of gluten with a narrow gap (2.5°) compared to a broader gap; adaptation of the rotational rate such that roughly the same shear rates were obtained in all systems, led to a comparable protein concentration at the apex of the cone, but the concentration profile over the whole cone was slightly different. These observations confirm the importance of the total deformation, calculated as the product of shear rate and processing time, as a process parameter influencing the gluten aggregate formation and the final separation of the shear-induced separation.

The results also indicate the importance of confinement of the dough in between the two cones. The gap space between the cones forces the growing aggregates to confine its shape to the gap space. It seems that the gluten starts to migrate, once the aggregates experience the confinement, leading to deformation of the gluten aggregates. That is why we concluded that the separation mechanism consists of three steps, rather than two, which was reported previously. The additional step describes the effect of confinement by the cones.

A conceptual map was constructed with variables shear rate, time and system geometry, which indicates in which regions only aggregation and in which regions also migration may be expected. Experimental findings confirm the conceptual map.

Introduction

Separation of agricultural products into their constituents is industrially and nutritionally important. The principles underlying separation processes are generally based on differences in density, particle size or solubility of the constituents. This is also the case in the current separation process for wheat flour into gluten and starch, as exemplified by the dough-batter washing process. (Van der Borght *et al.* 2005) This process requires large amounts of water and energy and leads to loss of protein into the waste water.

Some years ago, a new separation mechanism for wheat flour was proposed, using a conical separation device, which concept is inspired on a rheometer. Here, differences in rheological behaviour of starch and gluten were used to induce separation. It was hypothesized that the separation follows a two step mechanism. The first step is the aggregation of gluten, induced by deformation, which enables gluten patches to touch and then adhere. The total stress on the material limits the growth of aggregates, as too large stresses will cause the aggregates to break up again. In the second step, which describes the migration of gluten, the shear stress seems to play a positive role. A gluten-enriched fraction is formed in the tip of the cone, and a gluten-depleted fraction is observed in the outer region of the shearing device. Previous work described the influence of the process conditions on these steps, which led to the conclusion that both steps have different optimal process conditions. The aggregation step was promoted by high temperature, low rotation rate and higher water content (i.e., low shear stress). The gluten migration step benefits from high dough density and higher rotation rate (i.e., high shear stress). (Van der Zalm *et al.* 2009)

The separation of biopolymers by a well-defined flow was reported. Large DNA molecules could be separated by radial migration (Dill 1979), as well as migration of macromolecules (Agarwal et al. 1994).

This chapter extends the study on shear-induced separation by using a system in which the geometry of the shearing device can be changed. Cones with different angles were used to create different gap spaces in between the cones, while a radial inhomogeneity in shear field was introduced by having a certain gap at the centre of the two cones.

With this system the effects of shear rate and time, of which total deformation can be calculated, and system geometry on the separation mechanism were further elucidated. While the shearing device used gives the opportunity to verify and refine the hypothesis of separation that was formulated earlier, it also is the basis for a first outline of a qualitative operating window, giving the combinations of geometry, shear rates and processing times that lead to good separation. Also the types of structures that could be

formed were investigated. The results may lead to further understanding of the separation principles and may possibly lead to extension of the separation mechanism towards other raw materials and components.

Materials and Methods

Separation experiments were performed with Soissons wheat flour (Meneba, Rotterdam, The Netherlands) from a single wheat cultivar. The flour used had a protein content of 11.2% (w/w) and a moisture content of 14.5% (w/w). Water absorption of the flour was determined by the addition of tap water to 300 g of Soissons flour (14.5% moisture) to which 6 g of NaCl (Merck, Germany) was added on top of the sample (i.e., 2.0w% on flour basis). Farinograph water absorption of the Soissons flour, determined according to AACC-method 54-21, was 53.2% based on 14.5% moisture in flour, so 53.5% based on 14% moisture. The dough formed had a stability time of 14 minutes, a peak time of 23.5 minutes and a tolerance index of 30 BU.

<u>Shearing device</u>

The shearing device, in principle based upon the concept of a rheometer, applies a well-defined deformation to the material. The device used is an improved version of the devices made and described earlier (Peighambardoust *et al.* 2004; Manski *et al.* 2007; Peighambardoust *et al.* 2008). The shearing device has modifications in the closing system and the configuration of the system could be changed. First of all, the gap distance in the tip of the cone can be changed from 0, 1, 2 to 3 mm. Secondly, cones with several cone angles (parallel, 2.5°, 5.0° and 7.5°) could be inserted in the shearing device. The upper cone is the static cone that can be changed, and the bottom cone is the rotating one. A schematic drawing of the shearing device is given in Figure 7.1. The shearing device is connected to a Brabender Do-corder 330 unit (Brabender OHG, Duisburg, Germany).

The closing system is modified compared to the previous shearing devices. The shearing device is closed hydraulically; a PTFE (Teflon) ring closes the system by pressing perpendicularly on the lower cone. A more detailed description of the closing system can be found in. Van der Zalm *et al.* (2010b).

Slippage in the system is prevented by grooves in the stainless steel cones. The temperature is controlled by a circulated water flow and can be measured at two additional positions. Torque and temperature readings were recorded during the experiments by an interface and controlling unit.

Even though usually cone-cone or cone-plate systems are designed to have the same

1. Load cell
2. Thermocouple
3. Water circulation outlet
4. Water circulation inlet
5. Variable, stationary cone
6. Rotating cone
7. Water circulation inlet
8. Water circulation outlet
9. Shearing zone (sample material)
10. Connection to Brabender Do-corder
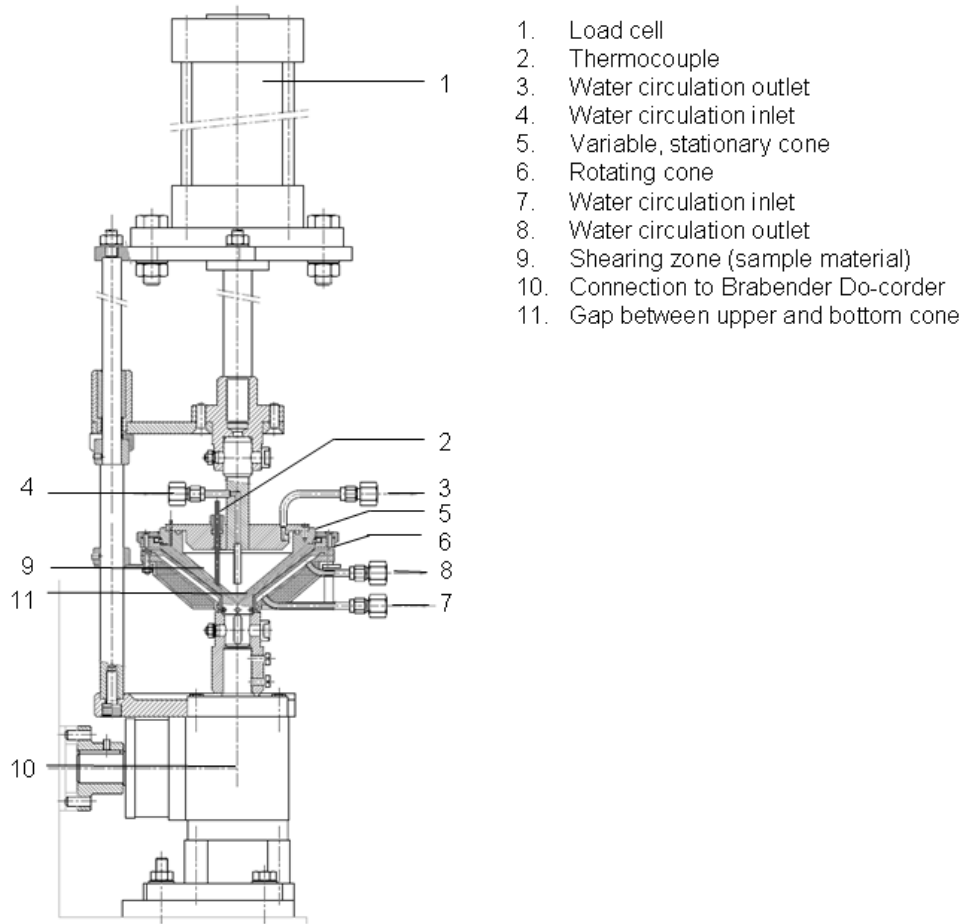11. Gap between upper and bottom cone

Figure 7.1: overview of the shearing device

shear rate everywhere in the system, we have extended their use here to inhomogeneous shear fields through the creation of a gap between the two cones. This gap can be adjusted and monitored during the process.

Figure 7.2 describes a schematic overview of the closing system of the shearing device. The local shear rate (s^{-1}) applied to the samples can be calculated using Equation 7.1.

$$\dot{\gamma} = \frac{2\pi \cdot \left(\dfrac{N}{60}\right) \cdot r}{\delta + r \cdot \tan\left(\theta\right)}$$
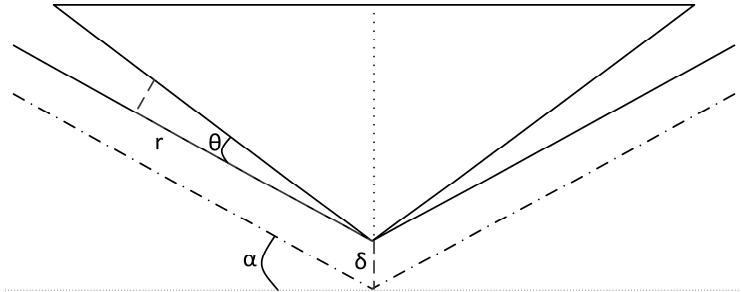
Equation 7.1

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Figure 7.2: Schematic drawing of the shearing device and its labels.

In this equation, N is the rotational speed (rpm), r the position on the hypotenuse of the shearing device (mm), δ the gap distance (mm) and θ the cone angle, (Figure 7.3). In case of δ = 0, the system applies homogeneous shear flow; this makes δ a parameter to control the in-homogeneity in the flow field. The changeable gap distance and cone angle had an influence on the shear rate created in the shearing device, which is applied to the material.

Dough preparation

A different configuration leads to a change in sample volume. This means that varying amounts of dough had to be used for the experiments. The formulations of the dough are given in Table 7.1. The ratio between flour: water: NaCl is in all cases 50.3 : 23.8 : 1.0. Before processing the material in the shearing device, the materials were manually mixed in a beaker glass. Water was added in 3 fractions during the mixing.

The mixture was manually spread in the bottom cone of the shearing device. After closure of the device, a vertical compression force was put onto the material. This force was approximately 2.5 bar (25 N/cm^2). To allow relaxation of the material, the dough

Table 7.1: Composition of the dough used for the shearing experiments. Gap distance is in all cases 2 mm.

Cone type	2.5˚ angle	5˚ angle	7.5˚ angle
Flour (g)	73.99	97.5	121.88
Water (g)	35.03	46.16	57.7
NaCl (g)	1.47	1.94	2.42
Volume Shear Zone (ml)	85	112	140

was rested for 15 minutes at 15 °C, prior to shearing.

Shearing process

All shearing processes started with 4 minutes shearing at 5 rpm to avoid wall slip. Then, the rotation rate was increased to the set rotational speed (rpm) within 1 minute. The total durations of the shearing processes used in this research were 15 or 60 minutes. After shearing, the material inside the shearing device was cooled in stationary position to approximately 5 °C. All shearing experiments were performed using a temperature of 15 °C.

Treatment of samples

After shearing, the material inside the shearing device was divided into 5 layers and encoded. The division of the 5 equal layers took place along the hypotenuse of the device. The material was immediately frozen using liquid nitrogen and stored until freeze drying. Samples were freeze dried for at least 48 h to a maximal moisture content of 3.5%. Subsequently, the samples were powdered with an IKA Basic mill (type A11, IKA, Staufen, Germany) and sieved using a pore size of 0.355 mm.

Protein content

The protein concentration in the various layers after shearing was determined by Dumas using a NA 2100 Nitrogen and Protein Analyser. (ThermoQuest-CE Instruments, Rodeno, Italy). Methionine was used as standard during analysis. A conversion factor of 5.7 was used. Duplicate measurements were performed for each layer. Variations of maximally 1% were observed. The protein content was expressed based on dry matter.

Dough structure

The structures formed during the various shear runs were analyzed. Digital photographs were made and structures were observed visually by eye.
The larger gluten aggregates formed obscured the smaller aggregates with confocal laser scanning microscopy, so this method could not be used for visualisation. Similarly, X-ray microtomography was unsuccessful due to the low resolution (i.e., the lack of a large enough density difference), between the starch and the gluten fractions. Therefore, the analysis of the spatial distribution of gluten other than the average composition over the five bands in the cell, was by eye and standard digital photo camera (Canon EOS 30D).

Results

As was mentioned in the materials-and-method section, we can induce in-homogeneity

in the flow field by creating a gap between the cones. While the shear rate in the centre is always zero, the shear rate will increase with the increasing distance from the centre. Figure 7.3 shows the trend in the shear rates in the systems for the three different cone angles used in this work. One can either run these systems at the same rotational speed (Figure 7.3a), in which case the system with the largest angle difference will exert much less shear, or run them at different shear rates by adapting the rotational speed (in rpm)
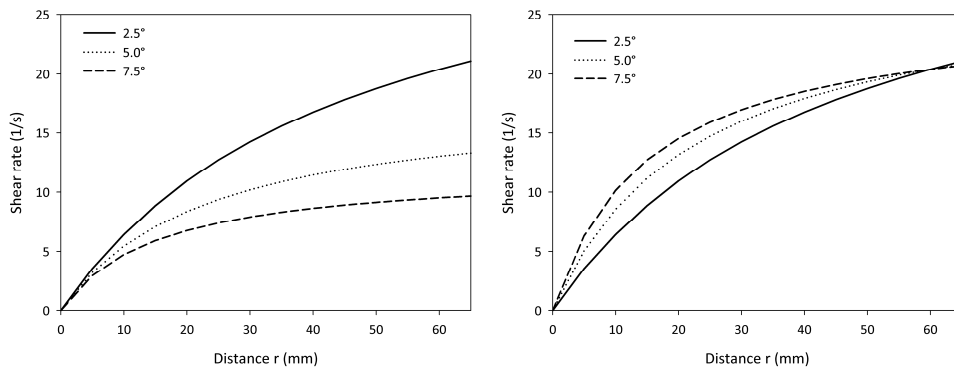

Figure 7.3: a) Shear rate development along the hypotenuse R of the shearing device (r = 67 mm total) for three cone configurations. N = 15 rpm, δ = 2 mm; b) The same, but with rotational rate adapted such that the shear rate at r = 60 mm is equal.

(Figure 7.3b).

Figure 7.4 shows the use of these two different settings in the shearing device. While a constant rotational rate leads to very different degrees of separation along the hypotenuse (with the best separation with the smallest angle), normalization of the
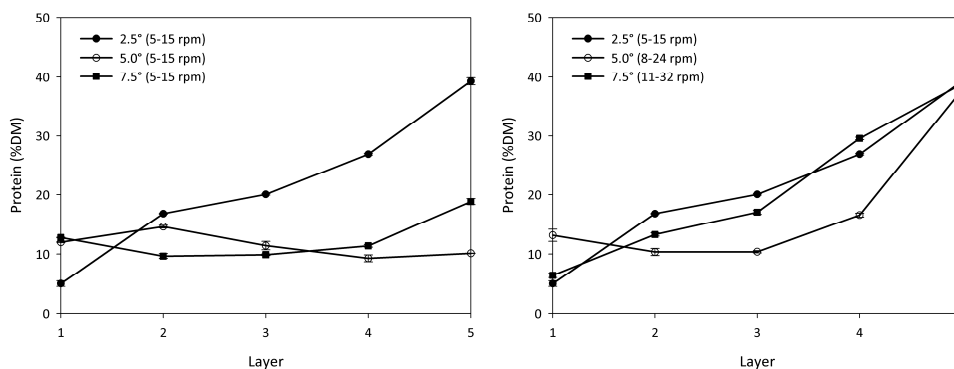

Figure 7.4: a) Protein concentration measured after performing the experiments according to Figure 7.3a (5 – 15 rpm for 15 min, δ = 2 mm); b) Protein concentration measured after performing the experiments according to Figure 7.3b (shear rate normalized at r = 60 mm, 15 min, δ = 2 mm). Initial protein concentration of freeze-dried Soissons flour is 11.2%.

shear rate at 60 mm leads to a more or less similar separation profile for the three situations. The total shear deformation apparently is important for the separation. It seems that increasing the gap size can be simply compensated for by increasing the rate of rotation, at least to a certain extent. During previous research the shear rate was influenced by changing the rotation rate (Van der Zalm *et al.* 2009), in this project the shear rate is influenced by changing the geometry. Contrary to what was reported in 2009, we see that a decrease of shear rate did not result in an increase in protein content at the apex of the cone. When changing the geometry a large variety of structures were found. As a result, we have to conclude that the shear rate or other factors than the shear rate play a role as well.

The change of the cone angle has a dramatic effect on the appearance of the system during shearing (see Figure 7.5). The 2.5° cone shows fast migration of relatively small aggregates to the apex after 15 minutes, while in the 7.5° cone, very large aggregates remained randomly dispersed throughout the cell. The larger cones yielded aggregates that became much larger, with the ones in the 7.5° cone by far the largest.

One should bear in mind, when reading Figure 7.4, that the material in each layer was freeze dried and grinded to a powder before the determination of the protein content by Dumas. Thus, the protein content given in Figure 7.4 is an average of the gluten aggregates and the rest of the material in that layer. The outcomes of the experiments are therefore difficult to compare on protein concentrations only. For example, Figure 7.5 shows that the system was very inhomogeneous for the large cone angles, while this is not evident from Figure 7.4. The aggregates formed did not migrate to the apex yet, but instead had grown very big (~1 cm), and contribute to the total protein content of
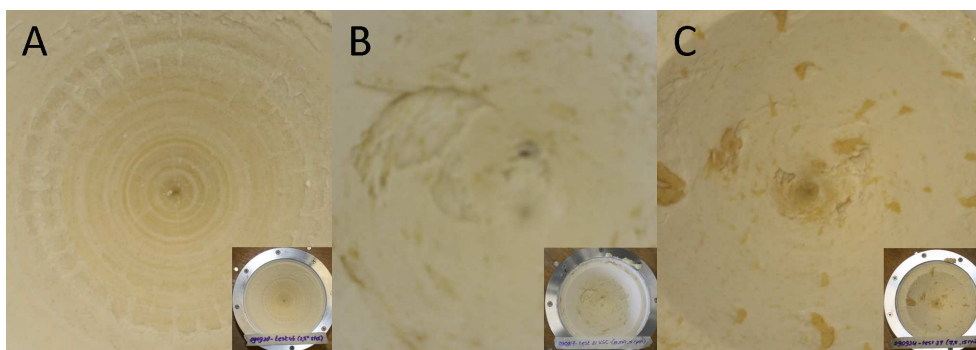

Figure 7.5: Top view picture of the structure formation in VSC for three cone configurations. (a) 2.5° cone (b) 5° cone (c) 7.5° cone. Rotation rate was 5-15 rpm (settings of figure 7.3a), processing time 15 minutes and the gap distance at the tip 2 mm. The magnification in each picture is comparable.
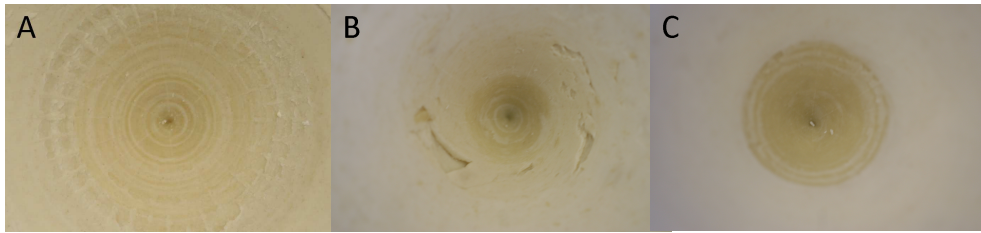
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Figure 7.6: Top view picture of the structure formation in the VSC for three cone configurations. (a) 2.5° cone (b) 5° cone (c) 7.5° cone Shear rate is adapted to 20.4 s^{-1} at 60 mm from the tip of cone (settings of Figure 7.3b). Samples were processed for 15 minutes, gap distance was 2 mm. The magnification in each picture is comparable.

that layer.

Subjecting the dough to more or less the same total shear rate in the three different geometries, by adaptation of the rotational speeds according Equation 7.1 (see Figure 7.3b), led to much more similar results in respect to the protein contents (Figure 7.4b) and top view pictures (Figure 7.6). Now, most of the aggregates and clusters that were formed in the larger cones had migrated as well, forming a clear gluten phase in the apex. Remarkably, the gluten phase in the 2.5° cone is much less clear than the one in the 7.5° system, which seemed to indicate better separation at larger angles. However, the overall protein concentrations in the layers could not confirm this observation as these were almost equal. The 5.0° cone resulted in a deviating protein concentration profile over the 5 layers. The reason behind this observation is not clear yet.

Measuring the protein content after separation does not provide enough information about the separation mechanism. As mentioned, the protein concentration in the layers is an average, and does not take into account the visual details observed by eye. Therefore, we had a closer look to the structures that were formed during the separation process. Figure 7.7 shows some examples of typical structures found after shearing wheat flour. With the 2.5° cone, small aggregates can be discerned after 5 minutes, which will migrate after prolonged shearing. This is different with the larger cone angle differences: in the 5° and 7,5° cones, large clusters were sometimes observed after 15 min, even though part of the gluten has already migrated to the centre. A further interesting observation is that the large clusters were deformed into a roll shape, which we suspect is due to the constraining caused by the upper and lower cone.

The large clusters shown in Figure 7.7 are not visible in every experiment. We expect that generally large clusters migrate rapidly to the centre.

In some situations, starch-gluten-starch layers could be observed in the vorticity direction instead of gluten rolls. Figure 7.8 shows a sample taken from a shear run in the
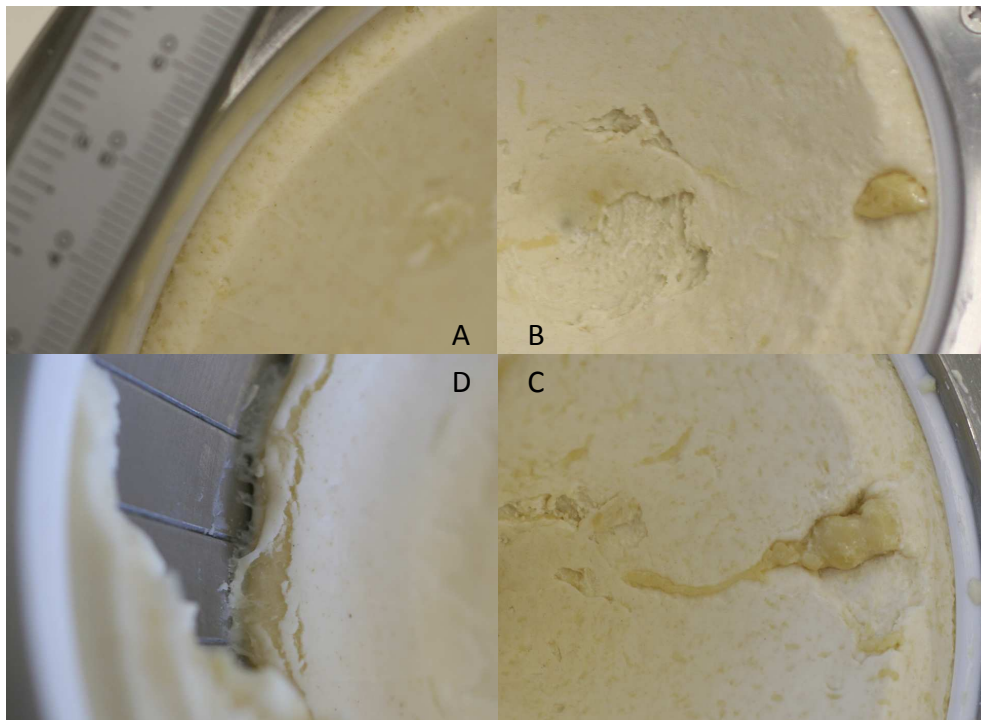
Figure 7.7: Structure overview after shearing. a) gap 2 mm, 2.5˚ cone, 5 m in, 5 rpm b) gap 2 mm, 5.0˚ cone, 15 min, 15 rpm c) gap 2 mm, 7.5˚ cone, 15 min, 15 rpm; d) gap 2 mm, 2.5˚ cone, 15 min, 5 rpm.

2.5° cone geometry, gap 2 mm, rotation rate 5-15 rpm which was sheared for 15 minutes (sample was oven dried). The sample had a clear layered structure. The size of the structure is approximately 1 cm^2. The layered structure consists of starch-rich outer layers, and a gluten-enriched inner layer. Even though it is not yet fully clear what how this structure was created, the gluten patches may well have become smeared out between an upper and a lower layer of the starch-rich phase. This might happen when

Figure 7.8: Starch, gluten, starch structure is visible after drying. Device geometry: 2.5˚ cone, gap 2 mm. Process conditions: 5-15 rpm, 15 min. The sample is taken from the intermediate layers of the shearing device.

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some of the patches come close to each other and merge. Whether this structure will later break up into smaller patches and migrate to the centre, is not known.

To summarize, it was found that the gluten first starts to form relatively small gluten aggregates which are then combined and compacted into larger clusters with high protein content. The maximum size of these clusters seems to be related by the gap space between the two cones. It seems that the cluster, formed by the accumulation of aggregates, can be deformed due to confinement of the geometry. The larger gluten clusters were only observed in the cones with larger gap space. Confinement by geometry seemed to favour migration.

Discussion

During this research, we experimentally investigated the structures formed during shear processing of dough. Previously, we hypothesized that the aggregation and subsequent migration were related to respectively the shear rate and shear stress applied to the system. (Van der Zalm *et al.* 2009) As a dough system with constant water content is used, the shear rate is the dominant parameter. Therefore the amount of separation as function of shear rate was investigated here.

The gluten clusters are quite concentrated in gluten protein, and increase in protein concentration over time. While the typical protein content in gluten clusters found to be 32-39% (dry weight) in the 5° cone angle system (Figure 7.7b), the gluten concentration in the further developed, the large gluten patch found in the 7.5° system (Figure 7.7c) was 48-52% (dry base).

Figure 7.4 shows that the dough system needs to have certain amount of shear before it can exhibit (overall) separation. The use of a cone angle of 2.5° already resulted in migration after 15 minutes shearing. This migration was not observed for the shearing system with a 7.5° cone yet, probably due to the fact that this system experienced in the same time approximately less than half of the total shear deformation. Adjusting the shear rate such that all systems exerted more or less the same shear rate gave similar average performance according to the amount of separation obtained. Comparable shear rate at a certain position in the shearing device gives the same type of structures, although it should be said that the geometry constraint plays an important role. The structures in the shearing devices with the larger gap distances were composed of clustered gluten.

So, the general overview is comparable; however, the details between the situations are different. This is agreement with the hypothesis that we posed earlier (Van der Zalm *et*

al. 2010b), that gluten aggregation is dependent on the total deformation (i.e., the product of shear rate and time). However, there are differences during the treatment, mainly influencing the structure formation. While in previous work the total processing time was 60 minutes, we used just 15 minutes in the experiments described here. Although the total deformation varies a factor 4, it was generally found to be sufficient for good separation. In both situations, a comparable amount of separated gluten protein could be observed. It is surprising, however, that while the aggregates need to grow until they are comparable in diameter to the local gap space, the 7.5° system gave good separation (after adaptation of the rotation speed to obtain similar shear rates). The later stages of the separation process (clustering and migration) therefore have to be quite fast.

This quick clustering and migration seems to be in line with the findings shown in Figure 7.8, which shows a sandwiched assembly, with rather pure gluten in between two starch -rich layers. This structure, which was found in a 2.5° system, is not common, even tough one does regularly see clusters that are highly extended and flattened. Such a structure may be a merger of many patches at the same time, which due to its sheer size prevented the formation of a roll. It is not known whether this structure will migrate in its entirety to the centre; however the good separation was normally observed in those experiments. Despite the quick formation of clusters from the initial aggregates, one sees very high protein content in these clusters. So, while they are formed by merging the smaller aggregates, there might be a simultaneous mechanism that causes a further concentration of the gluten (or expulsion of starch).

The general outline of the separation process can now be summarized as a sequence of stages, which is in agreement with the general hypothesis outline that we posed earlier (Van der Zalm *et al.* 2010b). The first stage is (orthokinetic) aggregation of gluten proteins, which approach due to the shear flow, and then merge due to the cohesive properties of the gluten, towards larger structures. These aggregates become rapidly larger by the same mechanism, while at the same time the internal starch is excluded by a mechanism that is not yet identified. Thus, large aggregates or clusters are formed, which can be seen by the naked eye.

Figure 7.9 may represent a schematic overview of the structure in between the first stages (more or less spherical initial aggregates), and the onset of the last stage (migration). The growing aggregates are more and more distorted by the constraining effect, and at some stage may form a layer due to elongation of the aggregates formed. The layers further away from the centre are still filled with gluten aggregates, while in the more central layers the gluten protein material fills the space between the two cones. The migration of the gluten protein material takes place in the vorticity direction.
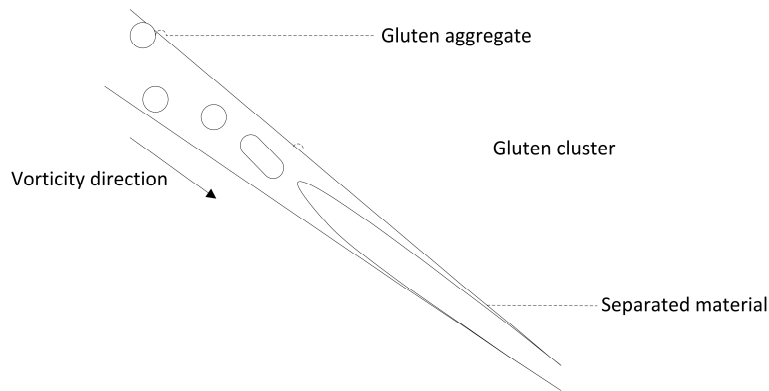
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Figure 7.9: Schematic overview of the formation of the structure formation in the shearing device. A cross-section in the middle of the device gave the typical starch-gluten-starch layer.

This phase then sets out to migrate, probably because of the differences in conditions over the cone. As the material migrates toward the centre, the constraining becomes more important as a result of which the diameter of the cluster is forced to become even smaller, which necessitates it to become longer and thus be extruded towards the centre. This mechanism would imply that the actual migration is dependent on normal forces (which cause the rolls to be extruded) and therefore is quite fast. Indeed, an experimental observation is that during shearing considerable forces has to be exerted on the device to keep it in place (in the range of 2.5 bar, which implies a normal force of about 105 N/m^2. Thus, most of the time is needed to form clusters that are large enough to be constrained. The border formed by the hyperbole in Figures 7.9 and 7.10 marks the amount of deformation needed to form aggregates that are large enough to span the (local) distance between upper and lower cones.

We therefore hypothesize that when the clusters become similar in size to the local distance between upper and lower cone, they become constrained. Then, the shear flow forces the clusters to rotate, while below and above the surrounding starch-rich phase needs to pass the aggregate. As a result, the gluten clusters become compressed and deform into a roll with its longer axis in the vorticity direction. This is evident from the large normal forces observed (Van der Zalm *et al.* 2010a). The new insights show that the separation mechanism can be divided in three steps: (I) aggregation of gluten to aggregates, (II) clustering of aggregates to larger clusters and deformation of these large clusters due to the confinement and (III) migration to the apex of the cone. Bearing in mind the hypothesized mechanisms behind these steps, together with the experimental evidence found, leads to a conceptual map of the expected system behaviour as function of shear rate, time and system geometry (while keeping the dough formulation constant), Figure 7.10 summarizes this.
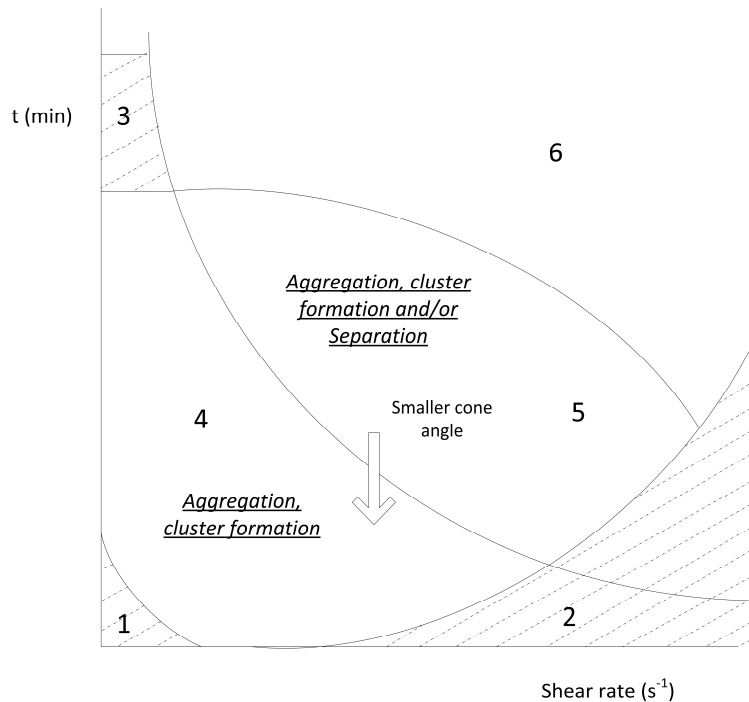
Figure 7.10: Schematic overview of the separation and migration in the shearing device. The arrow indicates the change of the line when the geometry is changed. Dough composition is not changed. Region 1: not sufficient deformation to cause significant aggregation; region 2: too high shear rate damaged the gluten; region 3: too long treatment causing enzymatic degradation; region 4: aggregation, but not yet sufficient deformation for cluster deformation and migration; region 5: sufficient deformation to have cluster deformation and migration; region 6: overmixing and gluten degradation.

At sufficient total shear deformation, the system will show aggregation and subsequent migration of the gluten towards the centre of the system. However, if the total shear is lower than a certain threshold (the hyperbole in Figure 7.10), aggregation will have taken place, but the aggregates/clusters have not yet reached the size that they are constrained due to the gap space, and consequently separation has not yet taken place. Figure 7.10 makes also clear that without aggregation, the separation step cannot be reached. Of course, the aggregate formation itself requires deformation as well, so below a certain limit no aggregation can have taken place (region 1). It was shown before that the shear rate should not be too high, because the material could be unable to follow the deformation and will be degraded as a result (region 2) (Van der Zalm *et al.* 2009). On the other hand, shearing at a very low rate, such that the material can completely relax, will probably mean that the gluten does not form any macroscopic aggregates. When applying a very high total shear rate (area 5 in the graph), the dough

will be damaged at molecular level, giving properties comparable to overmixed and/or overheated dough, and no separation will occur (Peighambardoust *et al.* 2008; Peressini *et al.* 2008). Area 3, indicating the combination of the application of a low shear rate for a long period, indicates the situation in which the dough enzymatic degradation takes place. This is observed by Peighambardoust *et al.* (2008) for very long treatment times (120 min).

When the configuration of the shearing device is changed, the boundary line between aggregation and migration will be positioned differently. Decreasing the cone angle of the shearing device is expected to result in a shorter time necessary for aggregation, and migration can start earlier. The formation of gluten aggregates is strongly dependent of the space between the two cones. In the 2.5° cone separation start fast due to fast aggregation, while in the 7.5° cone the separation goes fast as the aggregates are so large that high forces can be put on them. The 5.0° cone does not profit from one of these situations. (Van der Zalm *et al.* 2010b) When the cluster becomes (locally) similar in size to that of the gap space, migration will start. This may point to the possibility for further improvement in the performance of the system; for faster separation or for higher concentrations of gluten.
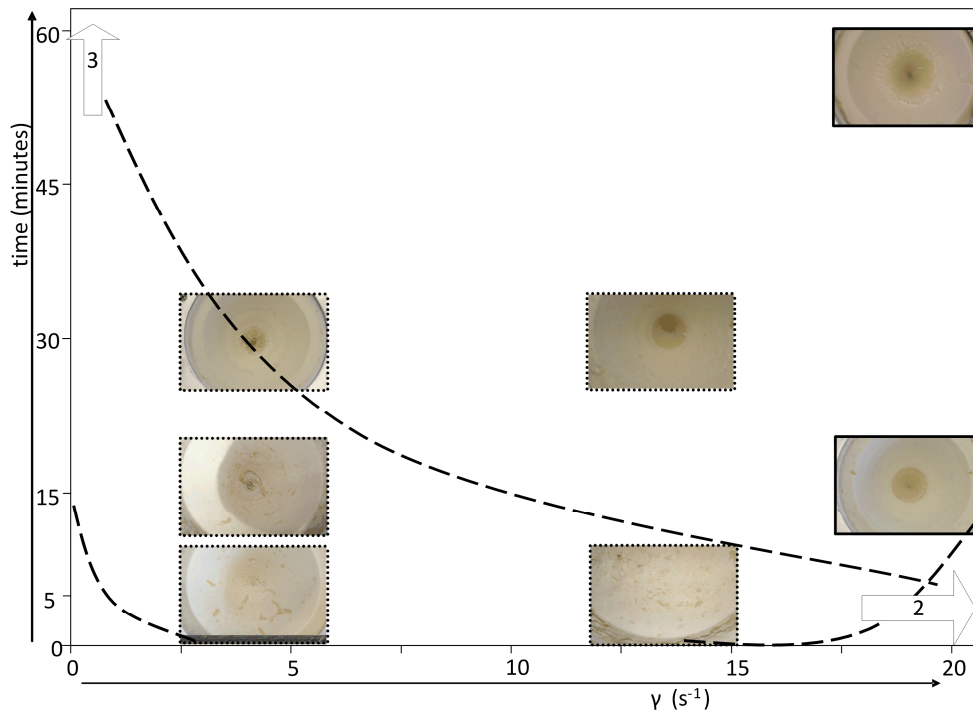

Figure 7.11: Overview of structures found in the 7.5° cone (gap = 2 mm) in time. Black dotted squares are shear runs with a rotation rate adapted at r = 30 mm and black squares are shear runs adapted at r = 60 mm. Boundaries lines for separation and gluten aggregation are depicted.

The conceptual behaviour as summarized in Figure 7.10 was matched to the experimental observations as given in Figure 7.11. Even though we could not identify some of the extreme regions (1, 2 and 3), the border between aggregation, and aggregation plus migration could be clearly distinguished.

Conclusion

Experiments were performed in a cone-in-cone shearing device, in which the cone angle between the two cones was altered, while keeping a certain gap distance between the cones at the tip of the cone. It was found that shearing for a specific time at the same rotational rate led to more separation with a narrow gap (2.5°) compared to larger gap spaces (5° and 7.5°). However, adaptation of the rotational rate such that all systems exerted more or less the same shear rates, led to more comparable overall separation behaviour.

Despite the similar overall separation efficiencies, the gluten structures formed during the processing were different; in some cases very large patches were found, especially with the larger cone angles. These patches had high gluten content, which indicates that during formation, the gluten concentration increases by a certain mechanism, which is not fully clarified yet. Secondly, the shape of these patches suggests that they were forced to assume an elongated structure perpendicular to the shear flow. A sandwiched structure that was found in one system may well represent the situation in which a number of the larger clusters merge while deforming.

The results show that parameters like shear rate and shear stress are important, but the confinement of the aggregates/clusters between top and bottom cone, plays a major role as well. The two step mechanism which was proposed earlier can now be extended to a three step mechanism: (I) aggregation of gluten to aggregates and larger patches, (II) deformation of the large patches as their size becomes equal to the gap space and (III) migration of these structures towards the centre.

A conceptual map was constructed with variables shear rate, time and system geometry, which indicates in which regions only aggregation and in which regions migration may be expected as well. The map was found to agree with experimental findings.

Acknowledgements

References

Agarwal, U.S., Dutta, A. and Mashelkar, R.A. (1994). Migration of macromolecules under flow: the physical origin and engineering implications. *Chemical Engineering Science* 49 (11): 1693-1717.

Dill, K.A. (1979). Theory for the separation of very large DNA molecules by radial migration. *Biophysical Chemistry* 10(3-4): 327-334.

Manski, J.M., van der Goot, A.J. and Boom, R.M. (2007). Formation of fibrous materials from dense calcium caseinate dispersions. *Biomacromolecules* 8(4): 1271-1279.

Peighambardoust, S.H., Hamer, R.J., Boom, R.M. and Van der Goot, A.J. (2008). Migration of gluten under shear flow as a novel mechanism for separating wheat flour into gluten and starch. *Journal of Cereal Science* 48(2): 327-338.

Peighambardoust, S.H., van der Goot, A.J., Hamer, R.J. and Boom, R.M. (2004). A new method to study simple shear processing of wheat gluten-starch mixtures. *Cereal Chemistry* 81(6): 714-721.

Peressini, D., Peighambardoust, S.H., Hamer, R.J., Sensidoni, A. and van der Goot, A.J. (2008). Effect of shear rate on microstructure and rheological properties of sheared wheat doughs. *Journal of Cereal Science* 48(2): 426-438.

Van der Borght, A., Goesaert, H., Veraverbeke, W.S. and Delcour, J.A. (2005). Fractionation of wheat and wheat flour into starch and gluten: overview of the main processes and the factors involved. *Journal of Cereal Science* 41(3): 221-237.

Van der Zalm, E.E.J., Van der Goot, A.J. and Boom, R.M. (2009). Influence of process conditions on the separation behaviour of starch-gluten systems. *Journal of Food Engineering* 95(4): 572-578.

Van der Zalm, E.E.J., Van der Goot, A.J. and Boom, R.M. (2010a). Influence of sodium chloride on shear flow induced starch-gluten separation from Soissons wheat dough. *Journal of Food Engineering* 99(3): 366-372.

Van der Zalm, E.E.J., Van der Goot, A.J. and Boom, R.M. (2010b). Starch-gluten separation by shearing: influence of the device geometry. *To be submitted*.

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General discussion

Introduction

In this chapter, we sketch the status of understanding of the shear-induced separation process of starch and gluten from wheat flour based on the results presented in this thesis. Starting from the aim of the study, we will continue with an overview of the conclusions described in each chapter. These conclusions will be assembled into a consistent model for the mechanism of shear-induced separation. Finally, we will discuss the societal relevance of the work by considering the reduction of water and energy use, and improvement in the use of the raw materials into high-value products that could be achieved with the process considered here.

Overall aim, approach and conclusion

The aim of this thesis was to unravel the underlying mechanisms of the shear-induced separation process for wheat flour as was previously described by Peighambardoust *et al.* (2008). The approach taken was to obtain better fundamental insight in the mechanisms of separation, and discuss its consequences to the process. Thus, first, the separation mechanism itself was further investigated to explain the influence, of the process conditions and dough composition. Second, the gluten quality obtained by the shear-induced separation process was tested. Third, the effect of the cell geometry was studied in more detail.

The separation process studied has the potential to save much water. Consequently, the energy consumption can be decreased as well. However, the shear-induced process has also a number of limitations at this stage. The shear-induced separation process is eased by the presence of NaCl. Also the purity of the fractions differs from the traditional process. In that process, extensive washing led to more pure fractions of gluten and starch; however it subjects the fractions to a harsher treatment. The functionality of the fractions from the shear-induced separation process therefore seems different, but not less.

Findings

Process conditions and dough composition

Chapters 2 and 3 describe the effect of the process parameters and dough composition on the separation process. It was shown that there are two important steps in the separation process: the aggregation of gluten protein, and the migration of gluten to the

tip of the cone. Process parameters such as shearing time, temperature, and rotation rate influence both steps, albeit differently. The aggregation was promoted by high temperature, low rotation rate and high water content, while the migration was positively influenced by a higher rotation rate and a higher dough viscosity (achieved through a lower water content).

In previous work, NaCl was added to the dough, as it was known that this improves the properties of dough aimed for bread making purposes. However, addition of NaCl to a large-scale industrial separation process would result in saline products, and saline side streams. It would obviously be better to avoid NaCl addition to the system. Therefore, we investigated the effect of NaCl on the separation process in detail. Separation was improved when increasing the NaCl content up to 4w% (on flour basis), but additional NaCl decreased the effect again. It was also found that separation of wheat flour dough without NaCl is in fact possible, but the process conditions should be chosen more accurately, because the material becomes less process tolerant.

Our initial expectations were that the separation would depend on the rheological properties of the dough matrix, and therefore the doughs with different NaCl content were characterised on their rheological properties. These measurements did not show clear differences in G' and tan δ value with varying NaCl concentration, but the shear stress and normal force at a constant shear rate were influenced. However, it should be mentioned that these experiments were difficult to carry out reliably, which explains that those obvious experiments have not been described in literature yet.

Previous research (Van der Zalm *et al.* 2009) showed that the separation mechanism can be applied to other wheat flour types than only the type studied in this thesis. A comparable amount of separation was observed for strong, weak and commercial flour. Thus, the separation process works for a broad range of wheat flour types.

<u>Gluten quality</u>

Chapters 4 and 5 describe the properties of the gluten-enriched fraction that is obtained after separation with respect to their molecular composition and functionality. First, a comparison between the gluten-enriched and gluten-depleted layer was made at two shearing times. As gluten is composed of protein fractions with different molecular weights, these fractions can be detected by SE-HPLC and SDS PAGE. All protein fractions take part in the aggregation and migration of gluten protein. Therefore, the composition of the gluten-enriched fraction is rather similar to the original flour (starting material), even though it is slightly enriched in large glutenin polymers.

Second, the gluten-enriched fraction was compared to commercial vital wheat gluten during kneading and baking experiments by enriching wheat flour with these gluten types. Gluten obtained by shear-induced separation showed a comparable to improved

gluten functionality compared to vital wheat gluten. The differences could not be related to the SE-HPLC chromatograms. Probably, the functionality could be different due to different drying techniques, or due to the inherent mildness of the shear-induced separation process.

<u>Geometry of the shearing device</u>

Chapters 6 and 7 describe the use of a new shearing device in which the cone angle and gap distance can be modified, so that a different shear rate profile is generated. For example, by applying the same rotation speed to a material, the shear rate profile was different for other geometry settings. While the overall separation was found to be mostly dependent on the overall deformation (shear rate multiplied by time), the geometry had a major effect on the final structure in the dough material inside the device as well. The migration and aggregation steps were observed, but we now noted that the aggregates first need to grow in diameter till the (local) gap space is reached, before gluten migration to the tip can take place. Thus, a third step in the separation mechanism was found.

Separation mechanism

Initially, a two step mechanism for separation of wheat flour into starch and gluten was used to describe the separation process. During the project, we concluded that this mechanism had to be extended with a third step. The three step mechanism consists of:

(1) the formation of aggregates;

(2) clustering and growth of aggregates up to the width of the gap space and deformation of clusters due to geometric confinement between upper and lower cone;

(3) migration of the clusters towards the apex of the cone.

The first step is probably driven by the aggregation or network forming properties of gluten. The starch shows a more dilatant behaviour at the conditions studied and is expected to have a passive role. The local gap space between the cones governed by the gap distance and the cone angles is important in step 2. When the clusters become of the same size as the gap space, the alignment of the clusters towards the apex of the cone starts, and extends into the vorticity direction. The alignment can be seen as the initiation of migration. While the initial aggregation is mainly dependent on the shear rate and total deformation, the local gap size (i.e., the shear cell geometry) determines at which moment the aggregates start to be deformed. Finally, the aligned clusters are pulled towards the tip of the cone. This step is probably based on the elastic properties

of the dough. It is clear that for all steps process conditions, like rotation rate, temperature and processing time, are important.

The separation process of the flour by shear is not influenced by the quality of the gluten protein. All constituents of the gluten are found in the final gluten fraction. One would expect that the soluble gluten proteins are only found in the gluten obtained by the shearing process, but surprisingly they are also present in the commercially available gluten source. This was not expected, as this gluten was obtained by a dough-batter type processing that includes a washing step. It could be that the soluble protein in the current industrial is quite rapidly entrapped by gluten. The baking quality of breads made with shear-induced gluten, was comparable or better than commercial vital wheat gluten.

The separation mechanism described assumes that the gluten is the active component in migration. Nevertheless, this is fundamentally an assumption. There is a possibility that the starch fraction is the actively separating phase. Possibly, the starch is packed in such a way that the gluten is pushed out the dough matrix. In Van der Zalm *et al.* (2009) we previously described the effect of enriching wheat flour dough by A-starch, so that the protein concentration became approximately 30 % of the protein concentration in the starting wheat flour. When this dough was processed in the shearing device, very large gluten domains were found in the dough. It seemed to be that these clusters were mainly formed in the upper and lower layers of the shearing device. The intermediate layers were lower in protein content. Due to the addition of starch, the mechanism for separation is strongly influenced. Obviously, considering starch as a completely passive component in the system might be an oversimplification.

The separation can be best described using a three step mechanism. The clustering and deformation of the aggregates step (step 2) depends strongly on the geometry of the shearing device. Process conditions play an important role during all steps. Shear rate and shear stress are important for the formation of the aggregates and their migration. In this thesis, the separation process was not divided into separate processing units for the steps of the separation mechanism, as all steps are combined in one device. Optimizing aggregation would be most likely favoured by low shear rates, increased water content, higher temperatures and a larger gap distance. As migration is positively influenced by high shear stresses, it will be improved by high shear rates and low water contents. This suggests that a two-step process may be more effective than the current one-step process.

We expect that the formation and strength of aggregates depends on the amount of water present in the system and the time available for the formation of bonds between the gluten proteins. The second step of the migration mechanism, the clustering and

deformation of aggregates, is limited by the gap space between the two cones in the conical device. The gap space is determined by the distance at the tip and the cone angle. Therefore the geometry of the shearing device plays an important role in the mechanism for migration. Decreased gap space creates faster migration, nevertheless this is probably undesired for industrial scale application separation as a high through-put is required. This would ask for a balance between these two considerations.

Methodology improvement

The results described in this thesis show that shearing wheat flour dough can lead to a broad range of different structures. Initially, the amount of separation was determined by measuring the protein content, while SE-HPLC and SDS PAGE were used to find which protein fractions are important in the process. Kneading and baking tests were performed to obtain more knowledge about the gluten quality. Visualisation of the situation after shearing was recorded by digital photographs.

Improvements could be made especially in the visualisation of the process. However, suitable visualisation methods for dense biopolymer structures are not common. It is of major importance that a visualisation technique dedicated for dense biopolymer mixtures will be developed that can handle a wide range of structures (not only in this project but in general). In this project, we have done several attempts to visualise the structure formation in the dough matrix. CLSM is less suitable for the visualisation of the separation process in the shearing device, because the separation process can lead to structures, e.g. gluten patches that are large (e.g. > 1 mm). NMR-imaging would be a useful tool to apply, as it can handle larger samples. The best option would be if the structure formation could be followed in-line, but this may well be complex, as the shearing device is made from stainless steel. An improved visualisation method would offer great opportunities to develop the understanding of the separation mechanism.

Controlling the filling of the shearing device with dough is important, as the stresses created in the system play an important role during processing. In this research, we did not explore this issue specifically, however, incomplete filling will influence the development of stresses in the system. A slight deviation already has a major impact on the separation process.

A model system that is simpler than wheat dough could be very useful to get more insight in the mechanisms driving the separation. However, it is hard to find a model system with comparable (rheological) properties. Simplification of the gluten-starch systems is difficult. For example, the addition of this wheat starch (which consists only of large A-starch granules) had already a major effect on the separation process. (Van der

Zalm *et al.* 2009). We tried to generalize the application of well-defined shear to other systems, such as glass beads - vital wheat gluten, A-starch - vital wheat gluten, and guar gum - glass beads. No aggregation or separation was found. None of these materials had the correct combination of rheological properties that made them comparable to the components in wheat dough. Those observations confirm that gluten is a unique product with viscoelastic and self-healing properties. We did not manage to find a material mimicking these properties. Nevertheless, after several attempts we think that the formation of aggregates lays at the heart of the process, and this may be induced in other systems as well, albeit with other means. With proteins, temperature and presence of ions, or pH may be used; with synthetic polymers, anti-solvents may have to be used, or any other way of obtaining phase separation. However, even then, the aggregated phase will need to have sufficient cohesive strength to withstand the stresses that are created in the shearing devices. Under continued shearing, an aggregating system will have the tendency to grow, so that larger aggregates can be formed as long as they can withstand the shear forces. The second and third step are thought to be more generic.

Conceptual design and comparison with conventional processing

Although the mechanism for shear-induced separation cannot be translated to industrial processing yet, it is useful to make some comparisons to the existing system, especially with respect to the requirements of water and energy of both processes, and the differences in product composition. Figures 8.1 and 8.2 give an overview of the material and energy flows involved with the old and the proposed new process for wheat flour separation. To simplify the system, a number of assumptions were made. First, the gluten fraction is assumed to consist only of starch and gluten. Besides, the flour added to the system does not contain moisture. The moisture normally present in flour is added through external feeding in the Sankey diagram. The traditional process combines 1 kg flour with 2.6 kg water. In the diagram, this water addition is represented as 3.0 kg water per kg dry flour (assuming 14% moisture in regular flour). For the shear-induced process, 0.72 kg water is added to 1 kg dry matter flour, in which it is taken into account the moisture present in the flour and the water added to make a dough. Furthermore, it is assumed that a continuous process based on shear-induced migration can create products with the same compositions as the extreme layers in our batch process. The moisture contents in the fractions obtained by the shear-induced process is assumed to be the same.

The conventional process requires a substantial amount of water to be added. Even though estimates vary, modern factories require a fresh water stream of about 3 kg
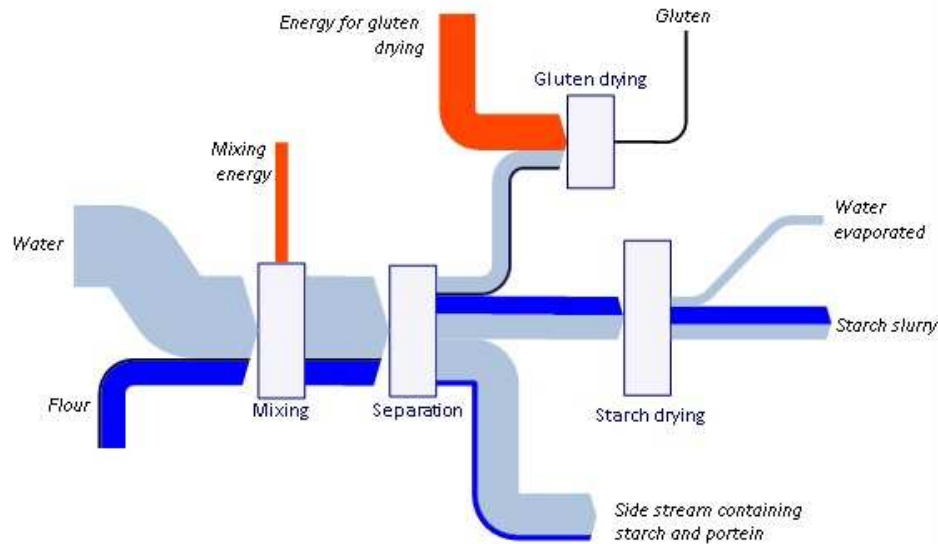
Figure 8.1: Production scheme of the current separation process. The thickness of the arrows represent the size of the stream. Dark blue is starch, black is gluten, light blue is water, and red is energy. Internal recycle streams are not shown.

water per kg flour (assumed to contain 11 w% protein and 89 w% starch in the further calculations). The first sep is a mixing step, in which a dough or batter is produced. More water is added, such that starch can be washed out of the gluten in the next step. About 20% of the water joins the gluten stream, 30% joins the starch stream, and 50% end up in the rest fraction (Cargill November 2010). Approximately 80% of the protein is recovered in the gluten fraction, while 20% of all valuable material is lost in the rest stream (Van der Borght *et al.* 2005). The starch fraction still contains 0.35% protein (on dry weight basis) before it is further processed, by example hydrolysed (Cargill November 2010). Approximately 75% of the starch is recovered in the starch stream; the rest ends up in the rest stream or in the gluten fraction. For matter of comparison, we assumed that the gluten fraction is dried, and the starch fraction will only be concentrated to a comparable moisture content as in our new separation process. This can probably be done with mechanical means without further heating, which requires much lower energy inputs. In addition, a slightly higher moisture content in the starch phase might not be a problem in practice, because the starch fraction might be hydrolysed enzymatically into glucose syrup directly after separation. However, Van der Veen *et al.* (2006) showed that it is possible to hydrolyse starch at much higher concentrations as well, which might lead to additional advantages for the new

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separation process, as the glucose obtained after hydrolysis has to be dried as well. In Figure 8.1, it was assumed that only the gluten fraction will be dried. The other streams will be processed differently and are therefore not taken into account for the energy
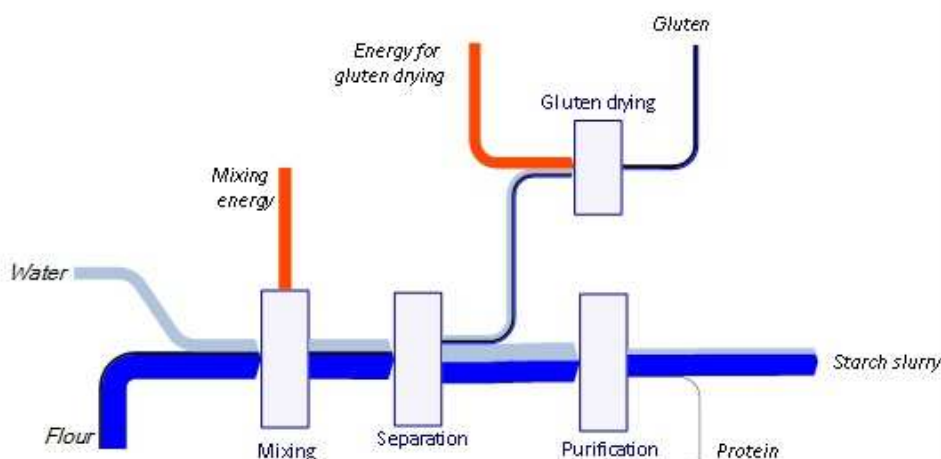

Figure 8.2: Production scheme of the new separation process. The thickness of the arrows represent the sizes of the streams; for comparison the same scale was used as in figure 1b. Dark blue is starch, black is gluten, light blue is water, and red is energy.

consumed by drying.

Figure 8.2 summarizes the situation of the new process, in which we assumed a continuous process that yields similar compositions as the outer layers of the shearing device studied in this thesis. This means that the gluten-enriched fraction has 60 w% protein (on dry basis) and that the starch-rich fraction has about 2.5 w% protein (on dry basis). The total amount of water added is assumed to be 0.72 kg/kg flour for the shear-induced separation process. The recovery of the gluten in the gluten fraction, which means the mass of gluten in the gluten fraction divided by the total amount of gluten, can be calculated using mass balances and turned out to be around 80%. The recovery of the starch in the starch phase is much higher, around 93%.

It seems that comparable amounts of both protein and starch can be recovered with the new process, when assuming current purities. Higher purities of both streams will lead to higher recoveries. The gluten fraction still contains about 40 % starch (dry basis), which is much higher than the non-protein fraction in vital wheat gluten (25 % on dry basis). Chapter 5 showed however that the fraction from the new process probably has excellent functional properties, and therefore it may not be necessary to remove the

starch from the gluten to obtain a similar effect as obtained with the currently available commercial vital wheat gluten. Another issue is that the current process produces a large side stream, which has relatively low value. This stream contains both protein, starch and other polysaccharides. When used for cattle feed, the water needs to be removed, which requires a large amount of energy. The starch in this stream consists partly of type B-starch, which in the new process ends up in the starch fraction. It is not clear whether this would pose a problem, or whether this would actually give the potential of having more complete use of the raw material. Further research on the effect of recovery B-starch is clearly needed.

The starch stream produced by the traditional process is less concentrated than that produced by the shear-induced separation process: 43 w% instead of 58 w%. It should be remarked that the last value is the same in the starting material, as well the gluten and starch stream. This stream would have to be diluted for conventional enzymatic starch hydrolysis process, however, Van der Veen *et al.* (2006) showed that it is in fact possible to intensify this process as well, such that in the end hardly any water needs to be removed. The new process will lead to a starch stream that still contains about 2.5% of protein and probably other wheat components such as non-starch polysaccharides. It is not clear yet, whether these components need to be removed before further processing to avoid for example Maillardation and other side reactions. For matter of completeness, this process step is shown, but we have not included any additional streams that might be needed to run this process (e.g., regeneration liquors).

The two figures show clearly that the new process produce less side streams and could therefore save a lot of water and energy. The energy used for the drying of the gluten fractions obtained varies between both processes. The total amount of water in both systems is 3 kg per kg flour for the traditional process, and 720 g per kg for the shear-induced separation process. The water amount added to the system is reduced enormously. We assume that only the gluten fraction is dried. Gluten obtained by the shear-induced separation process has 720 g water per kg gluten, while the traditional process produces gluten containing 6.1 kg water per kg gluten. Drying of the gluten fraction obtained from the new process requires therefore only 12% of the energy needed to dry the gluten from the traditional process. Drying consumes 4.8 MJ/kg water for the drying process. The value includes the energy for evaporation of water by heat (2.4 MJ/kg) and an energy efficiency of 50%. This has been considered to be a fair estimate for many drying processes (Schutyser *et al.* 2011). The differences in water usage between both processing techniques have a substantial impact on the energy consumption.

In conclusion, the new process seems to have major potential for reducing water and

energy input, but will require adaptation of the further starch processing. The typical recovery of the raw material into the primary product fractions is large as it can be expected that further understanding of the process will lead to higher purities.

Towards scale-up

The major challenge of further developing the separation principle will be the translation of the concept towards continuous processing, with processing times that are as short as possible. For this, an important conclusion from this thesis was that the three steps in the separation are all influenced differently by the processing conditions. It may therefore be wise for optimization on to have each step separately. For aggregation, low shear rates are essential, while shear stress is important in the third step (migration). In addition, the confinement is important in the initiation of the second step (elongation of the aggregates), and thus in the start of the final migration. Separate systems for each step may therefore lead to a more effective process than having all three phenomena taking place simultaneously (in 1 device). The estimate of the water and energy streams for the conventional and the new process shows that effort in developing a shear-based separation process will bear fruit: the potential energy savings are very large, as well as the difference in waste water treatment and reduction in lower-value side streams.

However, there are still some important issues to pay attention to. One is that the starch stream still contains some gluten. Even though the total gluten yield in the process is higher, the separation might induce an extra purification step, leading to extra processing costs. Secondly, the starch fraction also contains the starch that would normally be collected in the lower-value side stream. This may require an adaptation in the starch processing as well. Thirdly, it would not make sense to use the conventional starch hydrolysis process, as this would need a dilution of the starch stream. In addition, Van der Veen et al. (2006) and Baks et al. (2008) have already showed that it is possibly to intensify this enzymatic hydrolysis step.

Shearing as new unit operation in food processing

This project shows that well defined shear is not only useful to structure food products (Manski *et al.* 2007; Peressini *et al.* 2008), but can also be used to separate biopolymeric material. While commercial processes such as extrusion and calendaring have been very successful, they apply conditions that are inhomogeneous. Simplifying the flow into, for example, pure shear flow has now been found to lead to a wide range of phenomena. Although these phenomena do take place within certain areas of conventional

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equipment as well, their effects are not visible anymore due to the complex flow and other changes that take place. It is therefore very useful to simplify industrial equipment such that deformation pattern become more well-defined. Figure 8.3 summarizes that kneading is a process meant to have a chaotic pattern of shear and extensional flow, and thus can only give mixing at the end. Extrusion has a slightly better defined profile (depending on the configuration) and thus may also give macroscale structuring next to mixing. However, microscale structures are expected to be lost. The shearing device is designed to have only well-defined shear flow, and it is show in this and other theses that this can result in micro- and macroscale structuring or even separation. In this light, achievement of separation is very similar to structuring.

As is the conclusion in this chapter, the transition from batch-wise (shear cell) to continuous processing is far from trivial. Any type of already available equipment usually induces extensional flow as well. An inlet and outlet of the materials should be created, while the stress created in the system is not influenced too much. This creates a challenging task for further development towards a continuous operation of shearing devices. It is therefore important that attention be paid to this transition; not only for separation purposes, but just as well for structuring purposes of concentrated biopolymer materials.
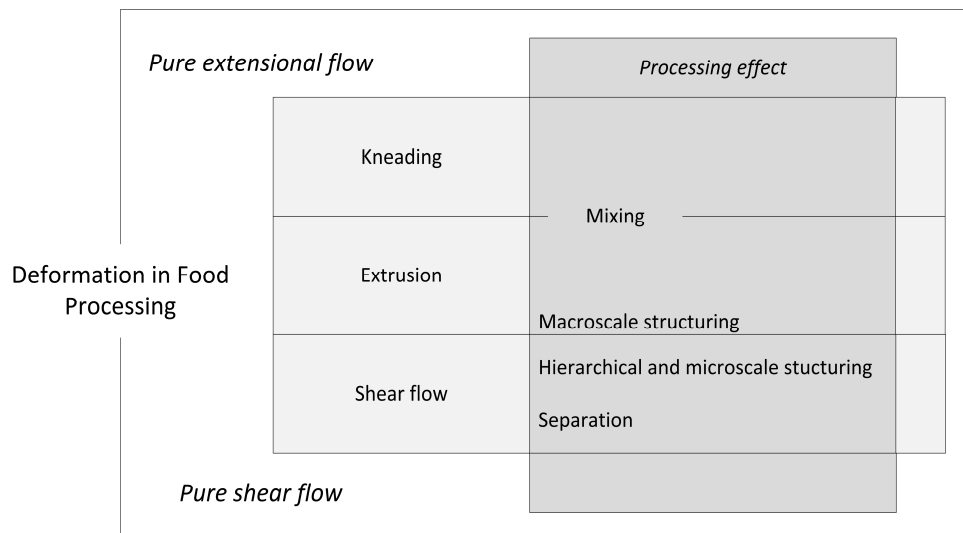

Figure 8.3: Food Processing and flow types.

General conclusion

This thesis has resulted in a better insight in the mechanisms behind the shear-induced separation of gluten and starch from wheat flour. Even though some significant steps still need to be taken (for example, scale-up, downstream purification and intensification of further processing), the results are promising.

The new, shear-based separation process may lead to a better use of raw material and waste reduction. Rough estimates on energy and water consumption, and on the product streams to be obtained showed that there is large potential for saving on energy and water, but also on improving the valorisation of the raw material into high-value products.

While the process so far was only successful for gluten-starch separation, the insight in the separation mechanism may well lead to adaptation of the process towards the separation of different (bio)polymeric systems in the future.

The results in this thesis show that there are many, industrially relevant opportunities will arise once process equipment is developed that accurately applies one specific flow field, even to complex materials as foods. This can be either for induction of specific structure in the product, or for separation of the biopolymer materials, without needing much energy or water.

References

Baks, T., Kappen, F.H.J., Janssen, A.E.M. and Boom, R.M. (2008). Towards an optimal process for gelatinisation and hydrolysis of highly concentrated starch-water mixtures with alpha-amylase from B. licheniformis. *Journal of Cereal Science* 47(2): 214-225.

Cargill (November 2010). Bergen op Zoom.

Manski, J.M., Van der Goot, A.J. and Boom, R.M. (2007). Formation of Fibrous Materials from Dense Calcium Caseinate Dispersions. *Biomacromolecules* 8(4): 1271-1279.

Peighambardoust, S.H., Hamer, R.J., Boom, R.M. and Van der Goot, A.J. (2008). Migration of gluten under shear flow as a novel mechanism for separating wheat flour into gluten and starch. *Journal of Cereal Science* 48(2): 327-338.

Peressini, D., Peighambardoust, S.H., Hamer, R.J., Sensidoni, A. and Van der Goot, A.J. (2008). Effect of shear rate on microstructure and rheological properties of sheared wheat doughs. *Journal of Cereal Science* 48(2): 426-438.

Schutyser, M.A.I. and Van der Goot, A.J. (2011). The potential of dry fractionation processes for sustainable plant protein production. *Accepted for publication in Trends in Food Science and Technology*.

Van der Borght, A., Goesaert, H., Veraverbeke, W.S. and Delcour, J.A. (2005). Fractionation of wheat and wheat flour into starch and gluten: overview of the main processes and the factors involved. *Journal of Cereal Science* 41(3): 221-237.

Van der Veen, M.E., Veelaert, S., Van der Goot, A.J. and Boom, R.M. (2006). Starch hydrolysis under low water conditions: A conceptual process design. *Journal of Food Engineering* 75(2): 178-186.

Van der Zalm, E.E.J., Sawalha, A., Van der Goot, A.J. and Boom, R.M. (2009). Separation of wheat flour into starch and gluten - Mechanisms and differences between flour cultivars. Proceedings International Symposium on Food Rheology and Structure, Zürich, 344-347

Summary

Summary

Starch and gluten are very important food ingredients, and hence separation of wheat flour into starch and gluten is an important industrial process. The current industrial separation process consists of two main steps. The wheat flour is mixed to a dough to obtain a gluten network, that is washed with large amounts of water to remove the starch. All water added has to be removed afterwards, which makes this separation process inefficient in the use of water and energy. This thesis describes an alternative separation process for wheat flour, based on well-defined shear flow, which was suggested by Peighambardoust *et al.* (2008). This so-called shear-induced separation process has the potential to be significantly lower in energy and water consumption, combined with increased gluten recovery. The shear-induced separation process is carried out in a conical shearing device, somewhat similar in configuration to a traditional rheometer. This thesis describes the new insights of principles driving this separation process. The impact of process parameters, dough composition and device geometry on the process as well as the gluten characteristics were investigated. By doing so, the next step towards the industrialisation of the shear process was made.

Process investigation

Chapter 2 describes the influence of the process conditions on the separation of Soissons wheat flour in the conical shearing device. Process parameters such as rotation rate, processing time, temperature and water content were varied. By exposing the dough matrix to a curvilinear shear deformation, gluten-enriched and a starch-rich (i.e. gluten-depleted) fraction were formed. The study confirms the hypothesis formulated by Peighambardoust *et al.* (2008), who proposed a two step mechanism for the gluten migration: (I) aggregation of gluten protein into gluten domains and (II) migration of the aggregates to the apex of the cone. In addition, it was found here that the two steps, aggregation and migration, are influenced in different ways by the studied process parameters. The first step, gluten aggregation, was favoured by higher temperatures, lower rotation rates and higher water content. The second step, the migration of the gluten aggregates, was promoted/supported with a higher dough viscosity and higher rotation rates.

In all previous research, sodium chloride (NaCl) was added to the dough, as is known to be important for the properties of the dough. However, addition of salt is undesirable in a large-scale production process, as it results in waste generation, and should therefore be minimized. **Chapter 3** describes a study into the influence of NaCl on the shear-

induced starch - gluten separation from Soissons wheat dough. The wheat dough was processed in the conical shearing device, with a variation of 0 - 7 wt% NaCl in the dough (on flour basis). The separation, defined as the changes in protein concentration in the various layers, compared to the starting material, was promoted up to a concentration of 4 w% NaCl addition. Wheat dough without any NaCl added gave limited separation, but this could be partly compensated by adapting the process conditions. Although, it is clear the dough without NaCl is less process tolerant. The rheological characterisation of the initial dough with different NaCl concentrations did not show clear differences in the storage modulus (G') and phase angle (tan δ) value. By applying a constant shear rate during rheological characterization, the accompanying shear stress and normal forces could be determined. These measurements were rather complex, applying these shear rates to undiluted dough disrupted the material within minutes. The values for shear stress and normal force varied for the dough materials containing various NaCl concentrations. The effect of NaCl on the other rheological parameters was rather limited. It was therefore concluded that the large differences in separation behaviour are more related to the influence of NaCl on the first step, i.e. the gluten aggregation, than on the differences in the rheological properties, which are probably more important in the second step.

Gluten composition and quality

During the shearing process, a gluten-enriched fraction is formed at the centre of the cone while a gluten-depleted (so a starch rich) fraction is formed at the outer rim of the shearing device. **Chapter 4** describes the composition of the gluten protein in these fractions. The main research question here was whether the separation is accompanied by a fractionation of the various proteineous components in the gluten protein fraction. Gluten is composed of glutenins, gliadins, globulins and albumins, which vary in molecular weight and size. These different fractions can be detected by SDS-PAGE and SE-HPLC. The compositions of the gluten-enriched and depleted fractions were compared with the compositions of the original flour and the kneaded dough.

All protein fractions migrate to the centre of the cone, and therefore the composition of the gluten-enriched fraction remains rather similar to that of the original flour. However, the larger glutenin polymeric fraction migrated slightly faster, so that the concentration of large polymers was increased by a factor 2.4 compared to that of the starting Soissons flour. The concentration of monomers in the gluten-enriched fraction was decreased to 70% of the original concentration in the original wheat flour. It is expected that the aggregates require a certain amount of polymeric protein to become sufficiently large, so migration can start.

Next to the molecular characterization described in Chapter 4, the functional properties of the gluten-enriched materials were investigated in **Chapter 5**. Here, focus was on the kneading and baking quality of flours enriched with gluten obtained by shear-induced separation, which were compared with the quality of commercial vital wheat gluten. Two tests were performed, namely kneading and baking tests. First of all, relatively strong wheat flour, Soissons, was enriched with gluten protein, and tested for kneading performance. In the second test, a weak flour, Kolibri, was enriched with shear-induced gluten and used for baking bread. The result was compared to breads baked with Kolibri flour enriched with commercial vital wheat gluten. It could be concluded that the wheat flour enriched with gluten protein obtained via the shear-induced separation process (SCG) performed at least comparable to commercial vital wheat gluten (CVWG). In terms of bread volume, it performed even slightly better. It was expected that these differences could be related to the protein composition, so therefore the composition of both gluten sources was compared by SE-HPLC. But these SE-HPLC measurements showed no clear differences in gluten composition between the two sources (SCG vs. CVWG). So, the differences in functionality can not be directly related to its composition. The differences in functionality may be related to the different drying methods applied (drying by heat for commercial gluten vs. freeze drying for SCG) used, or to the inherent mildness of the shear-induced separation technique, compared to the commercial process.

Shearing device geometry

Separation of wheat flour into starch and gluten is possible by shearing in a conical device. In Chapter 2-5 the geometry of the shearing device could not be changed. Chapters 6 and 7 deal with a new and improved version of the shearing device, which allow investigation of the influence of the geometry: the gap distance between the two cones and the cone angle were varied. A different geometry led to clear changes in the shear rate profile.

In **Chapter 6**, the influence of geometry on the aggregate formation and the following migration of the aggregates to the centre of the cone were investigated. It was found that the first step, aggregation, is mainly influenced by the local shear rate while the second step, migration, becomes effective when the aggregates have obtained a size that is on the order of the local gap space between the two cones. The migration starts when the aggregates are constrained by the walls of the cones. When constraining a gluten aggregate, it leads to elongation of that aggregate into a roll-like shape. The alignment is perpendicular to the shear direction, i.e. alignment of the aggregate towards the centre of the cone that then leads to migration.

In **Chapter 7**, the effect of the configuration was investigated further. By changing the

cone angle, while keeping the gap distance the same, the shear rate profile in the system could be changed. The same rotation rate and processing time led to more material migrated towards the centre for smaller gap distances. However, changing the rotational rate such that the shear rates became comparable resulted in a similar amount of separation. This shows that the total deformation, calculated as the product of shear rate and processing time, is a major determinant on the aggregate formation and migration. Nevertheless, the confinement of the aggregates due to the gap space was proven to be important as well. The results obtained in Chapters 6 and 7 required an extension of the separation mechanism into a three-step mechanism: (I) aggregation of gluten to aggregates and larger patches, (II) deformation of the large patches as their size becomes equal to the gap space and (III) migration of these structures towards the centre. In this chapter, these effects were mapped in a schematic process diagram.

<u>Impact shear-induced separation</u>

The overall potential of the process was investigated in **Chapter 8**. It was assumed that this batch process could be translated to a continuous process with outputs having similar compositions found in layers 1 and 5. That means that a protein fraction of 60% and a starch rich fraction containing 2.5% protein could be obtained. It was found that the shear-induced separation process needed 76% less water, and consequently requires a 88% less energy; mostly due to less energy required for dehydration.
As described in the thesis, it was found that the starch fraction contains a residual amount of gluten. This protein needs to be removed before continuing processing; however, the amount is relatively small and we therefore do not expect this to be a major problem. Often, the starch fraction is further processed, e.g. starch hydrolysis. The hydrolysis can also be carried out in more concentrated mode than currently done in industry. Previous research has showed already that this is possible.

The current thesis contributes with better insight into the principles of the separation process. The understanding of the separation mechanism is extended. The next hurdle to be taken is the conversion of the process into a continuous one. It is concluded that the process has ample potential for the future.

Samenvatting

Samenvatting

Zetmeel en gluten zijn belangrijke voedingsingrediënten die uit tarwe gewonnen kunnen worden. Het scheidingsproces voor gluten en zetmeel uit tarwebloem is daarom een belangrijk industrieel proces. Het huidige industriële scheidingsproces bestaat uit twee stappen. Eerst wordt de tarwe bloem met water gekneed tot een deeg waarin gluten een netwerk vormt. Vervolgens wordt dit deeg gewassen met een grote hoeveelheid water om het zetmeel te winnen. Aangezien al het toegevoegde water na afloop weer verwijderd moet worden, is het een inefficiënt proces qua water- en energieverbruik. Dit proefschrift beschrijft het onderzoek naar een alternatieve scheidingsmethode voor tarwebloem. De eerste ontwikkelingen van het proces zijn beschreven door Peighambardoust et al. (2008). Bij dit proces wordt gebruik gemaakt van gedefinieerde stroming. Dit zogenoemde shear-induced separation process biedt de mogelijkheid om significant minder water en energie te gebruiken, terwijl daarbij een hogere eiwitopbrengst wordt verkregen. Het scheidingsproces is uitgevoerd in een conisch afschuifapparaat (shearing device), waarvan de configuratie lijkt op die van een traditionele reometer. Dit proefschrift beschrijft de nieuwe inzichten over de principes van het scheidingsmechanisme voor tarwebloem door gebruik te maken van gedefineerde stroming. De invloeden van zowel de procesparameters, de deegsamenstelling en de geometrie van het afschuifapparaat op de functionele eigenschappen van de verkregen glutenfractie zijn onderzocht. Door dit onderzoek is de volgende stap naar industrialisatie van het afschuivingsproces voor tarwebloem gezet.

Het proces

Hoofdstuk 2 beschrijft de invloed van de procescondities op het scheiden van Soissons tarwebloem in het afschuifapparaat. Procesparameters zoals de rotatiesnelheid, verblijftijd, temperatuur en waterhoeveelheid zijn gevarieerd. Door de deegmatrix bloot te stellen aan een gekromd afschuifveld wordt een glutenverrijkte en een zetmeelrijke (dat wil zeggen een glutenverarmde) fractie gevormd. De studie bevestigt het tweestapsmechanisme geopperd door Peighambardoust et al. (2008): (I) aggregatie van gluteneiwit in glutendomeinen en (II) migratie van de aggregaten naar de punt van de kegel. Door dit onderzoek kan de hypothese worden uitgebreid met de vinding dat de twee stappen, aggregatie en migratie, verschillend beïnvloed worden door de bestudeerde procesparameters. De eerste stap, aggregatie van gluten, wordt bevorderd door een hogere temperatuur, een lagere rotatiesnelheid en een verhoogde waterhoeveelheid. De tweede stap, de migratie van gluten aggregaten, wordt bevorderd door een verhoogde deegviscositeit (dus minder water) en een hogere rotatiesnelheid.

In al het voorgaande onderzoek is natriumchloride (NaCl) toegevoegd aan het deeg, om-
dat bekend is dat NaCl een positieve invloed heeft op de deegeigenschappen. Echter,
NaCl is ongewenst bij industriële processen aangezien het reststromen lastiger te ver-
werken maakt. Hoofdstuk 3 beschrijft een studie naar de invloed van NaCl op het schei-
dingsproces van Soissons tarwebloem in zetmeel en gluten door de NaCl concentratie in
het deeg te variëren van 0 tot 7 w% NaCl (op bloem basis). De scheiding, gedefinieerd
als de verandering in eiwitgehalte in de verschillende lagen ten opzichte van het eiwitge-
halte in het startmateriaal, verbeterde tot 4 w% NaCl in het deeg. Deeg zonder NaCl gaf
een beperkte mate van scheiding, hoewel enige scheiding bereikt kon worden door aan-
passing van de procescondities. Het is echter duidelijk dat deeg zonder NaCl minder pro-
cestolerant is. Reologische metingen aan de initiële degen met verschillende NaCl-
concentraties laten zien dat de storage modulus (G') en fasehoek (tan δ) enigszins veran-
derde door een ander NaCl concentratie. De afschuifspanning en de normaalkracht zijn
bepaald bij een constante afschuifsnelheid. Deze metingen zijn complex; het toepassen
van deze afschuifsnelheden aan een deeg beschadigde het materiaal binnen enkele mi-
nuten. De waarden voor de afschuifspanning en normaalkracht variëren voor de degen
met andere NaCl concentraties. Het effect van NaCl op de andere reologische eigen-
schappen is beperkt. Daardoor is geconcludeerd dat de grote verschillen in scheidingsge-
drag meer gerelateerd zijn aan de invloed van NaCl op de eerste stap, dat wil zeggen de
aggregatie, dan op de reologische eigenschappen van het materiaal, welke mogelijk
meer van belang zijn voor de tweede stap.

Glutensamenstelling en -kwaliteit

Tijdens het scheidingsproces wordt een glutenverrijkte fractie gevormd aan de punt van
de kegel, en een glutenverarmde (dus een zetmeelrijke) fractie aan de buitenste randen
van het afschuifapparaat. Hoofdstuk 4 beschrijft de samenstelling van het gluteneiwit in
deze fracties. De belangrijkste onderzoeksvraag is of de scheiding ook leidt tot fractione-
ring van de verschillende eiwitcomponenten in de gluteneiwitfractie. Gluteneiwit is sa-
mengesteld uit glutenine, gliadine, globulines en albumine. Deze eiwitten variëren in
moleculaire massa en structuur. Deze verschillende fracties kunnen worden gedetec-
teerd met SDS PAGE en SE-HPLC. De samenstelling van de glutenverrijkte en de gluten-
verarmde fractie zijn vergeleken met de samenstelling van het startmateriaal (Soissons
tarwebloem) en gekneed deeg.
Alle eiwitfracties migreerden naar de punt van de kegel, en daardoor bleef de samenstel-
ling van de glutenverrijkte fractie vergelijkbaar met die van het startmateriaal. De grote-
re gluteninefractie migreerde iets sneller, zodat de concentratie van grote polymeren
met een factor 2.4 was verhoogd vergeleken met het startmateriaal (Soissons bloem). De
concentratie van monomeren in de glutenverrijkte fractie was verlaagd tot 70% van de

oorspronkelijke concentratie in het tarwebloem. Het lijkt daarom zo dat de aggregaten een bepaalde hoeveelheid polymerische materiaal moeten bevatten, zodat de aggregaten zo sterk zijn dat migratie kan plaatsvinden.

Hoofdstuk 5 beschrijft de functionele eigenschappen van de glutenverrijkte fractie. Daarbij is gefocust op het kneed- en bakgedrag van verschillende soorten bloem verrijkt met gluten verkregen met het scheidingsproces gebaseerd op afschuiving, welke vergeleken zijn met de kwaliteit van commercieel verkrijgbaar vitaal gluteneiwit. De kneedtesten zijn uitgevoerd met een relatief sterke tarwebloem, Soissons. De bloem is verrijkt met de verschillende glutenfracties en getest op het kneedgedrag. In de tweede test is Kolibri, een zwakke bloem, verrijkt met de verschillende soorten gluten. Het effect van verrijking van de tarwebloem met gluten verkregen door het afschuivingsproces, is minstens zo groot als met vitale tarwegluten. Het volume van de broodjes was zelfs wat groter. De verwachting is dat deze verschillen gerelateerd kunnen worden aan de eiwitsamenstelling. Echter, SE-HPLC metingen laten echter geen duidelijke verschillen zien in glutensamenstelling tussen beide soorten. De verschillen in functionaliteit zouden daarom ook gerelateerd kunnen worden aan de verschillende droogmethoden (drogen met behulp van warmte bij commerciële gluten in tegenstelling tot vriesdrogen bij gluteneiwit verkregen met het afschuivingsproces), of aan de inherente mildheid van het scheidingsproces gebaseerd op afschuiven vergeleken met het commerciële proces waarin gekneed wordt.

Effect van geometrie

Scheiding van tarwebloem in zetmeel en gluten is mogelijk in een conisch afschuifapparaat. In Hoofdstuk 2-5 is de geometrie van het afschuifapparaat niet veranderd. Hoofdstuk 6 en 7 beschrijven de invloed van een nieuwe en verbeterde versie van het afschuifapparaat, waardoor het mogelijk is om de invloed van de geometrie van het afschuifapparaat te onderzoeken. In de vernieuwde versie van het afschuifapparaat kunnen de afstand en de hoek tussen de twee kegels worden aangepast.

In Hoofdstuk 6 wordt de invloed van de geometrie op de aggregaatvorming en de daaropvolgende migratie van de aggregaten naar de punt van de kegel onderzocht. De experimenten bevestigen dat de eerste stap, aggregatie, voornamelijk beïnvloed wordt door de lokale afschuifsnelheid, terwijl de tweede stap, migratie pas begint wanneer de aggregaten net zo groot zijn geworden als de ruimte tussen de twee kegels. Dan worden de aggregaten vervormd, wat leidt tot migratie. De vervorming van een glutenaggregaat leidt tot uitrekking van de aggregaten in een rolletje. De uitlijning van een aggregaat is gericht naar de punt van de kegel, wat vervolgens leidt tot migratie in dezelfde richting.

In Hoofdstuk 7 is het effect van de geometrie verder onderzocht. De verandering van de hoek tussen de kegels leidt tot een andere afschuifsnelheid in het systeem, zelfs bij een

gelijke afstand tussen de punten van de kegels. Als gevolg van een andere hoek, zal echter de afstand tussen de kegels op andere posities veranderen. Eenzelfde rotatiesnelheid en verblijftijd leiden dan tot meer gemigreerd materiaal bij kleinere afstanden tussen kegels. Door het veranderen van de rotatiesnelheid kan de afschuifsnelheid gelijk blijven, wat resulteert in een vergelijkbare hoeveelheid scheiding. Dit laat zien dat de totale vervorming, berekend als het product van afschuifsnelheid en verblijftijd, een belangrijke factor in aggregaatvorming en migratie is. Desalniettemin speelt de vervorming van de aggregaten door de afstand tussen de kegels ook een belangrijke rol. De resultaten beschreven in hoofdstuk 6 en 7 hebben geleid tot een uitbreiding van het scheidingsmechanisme naar een driestaps mechanisme: (I) aggregatie van gluten naar aggregaten en grotere patches, (II) vervorming van deze grotere patches als het formaat gelijk is aan de afstand tussen de twee kegels en (III) migratie van deze structuren naar de punt van de kegel. In Hoofdstuk 7 zijn deze effecten weergegeven in een schematische proces diagram.

<u>Mogelijkheden en perspectief nieuw scheidingsproces</u>

In Hoofdstuk 8 is het scheidingsproces vanuit een breder wetenschappelijk en technologisch perspectief bekeken. Om de potentiële besparingen van het proces te berekenen is aangenomen dat het batchproces vertaald kan worden naar een continu proces, waarbij de uitgaande productstromen dezelfde samenstellingen hebben als die in de lagen 1 en 5 in het afschuifapparaat. Dit betekent dat er een eiwitfractie van 60% en een zetmeelrijke fractie met 2.5% eiwit verkregen kan worden. In dat geval verbruikt het afschuivingsproces 76% minder water, en heeft hierbij 88% minder energie nodig. Dit laatste wordt voornamelijk veroorzaakt doordat er minder energie nodig is voor drogen.

Zoals beschreven in dit proefschrift, bevat de zetmeelrijke fractie nog een klein deel van het gluteneiwit. Dit eiwit zal wellicht verwijderd moeten worden voordat het zetmeel verder verwerkt kan worden. Aangezien dit een kleine hoeveelheid is, verwachten we dat dit goed mogelijk is. Vaak wordt de zetmeelfractie verder verwerkt in een zetmeelhydrolyse proces. Deze hydrolyse kan ook uitgevoerd worden in een meer geconcentreerde stroom dan wat op dit moment in de industrie gebeurd. Voorgaand onderzoek heeft bevestigt dat dit mogelijk is. Dit betekent dat de waterbesparing die in het scheidingsproces bereikt kan worden, behouden blijft tijdens het verdere proces.

Dit proefschrift heeft bijgedragen tot een beter inzicht in de grondslagen van het scheidingsproces. De volgende stap die genomen moet worden is het continu maken van het proces. Het scheidingsproces biedt dan mogelijkheden voor een efficiënter proces en betere glutenproducten in de toekomst.