Development of Dough

under Shear Flow

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Abstract

Dough mixing involves a combination of different deformation flows, e.g. shear and elongation. The complicated nature of the mixing makes it difficult to understand dough processing on a mechanistic level. Apart from that, a quantitative relation between the type of deformation in the mixing and resulting dough properties is still lacking. This indicates the importance of unraveling the complex process into most relevant deformation patterns such as simple shear and extensional flow in dough mixing. Literature studies mostly focus on well-known parameters such as process time, temperature, energy (time, rotational speed), etc. in dough processing. The question is not fully answered is that how kneading (shear versus extensional deformation) affects structure formation (gluten development) in the dough.

The overall objective of this thesis is to generate understanding of the relation between relevant process parameters in dough mixing and changes in product properties. This thesis focuses on the effect of well-defined deformation on the structure formation and properties of dough on various levels of detail. To reach this aim, a zero developed (ZD) dough was used as starting material. The ZD dough was compared with a flour–water mixture as starting material in z-blade mixing trials. The ZD dough constituted a good model for dough mixing studies to help study the different aspects of mixing. The physical properties of glutenin macro polymer (GMP), as the most relevant quality criterion for end-use quality of wheat at, was used to detect changes in the properties of the dough. This analysis provided the characterisation of the dough at the length scale of glutenin particles (micro-scale). In line with this analysis, changes in microstructure of the dough (meso-scale) and large-scale deformation rheological characteristics of the dough (macro-scale) were elucidated under a well-defined simple shear flow. Finally, an attempt was made to compare above-mentioned characterisation of the dough at two different process conditions. In this respect, a Couette-type device was introduced to study dough properties upon changing the flow profile from steady into transient shear flow in the device. The benefits of using this methodology in understanding the conventional dough mixing on a mechanistic level have been discussed. Last but not the least, the implications of the findings for bakery industry, the overall conclusions and recommendations for future studies in this regard, are discussed.

To my loving family, Elham and Sana

Contents

General Introduction

General introduction

Wheat

Wheat has been used as a food since early history and it is among the oldest and most extensively grown of all crops. It is widely accepted that wheat was grown as a food crop about 10,000-8000 B.C. [27]. Wheat is the most important and widely cultivated cereal crop in the world. It is grown in very different climates, thereby leading to different appearance and characteristics. Different classes of commercial wheat cultivars are distinguished according to technologically relevant properties such as kernel hardness, bran colour, and protein content. Typical distinctions are hard or soft, strong or weak.

The structure of the wheat kernel is shown in Fig. 1. The kernel consists of the following tissues: bran (13-17%), endosperm (81-84%) and embryo or germ (2-3%). The bran coat consists of several distinct layers as is evident from the graphic details in Fig. 1. The endosperm cells are packed with starch granules embedded in a protein matrix. The protein is mostly, but not entirely, gluten, the poorly water soluble fraction, which is important for bread-making. In maturing wheat, gluten proteins are synthesized and deposited as protein bodies. As the grain matures, the protein bodies are compressed together into a matrix that appears mud- or claylike, and the protein bodies are no longer discernible [15]. Protein is distributed unevenly throughout the wheat kernel. The embryo and the aleurone layer are the richest tissues of the kernel in terms of protein content, consisting of about 30 and 20% protein, respectively. The endosperm has a lower protein concentration. However, since the endosperm is by far the largest tissue of the kernel, it contains about 70% of the total protein of the kernel.

Wheat endosperm varies both in texture (hardness or softness) and appearance (vitreous or opaque). In general, high-protein hard wheat varieties tend to be vitreous, and low-protein soft varieties tend to be opaque. However, the causes of hardness and vitreousness are different, and these two do not always go together [15]. Hardness and protein composition are heritable traits. The protein content is weakly heritable and strongly dependent on environmental factors. The physical characteristics of the wheat kernel influence its flour milling quality. For instance, flour from hard and vitreous kernels contains more damaged starch granules, which affects the water absorption properties of the flour.

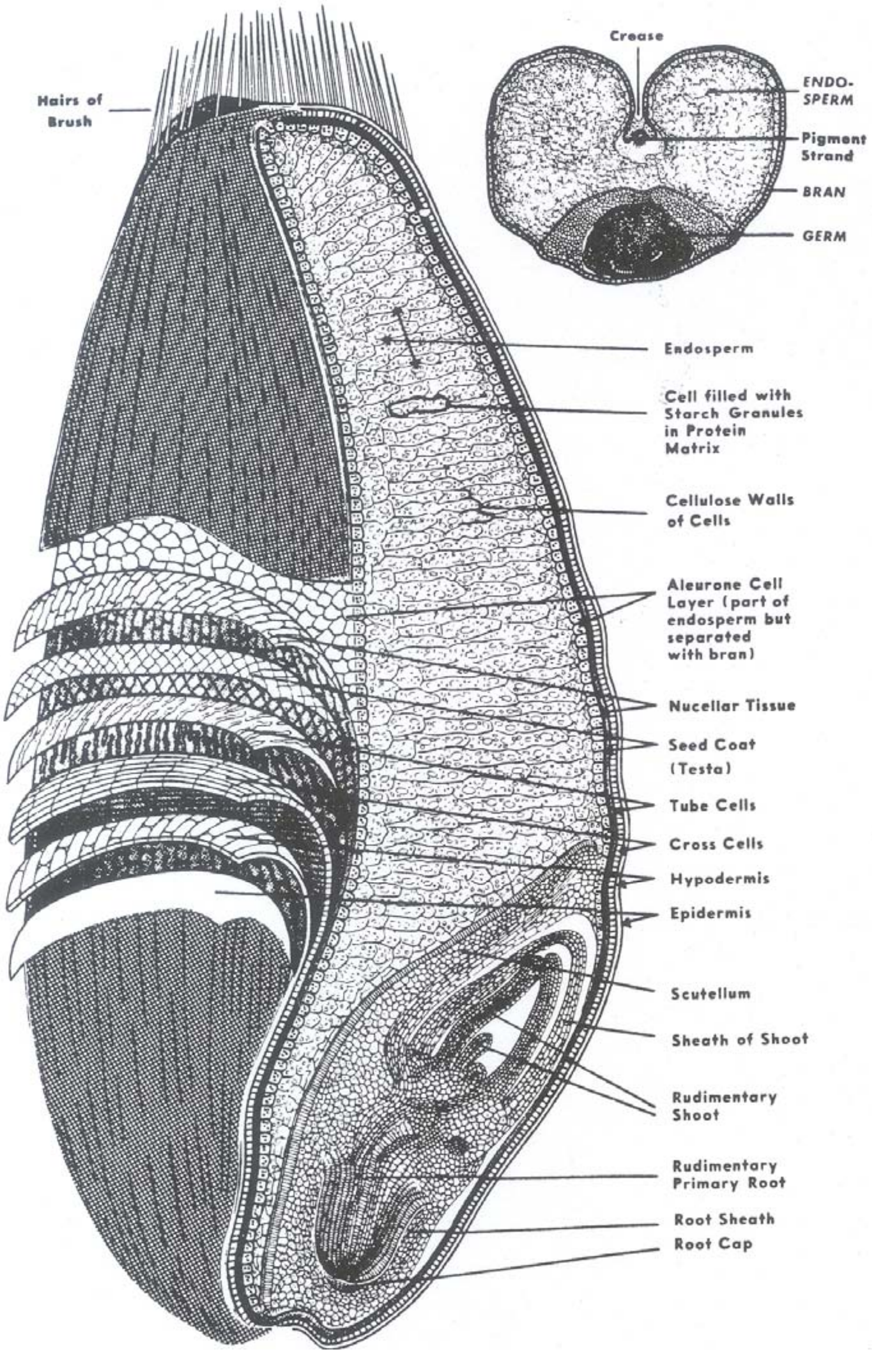

Fig. 1. The structure of wheat kernel (from Hoseney [15]) .

A major portion of wheat is utilized in commercial bakeries for making breads and similar bakery foods. Both artisanal and industrial bakers are continuously challenged to improve the quality of their products. It is their craft and technology to improve and ensure product quality by controlling the complex and sometimes subtle interplay between flour quality and processing.

Chemical composition of wheat flour

Wheat flour mainly consists of the starchy endosperm of the kernel. Fig. 2 illustrates a typical composition of wheat flour comprising carbohydrates (70-80%), proteins (8-18%), lipids (1.5-2.5%) and non-starch polysaccharides (2-3%) all expressed as weight percentage on dry matter basis [36]. The flour protein content and its composition have been shown to be the most important parameters determining the suitability of flour for the bread-making process.

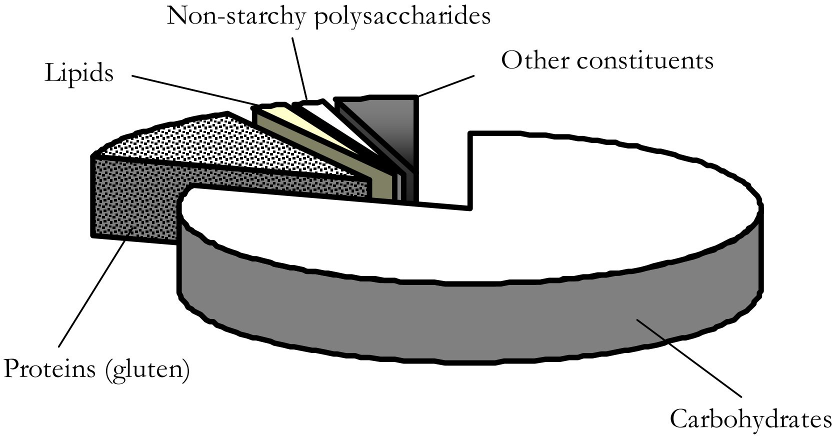

Fig. 2. Schematic illustration of the composition of wheat flour

Starch

Starch is the most abundant component of wheat flour. It consists of two populations of starch granules: large, lenticular (lens-shaped) granules of up to 40 µm across the flattened side, and small, spherical granules with a diameter ranging from 2-8 µm. In actuality, one can find granules of all sizes between these extremes, but these two sizes and shapes are preponderant [15].

Protein

Proteins are recognized as the most important components governing the bread-making quality of wheat [1]. According to Osborne [28], wheat flour proteins are classified into four groups based on differences in solubility: albumins, soluble in water; globulins, soluble in salt solutions; gliadins, soluble in 70% ethanol and finally glutenins, partly soluble in dilute acid or alkali. Albumins and globulins are minor wheat proteins

(20% of the total wheat proteins). The major part of wheat proteins (80% of the total proteins) consists of gliadins and glutenins, which together form the wheat gluten [29].

Other constituents

Lipids and non-starchy polysaccharides (NPS) are some minor components of wheat flour. Wheat germ (embryo) is rich in lipids. Among NPS components, arabinoxylans are more important than others such as inulins. Arabinoxylans positively influence water absorption of the flour. The higher the extraction rate (yield) of the flour in a milling process, the higher the pentosans content in flour.

Gluten and its composition

Undoubtedly, without the presence and unique properties of gluten it would not be possible to prepare the visco-elastic dough from wheat flour, suitable for bread making [29]. Gluten largely consists of two groups of proteins, the sulphur-rich glutenins, able to form a polymeric network by chemical cross-linking (called network proteins), and the sulphur-poor gliadins, largely present as monomers that interact with the glutenin in the network. The gliadin fraction contributes to the viscous properties of wheat flour dough; the glutenin fraction has a prominent role in the elastic properties of dough [22]. The glutenin fraction consists of high molecular weight glutenin subunits (HMWGS) and low molecular weight glutenin subunits (LMWGS).

Relevance of insoluble glutenins (glutenin macro polymer, GMP) to end-use quality

Today, glutenins are regarded as the key structuring proteins in gluten and dough. The HMW subunits of wheat glutenin are generally considered to play a key role in gluten structure and function and, consequently, are closely related to the quality of the wheat [37]. According to Chen and Bushuk [7], part of the glutenin is soluble in acetic acid, thus making the distinction between an insoluble and a soluble fraction. The importance of this distinction became clear when a positive correlation between the amount of acetic acid insoluble glutenin and bread loaf volume was reported [26]. The insoluble glutenin became widely recognised as the key protein fraction that correlated with differences in dough strength and bread-making quality. The use of detergents such as sodium dodecyl sulphate (SDS) and organic solvents like propanol allowed an even better fractionation and led to the conclusion that the insolubility was due to a high degree of polymerization

[12]. This polymerised fraction of glutenin was later renamed into Glutenin Macro Polymer (GMP) to reflect its highly aggregated nature [39]. Numerous studies [1, 2, 9, 12, 25, 30, 31, 33-35, 37-39] presented firm evidence that GMP quantity and composition are highly correlated with functional properties of wheat dough and end-use quality parameters, such as bread loaf volume.

Hamer and van Vliet [13] proposed a model for glutenin hyper-aggregation, which explains the impact of both physical and chemical interactions (covalent versus non-covalent processes) from the gluten(in) structure at various length scales. This model suggests the presence of mesoscopic (ca. 10-100 μm) glutenin *particles* as the main building blocks of the gluten(in) network in dough. The macroscopic scales (100-1000 μm) according to this hyper-aggregation model are solely based on physical (i.e. non-chemical) interactions. These are thought to be predominantly affected by process conditions. This is consistent with, for example, Weegels et al. [39] who reported that GMP from flour is not the same as GMP from dough (processed flour).

The colloidal particle approach for gluten as proposed by Lefebvre et al. [20] and strengthened afterwards [21], implies that the physical interactions at the mesoscopic (0.1-100 μm) length scale affect dough properties. In a recent study, Don et al. [8] confirmed the particulate nature of glutenins, demonstrating that GMP gel isolated from flour contains large glutenin particles (ca. 5-50 μm).

Belton [3] recently stated that the particle hypothesis of glutenins 'is weak'. Belton's criticism centres on his opinion that gluten should be regarded as a system of long, entangled flexible polymers, instead of as a colloidal particle system. According to Hamer et al. [14], dough or gluten can not be considered to be a system of flexible entangled soluble polymers, since strong protein–protein interactions will lead to stiff aggregates of protein. Studies of Don et al. [8-10] proposed glutenin particle properties as key to understand the link between GMP and dough properties. It was concluded that a strong correlation exists between properties of GMP and practical dough mixing requirements (energy).

Dough development upon mixing: the existing complication

In bread-making, the mixing of dough is generally considered a critical step that is important for the overall bread quality [6, 29]. Traditionally, dough is made by combining flour, water and mechanical energy through mixing. According to Bolksma [5] mixing of bread dough has three functions: (i) to blend and distribute the ingredients (flour, water, yeast, salt, and minor constituents) into a macroscopically homogenous mass (when examined on a size scale of around 0.5 mm; non-homogenous if examined at a finer resolution); (ii) to develop dough into a three-dimensional visco-elastic structure in which gluten forms a continuous network, and which has gas retaining properties, and (iii) to include air bubbles in the dough which will form nuclei for gas cells that grow during fermentation and the beginning of baking. To produce bread of optimum quality, dough needs to be formed with a developed protein structure for maximum gas holding capacity. Gluten proteins in wheat flour have the capacity to form such a structure when hydrated.

Generally spoken, the amount of mechanical work (mechanical energy) given to the dough in a certain period of time is critical to the quality of the baked product. Numerous studies have revealed large differences in bread quality when the dough was subjected to different work input levels [18, 32, 40]. It has also been shown that the work required to develop the dough varies with the type of mixer. Almost without exception, researchers use practical mixers such as the farinograph and mixograph to evaluate wheat flour (dough) properties [16]. Mani et al. [24] examined farinograph, mixograph, and Krups and Hobart mixers to test bread-making performance of dough obtained from these different mixers. They concluded that the bread quality was similar at the optimum dough mixing regimes regardless of mixer type. However, the optimum mixing times were different depending on the flour composition, mixer type, and dough formulation. Thus, the correct amount of mixing energy to achieve optimum bread quality depends not only on the characteristics of the flour but also on the type of mixer used in the process.

A great deal of research is being conducted on dough mixing with the aim of correlating process parameters such as mixing speed, mixer type (geometry) and capacity with dough properties. In this research, laboratory-scale farinograph or a mixograph type mixers are used [16]. However, the mixing requirements measured in these classical mixers are not well correlated with industrial-scale work input requirements. Indeed, there is a large difference between industrial-scale and laboratory-scale mixers, both in size (50–300 g vs. 100-200 kg) and geometry (for example, twin flat blades vs. single helix) [40]. Therefore, it seems unlikely that the (specific) work input requirements on the two systems are the same. Therefore, the results of dough investigations using conventional mixers can not be compared unless the mechanical history (for instance, shear rate and stress) of the mixing action is the same. [4]. If plant breeding efforts, which rely on laboratory-scale conventional mixing, are to yield flours with an energy requirement appropriate to industrial-scale bakeries, then we do need to know the relationship between work inputs measured in these two systems.

From the above-mentioned context, the importance of mechanical energy requirements and physical properties of dough mixing systems, e.g. mixer type (geometry) and capacity is clear. However, mechanical energy input alone can not characterise the mixing process; the way in which the energy is transferred to the material is also important. For instance, with the same amount of energy transferred, a dough mixed using only shearing will be totally different from dough developed using only elongational deformation [17]. According to Kilborn and Tipples [19], dough development by sheeting (which is elongational deformation) requires considerably less energy (3.1 kJ/kg) than development by mixing (22 kJ/kg).

Specifying the mechanical history of the mixing process requires the characterisation of the type of deformations involved in this process. During mixing, dough is deformed by a combination of shear and uniaxial elongation at high strain rates [23]. Zheng et al. [41] reported that in industrial mixers, dough is sheared and stretched by the mixer blades and subjected to both shear and elongational flow. According to Jongen et al. [17], a combination of rotational, shear and elongational components exists in a farinograph mixer, while a mixograph mixer mainly delivers the shear component. This is debated by Gras et al. [11] who assert that the mixing action of a pin mixer (such as a mixograph) is predominantly elongation. Such discussions illustrate the difficulties of mixing studies.

The complicated nature of the mixing process makes it a challenge to understand dough processing on a mechanistic level. Apart from that, a quantitative relationship between the type of deformation in the mixing and the resulting dough properties is still lacking. This indicates the importance of unravelling the complex process into the most relevant deformation patterns such as simple shear and extensional flow in dough mixing, and establishing the relations of these patterns with resulting dough properties. This will be the subject of this thesis.

Research objective

The overall objective of this thesis is to generate understanding of the relation between relevant process parameters in dough mixing and changes in dough properties. The thesis focuses on the role of the type of mechanical deformation in dough mixing to gain a mechanistic insight in the structure formation process of dough. Different structural levels that play an important role in evaluation of dough properties are considered. The major focus is the effect of well-defined shear flow on the physical properties of glutenin macro polymer (at a length scale of 10-100 μm) as building blocks for the gluten network, on the microstructure formation (at a length scale of 100-1000 μm), and rheological behaviour (at a length scale of more than 1000 μm) of the dough. To deal with this approach, an undeveloped dough (with zero energy mechanical addition) was used in the experimental part of this study.

The specific research (sub)questions can be formulated to reach the aim of this thesis are:

1. Does a zero-developed dough, as the starting material, behave like a flour–water mixture in mixing trials? If not, what is the difference?

2. Is a cone-and-plate shearing device useful to investigate changes in product properties under a well-defined shear flow?

3. How does a well-defined shear flow change the physical properties of glutenin macro polymer (GMP)?

4. How are the microstructure and rheological properties of the dough influenced by simple shear flow?

5. What are the effects of “steady” versus “transient” shear flow on GMP properties and microstructure formation of the dough?

Outline of the thesis

In order to show the general setup of this thesis and the various connections between chapters, a schematic outline of this thesis is given in Fig. 3. In the experimental methodology reported in this thesis, conventional flour-water mixture as starting material is replaced by undeveloped or so-called "zero-developed" (ZD) dough. It is not clear from the literature whether a zero-developed protocol really constitutes an undeveloped system and whether processing of such dough leads to the same results as conventional processing of flour. **Chapter 1,** therefore, describes the behaviour of zero-developed dough compared to that of a flour-water mixture in z-blade mixing trials. The chapter may lead to a better understanding of the use of ZD dough as the starting material in dough processing or characterisation studies. **Chapter 2** introduces a shearing device for processing of wheat gluten-starch-water mixtures with varying protein concentration. The chapter addresses the value of the introduced methodology to study the effect of simple shearing on dough development and properties under process conditions relevant to industrial processing. In **Chapter 3**, the effects of simple shearing (at steady rates) on physical properties of gluten are described at a more structural level, especially with respect to its most functional part, namely glutenin macro polymer (GMP). This chapter compares GMP properties of sheared dough with conventionally mixed dough at comparable levels of work input. Since it is thought that GMP particles are building blocks for gluten network structures, it is assumed that changes in the properties of this highly aggregated fraction be picked up by changes in dough microstructure and rheological properties. **Chapter 4** of this thesis, therefore, focuses on elucidation of the microstructure formation and large-deformation fracture properties of the dough processed under simple shear flow. A comparison is made with z-blade mixing at relevant work input levels.

Previous chapters (**Chapters 3** and **4**) investigate GMP properties and microstructure formation of the dough under steady shear flow. **Chapter 5** compares the effect of applying transient shear versus steady shear flow using an eccentric Couette type reactor on wheat dough microstructure and physical properties of GMP. The **General Discussion** provides questioning the validity of the approaches followed, the implication of the findings of this thesis in understanding of conventional dough mixing and the relevance of the findings for the wheat processing industry. The discussion finishes with an overview of the results obtained, general conclusions and recommendations for future studies.

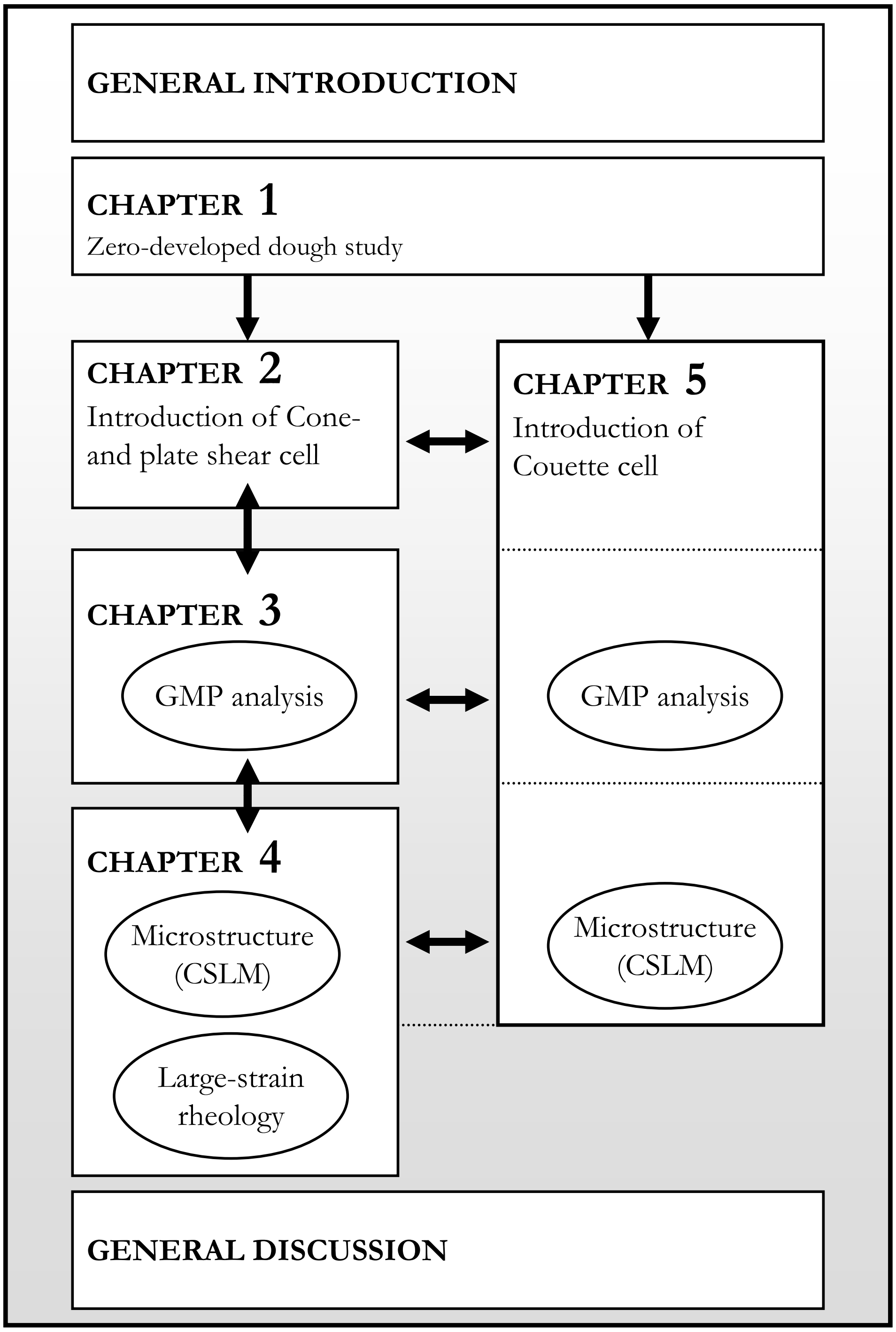

Fig. 3. Schematic illustration of thesis outline per chapter.

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Chapter 1

Mixing behaviour of a zero-developed dough compared to a flour–water mixture[♣]

Abstract

Z-blade mixing behaviour of zero-developed (ZD) doughs from the flours of two wheat cultivars of different gluten strength was compared to that of conventionally mixed dough of the same flours. In farinograph experiments, use of ZD dough led to shorter development time (with less energy requirement), less stability time, and consequently earlier breakdown compared to conventional mixing of the corresponding flour–water mixture. Mixing of ZD doughs led to an almost similar decrease of glutenin macro-polymer (GMP) wet weight as that of doughs prepared from flour–water mixtures. However, comparison of wet weight of re-assembled GMP revealed that until time-to-peak (TTP) mixing, there was no difference in GMP recovery with respect to the starting material used in the z-blade mixing experiments. Beyond TTP, recovery of GMP in doughs prepared from both starting materials was reduced. The results of large-strain deformation rheology showed strong visco-elastic behaviour as characterised by the highest values of fracture properties, followed by a decline in those properties upon further mixing for doughs mixed from both flour–water mixture and ZD dough from both types of wheat cultivars studied. It was concluded that at mixing regimes before TTP, there was no difference with respect to the use of ZD dough instead of a flour–water mixture in the mixer. When ZD dough is used as a starting material for dough preparation instead of flour, extra care should be taken not to over-mix the developing dough.

[♣] Peighambardoust, S.H., Van der Goot, A.J., Boom, R.M. and R.J. Hamer, 2006. **Journal of Cereal Science**, Accepted for publication.

Introduction

Dough development requires mixing of flour and other ingredients in the presence of water. Traditionally, dough is made by combining flour and water and mixing by an input of mechanical energy [4]. This energy input contributes not only to a uniform distribution of all ingredients but also to the hydration of the flour particles leading to the formation of a continuous gluten structure surrounding the starch granules [13]. In conventional dough mixers such as the farinograph or the mixograph, the distribution of dough ingredients and the hydration phase occur concomitantly with the mechanical energy input. To permit a rigorous analysis of the mixing process, effects of the distribution of ingredients, hydration and mechanical energy input should be examined separately. Decoupling of hydration from the mechanical energy input in dough formation can be achieved by using a non-developed dough system. This allows a careful and detailed study of dough processing, where the influences of flow field (e.g. simple shear versus extensional), strain history, and mechanical energy input levels on dough can be separately identified and quantified.

Earlier Olcott and Mecham [16] and Davies et al. [6] attempted to produce dough without mechanical energy input in studies focusing on lipid binding in the dough matrix, however, there was no systematic focus on the non-developed dough. Later a patent by Kageyama and Torikata [12] described the commercial production of bread dough with no energy input from powdered materials (flour, yeast, sugar and fats) and ice particles. Campos et al [3] introduced the production of "undeveloped" wheat dough. They defined undeveloped dough as a homogenous, fully hydrated wheat flour produced with no energy addition and showed that water added in the form of fine ice particles was evenly distributed in the dough. Several comparisons of the rheological behaviour and micro-structural characteristics of non-developed dough with a conventionally developed dough have been made [4, 14, 17]. These studies concluded that the non-developed dough possesses less resistance against deformation (in an oscillatory rheometer) and shows the lowest levels of "structure formation" compared to conventionally developed dough. Recently, Unbehend et al. [20] performed scanning electron microscopy (SEM) and dynamic oscillating measurements as well as large-strain deformation uniaxial extension studies on a hydrated unmixed flour (HUF) systems. Their SEM results revealed that proteins were present in the HUF system as a three-dimensional framework of gluten aggregates extending between the starch granules and partly surrounding them in a sponge-like structure. Moreover, they showed that their HUF system possessed pronounced visco-elastic behaviour as shown by a higher resistance to extension (R_{max}) and a higher elasticity at the beginning of dynamic oscillating measurements. These results appear to contradict earlier studies [4, 14, 17], where less resistance against deformation and the least gluten matrix was reported for undeveloped or non-developed doughs.

Due to these conflicting reports and the lack of systematic studies on the characteristics of the hydrated dough with zero mechanical energy input (zero-developed (ZD) dough), we have undertaken a critical assessment of the nature of this dough. It is not clear from the literature whether preparation of a ZD dough really leads to non-developed system and whether the processing of such dough leads to the same results as conventional mixing of flour–water mixture. Thus the main objective of the study was to elucidate the behaviour of ZD dough as a starting material in a z-blade mixer compared to conventionally prepared dough from the same flour.

Experimental

Wheat flour samples and physicochemical characteristics

Flour from Spring, a strong, hard Canadian wheat cultivar, and Soissons, a French wheat cultivar, were used in this study. Both flours were obtained from single (unblended) wheat cultivars. The physicochemical characteristics are presented in Table 1.

Preparation of zero-developed dough

ZD dough was prepared according to the method of Campos et al. [3] with modification described in **Chapter 3**. Farinograph optimal water absorptions of 47.8% and 58.7% (based on 14% moisture in the flour) were used to prepare the ZD dough from Soissons and Spring flours, respectively. ZD dough samples were kept for 20 h at 4°C to allow complete hydration and even distribution of water throughout the dough.

Mixing experiments

Z-blade mixing was performed using a 300 g farinograph mixer (Brabender OHG, Duisburg, Germany), as described in **Chapter 3**. Mixing experiments were performed either using a flour–water mixture or ZD dough. Table 1 presents farinogram data as a measure of the mixing behaviour of two starting material.

Table 1. Physicochemical analysis of the material used

	Soissons		Spring	
	Flour	ZD dough	Flour	ZD dough
Moisture (%)	12.7	nd[a]	13.3	nd
Ash (% db)	0.48	nd	0.57	nd
Protein (% db)	11.3	nd	16.1	nd
pH	6.7	6.6	6.4	6.7
Farinograph				
Water absorption (%) (14% moisture basis)	47.8	–	58.7	–
Arrival time (min)	10.5	5.1	7.9	9.3
Time-to-peak (TTP; min)	15.0	8.2	16.0	12.0
Stability (min)	21.0	9.0	25.1	12.2
Time to breakdown (min)	31.5	14.1	33.0	21.5

[a] not determined

Dough samples prepared from both flour–water mixture and ZD dough were mixed for different times, to different inputs of specific mechanical energy (SME). From a torque–time plot, an optimal development time (time-to-peak mixing; TTP) for dough was determined. Taking into account the TTP, three mixing regimes were arbitrarily assigned with respect to TTP: under-mixed (before TTP), at TTP, and over-mixed (beyond TTP). Table 2 shows the energy inputs for the three mixing regimes.

After mixing, three samples were taken for analysis: Sample 1; approximately 200 g of the resulting dough was frozen immediately in liquid nitrogen and held at –18 °C for pending analysis. Sample 2; approximately 200 g of dough was rested at 30 °C and ca. 85% RH for 120 min, and then frozen in liquid nitrogen for analysis of GMP after resting. Sample 3 was used for rheological characterization as follows: approximately 20 g dough was rounded gently into a small ball and placed in a pre-warmed (26 °C) Petri dish. The dough was covered with a glass cap to prevent moisture loss and placed in a humid atmosphere (ca. 85% RH) at 26 °C for 15 min and then moulded for the extension test.

Table 2. Energy requirements for different mixing regimes of the materials

		Time (min)	**SME** (kJ/kg)
Soissons			
Flour-water mixture	Under-mixed	2	6.6
	TTP	15	74.1
	Over-mixed	45	179.0
ZD dough	Under-mixed	2	8.3
	TTP	8	28.3
	Over-mixed	45	154.3
Spring			
Flour-water mixture	Under-mixed	5	4.8
	TTP	16	57.2
	Over-mixed	45	181.7
ZD dough	Under-mixed	5	16.9
	TTP	12	46.8
	Over-mixed	45	158.5

Isolation of GMP from flour and dough samples

Frozen dough samples (both non-rested and rested) were freeze-dried overnight to an average moisture content of ca. 6% (w/w). GMP from flour and freeze-dried dough samples was isolated as described in **Chapter 3**.

Extension test

Large-strain deformation rheological characteristics of dough samples were obtained using a uniaxial extension test (**Chapter 4**). The force–displacement curves were re-calculated into stress–strain data according to the method described by Dunnewind et al. [9], taking into account changes in the dimensions of the extended specimen and assuming a constant volume for the dough sample. The fracture properties were computed from the stress–strain data. The maximum stress or fracture stress (σ_{max}) sustained by the specimen, the Henky strain (ε_H) at fracture stress, and the integrated area (A) under the stress–strain curve were taken as a measure of the resistance to extension, the extensibility, and the energy required for extension, respectively. An *apparent* strain hardening value ($d\ln\sigma/d\varepsilon_H$) was also calculated from the stress–strain plot (**Chapter 4**). *Apparent* strain hardening is not the absolute value of strain hardening, but an arbitrary calculation of strain hardening in a strain interval of ε=0.45-2.17 equivalent to 20 to 95% of the fracture strain (for all test specimens). This range provided a good data fit (R^2=0.96-0.99) and allowed us to calculate $d\ln\sigma/d\varepsilon_H$ values. These values did not significantly vary with the selection of the strain interval. Differences between mean values for these parameters were assessed for significance using a t-test [5].

Results and discussion

Characteristics of torque–time behaviour of dough prepared from flour–water mixture versus dough mixed from ZD dough

The mixing experiments were performed for different processing times, leading to different mechanical energy inputs. Table 2 lists the energy inputs for z-blade mixing of the two wheat cultivars. The energy input is used as an indicator of practical dough development during mixing trials. Fig. 1 (Soissons) and Fig. 2 (Spring) illustrate variations in torque during the z-blade mixing of flour–water mixture (a) versus ZD dough (b). With a flour–water mixture used as starting material (Figs. 1a and 2a), a distinct hydration phase was recorded at the beginning of mixing, whereas the mixing curves of doughs prepared from ZD dough (Figs. 1b and 2b) did not show any hydration peak. In Figs. 1b and 2b, with ZD doughs as starting material, the torque line showed an initial decline (in the first 2 min of mixing) followed by a development peak at TTP. As is clear from these figures, the development peaks were shifted to the left compared to the mixing curves for the flour–water mixtures (Figs. 1a and 2a). Moreover, the use of ZD dough (at the same level of water addition) compared to the flour–water mixture in the z-blade mixer led to a reduction in average torque values. The average torque is a composite result of many factors, including for example the degree of water binding, protein/starch content and the stresses required to break the dough protein structures. Thus it is difficult to explain the recorded differences. With respect to the protein structures, the equilibrium between breakdown and reformation of gluten structures will have an effect. If this equilibrium is shifted towards the breakdown as is the case with ZD dough, the average torque may be lower. Moreover, the mixing of ZD dough compared to that of flour–water mixture resulted in an earlier and more enhanced breakdown.

Comparison of the two wheat cultivars (Figs. 1, 2 and Table 1) revealed a significant reduction in mixing performance (tolerance) of ZD dough compared to that of conventionally prepared dough, especially in the case of Soissons. The stability time for Spring dough (prepared from both flour-water mixture and ZD dough) was longer than that of Soissons, indicating that Spring is stronger than Soissons. As can be seen from Table 2, less energy is needed to reach TTP when ZD dough is subjected to mixing (for Soissons 45.8 kJ/kg and for Spring 10.4 kJ/kg less energy). Considering that mixing of

ZD dough is not accompanied by hydration and distribution of dough components, which requires some mechanical work, it is to be expected that in mixing of ZD dough the energy is transferred into the dough more effectively than with the flour–water mixture at the same processing time. This may explain the lower stability of ZD dough in mixing regimes. The lower tolerance of ZD dough upon mixing may be a general feature and applicable to other wheat varieties although the extent of weakening will depend on the quality of the flour.

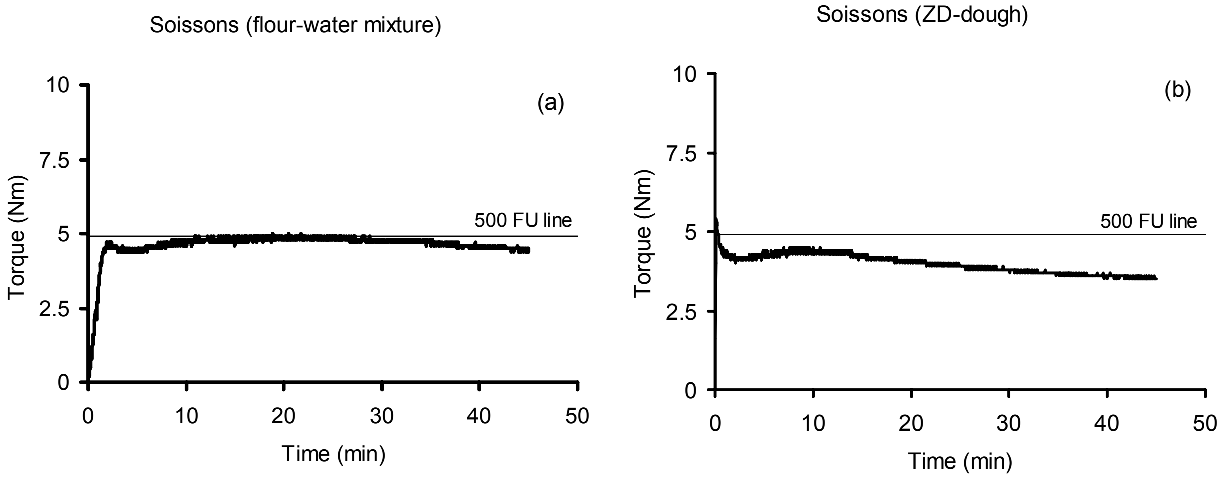


Fig. 1. Typical torque–time curves for Soissons doughs mixed from flour–water mixture (a) and mixed from ZD dough (b) in a 300 g mixing bowl. A torque value of 4.9 Nm corresponds to a consistency of 500 farinograph units (FU).

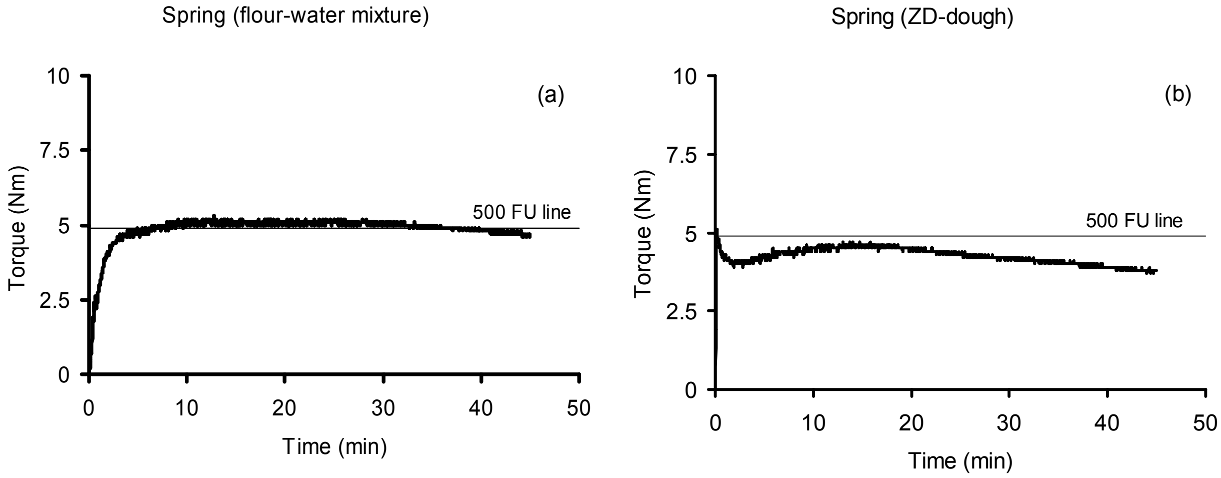


Fig. 2. Typical torque–time curves for Spring doughs mixed from flour–water mixture (a) and mixed from ZD dough (b) in a 300 g mixing bowl. A torque value of 4.9 Nm corresponds to a consistency of 500 farinograph units (FU).

Measurement of GMP wet weight before and after resting

The relationship between the amount of GMP and mixing state of a dough is well known (**Chapter 4**). The measurement of GMP wet weight allows an analysis of the mixing behaviour of ZD dough compared to its parental flour at the level of glutenin structures. GMP was extracted from the dough samples either before or after a resting time of 120 min as described above. GMP wet weight versus mixing energy for the non-rested dough samples is shown in Fig. 3a, b.

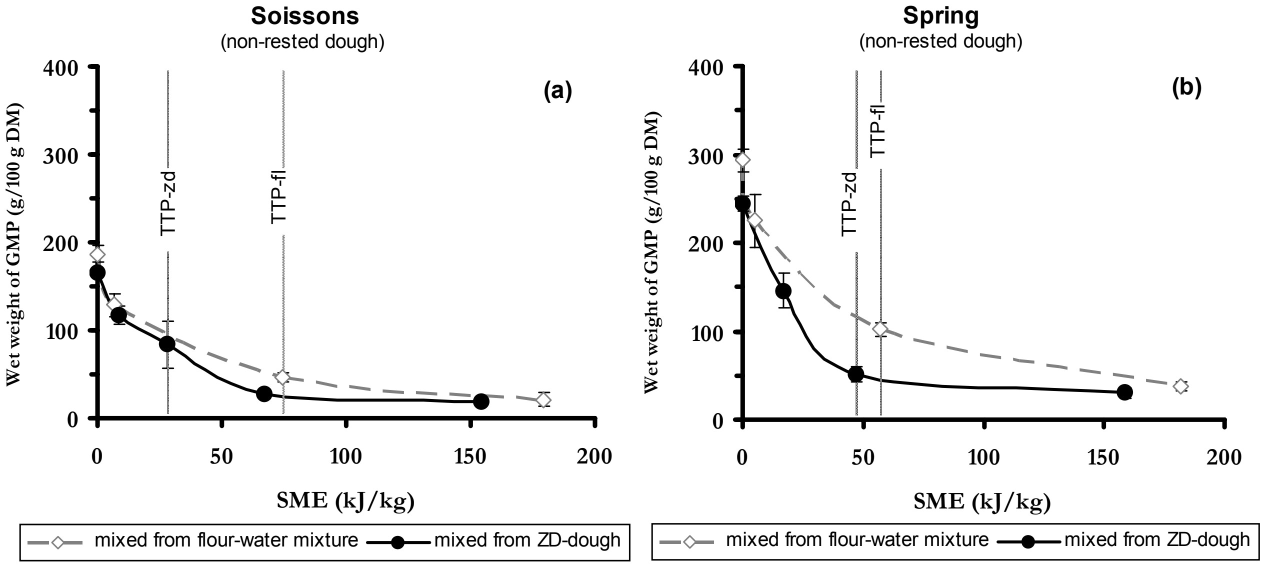


Fig. 3. The effect of mixing energy on GMP wet weight of (non-rested) doughs prepared from flour-water mixtures and doughs prepared from ZD doughs of Soissons (a) and Spring (b) cultivars. Error bars show 95% confidence intervals.

The amount of GMP in dough prepared from flour-water mixtures decreased as a function of energy input for both wheat varieties as shown previously [2, 7, 18, 19, 22]. As can be seen from Fig. 3a, b, the amount of GMP measured in ZD doughs (hydrated non-processed flours; SME=0) from both wheat cultivars was significantly (α=0.05) lower than that of the flours that used to prepare these ZD doughs. This difference can be explained by the hydration phase involved in the preparation of ZD dough, which obviously can affect the gel-forming capability of GMP. Upon mixing, a typical reduction in GMP wet weight (non-rested samples) was observed for both from flour–water doughs and from ZD doughs. Nonetheless, at a constant energy input, the GMP in doughs mixed from ZD dough samples was slightly lower than that of doughs mixed from flour–water mixtures of both wheat cultivars. This might be related to the fact that GMP in ZD doughs (non-processed) of both wheat cultivars was initially lower than that of the flours which used to prepare these ZD doughs. However, these differences decreased and disappear upon over-mixing (SME>100 kJ/kg) when almost all the GMP in dough prepared from both raw materials is gone.

Since rheological assessment can only be performed after resting the dough, we also measured the amount of GMP after resting doughs mixed from both flour–water mixtures and ZD doughs. This latter measurement provides a better understanding of the severity of the mixing process. The effect of mixing energy on wet weight of re-assembled GMP is shown in Fig. 4a, b. Mixing until TTP (for both cultivars) led to a complete recovery of GMP weight during resting. Until TTP, there were no differences in the recovery of GMP between doughs mixed from ZD doughs versus flour–water mixtures. However, mixing beyond TTP caused a rapid decline in the re-assembly capability of the glutenins. This is in agreement with the results reported in the literature [8, 24]. It should be noted that TTP varies with wheat quality and with respect to the type of starting material (ZD dough versus flour–water mixture). Since the maximum torque is not always pronounced (especially with strong flour varieties), this limits the usefulness of TTP as control parameter. Determination of TTP point from torque-time plot will only be useful if the TTP in a test charge with the same flour has been determined previously.

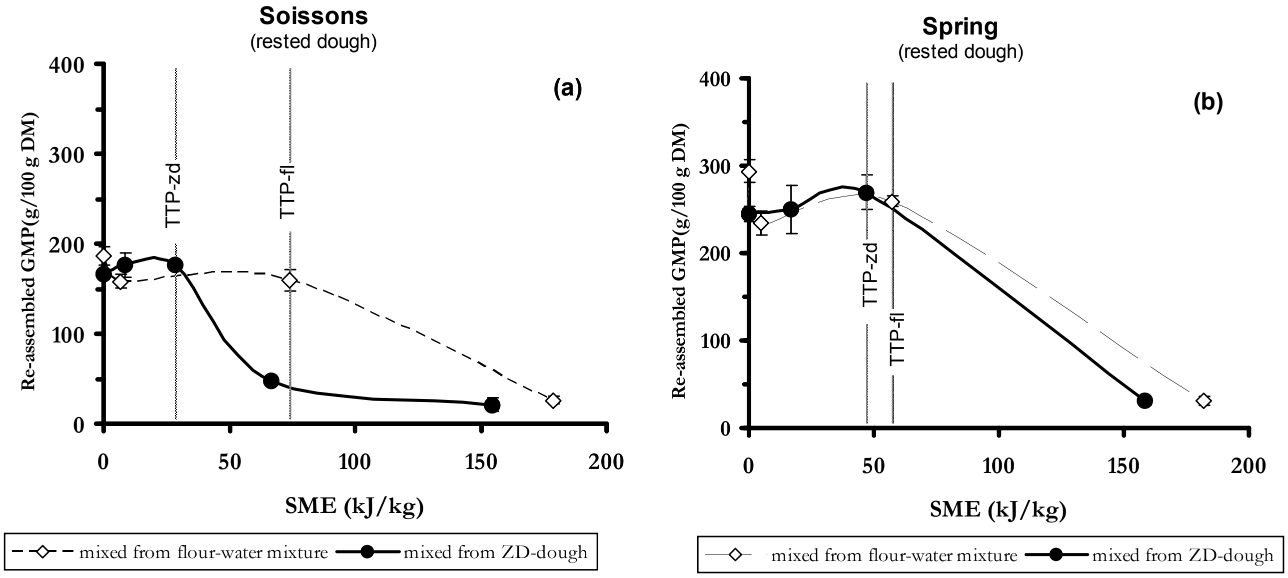


Fig. 4. The effect of mixing energy on re-assembled GMP wet weight in rested dough prepared from flour-water mixtures and from ZD doughs of Soissons (a) and Spring (b) cultivars. Error bars show 95% confidence intervals.

Rheological characterisation of dough using uniaxial extension testing

A comparison of the uniaxial extension properties of doughs mixed from flour–water mixture versus ZD dough are shown in Fig. 5a, b. Here the stress–strain behaviour of doughs mixed from flour–water mixtures and ZD doughs at different processing stages for Soissons and Spring cultivars, respectively is shown as the average curve of at least four replications for each experimental material used. Mean and confidence interval (α=0.05) values for data extracted from stress–strain curves are shown in Tables 3 and 4. As can be seen from Fig. 5 and Tables 3 and 4, unprocessed ZD dough of both wheat cultivars showed a strong visco-elastic behaviour as characterised by the highest values for fracture properties and *apparent* strain hardening. Extra care was taken in manipulation and sample preparation of unprocessed ZD dough for extension testing to prevent any unwanted deformation. Our results are in agreement with those of Unbehend et al. [20], who reported that a hydrated unmixed flour (HUF) with no mechanical energy input was firmer and showed higher resistance to extension (R_{max}) than dough prepared by a mixing and kneading.

Fig. 5 and Tables 3 and 4 show that the strain hardening effect of ZD dough was larger for the strong wheat cultivar, Spring, than for Soissons. In other studies it has been shown that the higher content and the better the quality of gluten fraction, the more pronounced the strain hardening effect [15, 21]. Microscopy of unprocessed dough shows a dispersed protein phase, covering the starch granules [20](see also **Chapter 4**) and it might be concluded that it is only the hydration of the protein phase around the starch granules that imparts the visco-elastic behaviour to the ZD dough. This is supported by the results presented in Tables 3 and 4.

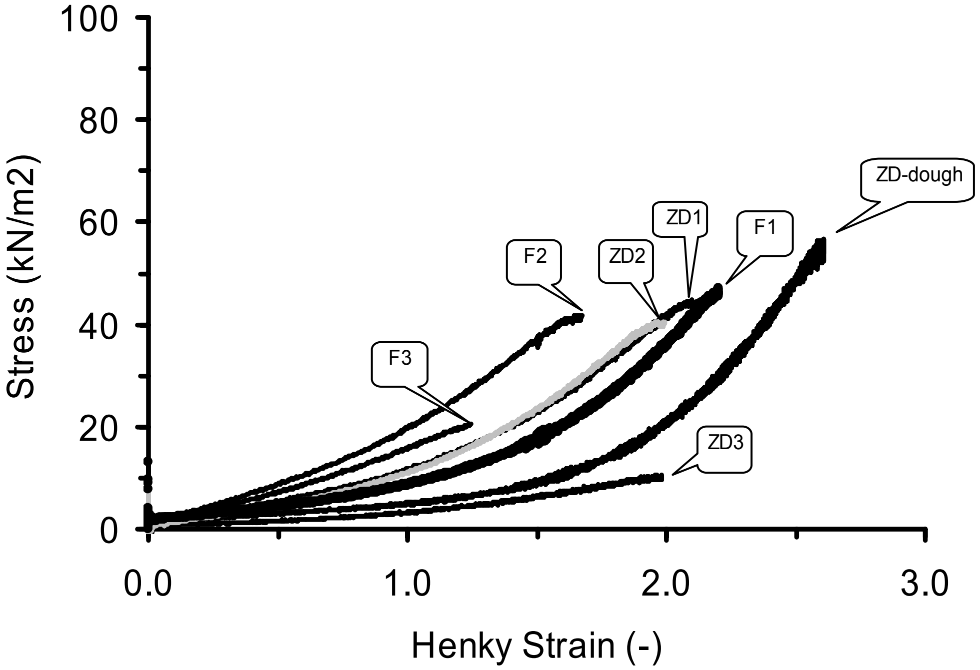
Table 3. Comparison of the fracture properties of unprocessed ZD dough with the dough mixed from either flour–water mixture or ZD dough for Soissons cultivar

	Fracture properties of the dough					
	SME (kJ/kg)	n^b	σ_{max} (kN/m²)	ε_H (–)	***A*** (kN/m²)	**d**lnσ/**d**ε_H (–)
Unprocessed ZD dough	0	5	56±7	2.6±0	40±4	1.6±0
Dough after mixing						
Under-mixed:						
Flour-water mixture	7	4	47±5	2.2±0	35±4	1.4±0
ZD dough	8	5	44±3	2.1±0	36±3	1.4±0
Mixed to TTP:						
Flour-water mixture	74	4	46±6	1.7±0	35±4	1.5±0
ZD dough	28	4	32±2	2.2±0	23±2	1.6±0
Over-mixed:						
Flour-water mixture	179	6	21±1	1.3±0	17±2	1.4±0
ZD dough	154	4	11±1	2.1±0	9±1	1.3±0

[a] Mean values ± confidence interval (α=0.05), [b] Number of replications in extension test.

Soissons

(a)



Spring

(b)

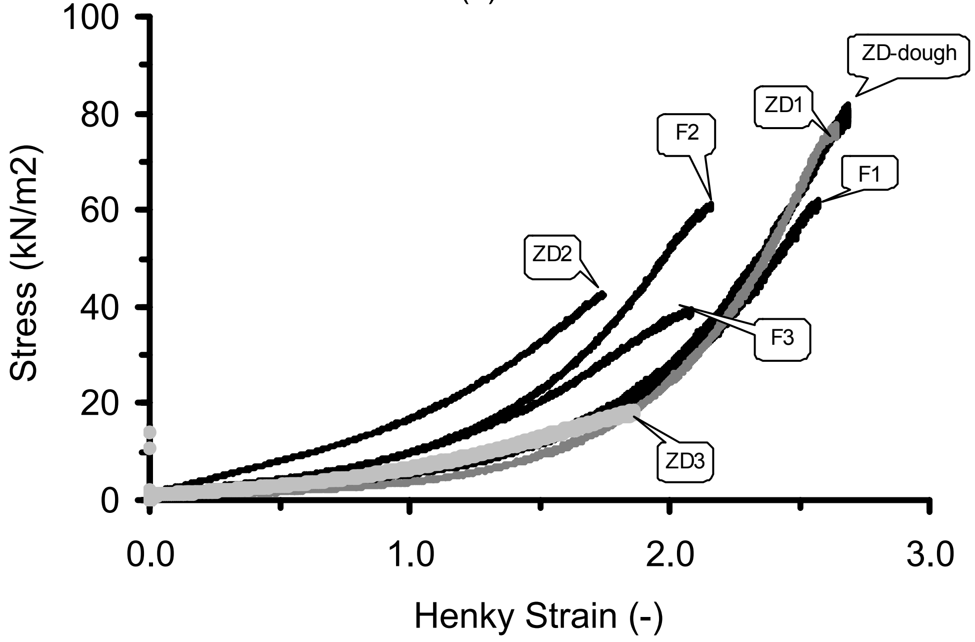


Fig. 5. Rheological behaviour of Soissons (a) and Spring (b) dough prepared from either flour–water mixtures or ZD doughs mixed at different times. F1, F2 and F3 represent dough samples mixed from flour–water mixtures at under-mixing (1), TTP (2) and over-mixing (3), respectively. ZD1, ZD2 and ZD3 represent dough samples mixed from ZD dough at under-mixing (1), TTP (2) and over-mixing (3), respectively.

Table 4. Comparison of the fracture properties of unprocessed ZD dough with the dough mixed from either flour–water mixture or ZD dough for Spring cultivar

			Fracture properties of the dough			
	SME (kJ/kg)	n^b	σ_{max} (kN/m^2)	ε_H (–)	A (kN/m^2)	$d\ln\sigma/d\varepsilon_H$ (–)
Unprocessed ZD dough	0	7	82±8	2.7±0	56±7	1.7±0
Dough after mixing						
Under-mixed:						
Flour-water mixture	5	5	61±3	2.6±0	42±3	1.6±0
ZD dough	17	5	76±4	2.6±0	45±3	1.9±0
Mixed to TTP:						
Flour-water mixture	57	5	59±7	2.2±0	40±3	1.6±0
ZD dough	47	5	45±3	1.9±0	38±2	1.4±0
Over-mixed:						
Flour-water mixture	182	4	32±3	1.7±0	21±4	1.4±0
ZD dough	159	5	20±1	1.9±0	18±2	1.3±0

[a] Mean values ± confidence interval (α=0.05), [b] Number of replications in extension test.

Hydration itself already leads to gluten development presumably by interfacial tension forces or swelling of the structures, which also occur with considerable forces. Together these observations suggest that hydration of gluten proteins can restore their ability to aggregate to form a continuous structure. This is in agreement with Amend and Belitz [1] who reported that visco-elasticity and aggregation behaviour are inherent properties of gluten in the starchy endosperm cells and that hydration restores these properties. It has also been reported that when flour is hydrated, the gluten fraction forms a visco-elastic network by its latent chemical potential and without mechanical energy input [20]. Hoseney [11] has also drawn attention to the importance of hydration in dough development. In his view, development is essentially the result of complete hydration of the flour particles, achieved by mixing the dough to an optimum level at which point all protein and starch in the flour are hydrated.

As presented in Tables 3 and 4, both flour–water mixture and ZD dough showed similar rheological behaviour at early stages of mixing (under-mixing). Nonetheless, at this mixing regime, σ_{max} and apparent strain hardening ($d\ln\sigma/d\varepsilon_H$) values for Spring dough mixed from ZD dough were significantly higher than those for the dough mixed from flour–water mixture (Table 4). For under-mixed Soissons dough, there was no significant difference between the fracture properties of the dough mixed from flour–water mixture versus the dough prepared from ZD dough samples (Table 3). As mixing reaches TTP, dough mixed from ZD dough showed significant lower σ_{max} values compared to dough mixed from flour–water mixture. For Soissons, dough prepared from ZD dough had higher ε_H and *apparent* strain hardening values compared to conventionally prepared dough. This was the other way around in case of Spring cultivar. This might be related to the lower energy requirement of TTP mixed ZD dough of Soissons compared to conventionally prepared dough (SME=28.3 kJ/kg at a mixing time of 8.2 min for ZD dough compared to SME=74.1 kJ/kg at a mixing time of 15 min).

Progressive mixing of dough prepared from both flour–water mixtures and ZD dough led to a significant (α=0.05) reduction in the values of fracture properties (except ε_H) for both wheat cultivars studied. Weakening of the rheological properties of the dough upon prolonged mixing is in line with the significant decline in GMP wet weight while over-mixing. This is in agreement with studies by Gras et al. [10] and Weegels et al. [23], who reported that the decrease in R_{max} as a result of over-mixing could be explained by the decrease in GMP content of dough. We therefore conclude that the loss of σ_{max} (comparable to R_{max}) in Tables 3 and 4 in over-mixed regimes corresponds to a weaker re-assembly of GMP in rested dough, as shown in Fig. 3a, b. With respect to the type of starting material, the use of ZD dough instead of flour–water mixture in mixing experiments (after TTP) resulted in a higher reduction in the fracture properties of the dough compared to dough mixed from flour. This indicates less tolerance of ZD dough with long mixing regimes. The lack of the hydration phase (Figs. 1b and 2b) in the mixing of ZD dough can describe the fact that the amount of energy transferred to the dough mixed from ZD dough is higher than that of the dough mixed from flour. This can explain less tolerance of material under mixing and consequently earlier breakdown of dough, as shown in Figs 1 and 2. Moreover, it is also possible that the production process of ZD dough combined with the initial hydration step to melt frozen powdered ZD dough may affect the “red–ox” system of the flour. Since the amount of GMP is the net result of breakdown and re-assembly, it is possible that the re-assembly process in ZD dough is slower. However, this aspect, concerning for example, the effect of freezing and thawing on the flour “red–ox” system was not explored in the present study.

Relation between GMP wet weight and rheological properties of the dough

The relationship between the GMP content of flour or dough and the maximum resistance (R_{max}) of z-blade mixed dough in uniaxial extension tests is well known. Thus a study was made of the correlation between the fracture properties of dough from both wheat cultivars used in this study under a uniaxial extension test (σ_{max}, ε_H and A) and the wet weight of GMP extracted from the rested dough with respect to the type of starting material (flour–water mixture versus ZD dough). The results are presented in Fig. 6. There was a good correlation (R^2=0.71–0.84) between σ_{max} and re-assembled GMP wet weight. The correlation line for the dough mixed from ZD dough samples (closed symbols) paralleled that of the dough mixed from flour–water samples (open symbols). Similar correlation was found between the area under the stress–strain curve (*A*) and the re-assembled GMP content (R^2=0.85) (results are not shown). Dough extensibility did not show a correlation with GMP in the dough mixed from ZD dough (R^2=0.23), whereas re-assembled GMP content of the dough prepared from flour–water samples showed rather better correlation with ε_H (R^2=0.68) (data not presented).

A weak correlation of GMP content with dough extensibility under uniaxial extension tests has been reported by Weegels et al. [23]. According to these authors, during mixing and resting of dough, changes occur in the GMP that are important for R_{max} but not for extensibility.

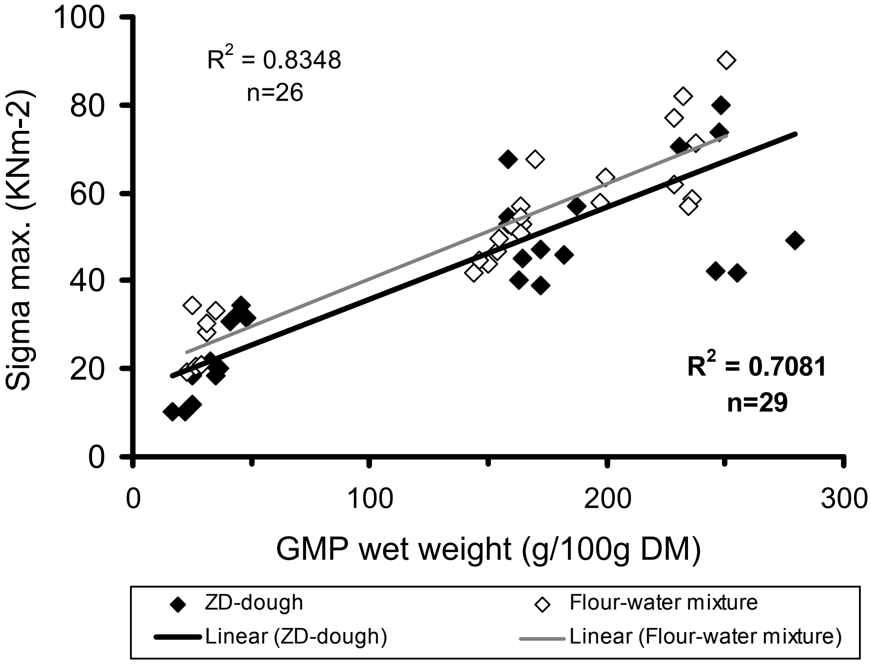


Fig. 6. Relationship between GMP wet weight of rested doughs (for 120 min) with maximum stress (σ_{max}). Both wheat cultivars are plotted together. Closed symbols represent doughs mixed from ZD doughs and open symbols show dough mixed from flour–water mixtures. Number of data points is shown by “n” in the graphs.

Conclusions

In this study z-blade mixing behaviour of ZD doughs from the flours of two wheat cultivars of different gluten strength was compared to that of conventionally mixed dough of the same flours. For both wheat cultivars, when ZD dough was used instead of flour–water mixture in z-blade mixing experiments: no hydration peak was observed, the development peak shifted to the left indicating faster development of the gluten structure, a lower stability and earlier breakdown were seen, and a lower average torque values were recorded.

In z-blade mixing of ZD dough there was a very similar breakdown of GMP wet weight compared to that of dough mixed from a flour–water mixture. Comparison of re-assembled GMP wet weight revealed that until TTP, there was no difference in GMP recovery with respect to the type of starting material. Beyond TTP, however, recovery of GMP in dough mixed from both ZD dough and flour samples was diminished.

Large-strain deformation rheological measurements for dough mixed from both flour–water mixture and ZD dough from both types of wheat cultivars showed strong visco-elastic behaviour as characterised by the highest values of fracture properties, followed by a decline in those properties upon further mixing. At mixing regimes before TTP, there was no difference between ZD dough and flour–water mixtures in the mixer. However, further mixing caused significant reduction in the values of fracture properties (except ε_H) of dough mixed from ZD dough (Tables 3 and 4).

The results show that ZD dough is a hydrated zero mechanical energy developed dough, exhibiting visco-elastic properties under uniaxial extension test. Further they suggest that the gluten structure in ZD dough is formed due to uniform hydration effect during the preparation of ZD dough. Moreover, when ZD dough is used as starting material for mixing, less energy is needed to reach optimal dough development. Therefore, extra care has to be taken not to "over-mix" the dough. In other dough processing studies where ZD dough is used, the mixing requirements of the system should be exactly determined. When these conditions are met, ZD dough constitutes a good model for dough mixing studies to help understand different aspects of mixing.

Acknowledgements

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Chapter 2

A new method to study simple shear processing of wheat gluten-starch mixtures[♣]

Abstract

This article introduces a new method that uses a shearing device to study the effect of simple shear on the overall properties of pasta-like products made from commercial wheat gluten-starch (GS) blends. The shear processed GS samples had a lower cooking loss (CL) and a higher swelling index (SI) than their unprocessed materials, suggesting the presence of a gluten phase surrounding starch granules. Pictures of dough microstructure by confocal scanning laser microscopy (CSLM) showed the distribution of proteins in the shear-processed samples. This study revealed that simple shear processing could result in a product having relevant cooking properties as compared to those of commercial pasta. Increasing gluten content in GS mixtures led to a decrease in CL and an increase in maximum cutting stress of processed samples whereas no clear correlation was found for SI values of sheared products. It was concluded that the new shearing device is unique in its capability to study the effect of pure shear deformation on dough development and properties at mechanical energy and shear stress levels relevant to industrial processing like pasta extrusion.

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Introduction

Traditionally, dough is made by combining flour, water, and energy through mixing. The addition of sufficient mechanical energy provides the distribution and hydration of flour particles, allowing the formation of a continuous protein matrix, holding starch and other components together [7]. During dough processing like mixing and extrusion, wheat constituents are not only mixed with other ingredients, but are also subjected to various shearing regimes. Bloksma and Bushuk [5] stated that mixing could be divided into three distinct stages: distribution of material, hydration, and energy input to stretch and align protein molecules. The energy input due to mixing, which involves generally shear and extensional deformation, is an important aspect of protein development. Mixing energy alone however does not completely characterize the mixing process. The nature of the mixing action is also important, but is more difficult to specify. Recently, Jongen et al. [18] using simulation study reported that dough mixing in kneaders consists of a combination of various flow patterns e.g. shearing and elongation. Moreover, they pointed out that the way in which these deformations are provided to the material during mixing is of crucial importance.

There is hardly any literature on the effect of a constant strain deformation using high shear stresses on dough properties. Information in literature [2, 9, 16, 17, 24, 27, 29-31] concerning the effect of shear on dough properties is often limited to the characterization of dough rheological properties under small amplitude oscillatory shear measurements with a shear stress range of 0.2 - 0.8 kPa. Lately, Schluentz et al. [28] and Lee et al. [20] produced partially developed dough using a well-defined shear or extensional deformation from undeveloped dough. They concluded that shearing or extensional deformation alone via a rheometer is not able to produce dough of a quality comparable to dough produced through a combination of shear and extensional deformation by a Farinograph mixer. These investigations however are limited to the application of significantly lower shear stresses (0.8 kPa) and mechanical energy input compared to those normally used in dough mixing and pasta extrusion. Therefore, it is likely that due to the different work input used, their material has been processed differently. We therefore developed a new shearing device that provides the possibility to study simple shear deformation on wheat gluten-starch mixtures. The cell is able to apply shear rates varying from 24 to 250 s^{-1} to high viscous materials, thereby allowing high shear stresses up to 50 kPa. These stresses are comparable to shear stress values obtained in extrusion trials performed by Leroux et al. [21]. A study by Van den Einde et al. [33, 34] showed that comparable product changes could be obtained by shear cell and extrusion trials in case of starch breakdown. We therefore concluded that the shear cell is a unique device to study changes of product properties under high shear conditions, which might help to improve the understanding of the effects of extrusion or mixing on pasta product properties.

A dough formula simplified to flour and water still encompasses a complex series of flour components and their interactions. Therefore a model mixture of gluten and prime starch (GS) is used, not only to allow us to control the protein content, but also to reduce the complex interactions of the flour constituents such as water soluble pentosans and soluble proteins including enzymes [25].

The goals of this study were therefore to: 1) Demonstrate the usefulness of the developed pilot-scale shearing device to investigate simple shear deformation of wheat GS dough; and 2) Investigate the effect of different protein/starch ratio on the overall properties of pasta-like products obtained from GS mixtures by a well-defined simple shear deformation.

Materials and Methods

Materials

Commercial wheat gluten and starch were obtained from Roquette Co. (Cedex, France). Moisture content of the starch and gluten were 12.3 and 6.5% (dm) respectively, determined by AACC Approved Method 44-15A [1]. According to the manufacturer, protein and fat content of starch sample were max. 0.4 and 0.1 %(w/w), respectively. Protein, starch and fat content of gluten were max. 86.0, 10.0 and 3.0 % (w/w), respectively. Commercial Italian spaghetti with a composition of starch 75.2% (w/w), protein 12.7% (w/w), moisture 8.9% (w/w) and fat 1.7% (w/w) was used as a reference product.

Methods

Preparation of GS blends

Starch was blended with different amounts of gluten to achieve 0, 5, 8, 11, 14, and 17% gluten based on dry weight of starch. All samples were adjusted to 40% (db weight) moisture before shear processing. Aside from the point that the moisture content in the current study provides a good handling of the material in the shearing device, it also is within the range of water content normally used in pasta extrusion (40-48%) [14, 21, 23]. The GS samples were encoded as G_0, G_5, G_8, G_{11}, G_{14} and G_{17}, where G represents gluten and the numbers show the percentage of gluten (starch db) in the mixture. A Waring blender with a modified mixing propeller was used to blend the dry ingredients.

Preparation of undeveloped dough

The method of Campos et al. [6] was used with some modifications described as below to prepare undeveloped GS dough. Powdered ice was prepared in the presence of solid carbon dioxide (dry ice) in a walk-in-cooler at +2 °C. Large pieces of solid CO2 were first pulverized with a Waring blender to generate a cold medium inside the blender. Ice flakes were then added and broken into smaller particles. Dry ice and ice particles were sieved to a particle size range of approximately 700 μm. The powder mixture was kept at -25 °C undisturbed overnight, allowing sublimation of solid CO2 while keeping the ice particles intact. The blending of starch, gluten and ice particles was performed inside a walk-in-freezer (-25°C). Materials were carefully weighed, placed in the Waring blender and distributed uniformly by mixing at a reduced speed. The resulting GS and ice mixture was placed in a closed container and kept frozen at –18 °C. The frozen gluten-starch-ice mixture was kept in a moisture tight container at 35°C for 60 minutes before each shear treatment. This holding period allowed the ice particles to melt, resulting in hydration of ingredients, and thus a homogenous undeveloped dough.

Shear cell

The simple shearing process was performed using a newly developed device in our laboratory. This new shearing device (shear cell) was based on a cone and plate rheometer concept. It was designed and sized at pilot scale. The maximum gap size between cone and plate was small compared to the diameter of the cone. A small angle between cone and plate resulted in a constant shear rate. The contact surface of both cone and plate (moving bottom cone) was roughened (serrated) to avoid possible slippage of material during shear processing. The motor used in the testing device was able to provide shear stresses up to 50 kPa. A schematic configuration of the shear cell is shown in Fig. 1.

The shear rate ($\dot{\gamma}$ in s^{-1}), shear stress (τ in Pa) and mechanical energy input (SME in kJ/kg) applied to the material during processing were calculated:

$$\dot{\gamma} = \frac{\omega}{\theta} \tag{1}$$

$$\tau = \frac{3M}{2\pi R^3} \rightarrow \tau = C.M \tag{2}$$

$$SME = \frac{\int_{t=0}^{t_f} \omega.M(t).dt}{m} \tag{3}$$

In these equations ω is the rotor speed (s^{-1}), θ is the angle between cone and plate (shearing zone in figure 1, in degrees), M is the torque (Nm), R is the projected radius of cone and “m” is the mass of material in the shear cell (kg). Equations 1 and 2 are based on the cone and plate rheometer [22]. C is a constant (m^{-3}) in Equation 2 and is equal to 379 considering the projected radius of the cone (R= 0.108 m).

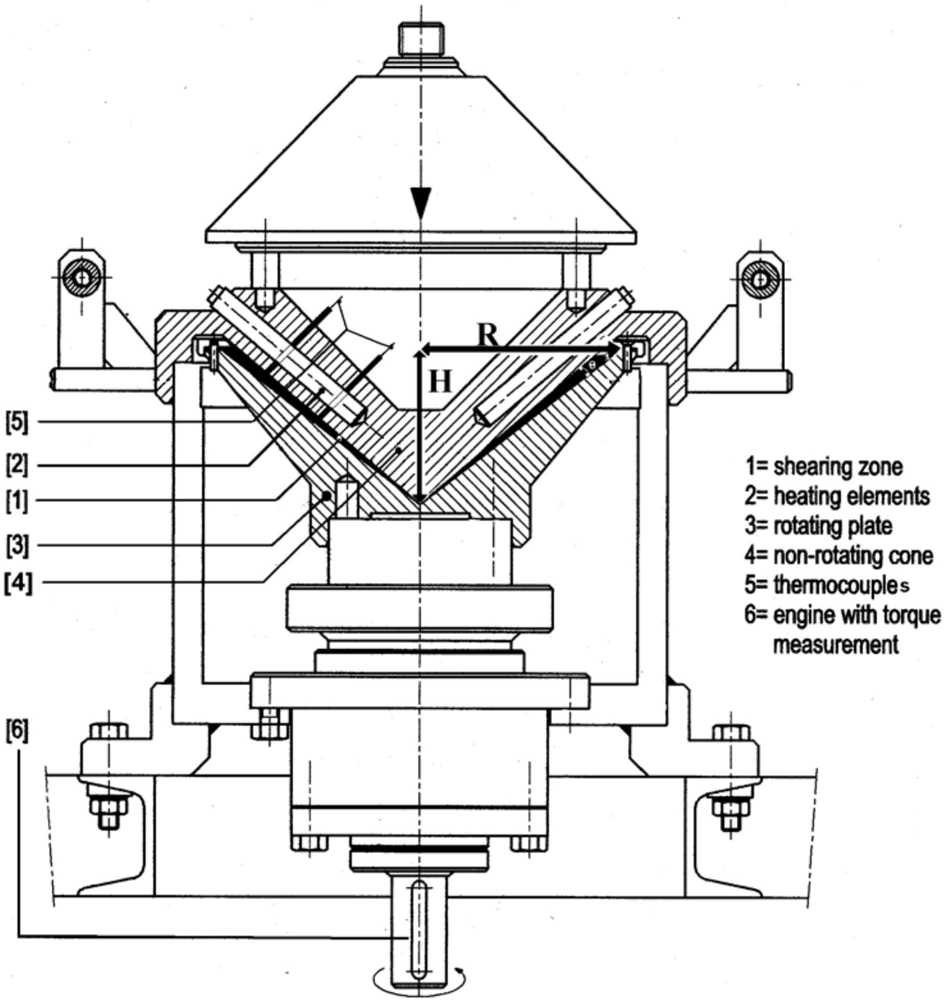

Fig. 1. Schematic illustration of the shearing device. Cone angle = 100°, angle between cone and plate (shearing zone) θ = 2.5°, R = 0.108m, H = 0.082m.

Shearing process

For all samples, constant amounts of undeveloped GS dough (215 g) were placed in the shear cell. The filling procedure was found to be a critical step in the shearing process, thus extra care was taken to evenly fill the cell. After filling the shearing zone (1 in Fig. 1) with undeveloped dough, the cone and plate cell was closed and a vertical pressure was applied and kept constant during the shear experiments.

Undeveloped dough was rested for 30 minutes to allow relaxation of stresses that result from the filling and pressurized closure of system. The system was heated to 40°C by electrical heating elements inserted in the cone (2 in Fig. 1). Shear was then applied to the "unprocessed" material between the rotating plate (3) and the non-rotating cone (4). Thermocouples (5) monitored the sample temperature during resting and subsequent shearing process for each experiment.

A constant shearing time of 900 s was applied for all samples at shear rates ranging between 24 and 48 s-1. The lower limit was set by the slowest possible rotation of the motor (10 rpm). This rate was kept for 180 s to avoid slippage. It was then increased to 20 rpm, corresponding to a shear rate of 48 s-1, until the end of the shear run. All shear treatments were done at least in duplicate.

After shearing the GS samples, a visual observation of a homogenous dough indicated the absence of slip during the run. If slippage had occurred a product only with a smooth surface would have been the result due to the lack of shearing action in the interior of the dough piece. Preliminary experiments showed that slippage yielded a product with a smooth surface. Under the process conditions reported in this study no slippage was observed. This was apparent from the presence of lines in the dough samples originated by the grooves in the cone and plate geometry.

Sampling of product for cooking experiments

As a consequence of the shear cell design, the thickness of the dough was 1.5-4.5 mm. We therefore selected material from the middle part of the plate, with an average thickness of 3 mm for sampling. Ring-shaped samples (concentric circles) were taken using a suitable sampling device. The diameter of outer and inner circles was 15.75 and 12.25 mm, respectively, which gave a wall thickness of 3.5 mm for ring-shaped samples. The resting of undeveloped dough inside the pressurized cell at a temperature of 38 ± 2°C for 30 min resulted in a complete hydration of the material. The resulting dough before the start of the shear run is called "unprocessed" dough. For comparison, sampling was done once before (unprocessed dough) and once after the shearing process for each sample. Ring-shaped samples were taken and immediately transferred to a freezer at –18°C. After freezing, the samples were freeze-dried overnight to an average value of 8% moisture. The dried samples were then stored in sealed plastic bags at ambient temperature until further analysis.

Characterization of product

Pasta cooking quality has been shown to be highly influenced by the protein network and starch gelatinization [26]. Water uptake (swelling) and starch gelatinization during cooking of pasta, are considered to be dependent on the quality of the surrounding protein network [14]. Cooking loss, swelling index and cooked firmness were the quality parameters used in this study to assess the quality of sheared GS products. Both unprocessed and shear processed samples were subjected to cooking. In addition, and as a matter of comparison, the quality of cooked GS samples was compared to that of commercial spaghetti.

Cooking test

The cooking procedure of Mestres et al. [23] was used with some modifications. Distilled water (100 mL) was heated to boiling under reflux in a 300-mL beaker. Freez-dried ring-shaped samples (ca. 5g) were added to the boiling water. The optimum cooking time was determined using a crushing test. A piece of sample was removed from the cooking water at various time intervals and squeezed between a pair of plexiglas plates. The optimum cooking time was reached when the white central core of the cooked sample had just disappeared. Cooking was continued 1 min more than the optimum cooking time defined as a normal cooking time. The sample was drained for 3 min in a Büchner funnel and weighed (W_1, g). The cooked product was dried in an oven at 130±1°C to a constant weight (W_2, g). The cooking and rinsing water was centrifuged (9000 × g) for 10 min. Then dry matter content of the supernatant (W_3, g) was determined. Total cooking loss (TCL), which is a combination of solid loss (dry matter content of sediment) and soluble loss (dry matter content of supernatant) during cooking, was calculated following Equation 4:

$$\text{Total cooking loss (TCL, \%)} = \frac{\mathrm{IW} \times DM - W_2}{IW \times DM} \times 100 \qquad (4)$$

Soluble loss (SL) was obtained using Equation 5:

$$\text{Soluble loss (SL, \%)} = \frac{W_3}{IW \times DM} \times 100 \qquad (5)$$

Swelling index (SI) after cooking was calculated by Equation 6:

$$\text{Swelling index (SI\%)} = \frac{W_1 - W_2}{W_2} \times 100 \qquad (5)$$

In these equations, IW (g) is the initial weight of samples before cooking; DM (g), dry matter concentration of crude samples; W_1 (g), drained wet weight, W_2 (g), dried weight od the samples, and W_3 (g) is dry matter content of the supernatant.

Cooked Firmness

A texture analyzer (TA-XT2, Stable Micro Systems, Surrey, UK) equipped with a 5-kg weight beam, was used to measure firmness of the cooked samples according to approved methods of AACC, 16-50 [1] modified for the TA application. The width of cooked samples before each measurement was determined carefully and the contact area between the sample and the edge of the tooth (0.11 cm) was calculated. Cooked samples or spaghetti strands were placed under the plastic tooth in a specially designed sample holder to avoid any unwanted movement of the sample during the cutting test. Samples were deformed (cut) at a 90° angle using a special plexiglas tooth [36] with a test speed of 0.17 mm/s. Penetration distances of 2.5 and 1.5 mm for GS and Spaghetti samples were selected, respectively. These distances were ca. 65% of total sample height. Seven replicates were performed for each sample. Generally, the maximum force (F_{max}) or maximum stress and the energy required to cut the sample are often used as a measure of pasta firmness [37]. Due to differences in the geometry and contact area of the cooked GS samples and commercial spaghetti, a cutting stress measured at a penetration 65% of the sample thickness was used in this study (maximum stress, σ_{max} in Nm^{-2}, Pa). This maximum stress was taken as a measure of the firmness of the cooked samples.

Characterization of dough microstructure by CSLM

Dough slices with a thickness of ca. 1 mm and dimensions of approximately 0.5 × 1 cm were obtained from unprocessed as well as shear processed dough. Unprocessed dough was taken from the shear cell just before starting the shear run. Specimens of shear processed samples were taken from defrosted frozen dough (at 4°C for 24 hr). A double staining technique (combination of FITC (1% w/v) and Rhodamine B (0.1% v/w)) was used. This method allowed simultaneous observation of protein and starch under CSLM. The stained slices were stored for 1-2 hr at ambient temperature before observation. The microstructure of dough was observed using CSLM (Leica TCS SP) equipped with an inverted microscope (model Leica DM IRBE) used in the single photon mode with an Ar/Kr visible light laser according to procedure described by van de Velde et al. [32].

Results and Discussion

Shear cell experiments

All shearing experiments were done in the pilot scale shear cell. Typical changes in torque values during shearing of GS blends with different gluten-starch ratio are shown in Fig. 2. In GS doughs, the torque curves were characterized by two main peaks (arrows in G_{17}), which are related to the two different rotational speeds applied during the tests and by a plateau after 5 min of shearing. In the shear processing of one of the G_5 duplicate samples an error accrued in the filling step of the cell and therefore that sample (G_5*) was discarded.

In general, torque measurements (Fig. 2) revealed good reproducibility of the shear treatments, especially for samples with high protein contents. This would indicate the potential of this device to apply well-controlled, simple shear deformations to materials. The effect of protein content on dissipation of the mechanical energy through the GS mixtures during shear processing was studied. Fig. 3 shows changes in specific mechanical energy versus gluten content.

Increasing the protein content in the GS mixtures led to a decrease in the torque (Fig. 2) and consequently in SME values (Fig. 3). It has been hypothesized that in a developed dough, starch granules are dispersed in a continuous gluten phase. In this hypothesis, the gluten plays the role of a lubricant for the starch granules in the dough [10]. This effect might explain the role of protein in reducing torque (and calculated SME) values. According to Watanabe et al. [38], a lower starch-to-gluten ratio in gluten-starch doughs leads to the formation of doughs (through mixing) with a rubber-like structure of low storage modulus (G') values. This conclusion is also supported by the rheological behavior of gluten-supplemented flour [4] and of wheat flour dough diluted with starch [19]. However, Fig. 3 shows that SME values leveled off at protein contents >11%. This result could be related to the fact that the additional lubrication effect of the protein will diminish at higher protein contents, as a result of which the stress and SME values measured will not decrease any more.

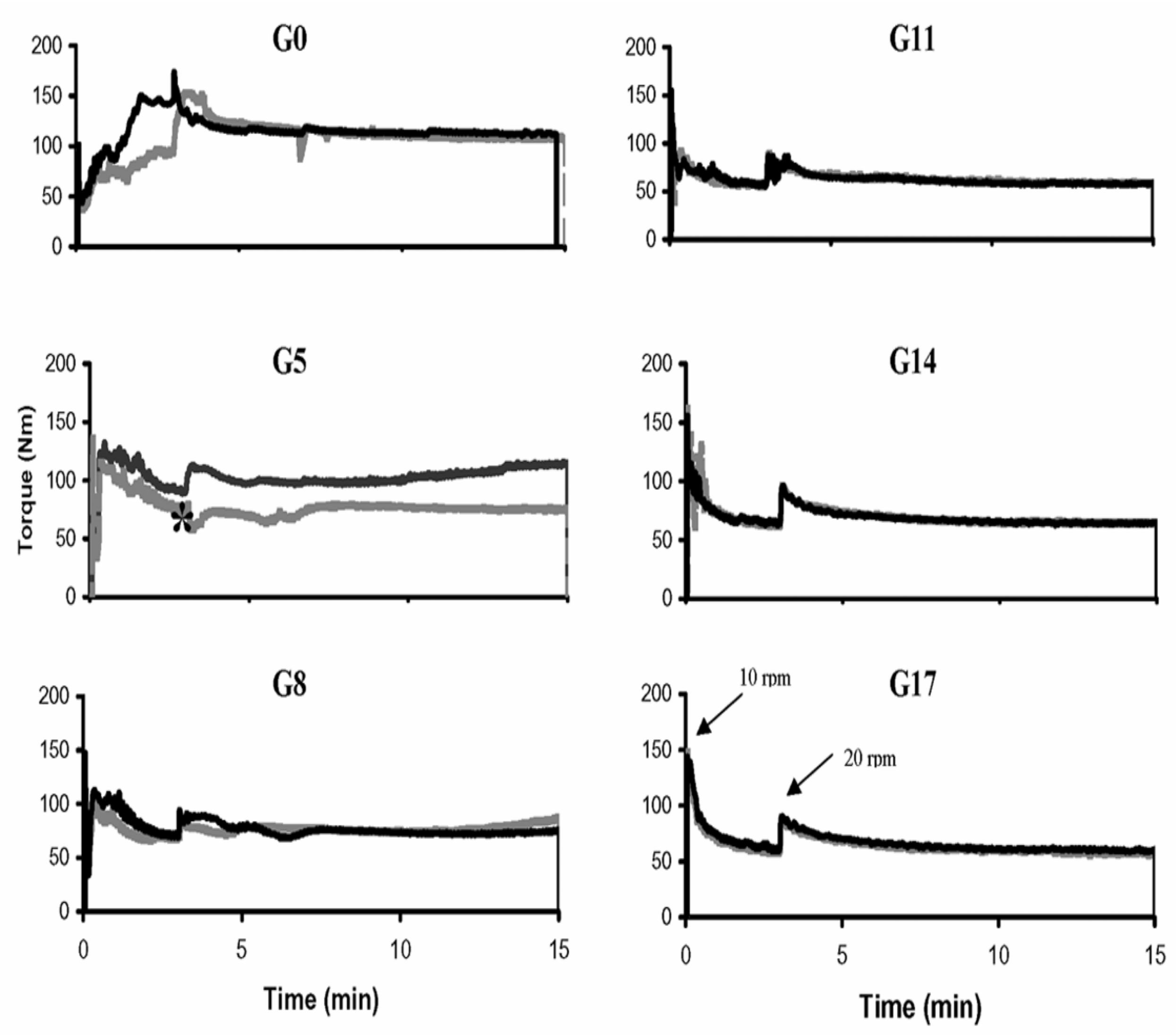

Fig. 2. Torque-time profiles of the shear processing of undeveloped GS samples (G_0 - G_{17}). The two curves given correspond to two replicates indicating reproducible shear processing of the samples (except for samples G_0). G_5* is a sample that was not produced properly and therefore discarded. Two curves in G_{17} are fully overlapped. Arrows in G_{17} indicate the two different rotational speeds used.

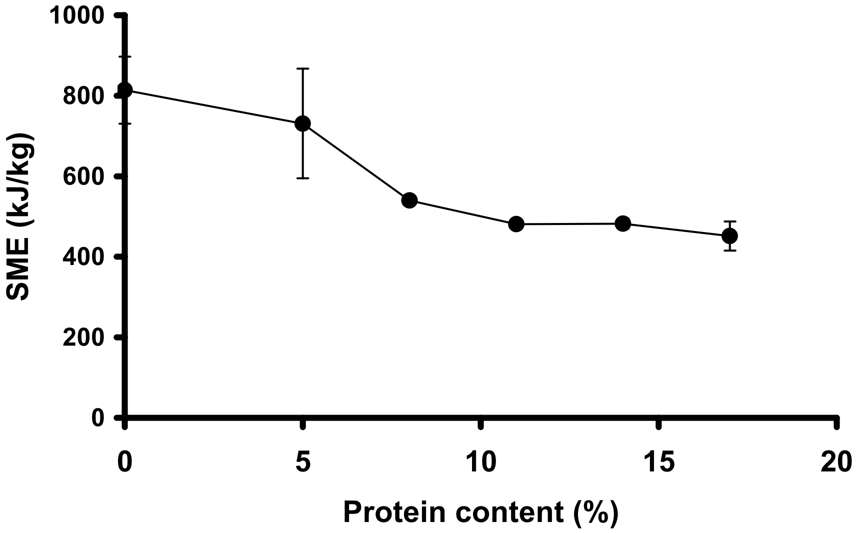

Fig. 3. Effect of gluten amount on specific mechanical energy (SME) during shearing process. Error bars show 95% confidence interval.

Product quality assessment

Cooking loss, swelling index and firmness were used to determine the quality of the cooked shear-processed pasta-like samples. We used these parameters to see the effect of simple shear processing on quality parameters of pasta-like products and compare it with a reference pasta product.

Cooking loss

Fig. 4 shows the cooking loss (CL) values for undeveloped and shear-developed samples. The shear-processed GS samples had lower CL values compared with unprocessed samples, indicating that the cooking quality of these products improved. It is well-documented that the cooking quality of pasta products is influenced by the formation of protein network surrounding gelatinized starch granules in cooked pasta [14, 15, 26]. This could imply that the shearing process develops a gluten network, thereby reduces CL. Both total and soluble CL values decreased in shear-processed GS samples compared with the unprocessed samples. For the starch sample, the total CL of the sheared G_0 was higher than that of the unprocessed sample. This might be related to shear-induced fragmentation of granular starch, which occurs at ambient temperatures at moderate shear rates [3, 35].

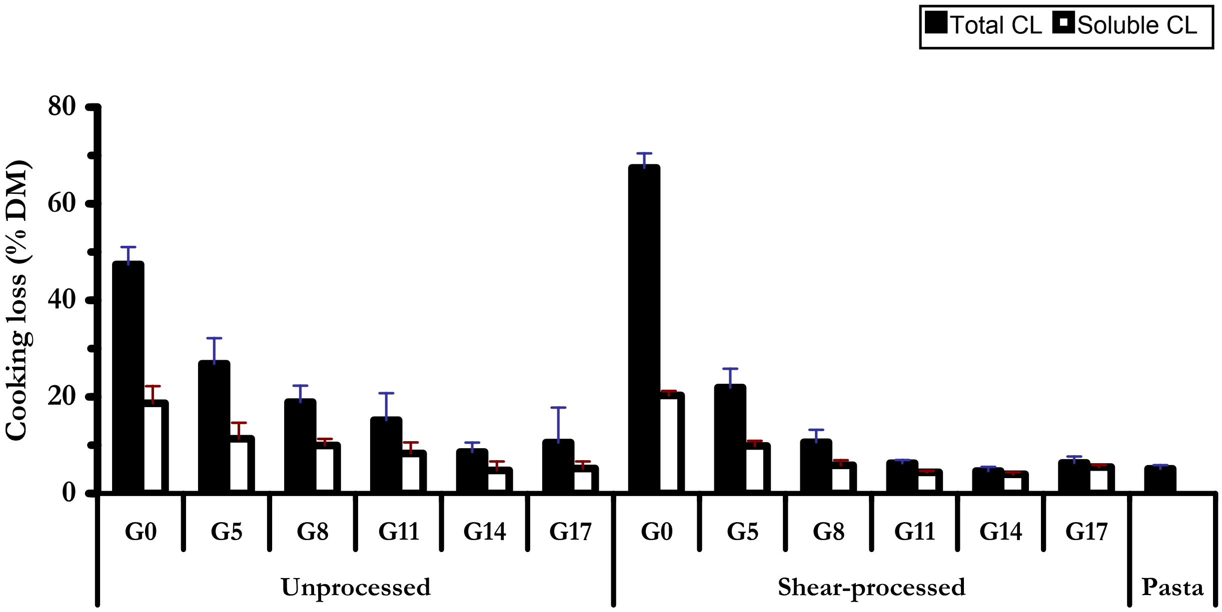

Fig. 4. Effect of shear processing and different gluten concentration on total and soluble cooking loss of cooked product. Error bars show 95% confidence interval.

Fig. 4 shows that increasing protein content in shear processed GS samples leads to a decrease in CL indicating that the cooking quality of sheared samples is related to the amount of protein in the mixture. However, CL data as a function of protein content appeared to level off for gluten content >11%. These results agree with numerous investigations indicating that the protein content contributes to the cooking quality of pasta products [8, 12-15].

The reduction of CL with gluten amount was also seen for unprocessed GS samples. This may be related to the covering effect of gluten protein on starch granules. A more discontinuous protein network was observed in unprocessed GS mixtures (CSLM results). According to Resmini and Pagani [26] the cooking of a weak or discontinuous protein matrix results in a loose protein structure. It is likely that increasing gluten content could provide better covering effect to the structure of the unprocessed GS samples.

Swelling index

The effect of shear treatment and protein concentration on the swelling index (SI) of the cooked samples is illustrated in Fig. 5. SI values of cooked processed samples were slightly higher than those of the unprocessed samples. This effect parallels the results of

CL and would indicate the effectiveness of the applied shearing regime in the structure formation of GS mixtures. There is a universal agreement that protein content is the primary factor influencing pasta quality (as measured by CL, SI and cooked firmness) and that gluten quality is an important secondary factor [12]. It has also been reported that swelling of pasta during cooking is dependent on the quality of the surrounding protein network [14].

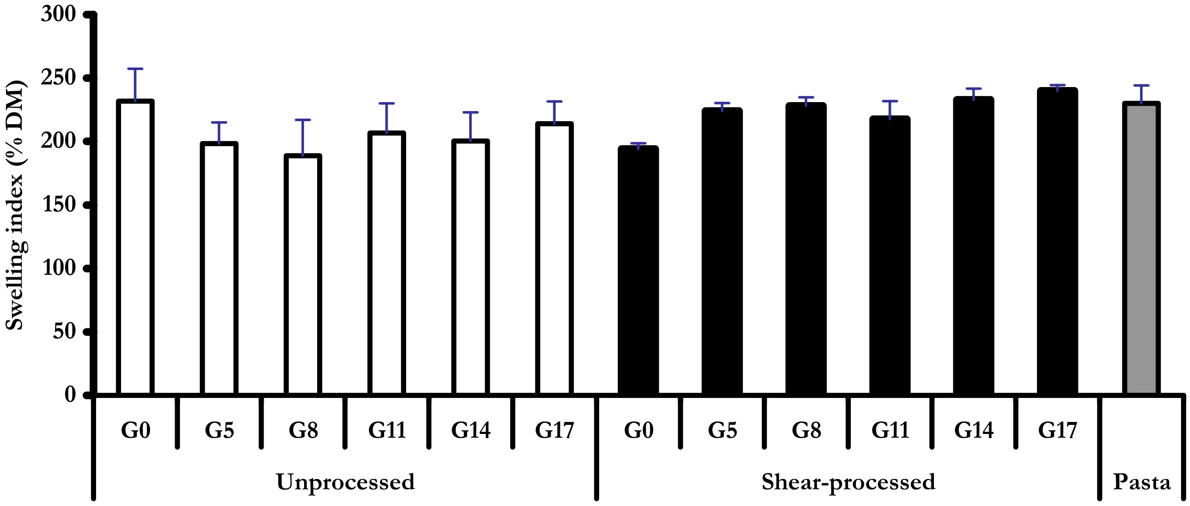

Fig. 5. Effect of shear processing and gluten amount on swelling index of cooked product. Error bars show 95% confidence intervals.

Fig. 5 shows an incremental trend for SI values as a function of gluten amount especially at a protein content of >14%. However, the differences are not significant (α=0.05). This agrees with the findings of earlier studies, which concluded that protein content does not affect SI of pasta products [11, 14]. The SI values of all shear-processed samples were in the range of commercial spaghetti.

Cooked firmness

Cooked firmnesses of both unprocessed and shear-processed GS samples were determined using a texture analyzer. For practical reasons (difference in the geometry and the size of GS samples with spaghetti), stress-strain data were extracted from force-deformation curves obtained by this instrument. Fig. 6 shows a stress-strain curve for the G8 sample (unprocessed vs. shear-processed) compared to spaghetti. A similar trend was observed for the other GS samples (results are not shown). According to Fig. 6, the maximum cutting stresses of shear-processed samples (G_8) are lower than those of the unprocessed samples, implying that the mechanical behavior of the product changed as a result of simple shear processing. The spaghetti sample is slightly firmer than shear processed GS samples. We are aware of the fact that, in principle, a direct comparison is not possible between freeze- dried samples and extruded durum pasta, which has been dried at higher temperatures. However, we were interested whether the mechanical behavior of the sheared product differed largely from the behavior of spaghetti. The mechanical behavior of GS samples and spaghetti was quite comparable.

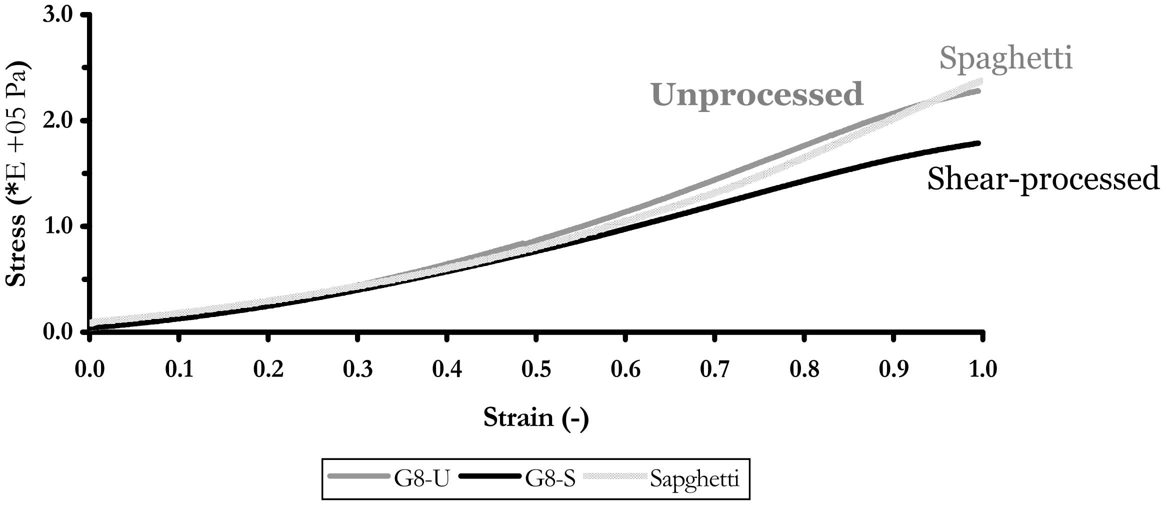

Fig. 6. Typical stress-strain behaviour of cooked G_8 sample (unprocessed vs. sheared) compared with spaghetti. Curves are mean of 7 replicates.

Maximum stress (σ_{max}) for unprocessed and shear processed samples are shown in Fig. 7. The maximum cutting stresses of all shear processed samples, except for G_{14}, were smaller than those of the unprocessed samples. Apparently, the shearing process led to a formation of a less firm structure. Cooking results (reduced CL) indirectly indicated the effect of simple shear in the formation of a protein phase in the product, which prevented the loss of starchy material during cooking. A less firm structure formed in the shear-processed samples might be explained by the lubrication effect of protein phase among starch granules in the dough [10], as discussed above. However, there is a reduction in σ_{max} after G_{14} sample. This is the point that the CL values also have reached to a plateau region, indicating ineffectiveness of further protein content increase in product behavior (possible change in the structure) after adding a given amount of protein.

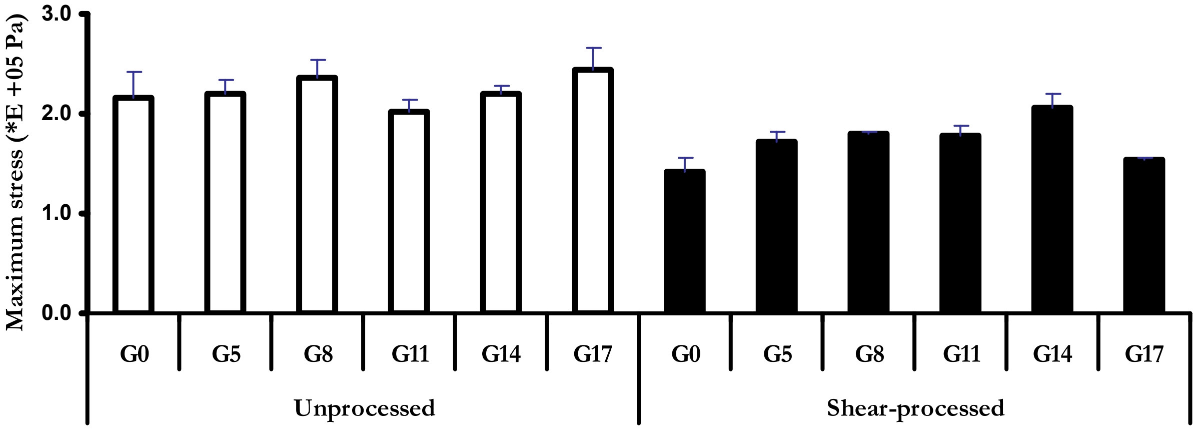

Fig. 7. Effect of shearing and gluten content on maximum cutting stress of cooked gluten-starch (GS) samples. Error bars show 95% confidence interval.

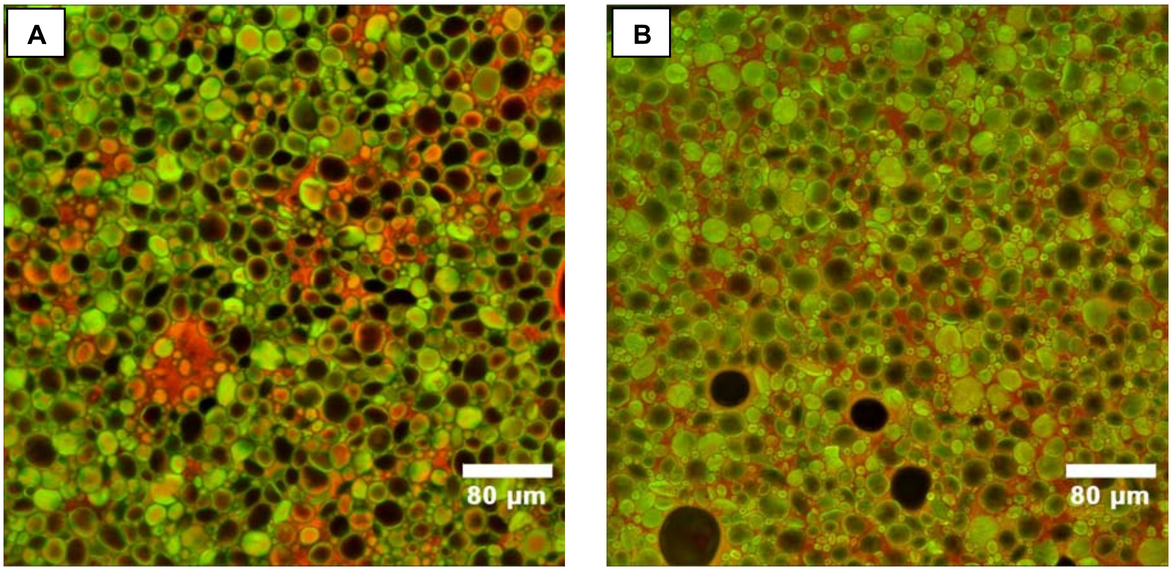

Fig. 8. CSLM images of unprocessed (A) and shear processed (B) gluten-starch (GS) dough. Green, starch granules; red, protein. Image size is 0.5×0.5 mm, bars=80 µm

Study of dough microstructure by CSLM technique

Aside from performing cooking experiments, the CSLM technique was used to visualize at a microscopic level the structure differences caused by the simple shear processing. Figs. 8A and B show the microstructure of unprocessed and shear processed G11 dough, respectively. In Fig. 8A, gluten proteins (in red) are in lumps and have not been distributed among the starch granules (in green) yet. Simple shearing resulted in a homogenous distribution of the proteins (Fig. 8B). It is clear from this picture that simple shear has resulted in the distribution of the gluten material throughout the sample. The starch particles are nicely dispersed in the gluten phase. The results of the CSLM pictures are in line with the results of the cooking experiments (CL, SI and cooked firmness), which also suggested a good dispersion of starch particle in the gluten phase. It can be concluded that simple shear is able to form a protein network.

Conclusions

A new method was introduced to study the effect of simple shearing processing on the cooking properties of pasta-like products obtained of wheat gluten-starch. The results of shearing experiments demonstrated the value of this method to study the effect of simple shearing on dough development and properties under process conditions relevant to industrial processing. The results of cooking experiments and stress-strain behavior of cooked products revealed the effect of both shear processing and protein content on cooking quality. The reduced CL values for shear-processed samples could be explained by the formation of a continuous protein phase in the sheared dough. This was confirmed by CSLM observation of dough microstructure.

Acknowledgement

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Chapter 3

Effect of simple shear on the physical properties of glutenin macro polymer (GMP)♣

Abstract

Gluten plays a key role in determining the end-use quality of wheat flour. The important role of the highly aggregated fraction of gluten, Glutenin Macro Polymer (GMP) in dough properties opens up new possibilities to reveal underlying mechanisms linking to dough processing. Using a new shear cell and advanced methodology to study the GMP, we investigated the effect of simple shear on physical properties of this fraction. Shear processing was compared with z-blade mixing, which involves both shear and elongational forces. Measurement of the amount of GMP and glutenin particle size distribution revealed large differences between simple shearing and z-blade mixing processes. In contrast to z-blade mixing, simple shearing at comparable levels of mechanical energy input, did not lead to a decrease in the wet weight of GMP or the size of glutenin particles. Confocal scanning laser microscopy of doughs showed that shear processing produced a homogeneously distributed protein matrix however in GMP extracted from the sheared dough, large clusters of particles were observed. On the other hand z-blade mixing led to disruption of these particles. Thus the type of deformation applied during dough processing is of crucial importance and in designing new equipments for dough processing the principles of different types of shear should be considered.

♣ Peighambardoust, S.H., Van der Goot, A.J., Hamer, R.J. and Boom, R.M. 2005. **Journal of Cereal Science**, 42:59-68.

Introduction

Proteins are recognized as the most important components governing bread-making quality of wheat [2, 34]. Gluten, composed of gliadins and glutenins, is the major wheat protein [20]. A main fraction of the glutenins can be isolated from wheat flour as a gel known as glutenin macro polymer GMP [6, 7, 10], which is insoluble in 1.5% (w/v) SDS solution. The importance of this highly aggregated glutenin and its composition in assessing wheat quality and predicting dough properties has been discussed in many recent studies [2, 7, 10, 19, 21, 24, 26, 32-34]. The composition and quantity of GMP are influenced by processing since the amount of GMP is strongly related to the mixing state of the dough [2, 6, 24, 34-36]. Don et al. [6] compared the effect of mixing energy (using a z-blade mixer and a pin mixer) on the physical properties of GMP. They concluded that the reduction in GMP content and glutenin size observed depended on the mechanical energy input in both mixing systems.

Mixing energy, however, does not completely characterize the mixing process. The nature of the mixing action is also important, but is more difficult to specify [3]. During mixing, dough is deformed by combination of shear and uniaxial elongation at high rates [18]. In a Farinograph mixer combination of rotational, shear and elongational components are involved, but in a Mixograph mixer the shear component is predominant [14]. However, it has also been reported that mixing action of a pin mixer is mainly elongational [9]. Thus the complicated nature of mixing makes it difficult to understand dough processing on a mechanistic level. Moreover, a quantitative relationship between type of mixing deformation and resulting dough properties is lacking. It is therefore important to take into account the individual impact of different types of deformation involved in dough mixing.

The effects of well-defined shear and elongational deformations on dough properties have been studied [5, 16, 22]. The different effects of shear or extensional, and their combination, on dough properties was demonstrated by Lee et al. [16]. They concluded that shear or extensional deformation alone did not produce dough quality, as judged by the amount of protein matrix comparable to that by a Farinograph mixer. However, comparable energy inputs for their different shearing regimes were not used and it seems likely that the energy input in their oscillatory tests were well below that occurring in, for example a Farinograph mixer. Consequently their results are difficult to interpret and no comparison with commercial processing is possible.

Mechanistic insight into the dough preparation process might be improved if comparable mechanical energy input and shear stress were applied when analysing the mixing process in terms of well-defined shear and extensional deformations. Van den Einde et al. [28] and Peighambardoust et al. (**Chapter 3**) introduced a new method based on a pilot-scale shear cell, which applied far higher shearing stresses (up to 50 kPa) and SMEs (up to 400 kJ/kg) comparable to the values used during industrial dough mixing and extrusion. Peighambardoust et al. (**Chapter 3**) using a well-defined shearing treatment obtained pasta like products with acceptable properties indicating formation of a continuous gluten network. Furthermore, even at high SME the products did not show signs of over-processing. This prompted us to study the effect of simple shearing on the physical properties of gluten at a more structural level, especially on its most functional part, the GMP fraction and compare it to the effect of z-blade mixing at comparable levels of energy input.

Experimental

Materials

Flours from Spring, a strong and hard Canadian wheat and Soissons, weak French wheat were kindly supplied by Wageningen Centre for Food Sciences (WCFS, Wageningen, the Netherlands). Both flours were from single wheat cultivars. Based on our previous study (**Chapter 3**), a gluten-starch (GS) mixture with 11% gluten (db) was added to the experimental material. Commercial wheat gluten and starch were from Roquette Co. (France). Sodium dodecyl sulphate (SDS) >99% purity was from Sigma. All other chemicals and staining agents used were at least of analytical grade.

Analytical methods

Moisture and ash content and the Farinograph characteristics of raw materials were determined using the AACC Approved Methods 44-15A, 08-01 and 54-21, respectively [1]. The protein contents (N × 5.7) of flour and freeze-dried GMP samples were determined by the Dumas method [23] using an NA2100 Nitrogen and Protein Analyzer (ThermoQuest-CE Instruments, Rodeno, Italy). Methionin was used as a standard. The chemical and physicochemical characteristics of the materials used in this study are presented in Table 1.

Preparation of zero-developed dough for shearing experiments

Zero-developed (ZD) dough was prepared in a walk-in-freezer (-18°C) by the method of Campos et al. [4] with modifications as described in **Chapter 3**. NaCl (2%, w/w) was used in the preparation of all ZD doughs. Moisture contents of 53.0, 48.9 and 51.0 % based on 14% moisture in flour were used for the preparation of GS, Soissons and Spring doughs, respectively. These values were selected to give a successful processing (no slippage) of material used in the shearing device and also a good handling (no stickiness) of product obtained. Frozen ZD doughs were kept in the freezer for shearing experiments.

Table 1. Chemical and physicochemical characteristics of wheat flour samples and GS mixture.

	Spring	Soissons	GS mixture
Moisture, % db	13.50	13.60	10.40
Ash, % db	0.57	0.48	0.41
Protein, % db	16.10	11.30	8.50
Farinograph:			
Optimal water absorption, % (14% moisture)	57.9	53.2	53.0
Development time or TTP, min	10.0	2.1	1.2
Stability time, min	18.0	1.0	0.2
Time to breakdown, min	33.0	2.5	1.2

Shearing experiments

Simple shearing was performed using the shearing device described in **Chapter 3**. The cone part of the shear cell filled in the freezer with 215-240 g amount of frozen ZD dough. The cone was sealed with a polyethylene film and the ZD dough held at 35°C for 60 min. During this period the ice particles melted, resulting in hydration of flours, producing a homogenous dough. The cone was then placed in the shear cell and the system was closed. The dough was rested for 30 min inside the cell to allow relaxation of stresses that result from the pressurized closure. At this stage, a dough sample was taken from the cell just prior to starting of shear run and designated "unprocessed" dough.

In all experiments the shearing process was performed at 30 ± 2°C for different processing times to obtain different torque curves, which resulted in different SMEs. The sheared samples were immediately frozen in liquid nitrogen after processing. The specific mechanical energy transferred to the material during shear processing was calculated from the following equation:

$$SME = \frac{\omega}{m}\int_{t=0}^{t_f} M(t).dt \qquad (1)$$

In this equation ω is the rotational speed (s^{-1}), m is the mass of material filled in the shear cell (kg), M (t) is the torque at time t (Nm), a measure of force applied to material to be deformed and continuously measured and recorded during the experiments and t_f is the shearing time (s). SME is expressed in kJ/kg and can be calculated at any time between t, 0 and t_f .

Mixing experiments

Mixing of flour as well as of the GS mixture was performed using a two-bladed, counter rotating batch mixer turning at a 3:2 differential speed (Do-Corder E330 equipped with a 300 g Farinograph bowl, Brabender OHG, Duisburg, Germany) and interfaced to a computer and controller unit (PL 2100, Brabender measurement and control systems, Duisburg, Germany). During mixing the torque was recorded continuously. Dough was prepared with flour and 2% (w/w) NaCl. Water was added according to method 54-21, AACC approved methods [1]. Mixing was performed at a speed of 63 rpm and a constant temperature of 30°C at different times. This resulted in different mechanical energy input on materials mixed. The processed samples were immediately frozen in the liquid nitrogen. The mechanical energy provided to the product during mixing was computed from the torque curve, following equation (1). In this equation, the rotational speed of slow mixing arm (63 rpm) was used for SME calculation.

Isolation of GMP from flour

A flour sample (5 g) was dispersed in 75 ml petroleum ether, mixed for 15 min and centrifuged (4150 g, 10 min at ambient temperature). Petroleum ether residues in the defatted flour sample were evaporated by keeping the flour overnight in a fume hood at ambient temperature.

GMP was isolated from flour as follows: the defatted sample was weighed (1.55 g) in an ultra-centrifuge tube of approximately 33 ml. The tube was then placed on a Vortex mixer. While vigorously stirring, 9.125 ml demineralised water was added with a calibrated dispensor. Mixing was continued for about 10 to 15 s until a homogenous, lump-free suspension was obtained. Immediately afterwards 3.875 ml 12% (w/v) SDS solution was added, followed by 9 ml demineralised water in two portions from a dispensor, to reach a final concentration of 1.5% (w/v) SDS. The tubes were centrifuged (80000 g, 30 min at 20°C) in a Kontron Ultracentrifuge. The supernatant (SUP) was decanted and the gel-like layer found on top of the starch sediment (GMP) was weighed immediately as GMP wet weight. This amount was expressed as grams of gel per 100 grams of sample (db). The isolation of GMP was repeated at least three times for each sample. The collected GMP in small tubes kept up to 24 h at ambient temperature before analysis. This storage of GMP in concentrated formhad no effect on the results.

Isolation of GMP from processed dough samples

The frozen processed samples were freeze-dried overnight to an average moisture value of 8% (w/w). The freeze-dried dough was then powdered on a Retsch mill using a sieve of 0.25 mm. GMP was isolated in at least three replications.

GMP dispersions

GMP dispersions were prepared through transferring ca. 1 g of GMP gel into a tube containing 10 ml 1.5% (w/v) SDS solution. Only the upper part of the GMP gel layer was carefully taken to minimise contamination of GMP gel with starch granules. The tube was sealed and installed on a Test-tube Rotator connected to an electrical rotor type RW20 (IKA-Labortechnik, Breisgau, Germany) and rotated at a speed of 60 rpm for at least 4 h at ambient temperature. After treatment, a visually homogenous, opalescent dispersion was obtained, which was used for further characterization.

Particle size analysis

The particle size distributions of GMP dispersions were determined by laser diffraction using Coulter LS 230 particle size analyzer (Beckman Coulter Inc., Hialeah, FL, USA) according to the method described by Don et al. [6].

Confocal scanning laser microscopy

GMP dispersions

Freshly prepared samples of GMP dispersions (ca. 0.1 mg/ml) were stained with 0.2% (w/v) Fluorescein isothiocyanate (FITC). FITC was added in a concentration of 1% (10 μl/ml dispersion). The stained GMP dispersions were incubated for least 1 h at ambient temperature before CSLM.

Dough samples

Frozen doughs were defrosted at 4°C for 24 h before CSLM observations. Slices with a thickness of ca. 1 mm and dimensions of approximately 0.5 × 1 cm were cut with a razor blade from both “unprocessed” and shear processed dough. Extra care was taken not to deform dough samples in this stage. A double staining technique using a combination of FITC (1% w/v) and Rhodamine B (0.1% w/v) in dimethylformamide (DMF) was applied. This allowed simultaneous observation of protein and starch under CSLM; FITC stains starch (green) and Rhodamine B stains protein (red). The stained slices were kept under small caps for 1-2 h at ambient temperature before observation. The microstructure of dough was observed using LEICA TCS NT (Leica Microsystems, Heidelberg, Germany) microscope according to the procedure described by Van de Velde et al. [27].

Results and discussion

Shearing and mixing experiments

The equipment used allowed continuous recording the torque during the processing of materials. Typical torque graphs for z-blade mixing and simple shearing experiments with a 45 min processing time are shown in Figs. 1A and 1B, respectively. Table 2 shows cumulative SME calculated based on Equation 1 from torque curves. The torque data for the other processing times followed the same trend (not shown), indicating good process reproducibility. Comparison of torque curves and SME data (Table 2) for two processes indicates that simple shearing resulted in much higher torque values (5-6 times in average torque) and SME inputs as compared to z-blade mixing.

Fig. 1A shows the typical Farinograph curves for Spring (a), Soissons (b) and GS-mixture (c). Spring is a strong flour with a long stability time, whereas Soissons flour and GS-blend are weak with short stability times. This figure also demonstrates an initial hydration phase of 3-5 min that is not seen in simple shearing (Fig. 1B) because ZD dough was used as the starting material.

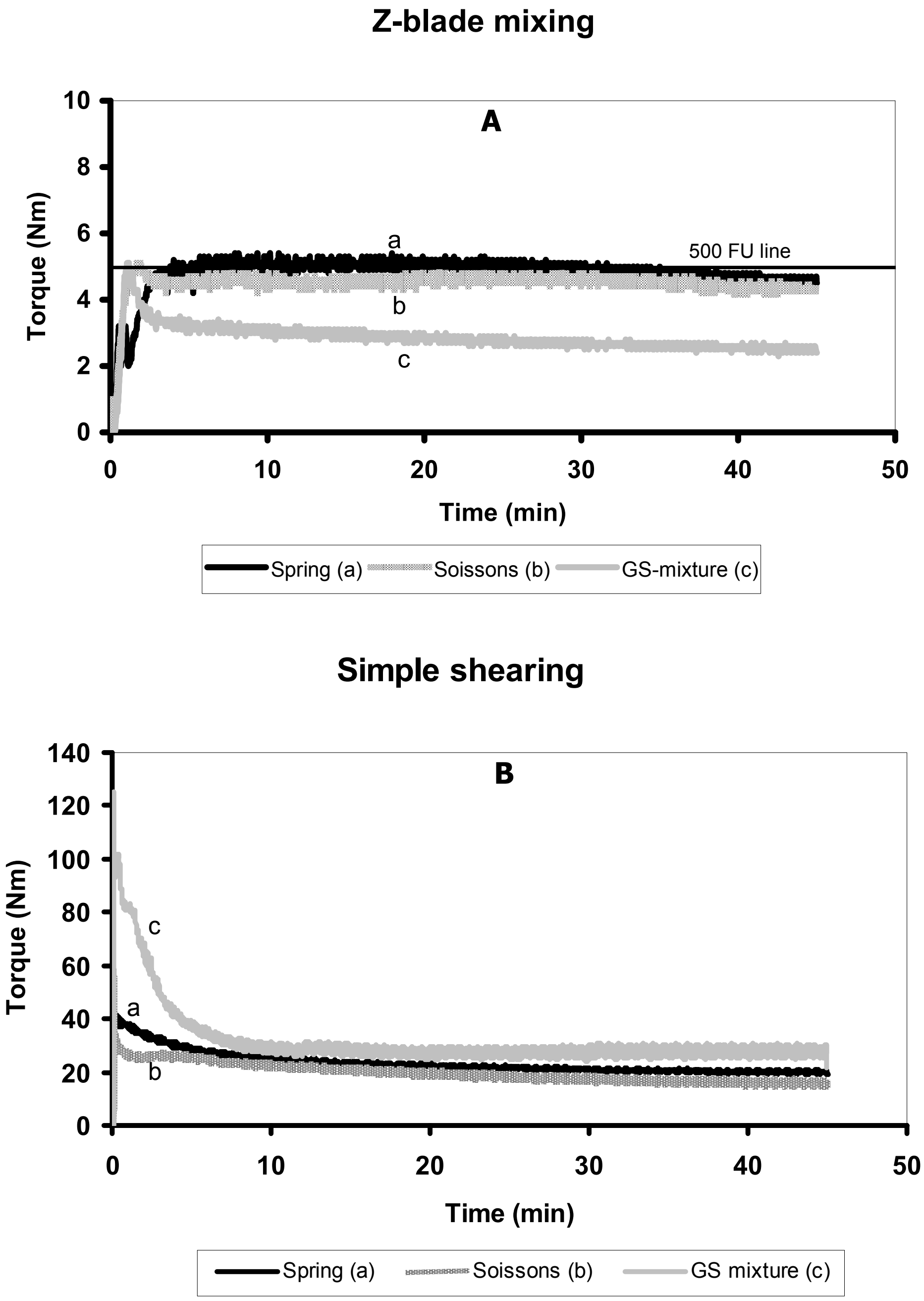

Fig. 1. Typical changes in torque values vs. processing time for the experimental materials processed via z-blade mixing (A) and simple shearing (B).

Since preparation of ZD dough at Farinograph moisture content led to difficulties in processing and handling the dough, the moisture content of wheat doughs was reduced by 6-7 %. For comparison, ZD dough at the same moisture content as the shearing experiments (6-7% less moisture), was processed in the z-blade mixer. The results (asterisks in Fig. 3B) showed that the lower moisture content in the range indicated in z-blade mixing had no influence on the breakdown of GMP.

Changes in the microstructure of dough as affected by simple shearing

The effect of simple shearing on the physical properties of GMP is the main interest of the current study. The CSLM was used to visualize the differences in the structure of dough before and after shear processing. Figs. 7A and B (in **Chapter 3**) show the CSLM images of the microstructure of the hydrated (MC=43%) GS dough (with no energy input) and the shear processed GS dough (for 15 min), respectively. In **Chapter 3**, Fig. 7A shows protein rich spots or aggregates (in red) as spread around starch granules (in green). This image represents the original non-deformed protein network in the hydrated GS dough with no energy input. Simple shearing resulted in formation of a continuous protein phase in the dough structure (Fig. 7B, **Chapter 3**). Thus simple shearing changes the protein aggregates in the hydrated GS dough into a continuous phase surrounding most of starch granules.

Table 2. SME data vs. processing time for experimental materials used. Moisture content of material based on a 14% standard moisture (in farinograph)

Simple shearing						Z-blade mixing					
Spring (m=51.0%)[a]T		Soissons (m=48.9%)[a]		GS mixture (m=53.0%)[a]		Spring (m=57.9%)[a]		Soissons (m=53.2%)[a]		GS mixture (m=53.0%)[a]	
Time (min)	SME (kJ/kg)	Time (min)	SME (kJ/kg)	Time (min)	SME (kJ/kg)	Time (min)	SME (kJ/kg)	Time (min)	SME (kJ/kg)	Time (min)	SME (kJ/kg)
5	39	5	44	2	41	5	15	2.5	6	2	6
9	68	15	108	4	60	12	44	6	21	4	12
15	92	30	194	6	95	20	76	12	45	9	24
30	175	45	232	9	125	33	128	20	78	15	40
45	277			15	195	45	181	30	117	22.5	62
				30	246			45	189	30	75
				45	413					45	110

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[a] Moisture content of material based on a 14% standard moisture (in farinograph)

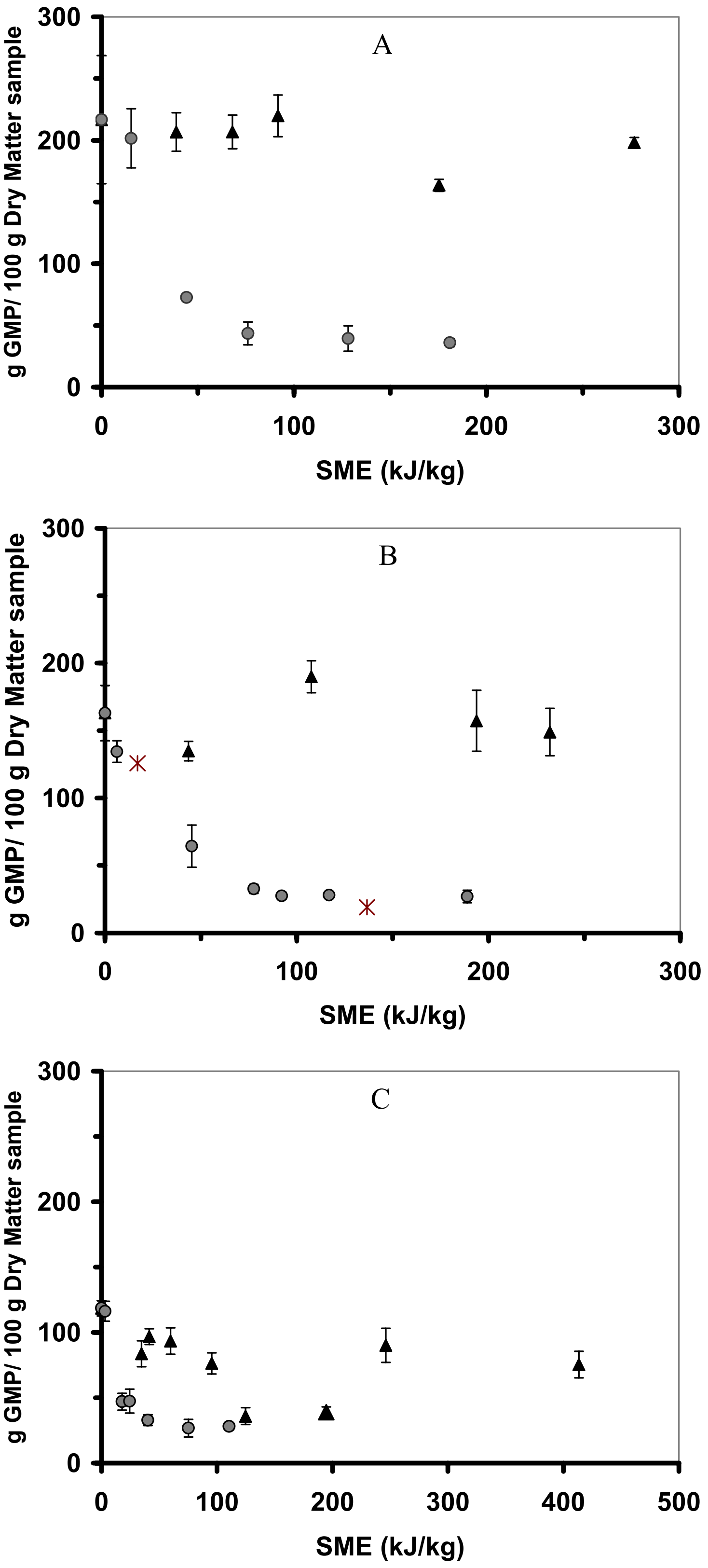


Fig. 3. The effect of simple shearing (▲) and z-blade mixing (●) on the GMP wet weight of Spring (A), Soissons (B) and GS mixture (C). Error bars show 95% confidence interval. In Fig B, asterisks indicate z-blade mixed samples from zero-developed doughs with lower moisture content comparable with the simple shearing experiments.

Effect of simple shearing and z-blade mixing energy on GMP wet weight

The effects of simple shearing and z-blade mixing on the wet weight of GMP extracted from samples taken at various SME values are shown in Figs. 3A, B and C. Z-blade mixing led to a decrease in the GMP wet weight for all varieties. In particular, the GS mixture was dissociated rapidly by z-blade mixing. The loss in GMP during z-blade mixing is in complete agreement with previous results [2, 6, 24, 25, 35]. Apparently the biaxial deformation or elongational flow during z-blade mixing causes break up of the glutenin particles, which are then found in the supernatant phase as shown in a recent study by Don et al. [8]. In contrast, simple shearing did not lead to a decrease in the GMP wet weight in either Spring (Fig. 3A) or Soissons (Fig. 3B) cultivars. However, the result for GS-mixture was slightly different as is seen from Fig. 3C that in simple shearing an initial decrease of GMP wet weight was followed by an increase. The mechanism behind this observation is not clear.

Changes in protein distribution between different phases of centrifuged samples as affected by simple shearing and z-blade mixing

The effect of simple shearing and z-blade mixing on flour protein was analysed using four arbitrarily selected samples. Ultracentrifugation of flour and processed dough suspended in an SDS solution leads to three distinct layers: SUP, GMP gel and starch sediment. The protein distribution between these three phases for the two wheat varieties after simple shearing and z-blade mixing is shown in Fig. 4. The GMP wet weight of the samples before and after processing is also indicated by dots (♦) in Fig. 4 (secondary Y axis). The amount of proteins (expressed as percentage) in the GMP gel layer for both varieties decreased considerably with z-blade mixing but remained constant or even increased (for Soissons) during simple shearing indicating that that the GMP gel becomes more concentrated after the simple shearing.

For z-blade mixing, a decrease in the amount of proteins in the GMP gel layer coincided with an increase of protein concentration in the SUP (Fig. 4). Z-blade mixing results in de-polymerisation of glutenins into smaller fragments. Due to their smaller size, these fragments remained in the SUP phase after ultra-centrifugation. This was not observed in simple shearing where the deformation is not likely to break up larger protein aggregates to the same extent as the z-blade mixing. They remain insoluble and stay in the GMP gel layer. Lee et al. [17] observed an increase in the amount of SDS-soluble proteins extracted from partially-developed (sheared) dough as compared to those present in the native flour. Even at the high level of energy input used, we observe only a slight increase in the protein content of SUP phase with simple shearing, and conversely the amount of proteins in the GMP fraction was not decreased by simple shearing. According to the mass balance (Fig. 4), the amount of protein in starch phase decreased with simple shearing. This could explain slight increase of the protein content in SUP of sheared samples. We expect that the results presented in Fig. 4 are representative for other processed samples.

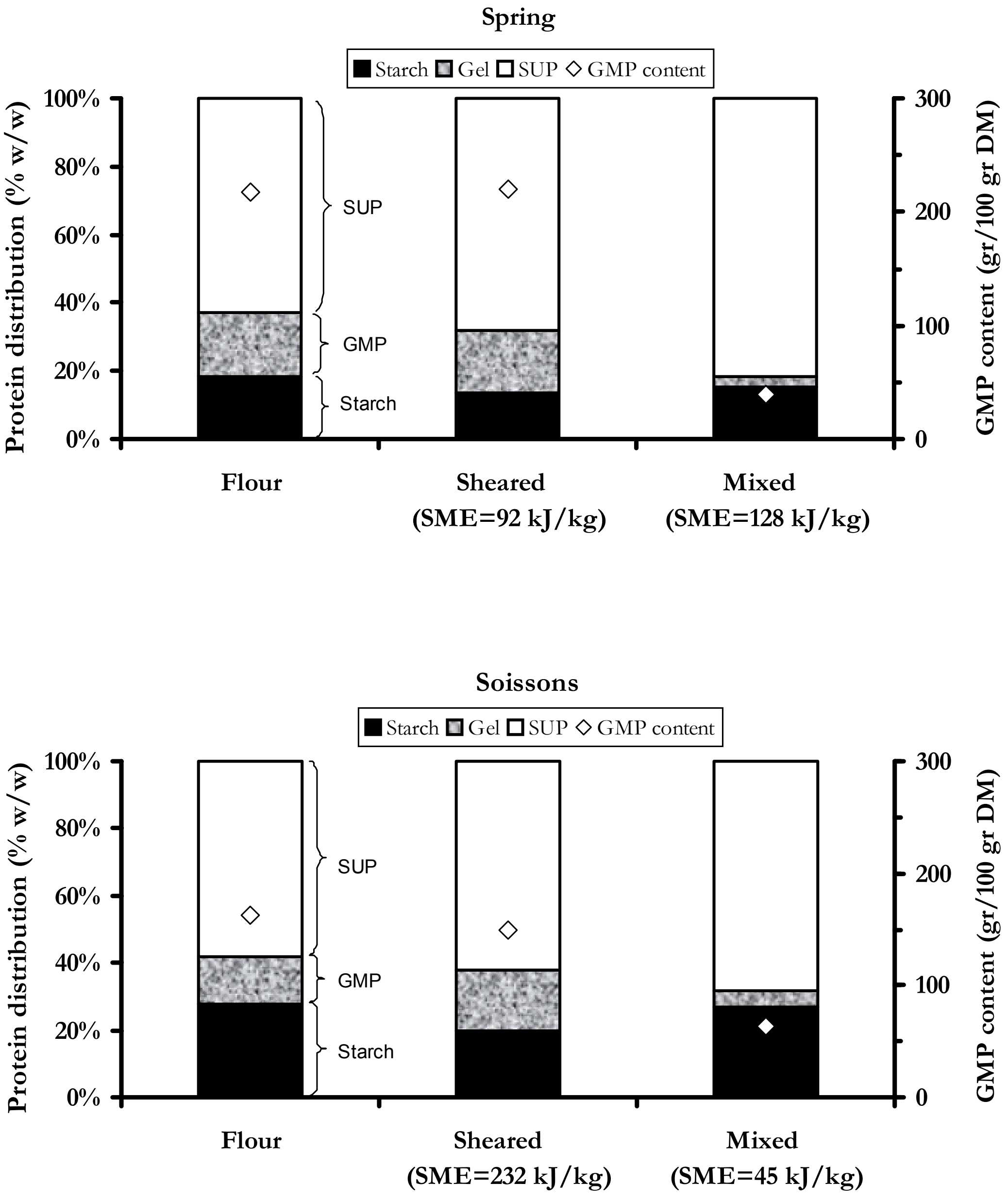


Fig. 4. The effect of shearing and mixing energy on the protein distribution (in three phases of centrifuged samples) and the GMP wet weight of Spring and Soissons varieties.

Changes in glutenin particles size distribution during z-blade mixing and simple shearing

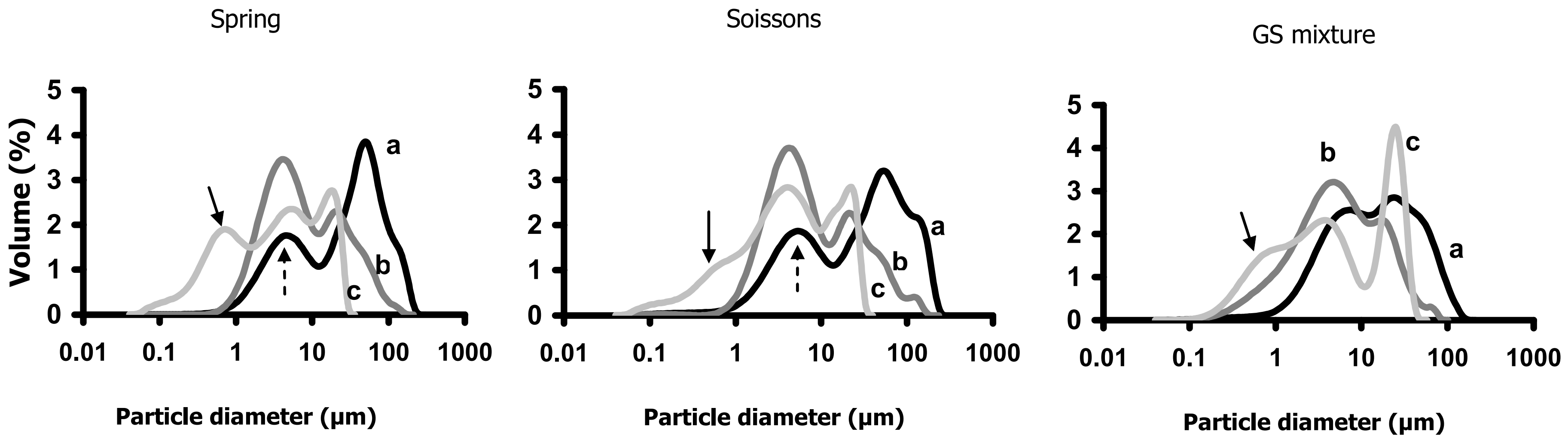
To further confirm that simple shearing did not affect glutenin particles, we analysed the glutenin particle size distribution in GMP dispersions extracted from experimental samples before and after processing. These are presented in Figs. 5 and 6. The particle size distribution curve for flour sample (curve "a" in Fig. 5) is characterized by two main peaks. The peak with smaller particles (1-10 μm) is generally considered to be mainly B-type starch particles [30, 31]. The peak with larger particles consists mainly of the glutenin particles. However, the particle size distributions of these samples show that starch particles are inevitably present in the GMP.

The composition of GMP fraction was estimated taking into account the measured protein content and the presence of SDS. The protein content in GMP gels extracted from flour and processed (sheared and mixed) dough was in a range of 16-27 % (w/w, db). If we exclude the possible interactions of SDS with other components of wheat flour, a calculation based on dry matter mass balance reveals that 22-30% (w/w) of GMP fraction consists of SDS. It seems likely that the remainder of GMP dry matter is starch.

We therefore may assume that the peak indicated by dotted arrows (Fig. 5) represents starch particles. However, all over-mixed samples show a third peak (solid arrows in curves "c" - Fig. 5) indicating that very small protein particles are formed. These represent glutenin particles with a diameter less than 10 μm.

Z-blade mixing led to a decrease in size of glutenin particles. Fig. 5 shows that the glutenin peak in three different materials shifted to the left, indicating a decrease in particle diameter, as shown by others [6, 11, 24, 35]. In contrast, Fig. 6 shows that shear processed samples have the same particle size as original flour; in some cases, even larger particles were observed (sheared Soissons and GS mixture).

The effect of mechanical energy on surface weighted mean diameter ($D_{3,2}$) of the glutenin particles is shown in Fig. 7. The $D_{3,2}$ of glutenin particles decreased rapidly with z-blade mixing as a function of SME, whereas with simple shearing the $D_{3,2}$ value increased. This confirms that the size of glutenin particles was not decrease by simple shearing, even at high levels of energy input. $D_{3,2}$ is a general parameter and thereby affected by all material present in GMP dispersions including starch. Nevertheless, the change in $D_{3,2}$ observed could be explained by changes in GMP starch content, but clearly from Fig. 6, the increase in $D_{3,2}$ values of sheared Spring and Soissons samples could also be a result of an increase in size of glutenin particles.

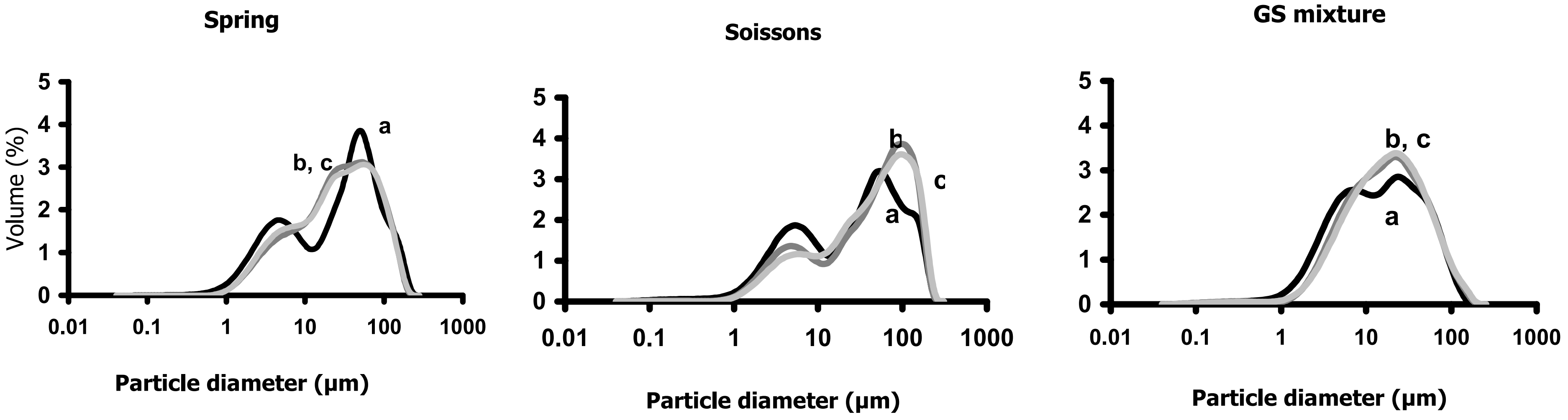


SME (kJ/kg): a) **0**, b) **44,** c) **18**

SME (kJ/kg): a) **0**, b) **45,** c) **189**

SME (kJ/kg):a) **0**, b) **24,** c) **75**

Fig. 5. Typical changes in the size distribution of particles in GMP dispersions for Spring, Soissons and GS mixture before (**a**) and after z-blade mixing (**b** and **c** at different SME inputs).



Spring

SME (kJ/kg): a) **0**, b) **175,** c) **277**

Soissons

SME (kJ/kg): a) **0**, b) **108,** c) **194**

GS mixture

SME (kJ/kg):a) **0**, b) **125,** c) **246**

Fig. 6. Typical changes in the size distribution of particles in GMP dispersions for Spring, Soissons and GS mixture before (a) and after simple shearing (b and c at different SME inputs).

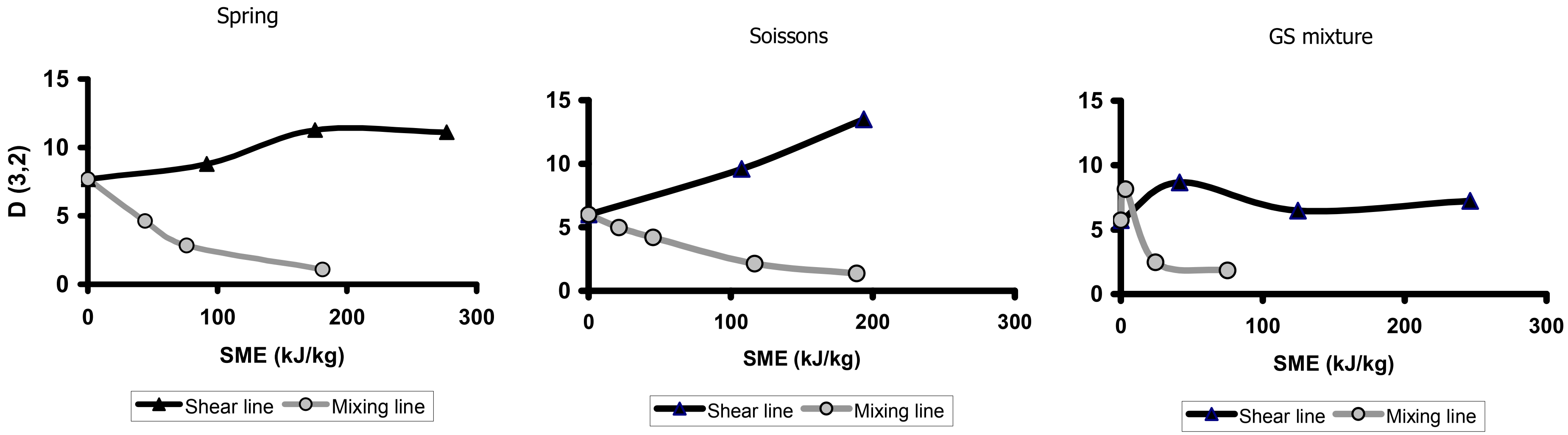


Fig. 7. Effect of the shearing and mixing energy on D3,2 value of particles in GMP extracted from Spring, Soissons flours and GS mixture.

The decrease in amount of GMP and glutenin particle size with z-blade mixing reconfirmed the findings of Don et al. [8], that the combination of shear and extensional deformations by a z-arm mixer leads to a reduction of the amount and size of glutenin particles. This is also in agreement with the study of Hamer et al. [12] who concluded that mechanical energy is the predominant actuator in GMP breakdown during mixing. The results presented in Figs. 5, 6 and 7 indicate that simple shearing is not effective in GMP breakdown. An explanation for this phenomenon may be provided by the results of Walstra [29] who showed that elongational flow is more effective in emulsion droplets break up than simple shear flow. Simple shear may lead to deformation of glutenin particles, thereby reducing the forces on the particles. Break up of the particles is then prevented, while the deformation may lead to aggregation of the glutenin particles into larger strands. This might result in an increased effective particle size and can explain the stability of glutenin particles in a simple shear field.

In this study, the SME values (Table 2) were greater than the values normally seen in over-processing of dough in z-blade mixing process. Don et al. [6] showed that in terms of GMP properties, over-processing may be well correlated to the mechanical energy input in the mixing system. With the high level of mechanical energy transferred to the material, it was expected that the sheared samples would qualitatively show signs of over-processing characterised by a typical wet, sticky dough with an "over-mixed" sheen [13]. However, shear processed material was clearly not over-processed. Moreover, no negative influence of the applied mechanical energy was seen on GMP physical characteristics (Figs. 3-8). Perhaps, this energy tolerant behaviour could be explained by the absence of extensional deformation in the shearing device used.

CSLM characterization of glutenin particles as affected by z-blade mixing and simple shearing

GMP dispersions extracted from Soissons flour and its processed dough were examined by CSLM. The images of glutenin particles isolated from the flour and processed samples are presented in Fig. 8. The arrows in Fig. 8A show some of the spherical particles in glutenin from Soissons flour. Fig. 8B shows that all particles disappeared after mixing (after TTP, SME=45 kJ/kg). In contrast, even at a higher mechanical energy input, simple shearing did not lead to the disruption of glutenin particles. Fig. 8C demonstares clusters of glutenin particles in GMP extracted from shear processed dough (at SME=194 kJ/kg).

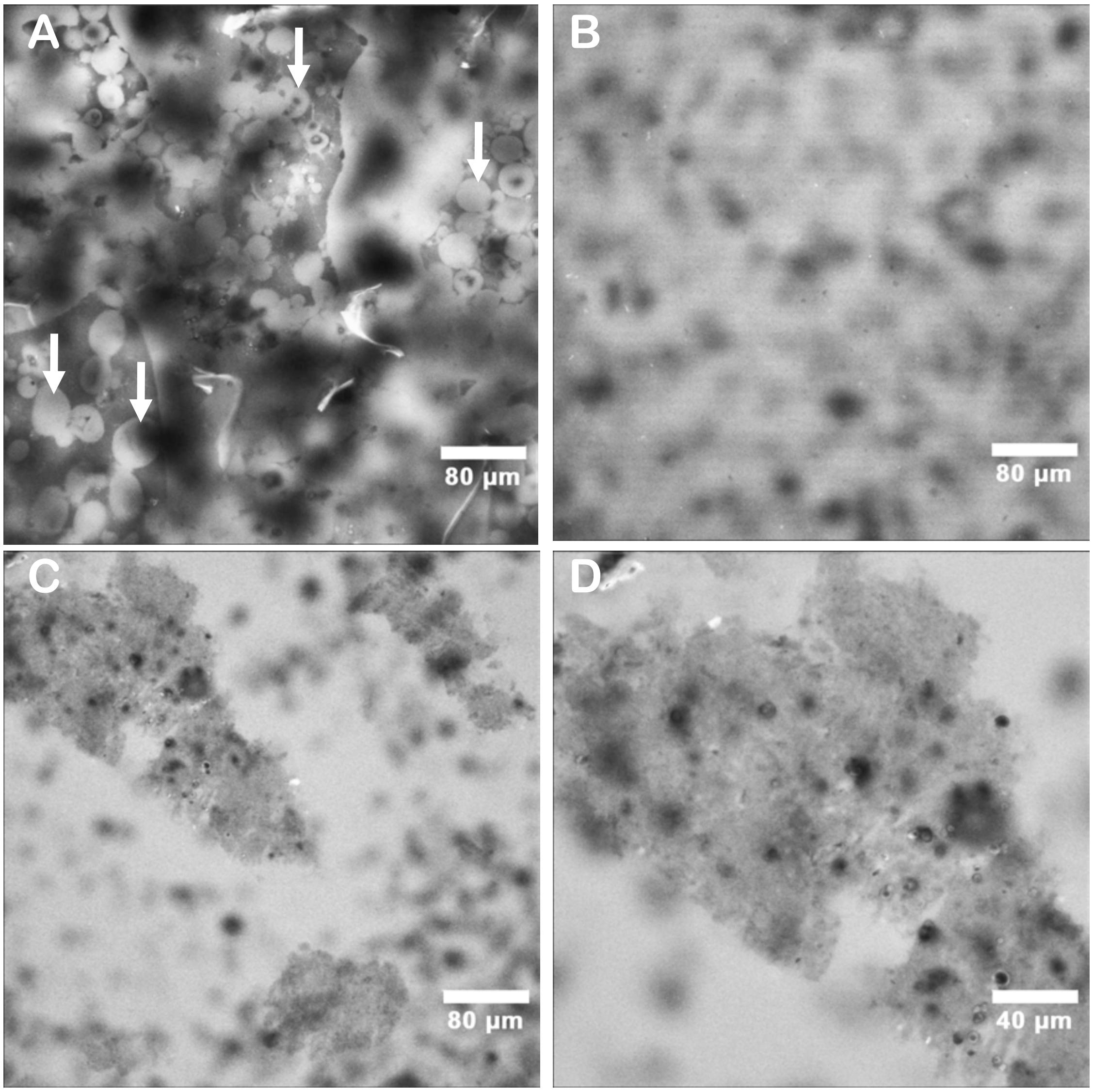


Fig. 8. CSLM images of glutenin particles isolated from Soissons flour (A), mixed dough for 12 min at SME = 45 kJ/kg (B) and sheared dough for 30 min at SME=194 kJ/kg (C and D). Scale bars in figures A, B and C correspond to 80 µm length (image size of 500×500 µm). In Figure D, scale bar shows 40 µm length (image size of 250×250 µm).

These findings may be compared with those of Ker and Chen [15] who reported shear-induced structuring of a concentrated soy protein isolate at high shear fields. The different structures formed with simple shearing may have consequences for practical applications. The resulting properties of the product will be an important subject for future research.

Conclusion

This study focused on the effects of simple shearing of dough compared with standard z-blade mixing, on the physical properties of the glutenin macro polymer. It was concluded that, in addition to the total energy input during dough processing, the type of deformation applied is of crucial importance. In contrast to z-blade mixing, simple shearing at comparable levels of work input, did not lead to a decrease in the wet weight of GMP or the size of glutenin particles. The CSLM study of the GS dough microstructure, showed that simple shearing changes the protein aggregates in hydrated GS dough into a continuous protein phase, which envelops most starch granules. In dispersions of GMP extracted from simple sheared dough large clusters of particles could be identified. Whereas these particles were disrupted in z-blade mixing. Additionally, shear processed materials showed a higher energy tolerant behaviour. All results could be explained by taking into account the reduced capability of simple shearing to break up particulate structures, compared to traditional mixing process.

Acknowledgement

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Chapter 4

Microstructure formation and rheological behaviour of dough under simple shear flow[♣]

Abstract

In a z-blade mixer, both shear and extensional deformations contribute to the development of structure in dough. We compared the effect of simple shearing versus z-blade mixing at similar levels of work input, on the microstructure and uniaxial extensional properties of dough prepared from two flours of different strength. With respect to microstructure, mixing initially increased the formation of coarse protein patches, leading to a heterogeneous dough structure with a high fracture stress (σ_{max}) and significant strain hardening. These parameters decreased with prolonged mixing. This was accompanied by loss of glutenin macro polymer (GMP) wet weight and formation of a more homogenous microstructure. Prolonged mixing typically led to an over-mixed state. In contrast, prolonged simple shearing did not affect GMP content, strain hardening and gave enhanced shear banding. CSLM revealed that short-term simple shearing induced structure formation in the direction of the shear flow for both flour types, followed by formation of shear-banded gluten structures both parallel and perpendicular to the direction of shear flow. Uniaxial extension of dough oriented parallel or perpendicular to the shear field did not reveal anisotropy. Apparently, the observed heterogeneity on a scale of "mm" was not relevant for this type of rheology. Nevertheless, a relative weakening of dough strength (reduced fracture stress) was observed as a function of long-term shearing. This seems to be related to a local segregation effect caused by differences in visco-elasticity between the gluten phase and the starch granules. The results of this study reveal important features of the dough processing and underline the importance of not only work input, but also the type of deformation applied.

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Introduction

Mixing flour with water results in an even distribution of the ingredients and the development of a continuous gluten network [22, 36]. According to Bloksma and Bushuk [6], mixing has three distinct functions in the development of the dough: distribution of materials, hydration, and energy input to develop a protein structure. Therefore understanding the role of mixing energy is an essential step towards optimising wheat dough development [38]. During the mixing process, dough is formed by a combination of shear and elongational deformations at high strain rates [31]. According to Jongen et al. [24], a z-blade mixer provides a combination of rotational, shear and elongational deformations on the dough, which makes it difficult to understand dough mixing on a mechanistic level. It is therefore interesting to compare the effect of well-defined shear deformation with z-blade mixing on dough properties.

Several researchers [10, 28, 38] have studied the effects of either well-defined shear or elongational deformations and their combination (using a Farinograph mixer) on dough properties. Lee et al. [28] showed different effects of shear or extensional deformation versus their combination on dough properties. They concluded that pure shear or extensional deformation alone cannot produce a dough quality (in which a high quality was defined as a high fraction of protein matrix), comparable to that obtained by a Farinograph mixer. However, they did not use comparable amounts of energy input for their different treatments.

We previously compared the effect of simple shearing with z-blade mixing on physical properties of a highly aggregated fraction of gluten, glutenin macro polymer (GMP), at a relevant work input level (**Chapter 3**). We found that, compared with mixing, both GMP wet weight and the size of the glutenin particles were stable upon simple shearing. The importance of the amount as well as composition of GMP in assessing wheat quality and predicting dough properties has been emphasised in many recent studies [3, 14, 32, 37]. Thus, the stability of GMP under simple shear conditions might lead to the formation of different structures in the dough compared to those normally seen in mixed doughs. On a structural level, we expect the formation of a gluten network that is relatively stable upon simple shearing. Moreover, the visco-elastic properties of gluten may not be drastically changed under simple shear conditions. This prompted us to further investigate the effect of simple shearing on the microstructure of dough using confocal scanning laser microscopy (CSLM). This technique allowed observation of dough microstructure in a depth of about 20-30 μm.

Changes in dough microstructure and its visco-elastic properties will influence the rheological behaviour of the dough. According to Bloksma [7], the macroscopic behaviour of a given dough, like that of any material, depends on its composition and microstructure (spatial arrangement of its constituents). It has also been reported that the molecular structure of a dough (i.e. type of bonds) directly affects its rheological properties [30]. Dobraszczyk and Morgenstern [13] showed a correlation between the molecular structure of dough (presence or absence of long-chain branching in glutenin subunits) and large-deformation rheology. Numerous studies have confirmed that the rheological behaviour of wheat flour dough at large deformation is dominated by the gluten fraction [13, 25, 39, 40, 42]. Moreover, according to Tronsmo et al. [42], large-deformation rheological methods are better suited for characterising flour doughs with respect to protein quality than small deformation methods (dynamic oscillatory testing and creep recovery). Thus, large-deformation rheology provides a basis to study structural changes in the protein phase of the dough microstructure, which has been shown to account for its visco-elastic behaviour and end-use quality [5, 17, 23].

In the current study, the effect of well-defined shear processing was compared with a z-blade mixing process using CSLM and large-deformation rheology. Specific objectives were: 1) to elucidate dough microstructure and gluten network formation as affected by simple shear and z-blade mixing, and 2) to study the large-deformation fracture properties of dough using the uniaxial extension test.

Experimental

Wheat flour samples and physicochemical characteristics

Flour from Spring, a strong and hard Canadian, and Soissons, a weak French wheat cultivars were used. Both flours were from single wheat cultivars. The physicochemical characteristics of these flours are shown in Table 1.

Preparation of undeveloped dough for experiments

An undeveloped dough was prepared according to the method of Campos et al. [9] with modifications described previously (**Chapter 3**). Moisture content 45% (based on 14% moisture in flour) was used in preparation of undeveloped dough. This moisture content allowed successful processing (no slippage) and handling (no stickiness) properties.

Table 1. Physicochemical analysis of the materials used

	Soissons flour	Spring flour
Moisture (%)	12.70	13.30
Ash (% db)	0.48	0.57
Protein (% db)	11.30	16.10
Farinograph:		
Water absorption* (%)	53.2	57.9
Arrival time (min)	10.5	7.9
Time-to-peak (TTP; min)	15.0	16.0
Stability (min)	21.0	25.1
Time to breakdown (min)	31.5	33.0

* 14% moisture basis

Simple shearing

Simple shearing (further abbreviated to shearing) was performed using a cone and plate shearing device (shear cell) as described earlier in **Chapter 2**. Frozen ZD dough (240 g) was put in the bottom cone of the shear cell inside the walk-in-freezer. The cone was then covered with a moisture tight plastic film and kept at 35°C for at least 60 min before each shear treatment. This holding period allowed the ice particles to melt, resulting in hydrated ZD dough.

Shear experiments were carried out at rotational speeds of 10-20 rpm corresponding with shear rates of 24-48 s^{-1} at temperature of 27-32°C for different processing time ranging from 9-45 min. The shear cell used could provide shear stresses up to 40 kPa. The specific mechanical energy (SME) applied to the material during processing was calculated from the following equation:

$$SME = \frac{\omega}{m}\int_{t=0}^{t_f} M(t).dt \qquad (1)$$

In this equation ω is the rotational speed (s^{-1}), m is the mass of material filled in the shear cell (kg), M (t) is the torque at time t (Nm), a measure of force applied to material to be deformed and continuously measured and recorded during the experiments and t_f is the shearing time (s). SME is expressed in kJ/kg and can be calculated at any time between t, 0 and t_f .

Mixing experiments

Mixing trials were carried out using a two-bladed, counter rotating batch mixer turning at a 3:2 differential speed (Do-Corder E330 equipped with a 300 g Farinograph bowl, Brabender OHG, Duisburg, Germany) and interfaced to a computer and controller unit (PL 2100, Brabender measurement and control systems, Duisburg, Germany). The Farinograph mixer will be called “z-blade mixer” or “mixer” afterwards.

Dough was prepared with flour, water and 2% (w/w) NaCl. Water was added at both optimal absorption levels (Table 1) according to AACC approved methods (method 54-21; [1]) and at reduced water contents (at the amounts as described for shearing experiments). Mixing was performed at a speed of 63 rpm and a constant temperature of 30°C at different times. This resulted in different mechanical energy inputs onto the materials mixed. The mechanical energy provided to the product during mixing was computed from the torque curve, following Equation 1. In this equation, the rotational speed of slow rotating arm (63 rpm) was used for SME calculation.

Sampling of dough for microstructural analysis

After shearing and mixing, half of the resulting dough was immediately frozen in liquid nitrogen and kept at –18°C for subsequent CSLM observations. A successful shear run (no slippage) in the shearing device was characterised by the presence of lines on the surface of the dough. Both the cone and the plate were serrated at contact surface with material. This gave dough having clear lines on the top and bottom surface after shearing. The direction of the shear flow was perpendicular to these lines.

Sampling of dough for extension testing

Two samples were taken from the sheared dough: one, taken parallel to the shear direction, was designated “P” and the other, taken perpendicular (transverse) to the shear direction, was encoded as “T”. A piece of dough, approximately 7 × 4 cm, suitable for studying in the SMS/Kieffer Dough and Gluten Extensibility Rig (Stable Micro Systems, Godalming, Surrey, UK) was cut using a razor blade. The dough sample was placed immediately in a pre-warmed (30°C) Petri dish. To prevent any moisture loss, the dough was covered by a glass cap and placed in a humid atmosphere (RH=85%) where it was kept at 26°C for 45 min. This period allowed handling and transporting of dough specimens for extension testing.

After mixing, a piece of dough (approximately 20 g) was rounded gently into a small ball and kept as described for the sheared samples. The sampling of the dough for both the shear and mixing processes was carried out with as little manual manipulation as possible.

Observation of dough microstructure

The frozen dough samples were transferred to a cryo-microtome (Micron CR50-H, ADAMAS-instrumenten, Rhenen, the Netherlands) at a temperature of –20°C. The cryo-microtome was used not only to remove dough surface irregularities and thereby to produce a very flat surface, but also to scrape off the outer layers of the dough. This made the inner part of each sample accessible for microscopic observation. The resulting dough slices, 3–5 mm thick, were then placed onto glass slides and kept at –18°C before CSLM observations.

Frozen dough samples were defrosted at ambient temperature. A combination of fluorescein isothiocyanate (FITC) and Rhodamine B (1% and 0.1% w/v, respectively) in dimethylformamide was used for non-covalent labelling of starch and proteins. This method allowed simultaneous observation of protein and starch by CSLM. FITC stains starch (green) and Rhodamine B stains protein (red). The stained doughs were stored at ambient temperature for at least 1 h before observation. A small glass cap was put over the sample to prevent moisture loss during staining and observation. The microstructure of the dough was observed using a LEICA TCS NT (Leica Microsystems, Heidelberg, Germany) microscope, according to the procedure described by van de Velde et al. [43].

All samples were studied at both low (image size of 2 × 2 mm) and high (image sizes of 1 × 1 or 0.5 × 0.5 mm) magnifications. Low magnification images normally show a better perspective of the overall dough structure, whilst higher magnifications provide better characterisation of the sample microstructure.

Image analysis

To quantify CSLM images with respect to the amount of protein matrix within the dough samples, Image J 1.32J freeware (a public domain, Java-based program developed at the US National Institute of Health) was used. CSLM images were loaded into the software and RGB (Red-Blue-Green) colour splits were made. In some cases the images had shading as a result of unwanted deviations in the laser/detector settings or variations in the background light from fluorescent tubes. To reduce this artefact, FFT filtering [46] was applied to all CSLM images. The resulting image (corrected shading) was then subjected to automatic thresholding, which converts the red image into binary (black and white) mode [18]. We used automatic thresholding for all images to allow a good comparison. The area fraction in each thresholded image was then calculated and reported as the protein matrix value (PMV) in percentage.

Analysis of GMP

Portions of the sheared and mixed doughs were rested for 60 min at 30°C and ca. 90% RH, and then frozen in liquid nitrogen. The frozen samples were then freeze-dried overnight to an average moisture content of maximum 7% (w/w). Isolation of GMP was performed according to the method described in **Chapter 3**.

Extension testing

A texture analyser (TA.XT2) equipped with a 5-kg load cell and a Kieffer Dough and Gluten Extensibility Rig was used to perform a uniaxial extension test on the processed dough samples according to the procedure of Smewing [41]. Dough test specimens were prepared by placing a piece of dough (4×6×2 mm) onto the grooved base of the Kieffer mould. The mould was pre-warmed to 30°C before mounting the dough. Using thin Teflon strips (2×60 mm) coated with silicon oil helped to prevent stickiness of dough to the grooves of the mould. The upper half of the mould was placed in position and tightly clamped, which distributed the dough over the channels to yield an equivalent number of dough strips of uniform geometry (3×5×53 mm). The dough was rested inside the mould at 26°C and 90% RH for 45 min. This resting period was followed 45 min resting of the dough after sampling and before moulding. Therefore all extension tests were carried out after a total resting time of 90 min

After the relaxation time, the tension in the clamp was released and the mould was transferred to a temperature and humidity controlled cabinet (26°C, ca. 90% RH), where the TA had been placed beforehand. Before doing the extension test, the mould was equilibrated to the atmosphere of the surrounding cabinet for 10 min. The individual dough strips were then separated from the Teflon strips, positioned across the SMS/Kieffer Rig dough holder, and immediately tested with the TA at a hook speed of 3.3 mm/s and a trigger force of 2 g.

After each extension test, maximum resistance to extension (R_{max}) and extensibility (Ext) were determined. The measurement of the extensibility was prone to error, as it was affected by the dough sticking to the hook. Therefore, as an alternative parameter, the distance at peak force (Ext_2) was used [42]. The force–displacement curves were transformed into stress–strain data according to the method described by Dunnewind et al. [16], taking into account changes in the dimension of the specimens being extended and assuming a constant volume for the dough sample. Uniaxial extension fracture properties were computed from the stress–strain data obtained. The maximum stress or fracture stress ($\sigma_{\max}$) sustained by the specimen; the Henky strain (ε_H) at fracture stress, and the integrated area (A) under the stress–strain curve were taken as a measure of the resistance to extension, the extensibility, and the energy required for extension, respectively. From the stress–strain plot it is possible to calculate strain hardening value ($d\ln\sigma/d\varepsilon_H$). In this study, an *apparent* strain hardening was computed in the strain interval 20 to 95% of fracture strain for all test specimens. This range provided a good data fit (R^2=0.96-0.99) to calculate $d\ln\sigma/d\varepsilon_H$ as an *apparent* strain hardening values. Furthermore, the *apparent* strain hardening did not significantly vary with the selection of the strain interval. Differences between mean values of fracture parameters calculated from stress–strain curves were assessed for significance using t-test [11].

Results

Shearing and mixing experiments

The equipments used allowed us to continuously record the torque during the processing of materials. Typical torque versus time graphs for mixing and shearing of Soissons and Spring flours are shown in Fig. 1(A-C). Farinogram data of these two flours are given in Table 1. Figs. 1A and B demonstrate the mixing behaviour of experimental materials at both Farinograph water absorption and at reduced moisture contents. As can be seen from these figures, reduction of moisture content led to higher torque and consequently higher specific mechanical energy (SME) values.

Fig. 1C demonstrates the behaviour of the materials under simple shear processing. As can be seen from this figure, the torque decreased right from the start of the shear run for both wheat cultivars. This can be explained by an inertia effect which exists at the starting point and caused a decrease of the torque value over several minutes because of the elastic nature of the system. According to this figure, Spring showed lower torque values than Soissons. This could be due to a more pronounced gluten–starch separation in case of Spring, leading to a reduced resistance against shearing. On the other hand for mixing trials, Spring showed higher torque values. Apparently, different break up mechanisms taking place in the shearing and mixing are responsible for the different torque behaviour. With z-blade mixing glutenin particles are disrupted and the energy required is related to the initial size of these particles [15]. The glutenin particles in Spring flour are larger in size ($D_{3,2} \approx 8$) than those in Soissons ($D_{3,2} \approx 5.5$) (**Chapter 3**). Therefore, more energy (torque × time) is required in the case of Spring.

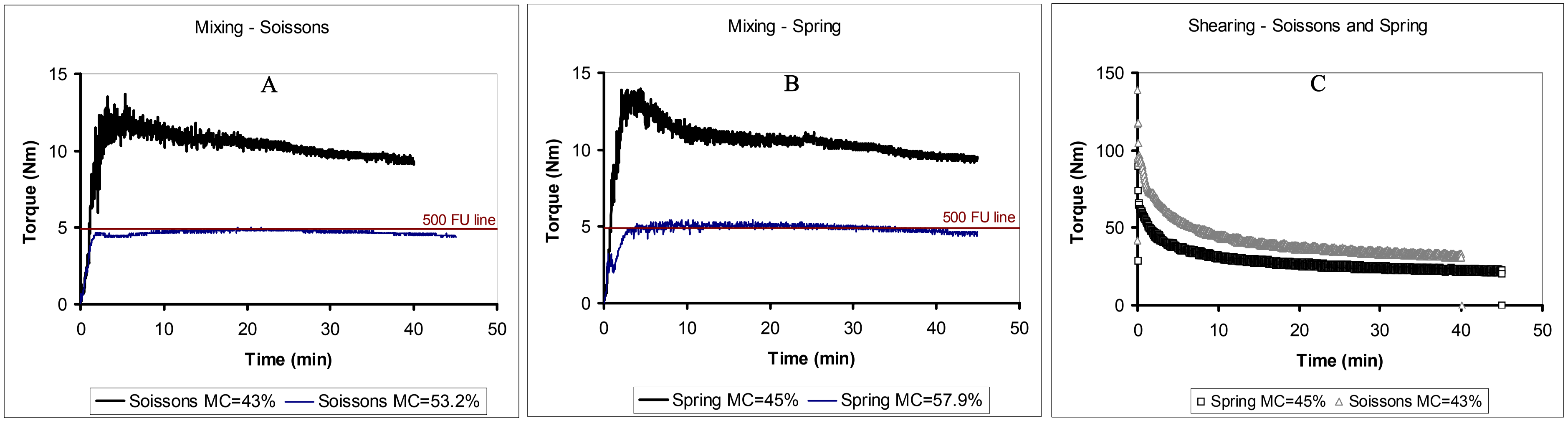

Fig. 1. Typical torque-time curve for Soissons (A) and Spring (B) doughs at Farinograph water absorption level as well as reduced water content in mixing, and at reduced water content in shearing experiments (C)

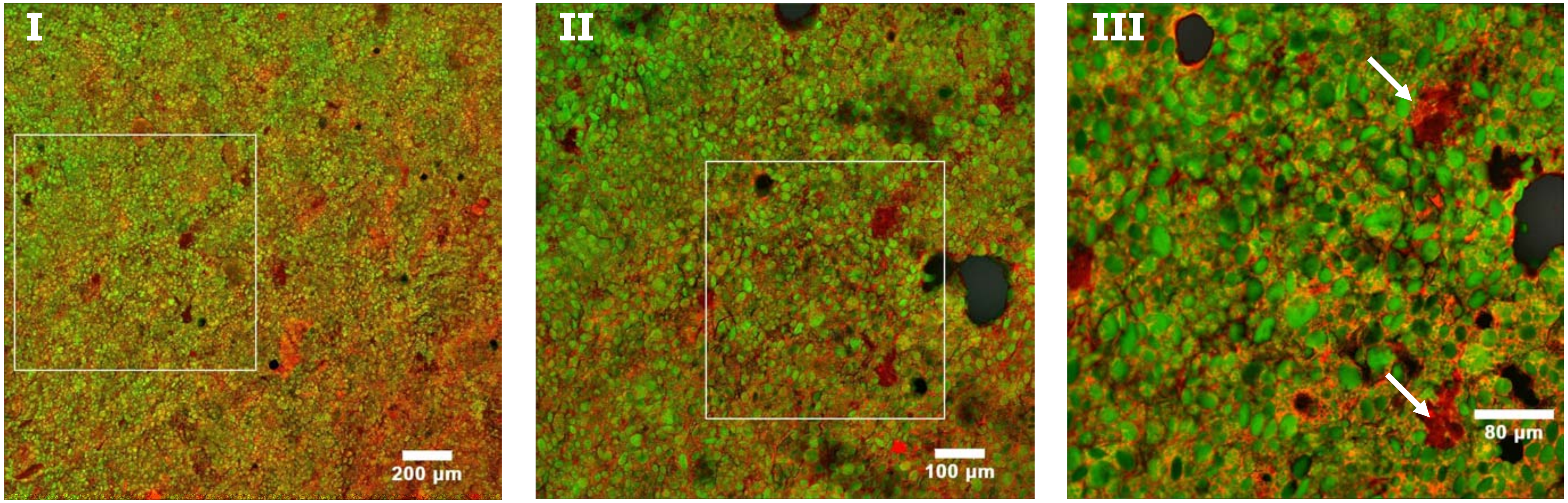

Fig. 2. CSLM images of zero-developed Spring dough at three different magnifications: (I) image size of 2 × 2 mm; (II) image size of 1 × 1 mm; (III) image size of 0.5 × 0.5 mm. Green, starch; red, protein. Squares in images I and II represent the positions that magnifications have been made. Arroows indicate protein rich spots.

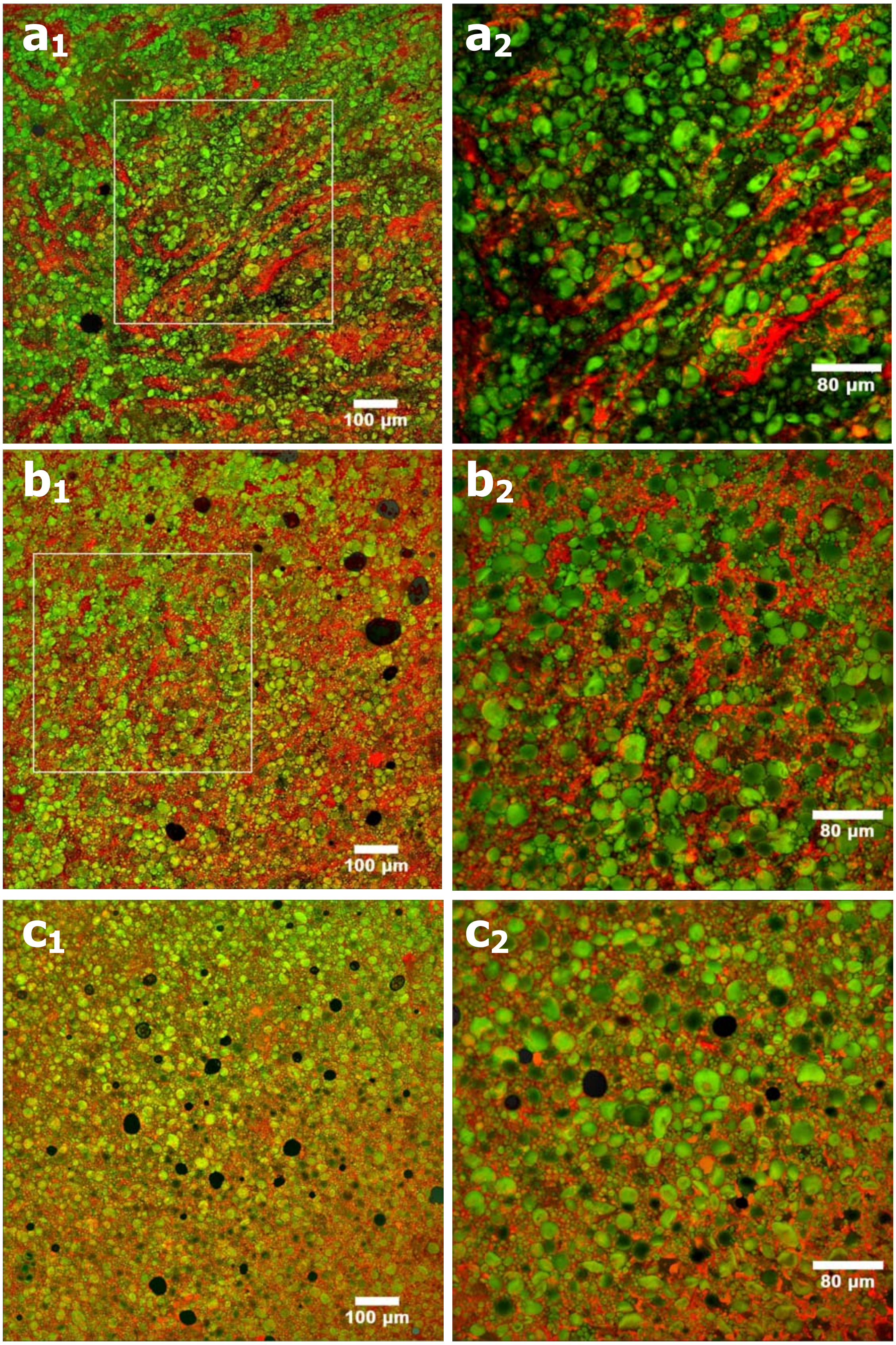

Fig. 3. CSLM images of Soissons dough after mixing at 9 min (a_1,a_2), 20 min (b_1,b_2) and 40 min (c_1,c_2). Green, starch granules; red, protein. Images a_2–c_2 (1×1 mm, bars=100 μm) are higher magnification images of a_1–c_1 (2×2 mm, bars=200 μm) at positions pointed by squares.

Both mixing and shearing experiments were carried out at different processing times, leading to different mechanical energy inputs into the dough. Table 2 shows the calculated SME values at different processing times for the two wheat cultivars (at lower moisture content).

Table 2. Variation of SME values as a function of processing time for two wheat cultivars used

Spring				**Soissons**			
Mixing		Shearing		Mixing		Shearing	
Time (min)	SME (kJ/kg)	Time (min)	SME (kJ/kg)	Time (min)	SME (kJ/kg)	Time (min)	SME (kJ/kg)
12	121.8	12	150.6	9	81.7	9	134.2
25	246.0	25	236.3	20	188.7	20	253.5
45	419.6	45	306.6	40	369.7	40	439.0

CSLM study of dough microstructure

Microstructure of "unprocessed" dough

ZD dough was used for shear experiments. The hydrated ZD dough with no energy addition just prior to a shear run was designated as "unprocessed" dough. CSLM images of unprocessed Spring dough at different magnifications (I–III) are shown in Fig. 2. Images I and II give an overall view of dough microstructure. Image III, taken at a relatively high magnification, demonstrates the dispersed protein phase (red) around the starch granules (green). The black spots are air bubbles. Protein rich spots (indicated by arrows) in this figure are formed due to aggregation of hydrated gluten. A similar structure was seen for Soissons unprocessed dough (results not shown). Unbehend et al. (2004) reported a three-dimensional framework of gluten aggregates extending between starch granules in a hydrated flour–water system prepared with no energy addition, which is also evident in Fig. 2III.

Microstructure of mixed dough

CSLM images of mixed Soissons and Spring dough as a function of process time are shown in Figs. 3 and 4, respectively. Two different magnifications for each processing time are included in these figures to allow a better interpretation of changes in dough structure. Short-term mixing of Soissons dough (Fig. 3a$_1$ and a$_2$) led to the appearance of coarse protein domains in the dough structure.

In these pictures (especially Fig. 3a$_2$), discontinuous protein rich domains, and areas with mainly starch granules are visible, demonstrating structure inhomogeneity in the dough. Increasing mixing time (Fig. 3b$_1$ and b$_2$) transformed the discontinuous protein domains to a homogeneous gluten phase.

Short-term mixing of Spring (Fig. 4a$_1$ and a$_2$) also transformed a fine and spread protein phase of unprocessed dough (Fig. 2) into a coarse and heterogeneous gluten structure. This is in agreement with the findings of others [8, 33, 35], who showed an initial extensive formation of coarse protein filaments at the early stages of dough mixing. Further mixing of the Spring dough (Fig. 4b$_1$) led to formation of a layered protein structures (better visible in Fig. 4b$_2$). Amend et al. [2] stated that the kneading process extends the gluten structures, thereby making the surface area larger and the depth smaller. Thus, a great number of extended protein fibrils transform into films, forming a continuous gluten phase.

Prolonged mixing of both Soissons (Figs. 3c$_1$ and c$_2$) and Spring (4c$_1$ and c$_2$) formed a fine gluten structure with maximum homogeneity at a length scale larger than 100 μm. This observation corresponds with the SEM study by Amend et al. [2] of an over-mixed dough. They reported that heavy stretching perforates the gluten films to form a closely interconnected network of fine gluten fibrils, which surround all starch particles.

Microstructure of sheared dough

CSLM images of sheared Soissons and Spring doughs as a function of processing time (a–c) are shown in Figs. 5 and 6, respectively. Short-term shearing of both Spring and Soissons doughs led to the formation of heterogeneous protein structures at a length scale of more than 200 μm. In these structures, distinct protein rich and protein poor (starch granules) domains can be seen. In the short-term sheared Soissons and Spring doughs, large protein patches were formed in the direction of the shear flow (the direction of the arrows). Despite the difference in the rate of the shear stress applied, this observation is in general agreement with the SEM study by Schluentz et al. [38], who reported the formation of a protein matrix in the direction of the shear flow in a partially developed dough subjected to a shear deformation.

Further shearing of Soissons dough for 20 min (Fig. 5b$_1$, b$_2$) led to further development of the protein phase, oriented in the direction of the shear flow. The large protein domains in Fig. 6b$_1$, b$_2$ (Spring dough sheared for 25 min) were not solely aligned in the direction of simple shear flow any more.

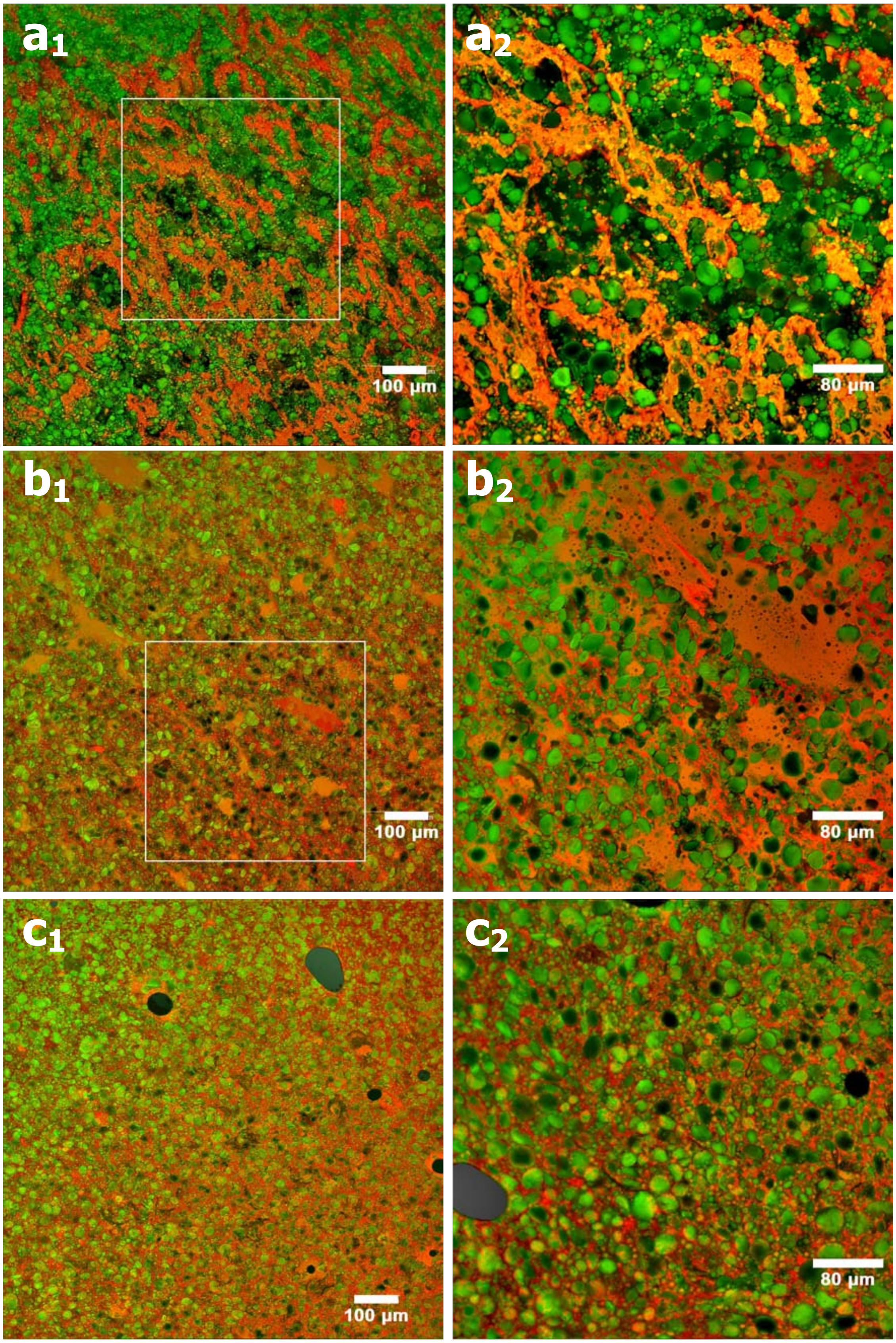

Fig. 4. CSLM images of Spring dough after mixing at 12 min (a_1,a_2), 25 min (b_1,b_2) and 45 min (c_1,c_2). Green, starch granules; red, protein. Images a_2–c_2 (1×1 mm, bars=100 µm) are higher magnification images of a_1–c_1 (2×2 mm, bars=200 µm) at positions pointed by squares.

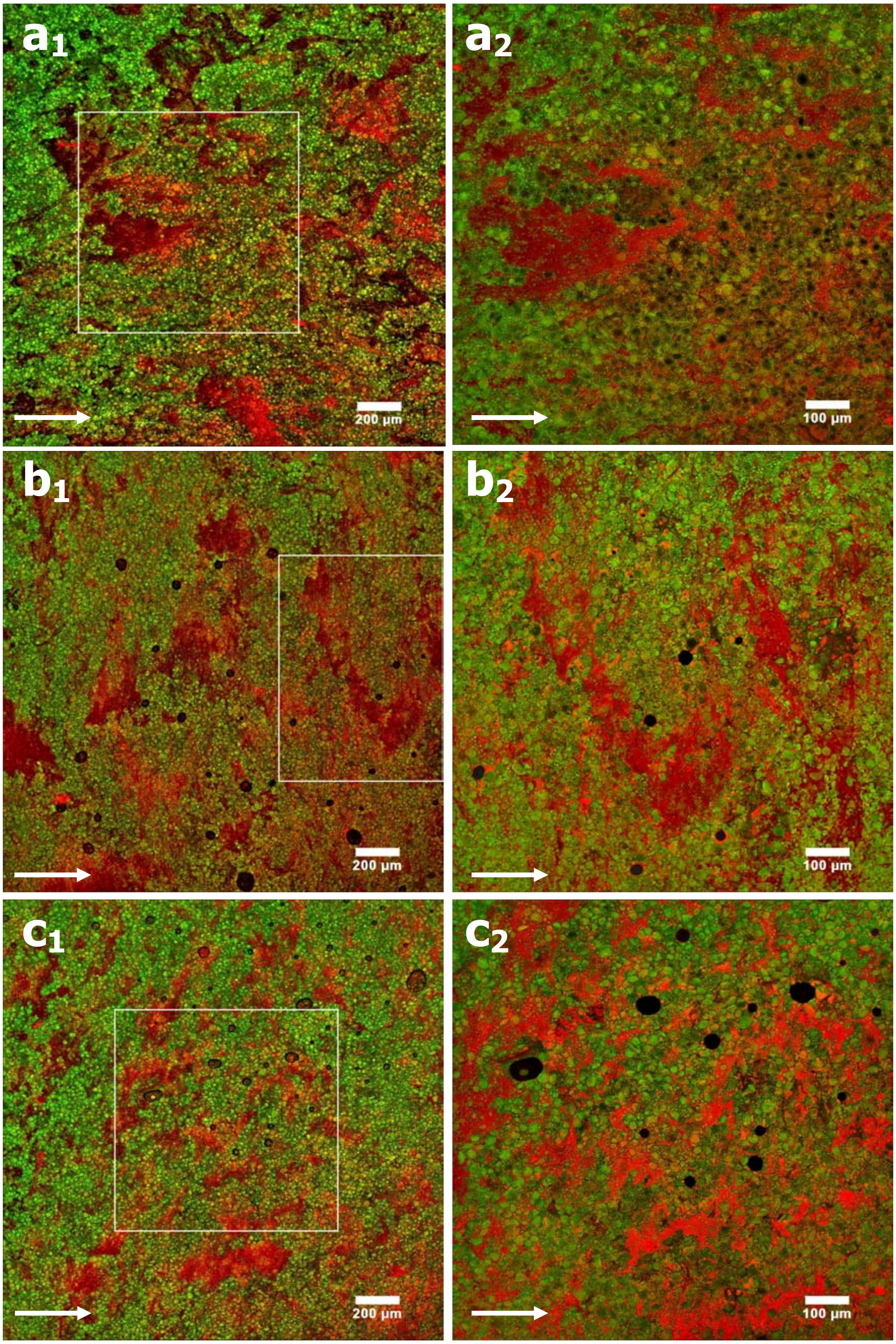

Fig. 5. CSLM images of Soissons dough after shearing at 9 min (a_1,a_2), 20 min (b_1,b_2) and 40 min (c_1,c_2). Green, starch granules; red, protein. Arrows indicate the direction of shear flow. Images a_2–c_2 (1×1 mm, bars=100 μm) are higher magnification images of a_1–c_1 (2×2 mm, bars=200 μm) at positions pointed by squares.

Long-term shearing of dough affected dough structure differently depending on the type of flour used. In the case of Soissons, a longer shear time (Fig. 5c$_1$, c$_2$) broke up the large protein domains into smaller fragments, which led to formation of a homogeneous protein phase, embedding the starch granules. Spring dough, sheared for 45 min (Fig. 6c$_1$), still showed large protein patches of a few hundred micrometers. Fig. 6c$_2$ shows one of those patches, formed perpendicular to the direction of the shear flow. Comparing the effect of long-term shearing of Spring (Fig. 6c$_1$, c$_2$) with that of Soissons (Fig. 5c$_1$, c$_2$), revealed a difference in the degree of dough homogeneity. It seems that the quality of gluten (its ability to aggregate) correlates with this phenomenon.

Image analysis

Image analysis was used to compare the area fraction of the protein matrix (PMV) in the dough samples (see experimental section), taking the common assumption into account that the area fraction correlates well with the volume fraction. The PMVs calculated from CSLM images of both Soissons and Spring dough before and after simple shearing at two image sizes of 1×1 mm (high magnification) and 2×2 mm (low magnification) are shown in Table 3. This table also shows PMVs for CSLM images of mixed doughs at different stages of mixing.

Table 3. Protein matrix values (PMV)[a] calculated from analysis of CSLM images (two different magnifications were taken only for simple shearing process)

	Image size of 1 × 1 mm		Image size of 2 × 2 mm	
Type of dough	**Soissons**	**Spring**	**Soissons**	**Spring**
Zero-developed	43±2.9	43±8.1	41±7.5	35±8.4
Sheared[b]				
S	40±5.7	28±4.6	29±2.9	30±2.3
M	42±1.3	40±1.5	37±1.7	41±1.8
L	34±1.1	44±3.4	35±0.9	33±1.4
Mixed[b]				
S	36±1.3	32±1.5		
M	49±0.8	49±0.9		
L	47±2.5	50±1.1		

[a] Means ± SD calculated using at least two CSLM images

[b] S, M and L stand for short-, middle- and long term processing, respectively, as described in Experimental section.

Short-term processed (S) dough samples of Soissons at high magnification and Spring at low magnification showed significant reduced PMVs compared to those calculated in the unprocessed doughs from these flours. This indicates aggregation of proteins at early stages of dough mixing and shearing. However, increasing the treatment time with both shearing and mixing increased the PMV (Tables 3, compare M with S), demonstrating an ongoing re-distribution of proteins in the dough. Long-term simple shearing of Spring dough (at low-magnification images) and Soissons dough (at high-magnification images) led to a decrease in PMVs (compare L with M), whereas there was no effect of further mixing on the PMV for both flours. This confirms the fine distribution of proteins in long-term mixed (over-mixed) doughs. Since we observed high PMVs for both unprocessed and over-mixed dough by mixing, a high PMV is not necessarily a measure of dough quality (development), in spite of the reported correlation between these parameters in the literature [28, 38]. In this study, image analysis in its current form was not sufficient to help to understand dough development as a function of process parameters.

Effect of simple shear and z-blade mixing on GMP wet weight

To investigate how the variations in dough microstructure relate to a scale closer to the molecular scale of gluten, we measured the amount of SDS-insoluble GMP fraction that can be extracted from the dough. Since microscopic observations as well as rheological tests were carried out after resting the dough, the amount of GMP gel was also measured in both sheared and mixed dough samples that were rested at 90% RH for 60 min. The results are presented in Fig. 7A, B for Soissons and Spring dough, respectively.

As can be seen from these graphs, there is a decline in re-assembled GMP wet weight for mixed dough of both varieties. However, at lower SME inputs, it appears that the GMP wet weight recovered from the dough is comparable to the GMP value of the original flour. It has been reported that resting of an optimally mixed dough can recover most of the GMP to its initial value in the flour [15, 47, 48]. However, the mechanical history of the mixing process strongly influences this reformation; we did not observe recovery of GMP for long-term mixed doughs in both flours used. Only 15–20% of the initial GMP wet weight could be recovered after long-term processing under the conditions used. This is different from the findings of Don et al.[15], who reported a recovery of about 70% in GMP wet weight for over-mixed samples after 90 min resting. This might be related to their milder process conditions (mechanical energy of 95 kJ/kg for the over-mixing regime of Soissons, instead of 370 kJ/kg in this study) as a result of the higher moisture content used in their experiments.

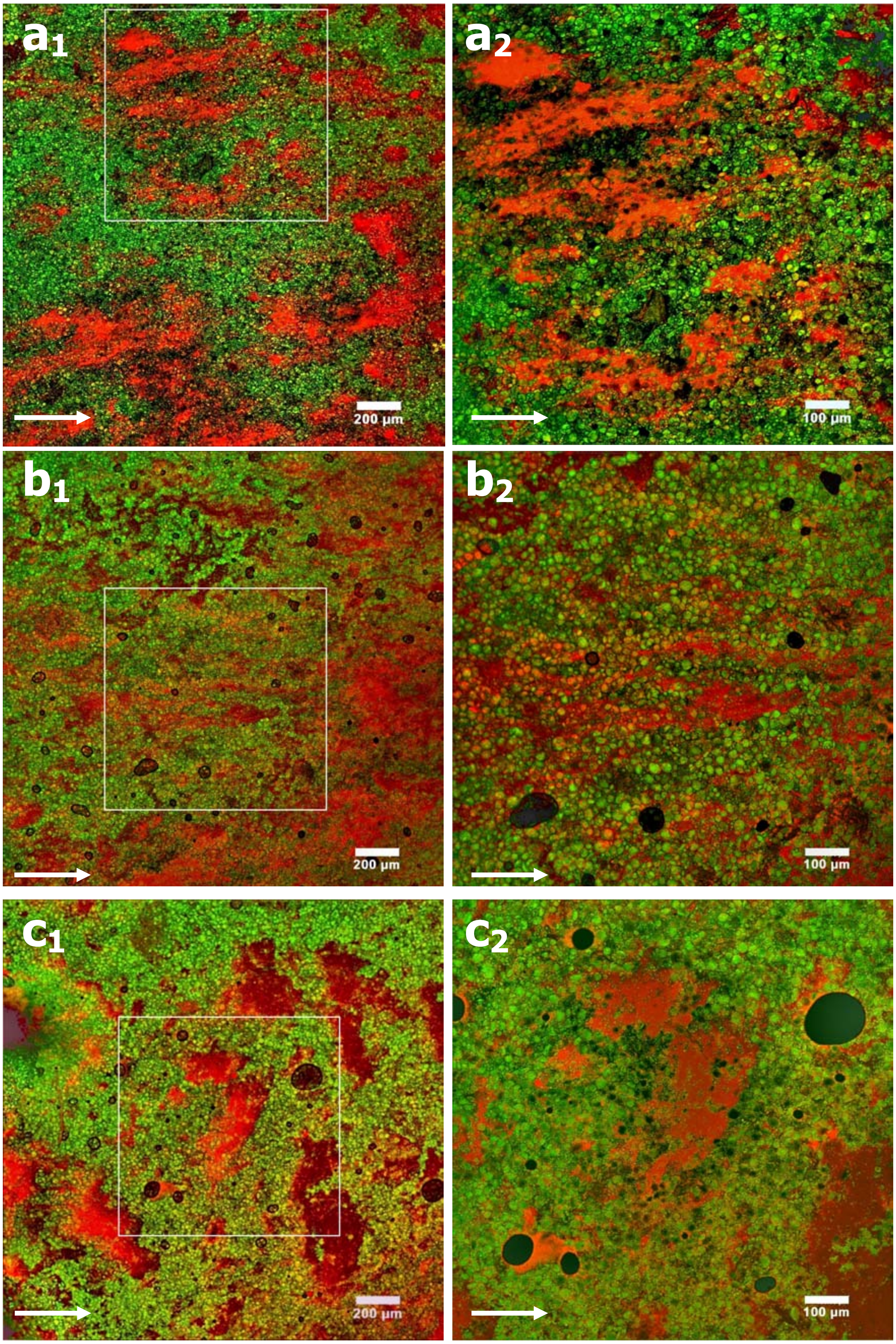

Fig. 6. CSLM images of Spring dough after shearing at 12 min (a_1,a_2), 25 min (b_1,b_2) and 45 min (c_1,c_2). Green, starch granules; red, protein. Arrows indicate the direction of shear flow. Images a_2–c_2 (1×1 mm, bars=100 µm) are higher magnification images of a_1–c_1 (2×2 mm, bars=200 µm) at positions pointed by squares.

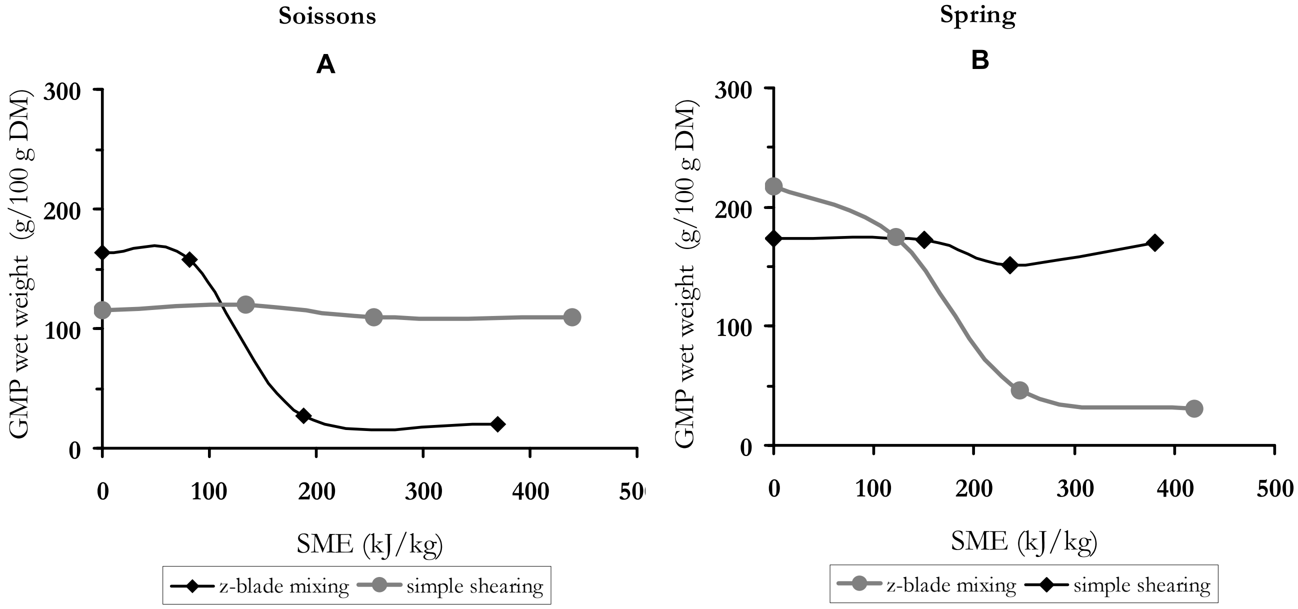

Fig. 7. Effect of specific mechanical energy in terms of simple shearing and z-blade mixing on the recovery of GMP wet weight from Soissons (A) and Spring (B) doughs after resting.

Fig. 7A and B show a constant value of the re-assembled GMP as a function of SME input for simple sheared doughs. **Chapter 3** showed that the amount of GMP wet weight was not decreased upon simple shearing. Therefore, it was to be expected that the amount of GMP wet weight remained constant during the resting period. These gels, however, appeared to be stiffer than those obtained from short-term mixed dough samples (data not shown).

Uniaxial extension rheology

We compared the effect of simple shearing with z-blade mixing on the rheological properties of dough under uniaxial extension. Fig. 8 shows a typical force–displacement curve for mixed Soissons dough (MC=45%, mixing time 9 min).

Force–deformation data were converted into stress–strain data. The results are shown in Fig. 9 for two wheat varieties during different stages of shearing and mixing. This figure represents a mean curve of at least four replicates for each treatment. Means and standard errors of values extracted from stress-strain data for both types of processing and flours are shown in Table 4.

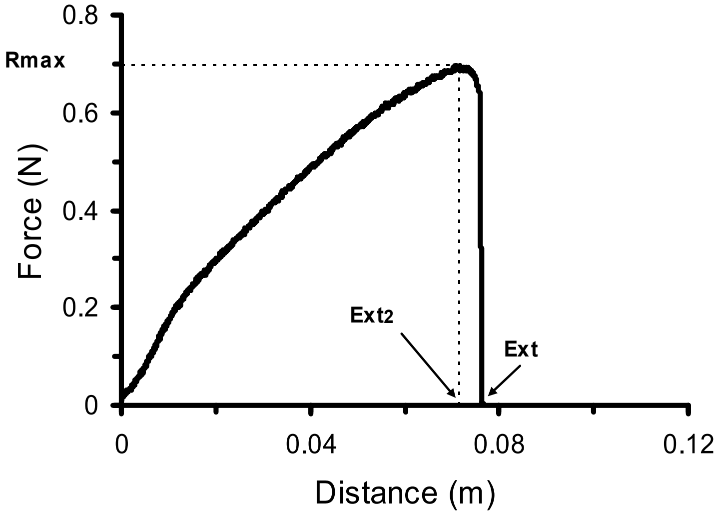

Fig. 8. Typical force–displacement curve for a mixed Soissons dough (MC=45%, mixing time 9 min) with indication of the following extensogram parameters: R_{max}, maximum resistance to extension; Ext, extensibility; Ext_2, extensibility at peak force.

Table 4. Mean values[§] of dough fracture properties[†] calculated from extension parameters on a texture analyser for Soissons and Spring dough subjected to mixing and simple shearing processes

Type of processing		Soissons					Spring				
↓		n	σ_{max} kN/m²	ε_H (–)	dlnσ/dε (–)	A kN/m²	n	σ_{max} kN/m²	ε_H (–)	dlnσ/dε (–)	A kN/ m²
Mixing											
S[‡]		9	96.7 ^{a}	1.40 ^{a}	1.46 ^{a}	63.4 ^{a}	5	141.8 ^{a}	2.12 ^{a}	1.73 ^{a}	70.7 ^{a}
M		5	49.8 ^{b}	1.31 ^{b}	1.41 ^{a}	33.5 ^{b}	4	72.1 ^{b}	1.45 ^{c}	1.67 ^{b}	45.1 ^{b}
L		4	24.1 ^{c}	1.18 ^{c}	1.08 ^{b}	16.6 ^{c}	4	39.2 ^{c}	1.77 ^{b}	1.25 ^{c}	30.0 ^{c}
Shearing											
S	P[¶]	5	70.7 ^{a}	1.98 ^{b}	1.44 ^{a}	47.5 ^{a}	6	86.5 ^{a}	1.97 ^{a}	1.61 ^{a}	56.0 ^{a}
	T	5	69.1 ^{a}	2.17 ^{a}	1.40 ^{a}	34.5 ^{b}	5	75.7 ^{b}	2.02 ^{a}	1.54 ^{a}	47.1 ^{b}
M	P	5	49.2 ^{b}	1.84 ^{c}	1.36 ^{a}	34.6 ^{b}	5	64.1 ^{c}	1.96 ^{a}	1.68 ^{a}	42.5 ^{c}
	T	4	43.1 ^{b}	2.01 ^{b}	1.30 ^{b}	29.3 ^{b}	4	56.7 ^{d}	2.02 ^{a}	1.58 ^{b}	36.4 ^{c}
L	P	4	39.2 ^{c}	1.67 ^{d}	1.40 ^{a}	27.0 ^{c}	5	42.9 ^{e}	2.06 ^{a}	1.68 ^{a}	24.9 ^{d}
	T	4	37.6 ^{c}	1.70 ^{d}	1.29 ^{b}	26.6 ^{c}	5	39.9 ^{e}	2.06 ^{a}	1.71 ^{a}	23.0 ^{d}

[§] Mean values are based on *n* replicates mentioned in the table. Differences between means were assessed for significance using the *t*-test. Values followed by different letters in the columns assigned for each process are significantly different ($P<0.05$).

[†] σ_{max}, fracture stress; ε_H, Henky strain at fracture stress; dlnσ/dε_H, *apparent* strain hardening value calculated in the strain interval 20 to 95% of fracture strain; *A*, the integrated area under the stress–strain curve.

[‡] S, M and L stand for short-, middle- and long-term processing, respectively, as described in Section 2.

[¶] P and T represent specimens taken parallel and perpendicular to the direction of shear flow, respectively.

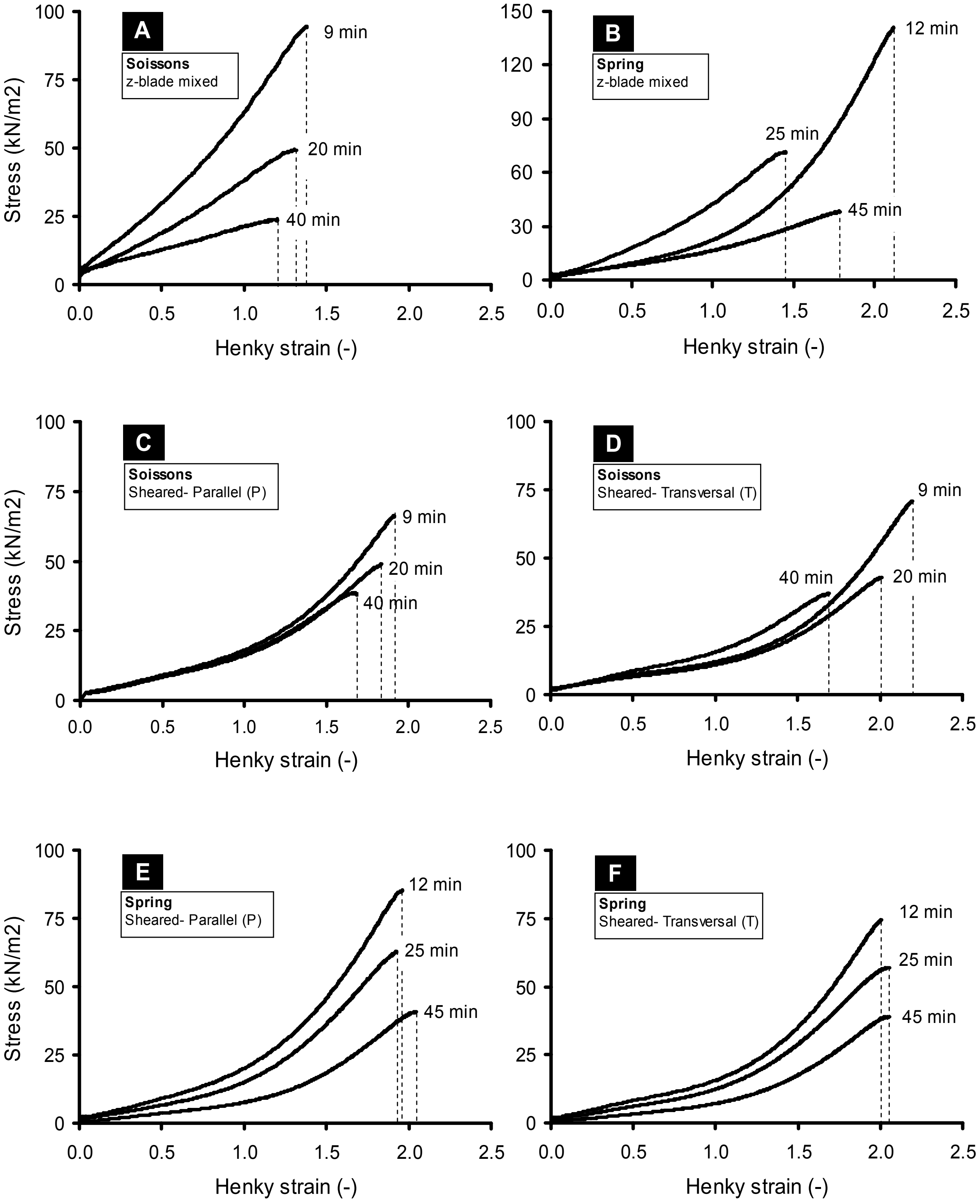

Fig. 9. Stress–strain curves for z-blade mixed Soissons (A) and Spring (B) doughs, simple sheared Soissons dough (C, parallel; D, perpendicular to the direction of the shear flow) and Spring dough (E, parallel; F, perpendicular).

As can be seen from Table 4, the fracture stress (σ_{max}), the fracture strain (ε_H), the apparent strain hardening value ($d\ln\sigma/d\varepsilon_H$), and the area under the stress–strain curve (A) were significantly ($P<0.05$) decreased by the progressive stages of mixing for both wheat varieties. This indicates the weakening of dough fracture properties upon further mixing. These results are in agreement with findings of Zheng et al. [49] and Gras et al. [19], who showed that over-mixing led to the diminishing of dough large deformation properties under extension tests. Table 4 also demonstrates that σ_{max}, ε_H and A for both wheat doughs except ε_H for Spring, were reduced somewhat as a function of the simple shear time. However, the rate of this reduction is far less than that of the mixing. Despite z-blade mixing, increasing process time with simple shearing did not lead to a significant ($P<0.05$) reduction in apparent strain hardening values (Table 4).

The fracture properties of short-term simple sheared dough (Table 4) from both wheat varieties were somewhat different with respect to sampling positions (P vs. T). For Spring dough, σ_{max} and A values were clearly lower for the T samples compared to P samples, while ε_H and $d\ln\sigma/d\varepsilon_H$ were not significantly different between P and T samples. In case of short-term sheared Soissons, only ε_H and A significantly varied with respect to sampling position.

As processing times increased, all above-mentioned differences diminished. It can be concluded from these data that simple shearing initially leads to formation of anisotropic gluten structures in the direction of the shear flow (as also clear from microscopic data). Regardless of the type of flour, long-term sheared dough samples had similar rheological properties independent of the sampling positions (P vs. T), indicating a more isotropic structure at the length scale relevant for uniaxial deformation.

Discussion

In this study, the effect of simple shearing and z-blade mixing on dough microstructure and uniaxial extension rheology was studied at comparable levels of work input. Comparison of the properties of the dough subjected to different types of deformation and subsequently rested for 60 min, revealed large differences between the two types of processing. Initial mixing changed the dispersed protein structure present in an unprocessed dough (Fig. 2) into a heterogeneous structure (Figs. $3a_1$ and $4a_1$). Apparently, the first stage of mechanical deformation provided by mixing leads to segregation of the flour constituents. According to Hoseney [21], mixing leads to an initial separation of starch and gluten. It has also been reported that short-term mixing forms a heterogeneous structure in the dough, which can be reduced by further mixing (over-mixing). From a technological point of view, over-mixed doughs are of little interest [4], due to a loss of product quality. Structural changes in the dough during the early stages of mixing are due to the formation of a gluten network as a function of extensional forces [5, 12]. Under these conditions, the total mixing intensity is not high enough to induce large changes in the smaller building blocks of the gluten network. This is in good agreement with results from Weegels et al. [47], who reported either no or minimal loss of GMP during the first stage of mixing followed by a complete re-assembly of GMP after resting for 90 min. Finally, these results are consistent with the results of large-deformation extension test that shows high fracture stress (σ_{max}) values for the short-term mixed doughs (Fig. 9A and B).

Further mixing (beyond the optimum mixing time) produced large changes in the uniaxial extension properties, dough microstructure and the amount of GMP recovered from the rested dough. At the macroscopic rheology scale, continued mixing led to lower σ_{max} values in dough rested for 60 min. This is in good agreement with studies by Grass et al. [19] and Weegels et al. [47] who reported that the decrease in R_{max} could be explained by the decrease in GMP content of dough. We therefore conclude that the loss of σ_{max} (R_{max}) in Table 4 corresponds to breakdown and concomitant loss of GMP, as shown in Fig. 7A and B.

Prolonged mixing caused a loss of apparent strain hardening ($d\ln\sigma/d\varepsilon_H$) in the dough (especially with the strong variety, Spring; Table 4). It is well known that strain hardening in dough depends on the amount and quality of the gluten fraction [29, 44]. This can explain higher values of the apparent strain hardening for Spring dough at different stages of mixing as well as shearing compared to those of Soissons cultivar (Table 4). We may conclude that changes in the gluten fraction as a function of long mixing lead to a reduction in both visco-elasticity and strain hardening in the dough. The results of CSLM observations (Figs. 3a–c and 4a–c) perhaps provide an explanation here. They show a change from a heterogeneous to a more homogeneous protein structure (increased PMVs, Table 3) upon further mixing. The loss of larger gluten structures upon over-mixing would facilitate a more homogenous distribution of dough components as observed in Figs. 3c and 4c. Upon extensive over-mixing, GMP recovery (after resting) is incomplete with both wheat varieties (Fig. 7A and B). According to Don et al. [15] over-mixing leads to a further breakdown of glutenin network structures. During resting, first, these structures have to be polymerised before they can physically aggregate to form larger structures. Since polymerisation under these conditions is much slower than physical aggregation, recovery of GMP is also incomplete. Taking these observations together, we propose that the loss of apparent strain hardening in the dough upon prolonged mixing is due to extensive breakdown of the gluten network structures.

From a rheological point of view, short-term sheared dough of both wheat varieties had higher fracture stress (σ_{max}) values and consequently higher energy requirements to extension (A) than long-term sheared dough (Table 4, Fig. 9C–F). This can be explained by the formation of a coarse gluten phase embedding compact clusters of starch granules (Figs. 5a and 6a), resulting in a heterogeneous dough structure. These structures showed a clear orientation in the direction of the shear flow and thus are probably the result of the shear flow. The inhomogeneous distribution observed with simple shear flow is a well known phenomenon for suspensions and other dispersed multiphase systems [20, 27, 34]. The phenomenon is hydrodynamically driven (minimization of hydrodynamic dissipation), and is for example used for transportation of suspensions. In other contexts, one often refers to this phenomenon as shear-banding [45]. Considering the dough as a two-phase system of starch granules and a visco-elastic gluten phase [26] may help us to understand that the orientation of the protein phase in the direction of shear flow is a shear-banding effect. Kieffer and Stein [26] reported that de-mixing is responsible for the dough-hardening phenomenon during rheological measurements. They concluded that uniaxial deformation during dough reshaping leads to de-mixing of gluten from starch due to dilatant behaviour of the starch granule suspension and the ability of gluten to aggregate. However, they did not systematically study this effect as a function of the amount of deformation and processing time.

The differences in the rheological properties of short-term sheared P and T samples (Table 4) can be explained by the formation of shear-banded gluten structures, leading to structural anisotropy in the dough at the early stage of simple shearing. However, prolonged shearing diminished these differences. No significant differences were seen in the mechanical properties of long-term sheared P and T dough samples (Table 4), indicating the formation of larger-scale isotropy in dough.

The time of simple shearing had a different effect on the properties of the dough depending on the type of flour used (the ability of proteins to aggregate). Clear changes in the rheological properties of Spring dough sheared as a function of time (Fig. 9E and F) reflect relevant differences in the degree of homogeneity of the dough structure. The formation of protein structures perpendicular to the direction of the shear flow (Fig. 6c) can be explained by the study of Vermant [45]. He reported that the formation of large structures both parallel and perpendicular to direction of shear flow is a typical feature of the shear-banding phenomenon. It is expected that protein patches slowly rotate by continuous simple shearing. If the internal strength (aggregation capability) of these protein patches is high enough to prevent physical breakdown, the rotational effect will lead to an orientation perpendicular to the flow direction (Fig. 6c).

CSLM images of long-term sheared Spring dough (Fig. 6c) showed inhomogeneity at the length scale of the microscopic images (ca. 1 mm). However, prolonged simple shearing did not lead to any significant difference in the rheological properties of P and T dough samples for both wheat cultivars (Table 4), indicating the formation of isotropic dough structures at the length scale of the extension tests. It can be concluded that at the scale determining the extensional rheological properties, the material structure remains homogenous upon prolonged shearing. This means that shear banding does not necessarily result in macroscopically anisotropic structures along the shear lines.

In contrast to over-mixed dough, long-term sheared dough of both Soissons and Spring still showed strain hardening (Table 4). This is likely related to the large protein structures found in long-term sheared dough (especially in Spring) and can probably be explained on basis of the plateau in GMP wet weight (Fig. 7A and B) as a function of the shearing energy. In **Chapter 3**, it was shown that prolonged simple shearing leads to the formation of large SDS-insoluble glutenin aggregates. We think that this is in line with the occurrence of the observed shear banding at the microstructural level at all shear times. The fact that the GMP wet weight, as a measure of protein quality, is hardly influenced by simple shear demonstrates that those structures are stable upon further shearing. Nevertheless, in this study the wet weight of GMP recovered from the sheared dough was at best 70% of the GMP wet weight in flour. This is probably related to the less effective hydration in preparation of the ZD dough, which was used as starting material for simple shearing treatments. Taking the relationship between GMP and R_{max} (Weegels et al., 1996a) could explain why the average σ_{max} values are ca. 70% of those in mixed (rested) dough. However, this does not explain why σ_{max} values decreased with prolonged simple shearing. A possible explanation for this reduction could be the effect of local segregation or shear-banding, which seems to be more manifest with strong flour (Spring), where more reduction in σ_{max} values upon long-term simple shearing was observed.

Conclusion

The results of the current study illustrate the importance of both the type and extent of processing on dough microstructure and large-deformation rheology as observed after resting. Z-blade mixing initially led to a heterogeneous dough with a higher fracture stress (σ_{max}) that exhibited a stronger apparent strain hardening. Both fracture stress and apparent strain hardening were reduced upon prolonged mixing. This was accompanied by a loss of GMP and the absence of segregation between gluten and starch. Taking all these observations into account suggests that long-term mixing induces extensive breakdown and consequently dispersion of gluten network structures, leading to structural homogeneity in the dough.

In contrast, simple shearing preserved the GMP content, apparent strain hardening and led to structure formation through shear banding, regardless of the process intensity (time). Long-term shearing led to macroscopic distribution, but not to disappearance of aggregated structures inside the dough. These structures obtained through shear banding are known to be quite stable.

Differences in dough microstructure and fracture properties obtained by simple shearing and z-blade mixing can therefore be explained by the different action of these two processes. The lack of relatively steady elongational flow, which is reported to be responsible for the break-up of large aggregates, makes the sheared dough more process tolerant. Nonetheless, the effect of shearing on bi-axial deformation properties of the dough, which is considered to be important for gas-holding properties of the dough, remains to be determined.

The results outlined in this chapter suggest that it is possible to compare structure formation in dough systems with studies describing flow induced segregation phenomena reported for non-biopolymer systems [34]. It is therefore likely that incorporation of those theories into the area of wheat processing will improve understanding of structure formation during dough mixing. Such comparisons will allow a more systematic approach to structure formation in wheat dough systems, and from this, may allow a wider range of properties to be realised in products; through sound fundamental theories on the behaviour of these complex and multiphase materials.

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Chapter 5

Dough processing in a Couette-type device with varying eccentricity: effect on glutenin macro polymer properties and dough micro-structure♣

Abstract

Dough mixing involves a combination of different deformation flows, e.g. shear and elongation. The complicated nature of mixing process makes it difficult to understand dough processing on a mechanistic level. A new Couette device allowed us to study the effects of shear flow on the physical properties of glutenin macro-polymer (GMP) and micro-structure formation of the dough. Steady shear deformation using concentric Couette type flow did not decrease GMP content and size of glutenin particles. Confocal scanning laser microscopy (CSLM) revealed formation of interconnected gluten domains indicating the development of a gluten network. Using an eccentric Couette configuration led to different results depending on the degree of eccentricity. A higher degree of eccentricity and a longer processing led to considerable reduction in GMP content and the size of glutenin particles. The micro-structural change inside regions of the narrow gap of the eccentric cell occurred at the early stages of processing, leading to break up of large protein domains, and dough homogeneity at a length scale of microscopic observations. Transient high shear flow leads to elongation and break up of gluten network structures. At low shear regions of the eccentric cell (wider gap settings), reformation or aggregation of protein domains was observed. Thus, the gluten aggregation–break up mechanism is strongly influenced by the local flow profile in a conventional mixer. The impact of different types of shear flow must be taken into account in the design of dough mixers.

♣ Peighambardoust, S.H., Van Brenk, S., Van der Goot, A.J., Hamer, R.J. and Boom, R. M. 2006. **Journal of Cereal Science**; Submitted for publication.

Introduction

Studies of gluten development during mixing have been hampered by the complexity of the mixing process, involving both disruption and reformation of the protein matrix [1]. Numerous studies have revealed large differences in bread quality at different work input levels [11, 17, 22]. These studies address the importance of the energy input but not the actual mixer properties such as geometry and mixing elements. Previous studies however showed that the way in which the energy is transferred to the material is of crucial importance (**Chapters 3, 4**). For instance, it has been reported that dough development by sheeting requires considerably less energy (3.1 kJ/kg) than development by mixing (22 kJ/kg) [12].

Specifying the mechanical history of the mixing process requires the characterisation of the type of deformations involved in this process. Consequently, mixing energy alone does not completely characterise the mixing process. During mixing, dough is deformed by a combination of shear and uniaxial elongation at high strain rates [14]. Zheng et al. [25] also reported that in industrial mixers, dough is sheared and stretched by the mixer blades and subjected to both shear and elongational flow. According to Jongen et al. [10], a combination of shear and elongational flow components exists in a farinograph mixer, while a mixograph (pin) mixer mainly delivers the shear component. This is debated by Gras et al. [6] who assert that the mixing action of a pin mixer (such as mixograph) is predominantly elongational. Such discussions indicate the difficulties of mixing studies. The complex nature of flows in mixing makes it difficult to understand dough processing at a mechanistic level. In addition, a quantitative relation between the type of deformation in the mixing and the resulting dough properties is still lacking.

We previously studied the effect of shear-only processing with steady shear rates of $\dot{\gamma} < 50$ s^{-1} on dough properties (**Chapters 3 and 4**). It was found that steady shear flow regimes did not result in dough showing signs of over-processing. We explained the differences in dough micro-structure and fracture properties obtained by simple shearing and z-blade mixing by the different actions of these two processes. It was suggested that the lack of elongation in the shearing device used probably makes the dough more process tolerant. This prompted us to further investigate the effects of transient shear flow on dough properties. To do this, we developed a Couette type device, which provided the possibility to vary eccentricity from zero (concentric) to the high eccentric position to apply either steady shear flow (at wide gap regions) or transient shear rate (at narrow gap regions), respectively.

The concept of the concentric versus eccentric annulus (rotor–stator) devices with rotation of the inner cylinder is well known. Numerous studies in literature are focusing on either numerical or experimental aspects of flow patterns of non-Newtonian fluids using these reactors [4, 5, 7, 9, 15, 16, 19, 23]. Nevertheless, there are hardly any data available on the processing of wheat dough in a Couette device with varying eccentricity. Therefore the main objective of the study reported here, was to investigate the effect of applying transient shear deformation using a Couette device with different eccentricity on micro-structure formation of wheat dough, and on the physical properties of glutenin macro-polymer (GMP). The relevance of these approaches has been previously reported in **Chapter 4**.

Experimental

Preparation of zero developed dough for experiments

A zero developed (ZD) dough was prepared according to the method of Campos et al. [2] with modifications as described previously in **Chapter 4**. Spring flour, a strong, hard, Canadian wheat cultivar with a protein content of 16 % (db) was used for preparation of ZD dough. Flour, NaCl (2%, w/w) and sufficient ice particles to provide a moisture content of 45% (based on 14% moisture in flour) were mixed inside a walk-in freezer. This moisture content allowed successful processing (no slippage) and handling (no stickiness) properties of the processed dough. ZD dough was kept at 27°C for at least 3 h to allow hydration and distribution of water throughout the dough. The hydrated ZD dough was used as starting material for shearing experiments.

Couette cell

To study the effect of either constant or transient shear flow we used a newly developed Couette type device (Couette cell). This device was based on the common concentric cylinder rheometer concept, but allows the introduction of eccentricity. A schematic diagram of the apparatus used is shown in Fig. 1. The inner cylinder rotates clockwise and is connected to the Brabender Do-corder® platform with an interface and controller unit (PL2100, Brabender measurement and control systems, Duisburg, Germany) to measure the torque required to maintain a constant angular velocity of the rotating cylinder. The position of the outer cylinder (stator) can be varied by a gap-setting device (Fig. 1, no. 7 or Fig. 2, no. 6) to provide different degrees of eccentricity in the cell. Therefore the cell can be used either with concentric annulus (Fig. 2) to apply a simple shear flow at a constant rate or as an eccentric annulus (Fig. 3A and B) to provide a combination of transient shear and elongational flows at narrow gap position. This design does not produce the same flow in all positions in the cell.

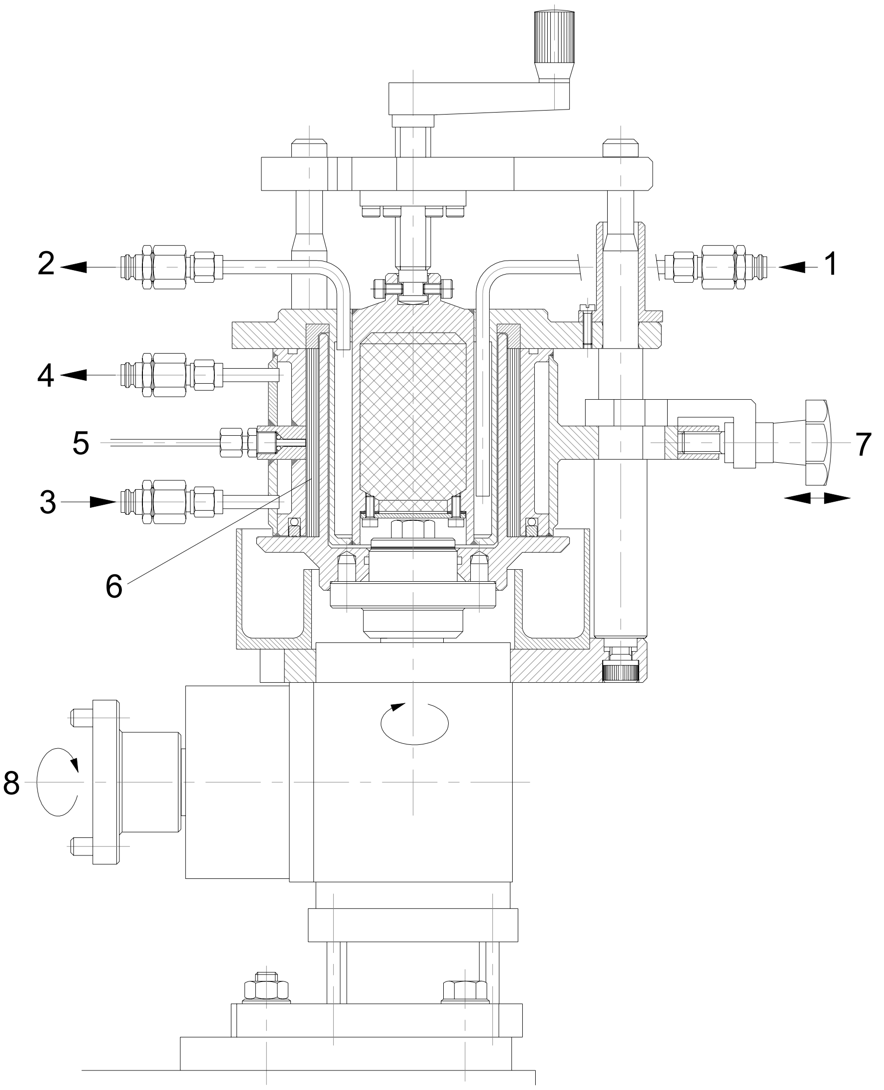

Fig. 1. Schematic diagram of the Couette cell. 1, 3: water circulation inlets; 2, 4: water circulation outlets; 5: thermocouple; 6: shearing zone (dough); 7: gap setting device; 8:conncention to torque measuring engine (Brabender® Do-corder)

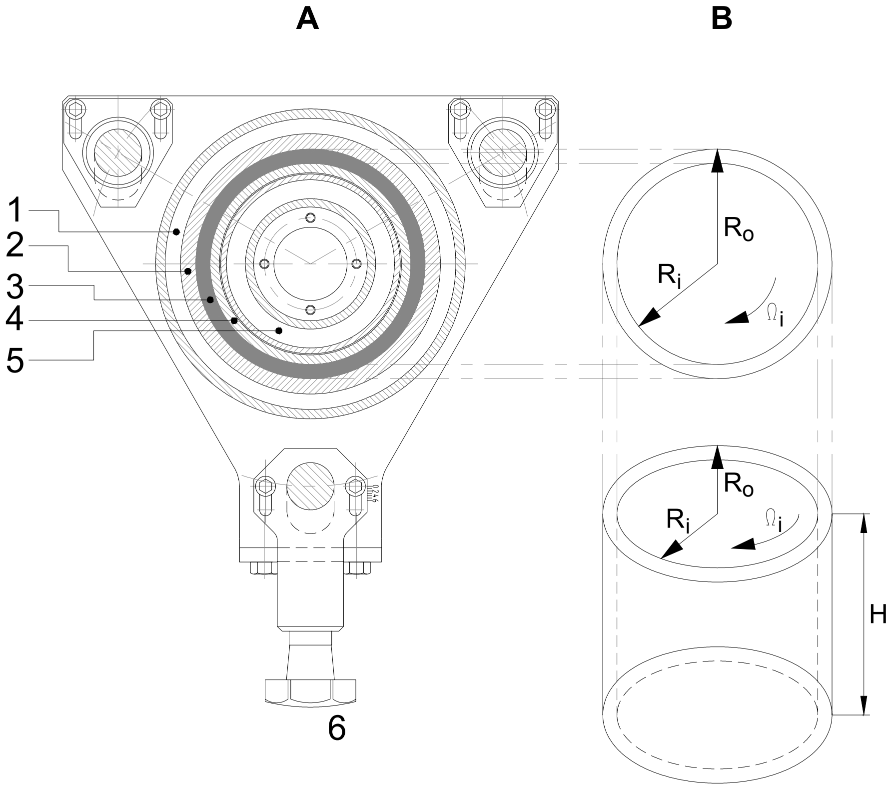

Fig. 2. Schematic diagram of the top view of Couette cell in concentric position. In figure A, 1: outer heating-cooling jacket, 2: outer cylinder, 3: shearing zone (dough), 4: inner cylinder, 5: inner heating-cooling jacket, and 6: gap setting device. In figure B, R_i (radius of inner cylinder) = 0.0425 m; R_o (radius of outer cylinder) = 0.0485 m and H (the height of both cylinders) = 0.085 m. The inner cylinder rotates clockwise with an angular speed of Ω_i (Rad.s^{-1}).

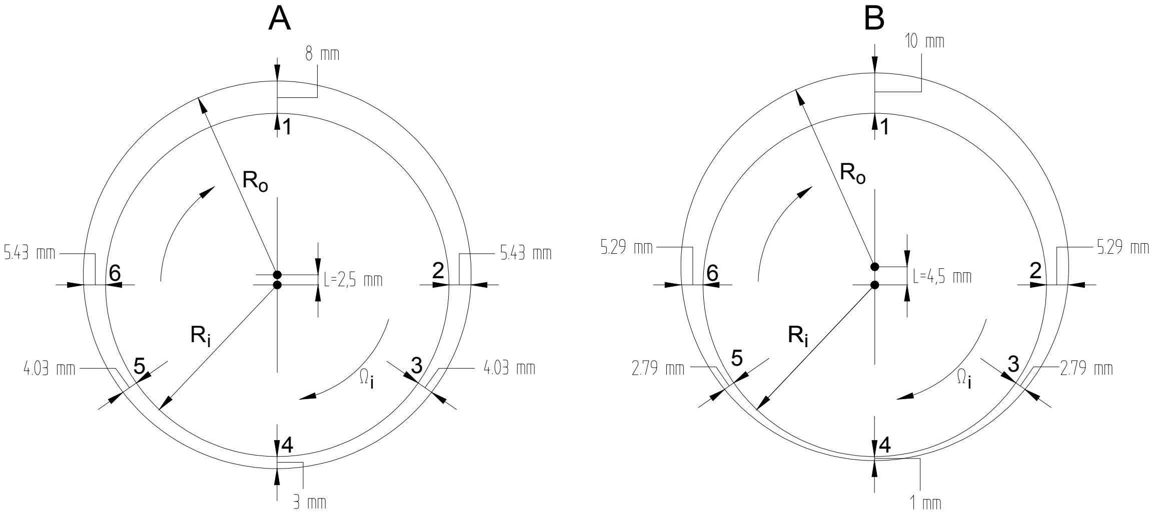

Fig. 3. Schematic diagram of the top view of eccentric Couette with different eccentricity: **A)** narrow gap of 3 mm; distance between centres of two cylinders; *L* = 2.5 mm (*e* = 0.42); **B)** narrow gap of 1 mm, distance between centres of two cylinders; *L* = 4.5 mm, (*e* = 0.75); numbers 1–6 indicate sampling positions selected for subsequent analysis; the size of gap at these positions is demonstrated in the figure.

To calculate the shear stress, shear rate and the specific mechanical energy (SME) supplied to the material following equations were used:

$$\tau = \frac{M_i}{2\pi R_i^2 H} \tag{1}$$

In this equation, τ (Pa) is the shear stress in a concentric Couette device, M_i (Nm) is torque measured on the inner cylinder, R_i (m) is the radius of the inner cylinder and H (m) is the height of the cylinder (see Fig. 2).

Many concentric cylinder rheometers use a fairly narrow gap, $0.5 < a < 1.0$, where: $a = Ri/Ro$, (in the case of our cell, $a = 0.88$). For very narrow gaps ($R_o - R_i << R_i$), the curvature of the walls can be neglected and the system approaches simple shear [13, 18]. Assuming a uniform shear rate across the gap gives:

$$\dot{\gamma}_i = \frac{\Omega_i R_i}{R_o - R_i} \tag{2}$$

where, $\dot{\gamma}$ (s^{-1}) is the shear rate in the concentric Couette, $\mathbf{\Omega}_i$ ($Rad.s^{-1}$) is the angular velocity of the inner cylinder, R_i (m) and R_o (m) are the radius of the inner and outer cylinders, respectively.

The flow pattern in an eccentric Couette was described by Windhab et al. [23] using computational fluid dynamics (CFD). They showed that the flow in the cell is mainly simple shear flow (even near the restricted zone of the cell). This prompted us to estimate the shear rate in the different zones of the eccentric cell geometries using the following equation:

$$\dot{\gamma}_i = \frac{\Omega_i R_i}{\Delta x} \tag{3}$$

where, $\mathbf{\Omega}_i$ (rad s^{-1}) is the rotational speed of the inner cylinder, R_i (m) is the radius of the inner cylinder and x (m) is the gap size between two cylinders.

The specific mechanical energy transferred to the material during shear processing was calculated following equation:

$$SME = \frac{\Omega_i}{m} \int_{t=0}^{t_f} M(t).dt \tag{4}$$

where, Ωi (Rad.s^{-1}) is the rotational speed of the inner cylinder, *m* (kg) is the mass of material filled in the Couette cell, *M*(t) is the torque (Nm) at time *t*, and t_f (s) is the shearing time. *SME* (kJ/kg) and can be calculated at any time between t=0 and t_f .

Experiments with the concentric cell geometry

For simple shear experiments, frozen ZD dough (190 g) was filled in the gap between concentric cylinders (filling time of 2 h). To achieve a complete filling of the cell, material was pressurized by a suitable filling device. Filling was followed by a resting time of 1 h to allow relaxation of stresses due to the pressurized filling. The cell was heated using a water bath set to 27 °C during filling and subsequent resting. Shear was then applied to the material between the rotating inner cylinder and the stationary outer cylinder. The inner cylinder was rotated clockwise at a speed of 15-30 rpm for 15 min and 30 min experiments, and at a speed of 30-40 rpm for 45 min processing trial. Therefore, the shear rate varied on the order of 11-30 s^{-1}.

Experiments with the eccentric cell geometry

In annulus cylinders, eccentricity is defined with a dimensionless number, which is equal to zero for concentric and is equal to one for fully eccentric position [15]. The centricity (*e*) is given by: $e = L/(R_o\text{-}R_i)$, where *L* is the difference between centres of the inner and outer cylinders and R_i and R_o are the radius of inner and outer cylinders, respectively. As schematically illustrated in Fig. 3, we used two different eccentricity values of e = 0.42 with narrow gap size of 3 mm and e = 0.75 with narrow gap size of 1 mm.

The filling conditions and process temperature in experiments with the eccentric cell were the same as described for the concentric geometry. For both eccentric geometries we used a rotational speed of 15-30 rpm. The lower speed (for 4 min) was set to avoid slippage of dough between the cylinders. The shear rate was calculated in eccentric Couette cell geometries at two settings of the wide and narrow gap using Eq. (3) (see Table 1).

Table 1. Variation in the shear rate (s^{-1}) at different eccentricity geometries and rotational speeds. A maximum shear rate is obtained in the narrow gap (hat a higher rotational speed) and a minimum obtained in the wide gap settings (at low rotational speed).

Shear rate (s^{-1})	**Eccentricity of *e* = 0.42**		**Eccentricity of *e* = 0.75**	
at:	Gap 8 mm	Gap 3 mm	Gap 11 mm	Gap 1 mm
15 rpm	8	22	6	67
30 rpm	16	44	12	134

Sampling of dough for subsequent analysis

Due to the constraints involved in the design of our Couette cell, the assembly and disassembly of the lid and the rotor–stator is time consuming. In the experiments reported in this study, complete disassembly of the cell required a 3-5 min time. Since this study focuses on GMP properties immediately after processing (no resting effect), the possible effect of this short lag period will be discussed in the results section.

After removing the lid, the area inside the inner cylinder (area I in Fig. 4) was filled with liquid nitrogen to facilitate disassembly of the two cylinders and fix the dough. Fig. 4 illustrates three different sampling positions for the concentric cell geometry (top, middle and bottom). The surface of the inner and outer cylinders at contact area with dough is serrated and roughened by dots to avoid slippage. This resulted in a dough with clear lines and dots on both surfaces after shearing. The direction of the shear flow was perpendicular to the lines. Dough samples were immediately frozen in liquid nitrogen and kept at –18 °C for subsequent analysis.

With the eccentric cell geometry, sampling of dough was performed at different gap positions as depicted in Fig. 3. At lower eccentricity geometry trials (e = 0.42 in Fig. 3A), six dough samples were taken at the middle part of the cell after processing (at positions number 1-6). However, in the eccentric experiments with e = 0.75 (Fig. 3B), due to processing constraints and slight dough leakage, no dough was sampled from position no. 6. All the samples collected were marked with regard to the direction of flow and kept frozen until subsequent analysis.

Analysis of GMP

Frozen samples were freeze-dried overnight to an average moisture content of 7% (w/w). Isolation of GMP was performed according to the method described previously (**Chapter 3**).

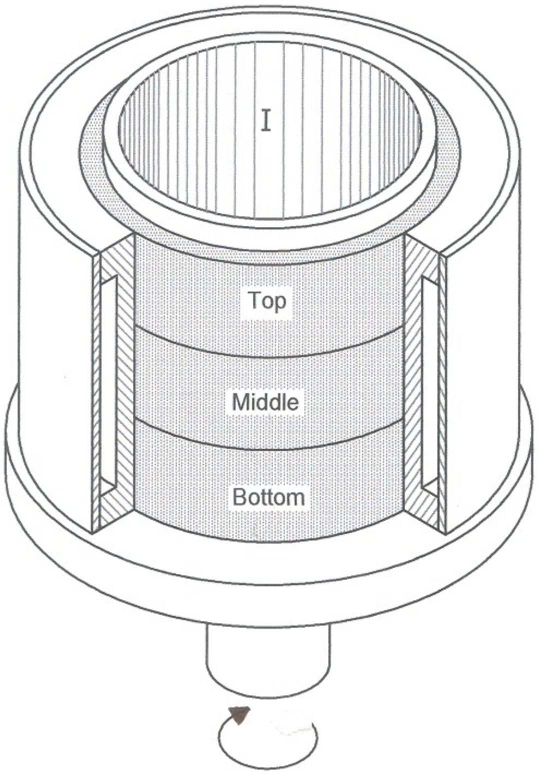

Fig. 4. Schematic illustration of concentric Couette with different positions for sampling.

Particle size analysis

GMP dispersions were prepared by transferring ca. 1 g of GMP gel into a tube containing 10 ml 1.5% (w/v) SDS solution. The upper part (ca. 80%) of the GMP gel layer was isolated carefully to minimise contamination of GMP gel with starch granules. The tube was sealed and mounted on a test-tube rotator connected to an electrical rotor type RW20 (IKA-Labortechnik, Breisgau, Germany) and rotated at a speed of 60 rpm for at least 4 h at ambient temperature. After treatment, a visually homogenous, opalescent dispersion was obtained, which was used for measuring the particle size distribution. The particle size analysis was performed according to method described in **Chapter 3**.

Observation of dough micro-structure

Observation of dough micro-structure was performed using confocal scanning laser microscopy (CSLM) as described in **Chapter 4**.

Results

Characterisation of the starting material

In **Chapter 1** it was shown that the initial hydration step provided in the preparation of ZD dough changes the properties of this dough. In this study, the GMP wet weight and particle size distribution of glutenin particles were determined in the ZD dough before and after hydration and compared with those of the parental flour. The results are shown in Fig. 5. The amount of GMP and the size of glutenin particles (as shown by $D_{4,3}$ number) in frozen ZD dough (ZD_1) was significantly less than those of the flour sample. It seems that freezing of the flour has affected glutenin particle size distribution, leading to less gel-forming ability. This effect was more pronounced with hydrated ZD dough (ZD_2). There are a couple of possible explanations for this: (1) the effect of hydration on less-gel forming ability of glutenin particles, as discussed in **Chapter 1**; (2) the pressurized filling process of the cell may cause reduction in GMP wet weight and the size of glutenin particles; Finally GMP analysis in ZD_2 involves another freezing stage of hydrated dough in the freeze-drying process. This may lead to a lower GMP content in ZD_2 compared to ZD_1 sample. The reduction in GMP wet weight was accompanied by a reduction in the size of large glutenin particles from flour towards ZD dough, as can be seen in Fig. 5B. This is clearer from data of surface-weighted mean diameter ($D_{4,3}$) in Fig. 5C.

Characteristics of torque–time behaviour of dough

The equipment used allowed us to continuously record the torque during the processing of dough. Typical torque versus time graphs of Spring dough processed with either concentric or eccentric Couette geometries with different eccentricity are shown in Fig. 6. The experiments with the concentric cell geometry ($e = 0$) gave the lowest average torque values compared to the other eccentric geometries. Increasing the degree of eccentricity led to an increase of the average torque values. However, this observation is contrary to Macosko T[13], who reported that increasing eccentricity leads to a decrease in torque values for a Newtonian fluid. A possible explanation is the higher viscosity in the narrow gap due to a higher concentration of starch combined with its dilatant behaviour.

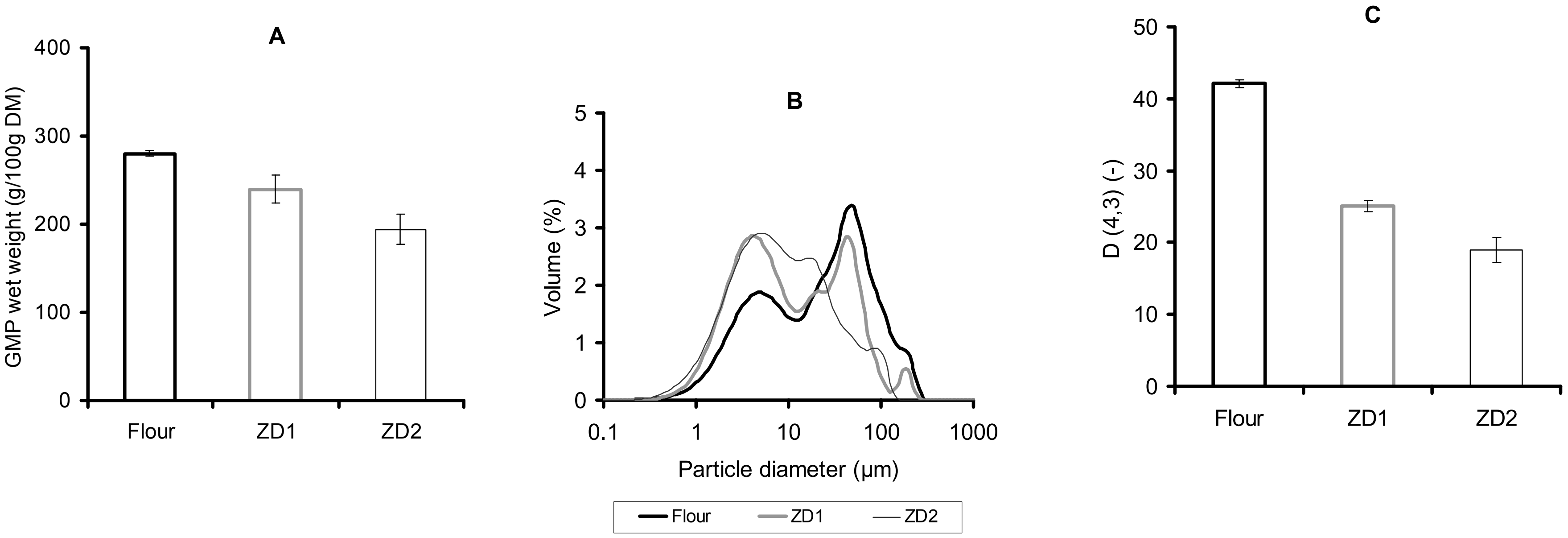

Fig. 5 Comparison of GMP wet weight (A), particle size distribution (B) and $D_{4,3}$ values of Spring flour compared with zero-developed dough [ZD_1: frozen zero-developed dough (before hydration), and ZD_2: zero-developed dough after pressurized filling in the cell (hydrated). Error bars show 95% confidence intervals.

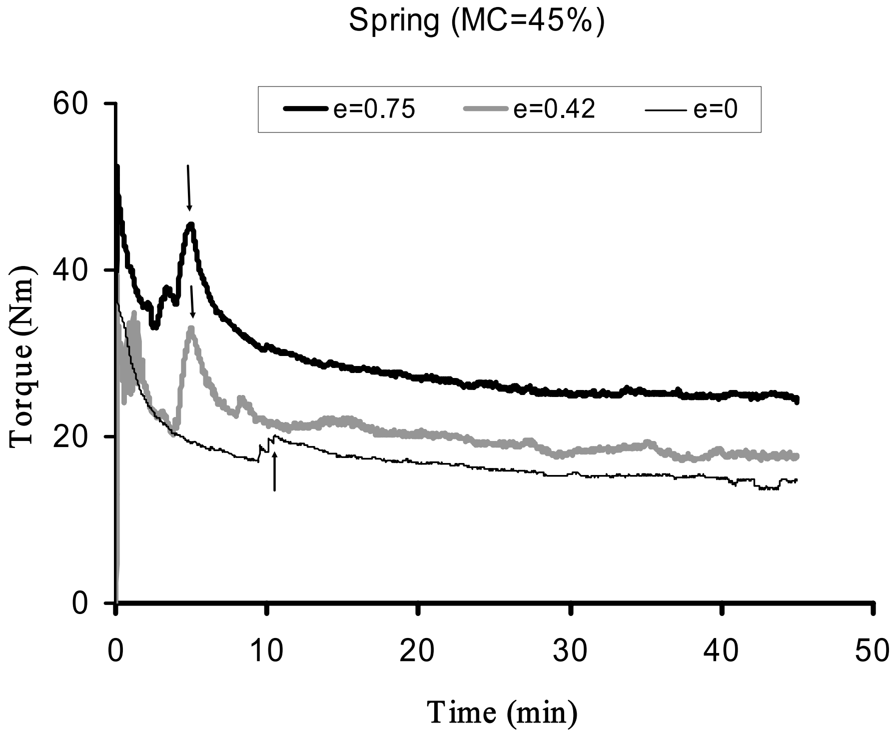

Fig. 6 Typical torque–time curves of Spring dough processed in the Couette cell with different eccentricity (*e*): *e* = 0.75 or narrow gap size of 1 mm, *e* = 0.42 or narrow gap size of 3 mm, and *e* = 0 or concentric cell (gap size of 5.5 mm). Arrows indicate the points at which the rotational speed of inner cylinder was increased. (for eccentric geometries, 15 to 30 rpm and for concentric geometry from 30 to 40 rpm).

All experiments with the Couette cell were carried out at different processing times, leading to different mechanical energy inputs into the dough. For the concentric cell geometry, the processing time varied between 15 and 45 min, corresponding to SME values of 247-963 kJ/kg. Under these conditions, the shear stress varied between 20 and 50 kPa. For eccentric cell geometries, processing times ranging between 7 and 45 min, corresponding to SME values of 165-1239 kJ/kg were used. Under the process conditions used, the shear stress applied onto the material varied between 18 and 28 kPa. Fig. 6 shows the results for 45 min runs for the different experiments. The details of energy requirements for experimental settings used are shown in Table 2.

Table 2. Variation of SME values as a function of processing time for different positions of Couette cell giving varying degree of eccentricity

Concentric Couette		Eccentric geometry		Eccentric geometry	
$e = 0$		$e = 0.42$		$e = 0.75$	
Time (min)	SME (kJ/kg)	Time (min)	SME (kJ/kg)	Time (min)	SME (kJ/kg)
15	247	07	165	10	185
30	364	25	518	25	746
45	963	45	856	45	1239

To investigate whether or not processing of ZD dough in the eccentric Couette leads to a homogeneous mixture, carbon powder was added to the large gap area at three different vertical positions during filling stage. Fig. 7A illustrates a control dough (no carbon powder) processed in the eccentric geometry (e = 0.42) with a rational speed of 15-30 rpm for 25 min. Fig. 7B shows a uniform distribution of carbon powder, under the conditions described, resulting in a dark grey colour in the dough. This experiments confirms a successful processing with the absence of internal or wall slippage .

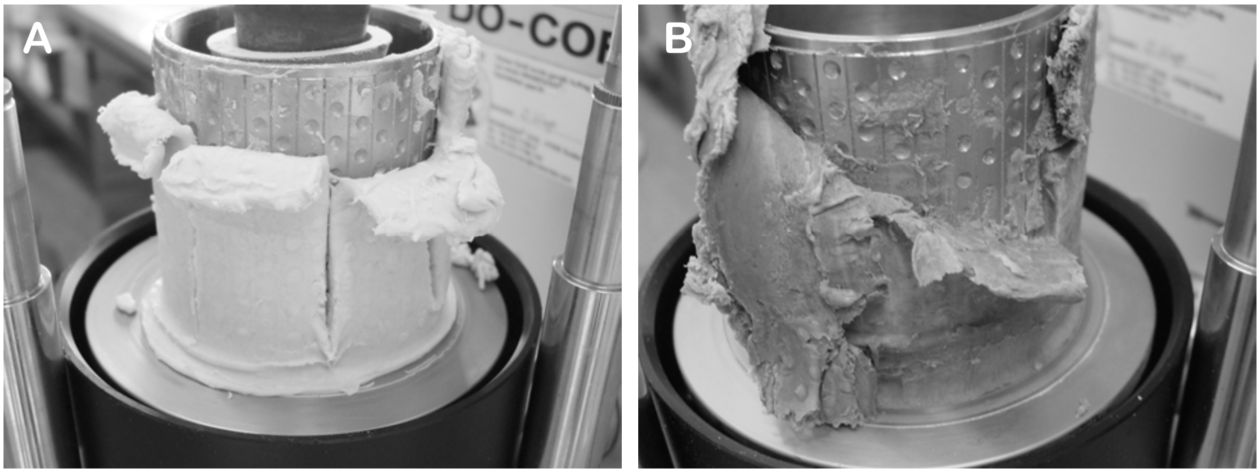

Fig. 7 Distribution of carbon powder in the Couette cell. A) control dough with no carbon powder addition, and B) dough with carbon powder. The cell eccentricity was e = 0.42. A rotational speed of 15-30 rpm and a processing time of 25 min were used for these runs.

Characterisation of GMP from sheared dough in a concentric cell geometry

GMP wet weight

The relation between the processing of dough and the quantity of GMP was pointed out previously (**Chapter 3**). The measurement of GMP wet weight allows characterisation of the starting material (ZD dough) compared to the dough after processing at the level of glutenin structures. The effect of simple shearing time on GMP wet weight of dough processed in the concentric Couette cell at three different sampling positions (top, middle and bottom) is shown in Fig. 8A.

There was no significant difference in GMP content of dough along the height of the cell. Therefore, we used only samples from the middle part of the cell for particle size analysis. As can be seen from Fig. 8A, the GMP content was not influenced by the shearing time. This supports our data published earlier with a cone and plate shearing device (**Chapter 3**) indicating that simple shear flow at steady shear rates ($\dot{\gamma}$ = 22-30 s^{-1}) does not reduce GMP wet weight.

Particle size analysis

Fig. 8B and C illustrate the particle sizes of glutenin particles extracted from sheared dough in the concentric geometry. As can be seen from this figure, there is a shift from smaller to large particle size by increasing shearing time. In Fig. 8C, the surface-weighted mean diameter ($D_{4,3}$) values were significantly increased upon simple shearing, indicating shear-induced aggregation of glutenin particles, which was also reported in **Chapter 3**.

Properties of GMP in an eccentric cell geometry with varying eccentricity settings

GMP wet weight

Fig. 9 (A and B) shows the effect of two eccentricity settings (e = 0.42 versus e = 0.75) on GMP wet weight of the dough at different sampling positions (numbered 1-6, referring to Fig. 3). The results presented in Fig. 9A resembled the GMP data of concentric cell (Fig. 8A), indicating that under process conditions used and regardless of processing time, the eccentricity of e = 0.42 did not lead to a change in the solubility of glutenin macro polymer in SDS solution.

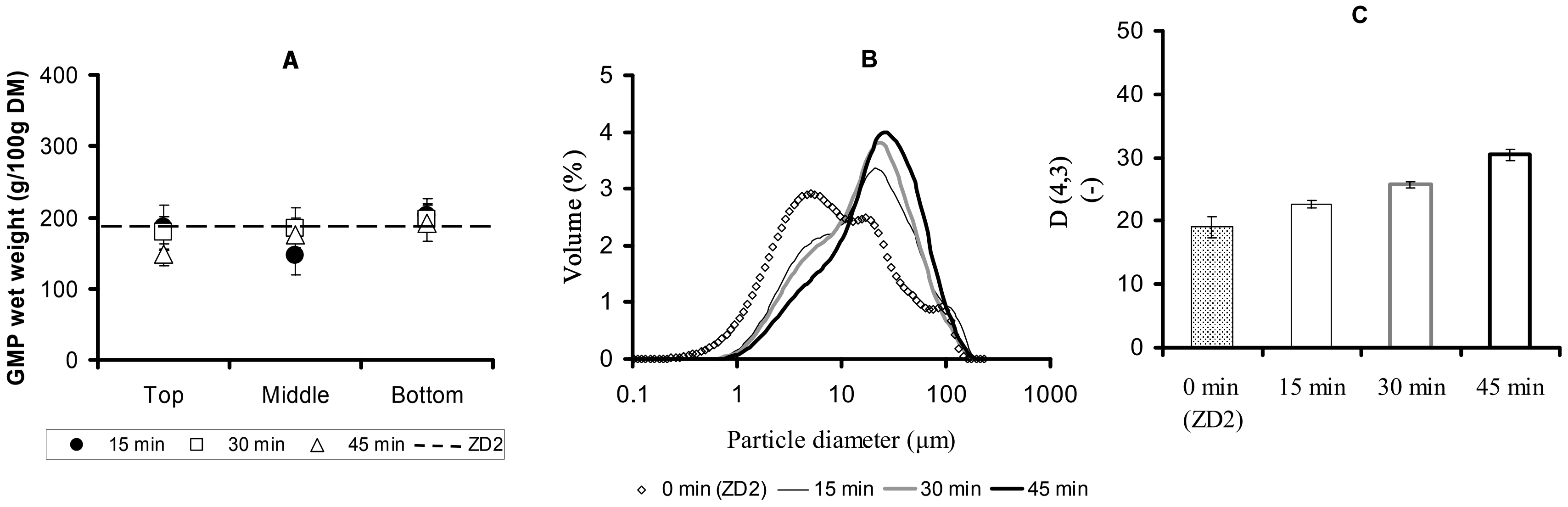

Fig. 8 GMP properties in concentric Couette. A) GMP wet weight as function of processing time and different sampling positions in concentric; B) particle size distribution of glutenins as function of processing time, and C) $D_{4,3}$ values of glutenin particles as function of time. Error bars show 95% confidence intervals.

Furthermore, there was no significant difference in GMP content of the dough at different sampling positions in this eccentricity geometry.

As can be seen in Fig. 9B, when the degree of eccentricity in the cell increased (*e*=0.75) a noticeable decline of GMP gel content was seen at narrow gap positions (No. 4) compared to other sampling positions for all processing times. Interestingly, 25 min processing led to more GMP content at all sampling positions than 10 min processing. Increasing the process time to 45 min led to a considerable reduction in GMP wet weight of the dough at all sampling positions, especially at the narrow gap setting (number 4).

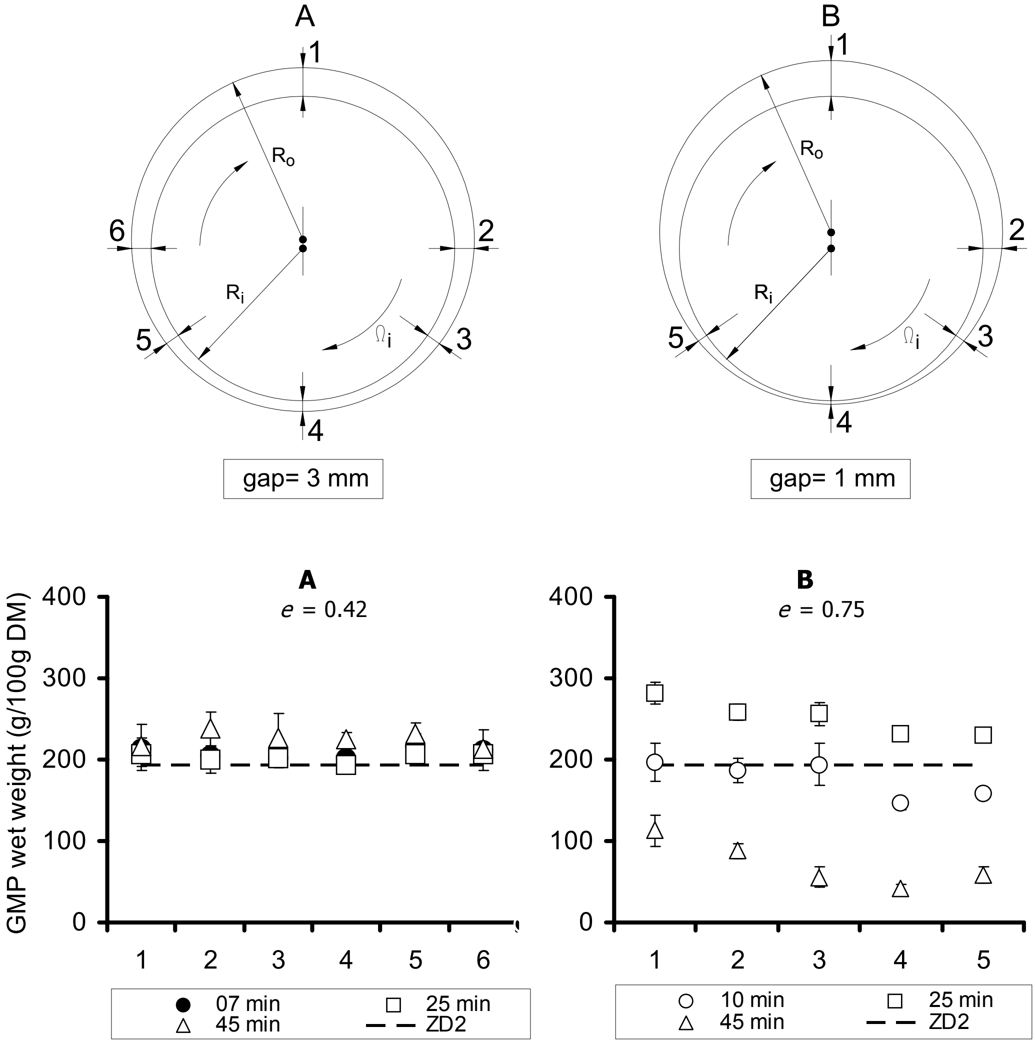

Fig. 9. Measurement of GMP wet weight as function of processing time in eccentric Couette with *e* = 0.42 (A) and *e* = 0.75 (B). Schematic illustrations in top row indicate different sampling positions (1-6). Error bars show 95% confidence intervals.

Particle size analysis

The effect of processing time on particle size distribution and surface weighted mean diameter ($D_{4,3}$) of glutenins extracted from the dough processed in the eccentric Couette cell is shown in Fig. 10 and Fig. 11.

The figures show the influence of different eccentricities (e = 0.42 versus e = 0.74) is shown on particle size distribution as function of processing time and sampling positions. As can be seen from Fig. 10, a processing time less than 25 min did not change the size of the glutenin particles with respect to the sampling locations (1-6). $D_{4,3}$ values were also comparable to those of unprocessed dough just before starting the process (ZD_2) (Fig. 5C). This means that the deformation patterns provided in the narrow gap (No. 4 in Fig. 9A) were not effective in break up of glutenin particles. This effect was discussed in the previous section in case of the GMP wet weight.

Under the process conditions used with this gap size, further processing (45 min) led to an increase in the amount of large glutenins (>100 μm). As is clear from Fig. 10, $D_{4,3}$ values of glutenins at processing time of 45 min were significantly (α = 0.05) higher than at short processing times. Nevertheless, long-term processed samples showed some reduction in size of glutenins at sampling positions after the narrow gap (4-6) compared to the positions before the gap (1-3).

The results presented in Fig. 11 show that increasing the eccentricity of the cell to e=0.75 for short (10 min) processing did not lead to any significant change in the particle size distribution results. The $D_{4,3}$ values of the glutenin particles were comparable to those of unprocessed dough (ZD_2 in 8C). Increasing the process time at this eccentricity led to further increase in the size of glutenin particles. This agrees with the results of measurement of GMP content. However, there was a significant reduction in the size of glutenins extracted from the dough sampled at the narrow gap (location 4) compared to the other sampling positions at processing time of 25 min (Fig. 11). This effect can also be seen with an eccentricity of e = 0.42 in Fig. 10.

Long-term processing (45 min) caused a considerable reduction in the size of glutenin particles, especially at the narrow gap (No. 4) as depicted in Fig. 10. $D_{4,3}$ data also show a trend of reduction in size from large gap (No. 1) to narrow gap (No. 4). These results support the reduction of GMP wet weight that was noted earlier (Fig. 8C) as function of long-term processing in a Couette cell with higher eccentricity.

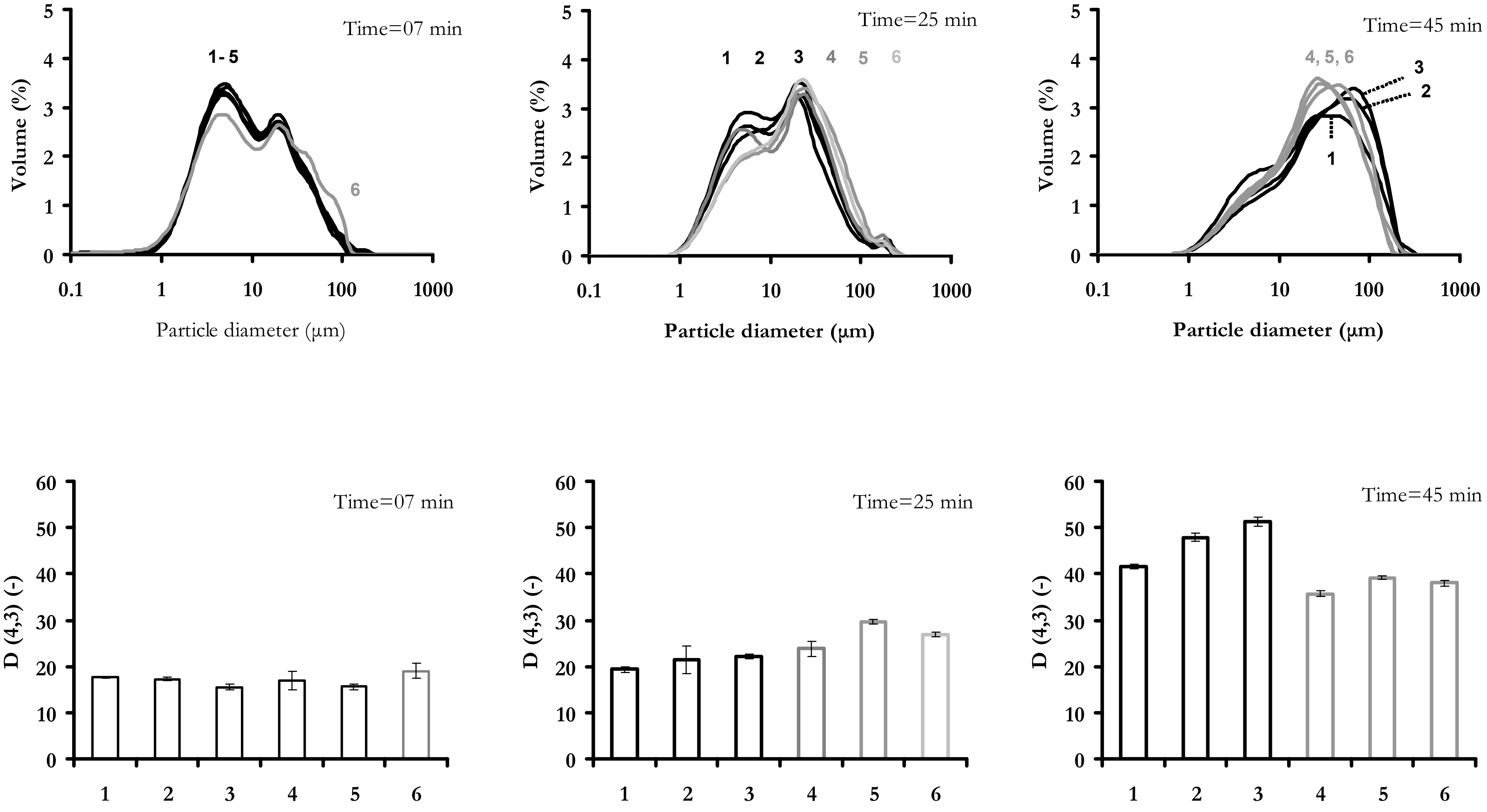

Fig. 10. Effect of processing time on particle size distribution (top row) and $D_{4,3}$ number of glutenin particles (bottom row) in eccentric Couette cell with ***e* = 0.42** at different sampling positions (1-6). Error bars show 95% confidence intervals.

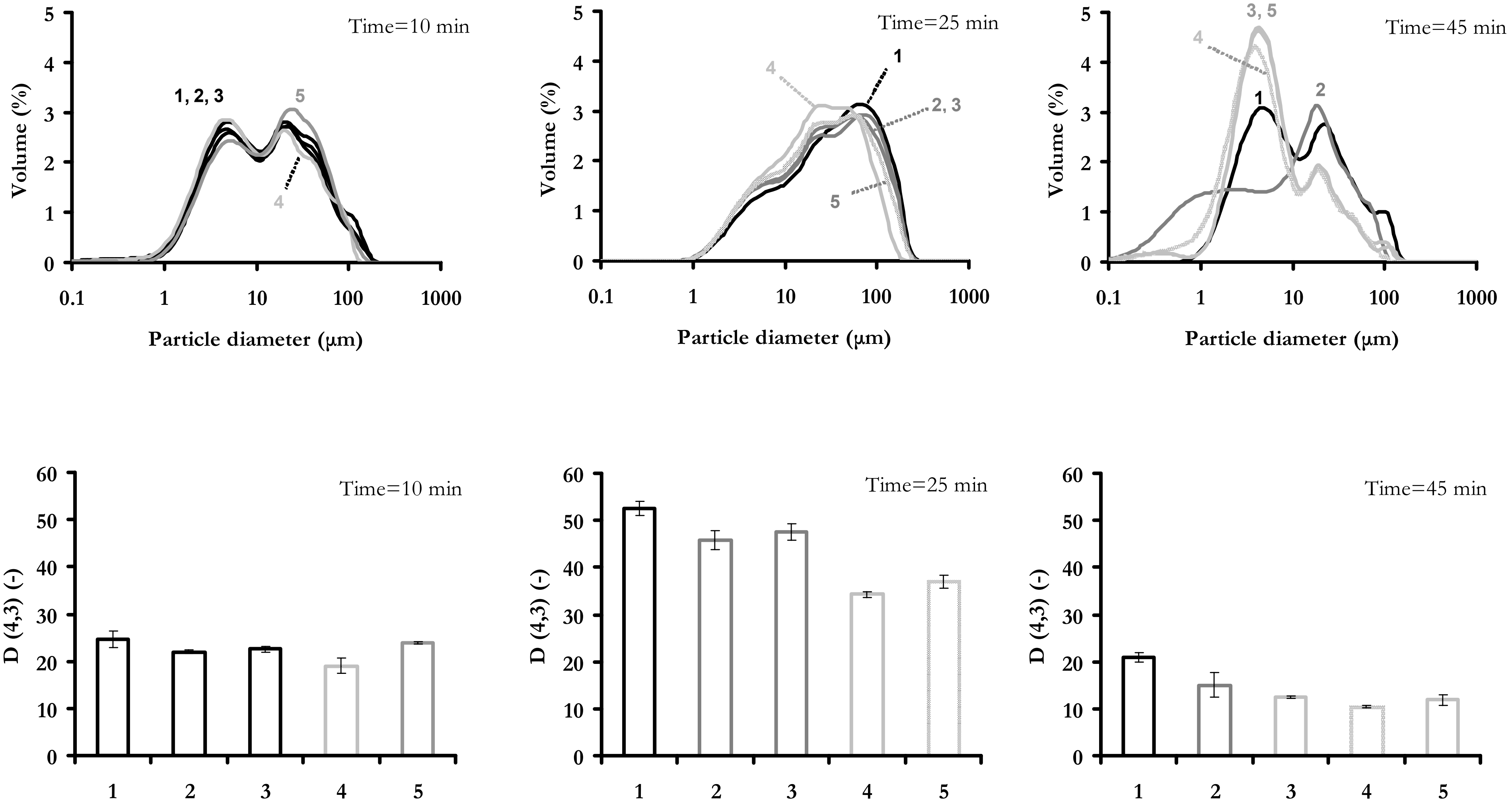

Fig. 11. Effect of processing time on particle size distribution (top row) and $D_{4,3}$ number of glutenin particles (bottom row) in eccentric Couette cell with ***e* = 0.75** at different sampling positions (1-5). Error bars show 95% confidence intervals.

Study of dough micro-structure in the Couette cell with concentric geometry

CSLM images of dough sheared in the concentric geometry as a function of processing time are shown in Fig. 12A-C. The micro-structure of the dough in short-term sheared dough (A) is heterogeneous; large protein patches (>200 μm) have been formed. If we compare this structure to the micro-structure of the dough before shearing [see **Chapter 4**], it can be concluded that short-term shearing changed the homogenous protein structure in ZD dough into a heterogeneous structure with coarse protein patches embedding clusters of starch granules. Increasing the process time to 30 min (Fig. 12B) caused further development of the protein domains, oriented in the direction of the shear flow (the direction of arrows). Further simple shearing led to development of more protein network structures. Long-term sheared (Fig. 12C) led to formation of an interconnected gluten network embedding most of the starch granules. These structures are in accordance with the stability of GMP wet weight (Fig. 8A) and shear-induced aggregation of glutenin particles (Fig. 8B and C). The fact that the GMP wet weight and the size of glutenin particles are not negatively influenced by simple shearing explains the stability of gluten network structures under steady simple shear flow.

Study of dough micro-structure in the Couette cell with eccentric geometry of $e = 0.42$

CSLM images of the dough processed in the eccentric cell ($e = 0.42$) as function of both time (A, B and C) and gap size (1, 3 and 4) are presented in Fig. 13. Images taken from the wide gap location showed highly aggregated protein domains (at length scale of mm), regardless of the processing time (A-1, B-1 and C-1). Fig. 13 shows that aggregated gluten structures only occur in the wide gap of the eccentric cell geometry, where steady simple shear deformation is likely to occur. These large protein domains resembled the dough micro-structure obtained in the concentric cell geometry (under simple shear flow).

First, as is clear from Fig. 13, the micro-structure of the dough varied considerably with the location of the gap (from no. 1 to no. 4). The decrease in the gap size led to break up of large gluten domains at all processing times (A-C). The narrowing the gap introduces transient shear flow onto the material, leading to break up of the large domains into smaller and distributed protein fragments in the narrow gap location. Second, Fig. 13 demonstrates that increasing process time at the wide gap location (A-1 to C-1) did not cause major changes in dough micro-structure, except some orientation of protein domains to the direction o the flow, which is clear in images B-1 and partly in C-1 in this figure. However, increasing processing time at narrower gap position (from A-3 to C-3) led to smaller protein fragments. This effect was more enhanced at location no. 4 in Fig. 13 (from A-4 to C-4). Break up of protein structures at the location of the narrow gap (no. 4) occurred already in the beginning of the process (A-4). This interesting observation indicates that elongational deformation caused by transient shear flow, regardless of process duration, led to break up of gluten structures, regardless of process duration.

Study of dough micro-structure in the Couette cell with eccentric geometry of e = 0.75

Fig. 14 shows the micro-structure of dough processed with the highest possible eccentricity (*e* = 0.75) in the cell. In this figure, the CSLM images are presented as function of processing time (A, B) and different gap size (1, 3 and 4). The details of set-up are shown in Fig. 3B. Comparing images taken at the wide gap location in Fig.14 with those at the same location in Fig. 13 demonstrates that when the degree of the eccentricity increased, large gluten aggregates even at wider gap positions became smaller. As can be seen from Fig. 3B, the size of the gap no.1 with eccentricity *e* = 0.75 is larger (11 mm) than that with *e* = 0.42 (8 mm). However, a change from large to smaller gluten domains in dough micro-structure in this gap setting with increasing the degree of eccentricity is obvious.

Another observation in Fig. 14 is that there is a change from "coarse and heterogeneous" dough structure in the wide gap position (A-1 or B-1) towards a "fine and homogeneous" structure in the narrow gap (A-4 or B-4) at all processing times. This effect paralleled the structures explained in previous section at eccentricity of *e*=0.42. However, protein aggregates became smaller at higher eccentricity. In Fig. 14, the micro-structure of dough at the narrow gaps (A-4* and B-4*) resembled the micro-structure of an over-mixed dough as shown previously (**Chapter 4**).

An interesting observation is that changes in dough micro-structure at different locations in the cell occurred already at relatively short processing time (10 min). This implies that there is hardly any time effect in the breakdown of gluten structures in these figures. This is evident from similarities between images A-1 and B-1, A-3 with B-3, and A-4 with B-4.

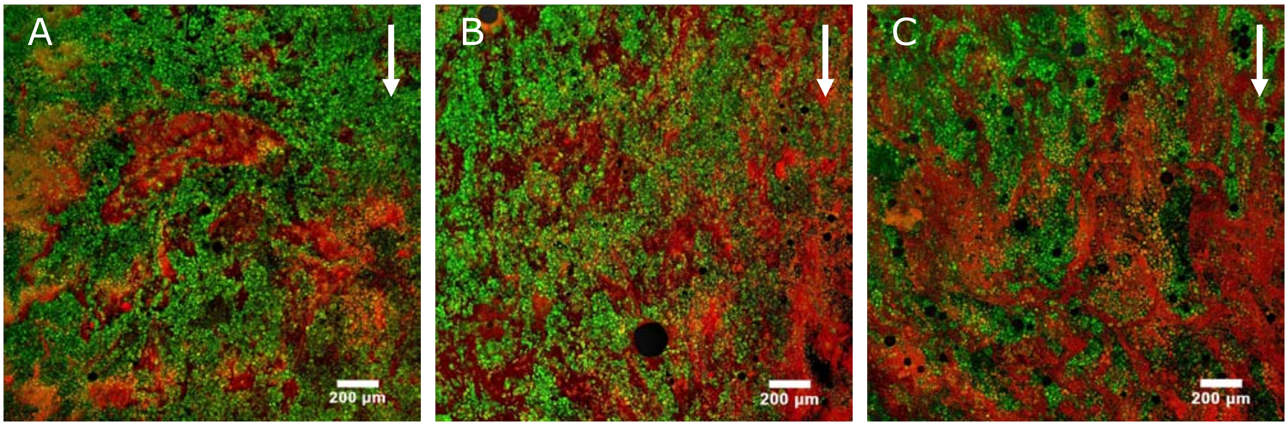

Fig. 12 CSLM images of dough processed in concentric Couette cell for A) 15 min, B) 30 min, and C) 45 min. Green, starch granules; red, protein; black holes, air bubbles. Arrows indicate the direction of shear flow. Images are given in a size of 2 × 2 mm (bars = 200 µm).

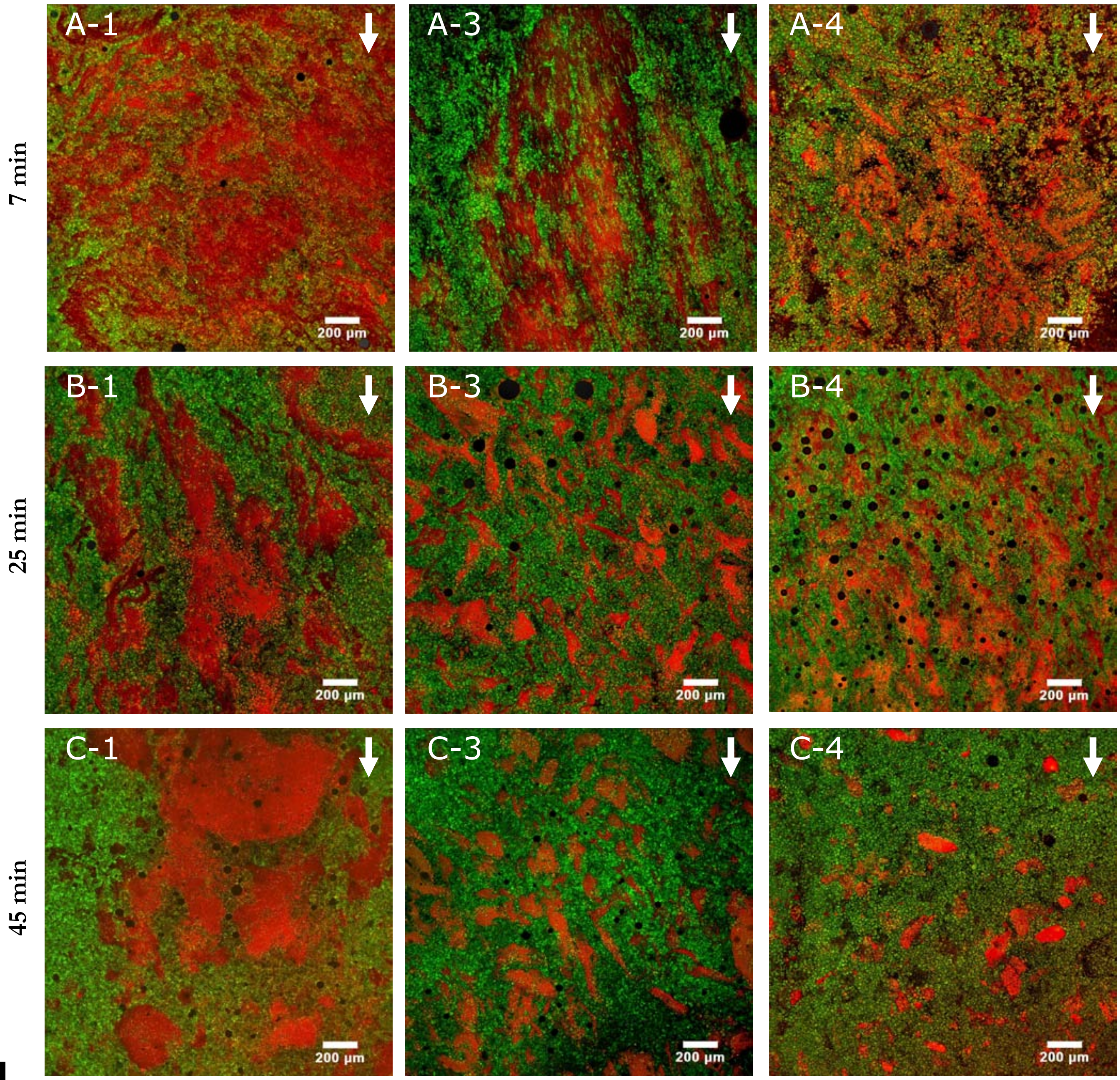

Fig. 13. CSLM images of dough processed with eccentric Couette cell (e=0.42) for A) 7 min, B) 25 min, and C) 45 min. Numbers 1, 3 and 4 are indicating different sampling positions (see Fig.3A). Green, starch granules; red, protein; black holes, air bubbles. Arrows indicate the direction of shear flow. Images are given in a size of 2×2 mm (bars = 200 µm).

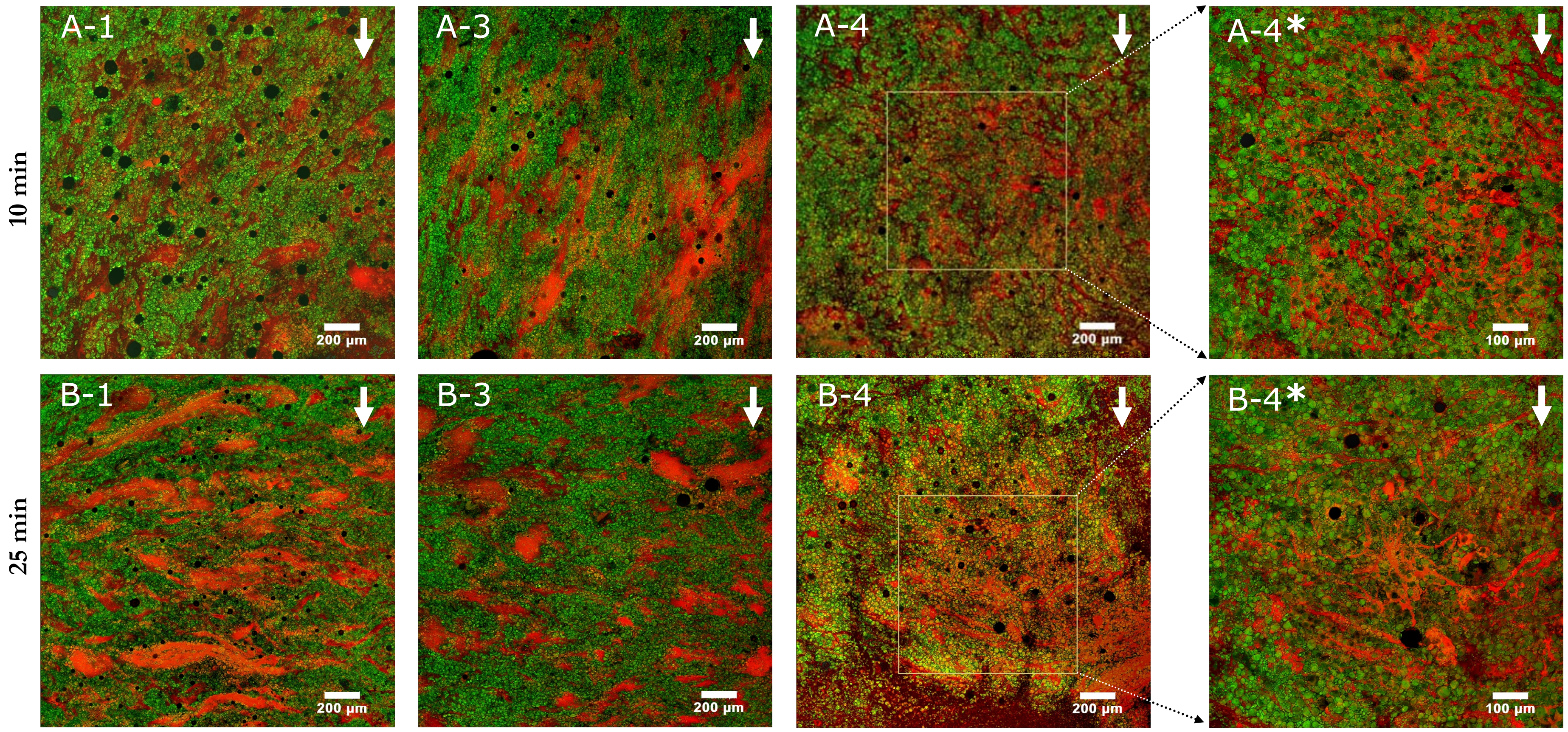

Fig. 14. CSLM images of dough processed with eccentric Couette cell (e=0.75) for A) **10 min**, and B) **25 min.** Numbers 1, 3 and 4 are indicating different sampling positions (Fig. 3B). Green, starch granules; red, protein; black holes are air baubles. White arrows indicate the direction of shear. Images A-4 and B-4 are given in two different magnifications: right columns (image size of 1×1 mm, bars=100 µm) are higher magnification of images in the left columns (image size of 2×2 mm, bars=200 µm) at positions pointed by squares.

Comparison of image A-1 in Fig. 13 with image A-4 in Fig. 14, which have more or less the same energy input levels (SME=165 kJ/kg versus SME=185 kJ/kg), reveals a significant difference in dough micro-structure. Increasing the degree of eccentricity in the cell combined with a decrease in gap size accounts for this difference, due to different flow profiles from steady shear towards transient shear flow. Thus, the type of flow has great influence on the dough structure.

Discussion

The previous chapters (**Chapters 3 and 4**) led us to infer that simple shear flow at steady rates does not lead to break up of glutenin structures, leading to process tolerant behaviour of the dough. In this study, we originally assumed that it is not the mechanical energy but the type of deformations applied to the material that strongly influences structure formation of the dough. In line with this hypothesis, we assumed transient shear flow and consequently elongational deformation provided by an eccentric Couette device could change GMP properties and dough micro-structure rather than steady planar shear flow. Comparison of the properties of the dough processed in the Couette cell with different degrees of eccentricity revealed clear differences in terms of GMP characteristics and dough micro-structure. Moreover, the differences were strongly affected by the local flow profile in the cell (different sampling positions).

The methodology introduced in this study provided an elegant way (using three different geometries: concentric versus two different eccentric geometries) to study the effect of flow profile on structure formation of the dough. By systematically comparing our studies with z-blade mixing experiment, this methodology offered an approach to understanding structure formation of dough. In this chapter, the effect of simple shear flow on gluten structure formation and the properties of GMP can be discussed with respect to different rates of shear flow obtained in different cell geometries used. Three main shearing regimes can be addressed in this respect as follows:

1) A low shear regime in the Couette cell with concentric geometry ($e = 0$):

The results obtained resembled our previous findings using a cone and plate shearing device (**Chapters 3 and 4**). It was concluded that steady simple shear flow ($\dot{\gamma} < 48$ s^{-1}), regardless of the device geometry, leads to shear-induced aggregation of glutenin particles and formation of stable gluten structures through shear-banding (de-mixing), confirming shear rate as process parameter. Under this processing regime the shear stress applied was not effective in breaking up gluten structures.

2) A medium shear regime in the Couette cell with eccentric geometry of $e = 0.42$:

When the degree of cell eccentricity increased, shear stress applied caused a partial (incomplete) break up of protein domains at the narrow gap positions at all processing times. Studies reported by Feigl et al. [5] and Windhab et al. [24] showed that elongation and thereby break up of droplets occurs as they pass the narrow gap of an eccentric cylinder ($e = 0.2$-0.4). However, analysis of GMP revealed a shear-induced aggregation of glutenin particles upon a certain period of time, characterised by large particle sizes. When the size of these aggregates reach a maximum value (e.g. $D_{4,3} = 40$) they become broken up at the narrow gap position, where a relatively high shear stress dominates. This interesting observation suggests a size criterion for glutenin aggregates, which make them sensitive for subsequent breake up under these conditions. However, no local differences in the cell in terms of GMP properties were observed in the cell with an eccentricity of 0.42. The break up of gluten domains in the narrow gap position did not parallel changes in GMP properties, suggesting that re-assembly of glutenin particles at micro-scale is faster than re-assembly of protein domains at meso-scale (a length scale of microscopy observation). This might be also due to the delay of 3-5 min in disassembling of the cell (as described in the experimental section), which may lead to a partial (up to 20% [21]) recovery of the GMP-gel.

3) A high shear regime in the Couette cell with eccentric geometry of $e = 0.75$:

This geometry provided high shear stresses at the narrow gap position, which led to significant break up of gluten domains. Moreover, analysis of GMP showed that this shearing regime caused a significant reduction in the wet weight of GMP at the narrow gap (1 mm) compared to other sampling positions at all processing times. This effect became more pronounced upon further processing. However, a maximum was found for the GMP content and for the glutenin particle size at a processing time of 25 min. This suggests that subjecting the dough to an elongational flow regime initially made a large interaction surface (elongated particle) favouring (hyper-)aggregation. It means that the initial break up leads to a strong reformation. This observation is in agreement with a recent study by Don et al. [3], who showed that mixing until TTP positively affects the tendency of glutenin particles to re-aggregate. They found that mixing initially increases $t_{1/2}$ values of the dough, leading to a stronger visco-elastic behaviour followed by a decrease upon over-mixing. The model proposed by Hamer and Van Vliet [8] might provide an explanation here, indicating that hyper-aggregation is predominantly influenced by process conditions.

Longer processing, however, led to strong reduction in wet weight of GMP and particle size of glutenins. At this point, the glutenin particles gradually lose their ability to re-aggregate and the system degrades towards over-mixing. Gluten structure reformation requires both physical and chemical interactions. Apparently, this regime negatively influenced dough chemistry in such way that it could no longer favour structure build up. A longer processing regime in the Couette cell with a higher degree of eccentricity exhausts this re-aggregative system, as is the case in an over-mixed situation in a z-blade mixing. In this situation, broken structures have to be polymerised before they can physically aggregate to form larger structures. Since polymerisation under these conditions is much slower than physical aggregation, reformation of gluten network structures is also incomplete.

Conclusions

The results of this study revealed the influence of the type of deformation in dough processing. It was concluded that both gluten aggregation and break up are strongly influenced by the local flow profile in the mixer. In a concentric Couette cell geometry, both gluten structure formation and GMP properties (amount and size) were positively influenced by the flow profile in the cell. When the cell was set to eccentric geometry: a medium shear regime at the narrow gap setting did not influence GMP wet weight and the size of glutenin particles. However, under this shearing regime significant changes were observed in the micro-structure of the dough as function of different sampling locations in the cell. A high shear regime at the narrow gap significantly influenced both micro-structure of the dough and GMP properties.

This chapter confirmed our previous study (**Chapters 3 and 4**) with a cone-and-plate type shearing device, indicating that, regardless of device geometry, steady shear flow leads to gluten structure formation (development). However, this chapter shows that transient shear in the narrow gap positions of an eccentric Couette cell causes break up of glutenin structures. This study concludes that break up mechanism is a time-independent process, confirming the results of Van den Einde et al. [20] in case of high-shear processing of starch. We also conclude that break up action is governed by the shear stress (as a relevant process parameter) onto the material and that this break up is also dependent on the initial size of protein domains.

This study proposes shear rate and thereby shear stress as relevant key process parameters in dough mixing studies, which open up interesting possibilities for innovative mixer designs.

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General Discussion

Overview

This thesis elaborates on the role of the type of mechanical deformation to gain knowledge on understanding mechanistic insights in dough development. This thesis shows that using a well-defined shearing regime in dough processing can lead to interesting and promising results. The methodology used offers interesting possibilities for future research. First, the function of hydration-only on gluten properties, which leads to remarkable properties of the hydrated dough (with "zero energy" addition); second, the major impact of the type of deformation in structure formation of the dough. These two main points will be further addressed in this chapter and overall implication of the findings for wheat processing industries and studies will be discussed.

General discussion

The overall objective of this thesis is to generate understanding of the relation between relevant process parameters in dough mixing and changes in dough properties. Different structural levels that play an important role in evaluation of dough properties are considered. The major focus is the effect of well-defined shear flow on the physical properties of glutenin macro polymer (at a length scale of 10-100 μm) as building blocks for the gluten network, on the micro-structure formation (at a length scale of 100-1000 μm), and rheological behaviour (at a length scale of more than 1000 μm) of the dough. This approach led to interesting and promising results, which were discussed in previous chapters. It was concluded that structure formation in dough is strongly influenced by the type of deformations applied. This chapter initially addresses the research questions raised in the *General Introduction*. Then, a critical review of the approaches used in the reported research is presented. Finally, the implications of the findings for the wheat processing industry, and the overall conclusions and recommendations for future studies in this regard, are discussed.

At this stage, the research questions formulated in the *General Introduction* chapter of this thesis can now be answered:

1. Does zero-developed (ZD) dough, as the starting material, behave like a flour–water mixture in mixing trials?

Yes, it does; as long as mixing regime does not exceed time-to-peak (TTP) region. The results presented in **Chapter 1** indicated that ZD dough possesses strong visco-elastic behaviour under uniaxial extension, which implies that this system is not entirely undeveloped or "zero developed" system. **Chapter 1** showed that ZD dough constitutes a good model for dough mixing studies to help study the different aspects of mixing (homogenisation, hydration and gluten development).

2. Is the introduced cone-and-plate shearing device useful to investigate changes in product properties under a well-defined shear flow?

Yes, it is; **Chapter 2** demonstrated the value of the introduced methodology to investigate the effect of simple shear flow on gluten properties under process conditions (shear stress) that are relevant to industrial processing.

3. How does a well-defined shear flow change the physical properties of glutenin macro polymer (GMP)?

Chapter 3 showed that, in addition to the total (specific) mechanical energy input during dough processing, the type of deformation applied is of crucial importance. It was concluded that simple shearing does not break up glutenin macro polymer (GMP), but instead induces aggregation of glutenin particles.

4. How are the micro-structure and rheological properties of the dough influenced by simple shear flow?

Chapter 4 confirmed the importance of the type of deformation in dough processing studies. The confocal scanning laser microscopy study showed that simple shearing leads to formation of larger gluten structures. Longer processing times enhanced this effect by the formation of shear-banded protein domains both parallel and perpendicular to the direction of the shear flow. The resulting structural anisotropy was only seen at the length scale of microscopic observation, but not at the length scale relevant to large deformation rheology measurements. In contrast to z-blade mixing, prolonged simple shearing did not lead to a reduction in the values of the fracture properties (e.g. strain hardening) of the dough under uniaxial-extension.

5. What are the effects of steady versus transient shear flow on GMP properties and micro-structure formation of the dough?

Dough processing in a new Couette-type device (**Chapter 5**) showed, in agreement with chapter 4, that simple shear leads to the formation of gluten structures. Under these conditions, no over-processing was observed with respect to GMP properties (amount and size). Introduction of transient shear flow, by using an eccentric Couette geometry, led at longer processing times to a considerable reduction in GMP content and the size of glutenin particles. Micro-structural changes at the narrow gap position of the eccentric cell occurred already at early stages of processing, leading to disintegration and thereby distribution of gluten aggregates. Thus, transient shear flow leads to elongation and thereby break up of gluten structures at a length scale of few hundred μm.

Review of the research approach

Why shear-only processing?

Dough mixing or kneading involves a combination of different tyupes of deformation, such as shear and elongation. This makes mixing already a complicated process and leads to difficulties in understanding dough processing on a mechanistic level. Literature studies mostly focus on well-known parameters in dough mixing such as mixing time, mixing speed, temperature, energy, etc. Yet there is a lack of studies on the characterisation of dough mixing with respect to the type of different deformations such as simple shear flow or elongation shear. As a consequence, it is not fully understood how mixing (shear and extensional deformation) affects structure formation (gluten development) in the dough.

Therefore, the objective of this thesis was to improve the understanding of gluten development during mixing by focusing on the effects of well-controlled deformations such as steady versus transient simple shear flow on wheat flour dough properties. A novel methodology was introduced for this, which yielded insight into dough structure formation (development) and provided leads towards improved dough processing. However, the translation of our results to phenomena occurring in conventional mixers requires the inclusion of other mixing aspects such as homogenisation and mechanical aeration functions to explore the effect on final product.

What are the relevant length scales in product characterisation?

In this thesis, the effect of a well-defined shear flow was studied on dough properties. The characterisation of dough was performed with respect to three different length scales as shown in Fig. 1. In this figure, the micro-scale represents glutenin particle structure in a range of 10-100 μm, the meso-scale stands for structures to be characterised with microscopic technique with a length scale of 100-1000 μm. The macro-scale deals with structures relevant for large-scale deformation characterisation of the dough (more than 1000 μm).

In **Chapter 3**, the effect of simple shear flow was studied on the properties of glutenin macro polymer (GMP). The measurement of the amount of GMP gel and of the particle size distribution of glutenins provided characterisation of the dough at a micro-scale. In **Chapter 4**, changes in micro-structure of the dough as characterised by microscopic observation (mesoscopic scale) were studied under simple shear flow. In the same chapter, analysis of uniaxial large-scale deformation rheological characteristics of the dough provided a macro-scale type characterisation.

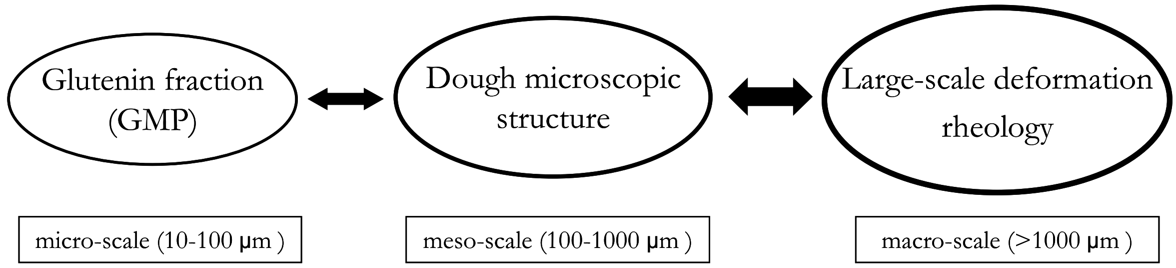

Fig. 1. Schematic representation of three structural levels used

The relevance of GMP analysis concept in understanding dough processing was already discussed in the *General Introduction*. Don et al. [8] proposed the use of glutenin particle properties as a key parameter in the link between GMP and dough quality. They concluded that a strong correlation exists between the physical properties of GMP (wet weight and size of glutenin particles) and practical mixing requirements (energy). Nevertheless, to obtain more knowledge on gluten structure formation, microscopy can directly visualize changes in dough micro-structure upon processing. Numerous researchers examine the micro-structure of dough during different stages of development using scanning electron microscopy [6, 23, 27, 29, 34] or light microscopy [12, 20, 25].

It is expected that any changes at the micro-scale as well as at meso-scale influence the visco-elastic properties of the dough and thereby its rheological behaviour (macro-scale). According to Bloksma [3], the macroscopic behaviour given dough depends on its composition and micro-structure (spatial arrangement of its constituents).

Numerous studies have confirmed that the rheological behaviour of wheat flour dough at large deformation is dominated by the gluten fraction [7, 19, 31, 33]. According to Safari-Ardi and Phan-Thien [28], small deformation rheological measurements (dynamic oscillatory testing and creep recovery) cannot be used to explain the rheological behaviour relevant to bread-making, since the rheological behaviour of dough is highly non-linear. Moreover, it is reported that protein-protein interactions in dough during small strain deformations are masked by the starch-starch and starch-protein interactions.

Thus, large-deformation rheological methods are better suited for characterising flour dough with respect to protein quality [17, 18, 33]. Nonetheless, it has been also reported that small-scale deformation rheology is a suitable method to discriminate between structural components in gluten network structure such as chemical cross links, physical interactions (hydrogen bonds, hydrophobic or electrostatic interactions) versus physical entanglements of polymeric chains [9, 35].

The most commonly applied type of large deformation characterisation of flour dough is the uniaxial extension [2, 14]. Thus, in this thesis uniaxial large-deformation rheology was used for the characterisation of the dough (not leavened) under different processing treatments. However, the relevant deformation around a growing gas cell during fermentation of a leavened dough is primarily biaxial, rather than uniaxial extension [13, 14]. The uniaxial large-deformation rheology used in this thesis is therefore only regarded as a first step.

In **Chapter 4** we showed that prolonged simple shearing leads to the formation of an anisotropic structure at a length scale relevant to microscopic observation. This anisotropy was however not detected using large deformation uniaxial rheological characterisation. Generally, the flow-induced anisotropy that was observed can be explained by segregation or shear-banding. These phenomena are, in turn, dependent on starch-protein interactions [20]. Therefore, characterisation by a combination of small and large deformation is recommended for further studies.

Implications of the findings of this thesis

Understanding of shear-induced structuring in wheat dough systems

In **Chapter 4,** the effect of simple shearing and z-blade mixing on dough micro-structure and uniaxial extension rheology was studied at comparable levels of work input. Comparison of the properties of the dough subjected to different types of deformation and subsequently rested, revealed large differences between the properties resulting from the two types of processing. Differences in dough micro-structure and fracture properties obtained by simple shearing and z-blade mixing can be explained by the different action of these two processes. The lack of extensional flow made the sheared dough (gluten) more process tolerant. Simple shearing of wheat flour did not damage the gluten in terms of GMP. As a result of that, we expect no effect on the visco-elastic properties of gluten. This opens up opportunities to simplify the wheat dough into two phases of dilatant starch granules and visco-elastic gluten phase, as also described by Kieffer et al [20]. The observed local segregation of starch and gluten, probably as a result of the differences in viscosity for both phases might thus be compared to segregation of polymeric systems under shear flow, which is a known effect with synthetic (non-food) polymer blends [11, 26, 37].

Mixtures containing one or more visco-elastic phases can feature shear-banding. This phenomenon is the tendency of a flowing liquid to form adjacent regions that have a shear-rate discontinuity at their interface. This can occur if the flow curve (shear stress versus shear rate relation) has a negative slope over some range of shear rates [37]. This can lead to unstable flows giving bands with different shear rates. In a cone and plate geometry, shear-banding was observed in the case of a surfactant solution by Kumar and Larson [22]. Hobbie et al. also [11] studied the shear-induced segregation behaviour of three completely different polymeric systems in a cone and plate system and found that the patterns obtained were quite similar. The structural elements obtained by them were in the range of 5 to 50 μm. Jupp et al. [16] obtained interesting results by modelling shear-induced phase transitions. They derived a relation between rheology and micro-structural evolution.

Shear banding occurs in solutions containing worm-like micelles, entangled polymer solutions and in colloidal systems, and solutions containing surfactants, as was studied by Vermant [37]. Generally, shear bands are stable upon further shearing. In a Couette geometry, coexisting structures were found in parallel as well as orthogonal to the cell walls. He concluded that non-homogeneous materials under flow can yield a wide variety of shear-induced structures. Orientation in the flow is no longer observed after some time; rather a development of the structure perpendicular to the flow direction is obtained. This absence of clear shear-induced alignment of structures was also noted by Hobbie et al. [11].

The results outlined in **Chapter 4** indeed suggest that it is possible to compare structure formation in dough systems with studies describing flow induced segregation phenomena reported for non-biopolymer systems. It is therefore likely that incorporation of those theories into the area of wheat processing will improve understanding of structure formation during dough mixing. Such comparisons will allow a more systematic approach to structure formation in wheat dough systems, and from this, may allow a wider range of properties to be realised in products.

Understanding of gluten development/break up upon mixing

As already mentioned in the "General Introduction" chapter, mixing has three main functions in structure formation of dough: homogenisation, air inclusion and gluten development. The latter function is main focus of this thesis, although the other functions are of course relevant as well. It is generally believed that the gluten development phase of mixing is governed by the amount of work or (mechanical) energy input [5]. Numerous studies related the amount of work input during mixing to bread quality [21, 30, 38]. Nevertheless, only a limited number of publications characterized dough mixing with respect to the role of the type of deformation [10, 15, 21, 24, 39], due to the complexity of the mixing process, involving both disruption and reformation of the protein matrix [5]. There is a debate in current literature about the mechanism of dough development during kneading. It is mostly believed that gluten development i.e. the build up of a continuous network, is achieved during dough mixing [32]. However, it is also reported that mixing leads to break up of gluten network structures [4]. Apparently, the application of mechanical work in dough mixing or kneading can act both in the direction of structure formation, and in the direction of structure break up. Since the motion of a particular piece of dough in a mixer is not purely deterministic but contains a stochastic element as well, a good quantitative description of the deformation of dough in a mixer is difficult. We therefore developed a simplified approach.

The question to be asked here is: Can we translate the results provided by this thesis to conventional mixers? We do think that the shear processing approach introduced in this thesis allowed us to better understand conventional dough mixing systems. Among other effects, it was shown that specific mechanical energy (SME) is not the governing factor that characterises dough mixing. In the bakery industry, mechanical energy or work input, which is related to a measurable increase in the temperature of the dough, is normally used to characterise and control mixing operations. However, we showed that the way that this energy is transferred into the material is of crucial importance. The results discussed in **Chapter 5** indicated that the break up of large gluten domains (at length scale of CSLM images; 100-1000 μm) occurs in zones of high shear stress (the narrow gap inside an eccentric Couette geometry) already at early stages of mixing. In the low shear stress zones (the wider areas in the eccentric Couette geometry), either no break up occurs or the broken gluten domains could re-form (re-aggregate). This reformation seems to be time-dependent, since prolonged processing led to further development of protein domains.

Fig. 2 schematically illustrates the above-mentioned features. In this figure, the mechanisms shown by solid arrows were directly and separately observed using Couette cell. However, when these mechanisms act simultaneously, as is the case in conventional mixers, the model predicts those features that are observed during conventional mixing. Indeed, in conventional mixing, low shear stress (no. 1 in Fig. 2) and high shear stress (no. 2) zones are present simultaneously, and one would expect simultaneous action of these mechanisms (indicated in the figure by the dotted arrows). The exchange of material between low shear stress and high shear stress regions in time would result in a seemingly time-dependent break-up process, even though locally the break-up is practically instantaneous. Thus, Fig. 2 forms the basis of a hypothesis that may help to understand such break up and reformation mechanisms that are occurring in a conventional dough mixer, leading to over-mixing. Of course, in addition to the flow-induced structure formation/break up concept, the physico-chemical characteristics of the dough, e.g. gluten content and composition [e.g. the presence of certain low molecular weight glutenin subunits, which block free sulfhydril (–SH) groups], etc. play a major role as well.

The type of deformation, more specifically the shear stress applied onto the material, is generally applicable and seems to be the driving mechanism for gluten structure break up. Using the cone-and-plate shear cell, Van den Einde et al. [36] were able to relate (thermo-)mechanical break up of starch under high-shear treatment to break up in extrusion process. They found that whereas break-up in an extruder seems to be time-dependent, in fact the local mechanism of break-up is instantaneous, due to the forces exerted on individual amylopectin molecules. In case of wheat flour, we expect a more complex correlation between the shear cell and a mixer due to the complexity of the dough (hydrated gluten network embedding the starch granules giving rise to visco-elastic properties).

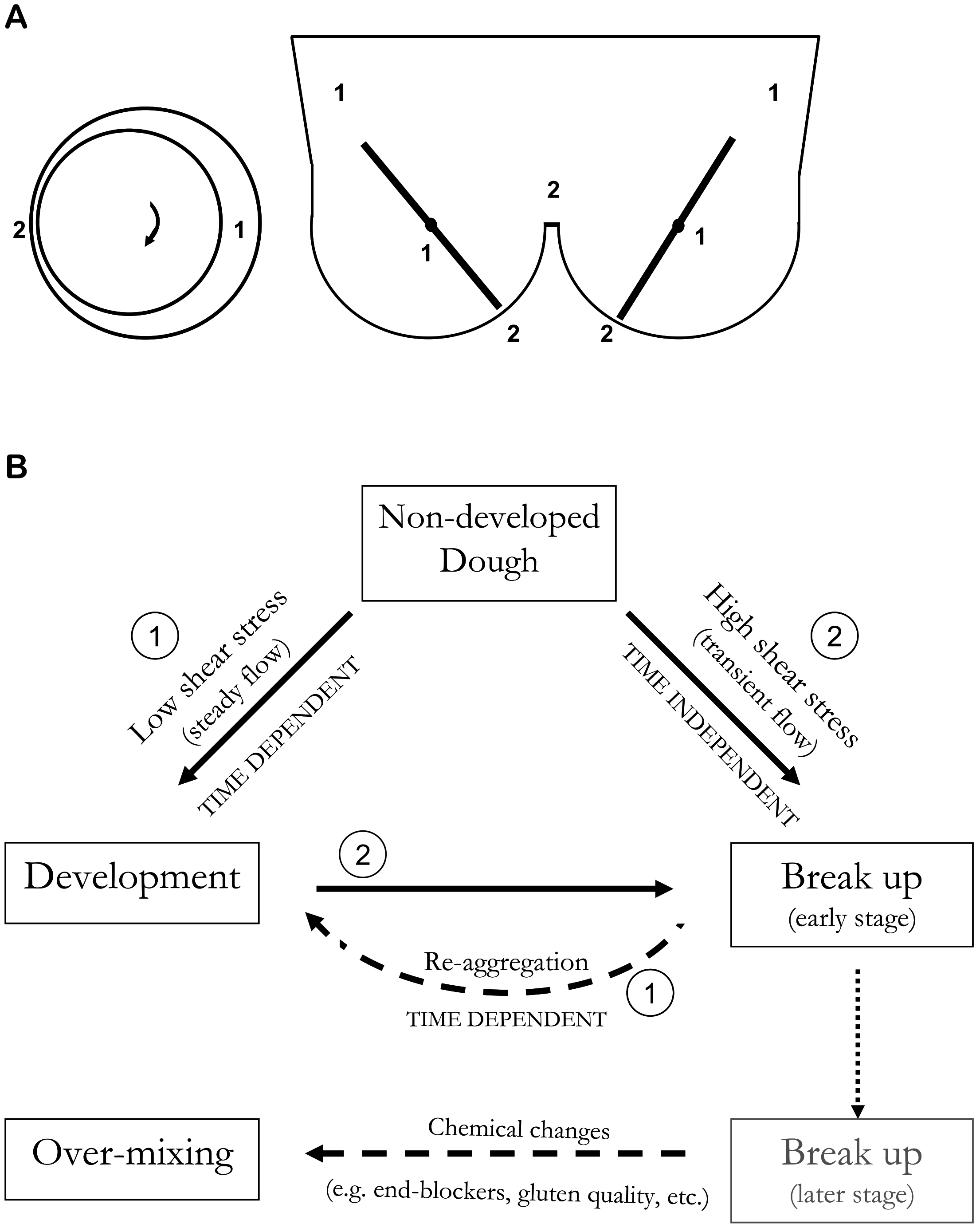

Fig. 2. A) schematic drawings of an eccentric Couette cell with rotating inner cylinder (left) and a z-blade mixer with counter-rotating blades (right); numbers 1 and 2 indicate low shear and high-shear zones, respectively.
B) schematic overview of gluten development/break up mechanisms occurring in a conventional mixer; numbers 1 and 2 indicate low shear and high-shear zones, respectively.

Relevance of our findings for wheat dough processing

The methodology used in this thesis was aimed at unravelling mechanisms underlying the phenomena observed in dough mixing, in terms of a well-defined shearing regime that used hydrated flour with "zero mechanical energy" addition (ZD dough) as starting material. Results obtained in **Chapter 1** showed that the initial hydration step changed the gluten properties in such a way that ZD dough already showed viscoelasticity (including strain hardening) under uniaxial extension tests. It was shown that less energy is needed to mix the dough from hydrated ZD dough compared to a conventional flour-water mixture. However, the mixing of such system is critical, indicating that also other aspects are changed. Decoupling of the hydration phase from energy input of dough formation can be achieved by working with ZD dough. This would facilitate a careful and detailed study of dough processing, where the influence of flow fields (e.g. simple shear versus extensional), strain history, and energy input levels on dough could be determined.

In the bread-making, kneading provides mechanical aeration to include air bubbles in the dough which will form nuclei for gas cells that grow during fermentation and the initial stages of baking. Nevertheless, in the wheat dough processing industry, there are some applications where kneading is primarily used to hydrate wheat flour and to homogenise other dough ingredients to produce a cohesive dough. In such conditions, kneading is normally followed by moulding, depositing, extrusion, sheeting (rolling), or laminating processes, which mainly determine the overall structure of the final product. In these applications, air inclusion seems to be less important for the physical structure (volume) of the final product. As example, a wide range of flat bread products (such as Mexican tortilla, Naan, Pita, Chapati, Lavash, pancakes, pizza crust, etc) or pasta products can be found throughout the Middle and Far East as well as the Caribbean countries. In fact, almost every country in the world has a variety of flat breads and today more than 1.8 billion people worldwide eat these traditional bread products [1]. Thus, if a homogenized and fully hydrated dough (with no energy addition) is subjected to a well-controlled deformation (for instance simple shearing, **Chapters 2-5**) capable of forming a good gluten network, the kneading process in its current form might be skipped or modified. Therefore, the combination of hydration and homogenization of dough ingredients with a well-controlled deformation process would offer potential benefit to pasta and flat bread products market. On the one hand, this can save energy and time (production costs), and on the other hand it may better preserve the quality of the final product, which might be damaged due to improper kneading.

The relevance of our findings in understanding phenomena occurring in conventional dough mixers was discussed. However, there are clear limitations in this approach: We introduced a model system to elucidate the different effects of well-defined flow, however the aspects of air inclusion (mechanical aeration) were not considered in this study. Although the equipment used provided a possibility to apply elongational deformation onto the material at specific locations in the cell, the establishment of constant elongational flow seems to be impossible.

Nonetheless, the results presented in thesis open up possibilities for innovative mixer designs. In this respect, the type of deformation should be considered as one of the main design parameters.

General conclusions

- Zero developed dough constitutes a good model for studies in which the different aspects of dough processing should be identified and quantified.
- The shearing device introduced is effective for investigating the shear-induced structure formation of wheat dough.
- Simple shearing at relevant levels of mechanical work input compared to mixing, does not lead to break up of glutenin macro polymer (GMP)
- The type of deformation applied during processing is of crucial importance for the structure formation of wheat dough.
- Gluten aggregation–break up mechanisms are strongly influenced by the local flow profile in a mixer.
- This thesis introduce shear rate and thereby shear stress as relevant key process parameters in dough mixing studies, which open up interesting possibilities for innovative mixer designs.

Outlook

There are three main leads from this thesis to be addressed as follows:

1) Improvement of the mechanistic understanding of dough development

In view of the discussions mentioned in **Chapters 2-4**, the importance of the type of deformation rather than the amount of work input in dough processing becomes clear. Furthermore, as mentioned in **Chapter 4**, a comparison of the structure formation in dough systems with studies describing flow-induced segregation phenomena reported for non-biopolymer systems [11, 22, 26, 37] will improve the existing knowledge of understanding dough structure formation upon mixing. Such comparisons will allow a more systematic approach to structure formation in wheat dough systems, and from this, may allow a wider range of properties to be realised in products through sound fundamental theories on the behaviour of these complex and multiphase materials.

2) Optimization of current mixers and design of new dough mixer

With regard to the conclusions made in **Chapter 1**, the concept of 'homogenization and hydration of dough ingredients without kneading' can be regarded as good starting point for dough processing. This can lead to new insights in the optimization of current mixing process as well as the design of new dough mixer.

3) Design of novel structures in food products

The results discussed in this thesis might suggest possibilities in application of equipment that subjects the dough to a well-defined shear flow for the design or creation of novel structures in food products. For instance, shear-banding might lead to new types of micro-structures, yielding products with different sensory properties.

In retrospect with the discussed points in this thesis, the following topics can be of interest for future research:

- Further study into the role of hydration only process (non-frozen conditions in the ZD dough preparation) on gluten development and dough properties.

- More exhaustive studies on the properties of dough mixing resulting from a combined (adjustable) action of steady and transient shear flow should be carried out.
- More insight should be gained on understanding the different structural length scales in dough development, whose relevance is evident from the hyper-aggregation model
- Considering bread as the most important bakery product, more studies on the role of shear-only processing on mechanical aeration during mixing which influence the final loaf volume are recommended..

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Appendixes

English Summary

Dutch Summary (Samenvatting)

Acknowledgement

Training and Supervision Plan

List of Publications

About the Author

Farsi Summary (خلاصه فارسی)

Farsi Appendixes

Summary

Dough mixing involves a combination of different types of deformation, such as shear and elongation. The complicated nature of the mixing process makes it difficult to understand dough processing on a mechanistic level. Most of studies in literature focus on well-known overall parameters such as process time, temperature, energy input (time, rotational speed). A limited number of publications address the fundamentals of dough mixing with respect to the type of different deformation flows involved in this process. It is therefore still not clear how kneading (shear versus extensional deformation) affects dough properties such as gluten development. Thus, the objective of this thesis was to investigate the effects of well-defined shear flow on the properties of wheat flour dough and to establish a correlation between relevant process parameters and the changes in product properties.

To reach this aim, a zero developed (ZD) dough had to be used as starting material for shearing experiments. The results presented in **Chapter 1** showed that mixing regimes before time to peak (TTP) gave similar dough properties when using a ZD dough instead or a flour–water mixture in the mixer. However, further mixing with ZD dough caused significant reduction both in the ability of glutenin particles to re-aggregate and in the large-strain deformation rheological behaviour of the dough. When ZD dough was used as the starting material, less energy was needed to reach TTP. Therefore, extra care has to be taken to not "over-mix" the dough system. In future dough processing studies with ZD dough, the mixing requirements of the system should be accurately determined. However, with these constraints, ZD dough does present a good model for dough mixing studies to help study the different aspects of mixing.

Chapter 2 demonstrated the value of using well-defined flow regimes to investigate the effect of simple shear flow on dough development and properties under process conditions relevant to industrial processing. The properties (cooking quality) of pasta-like products obtained by shear-only processing revealed that the new shearing device is unique in its capability to study the effect of pure shear deformation on dough development and properties at mechanical energy and shear stress levels relevant to industrial dough kneading and extrusion. This provided a basis for further studies using simple shear as key process parameter in determining product properties.

The study carried out in **Chapter 3** focused on the effects of simple shearing of dough compared with standard z-blade mixing on the physical properties of glutenin macro-polymer (GMP). It was concluded that, it is not the mechanical energy input during dough processing, but the type of deformation applied that is of crucial importance. In contrast to z-blade mixing, simple shearing at comparable levels of work input, did not lead to a decrease in the wet weight of GMP or the size of glutenin particles, evident in process tolerance of the glutenin particles.

The effect of simple shearing versus z-blade mixing on dough microstructure and rheological properties was discussed in **Chapter 4**. Mixing for short times led to formation of coarse protein patches, leading to a heterogeneous dough structure. Longer mixing, however, caused break up of large protein aggregates, leading to a homogenous dough micro-structure at a size scale of few hundred μm. Longer mixing times caused a significant loss of GMP wet weight and a reduction in the size of glutenin particles. This was accompanied by a reduction in the values of fracture properties of the dough under uniaxial extension testing. In contrast, prolonged simple shearing did not negatively affect the GMP content and the rheological behaviour and gave larger gluten structures both parallel and perpendicular to the direction of shear flow. This chapter thus confirmed the importance of the type of deformation in dough processing studies.

A new Couette-type device introduced in **Chapter 5** allowed us to systematically study the effects of shear flow in steady versus transient conditions on dough properties. In steady state, the shear rates remains constant at different locations of the cell, whilst in transient state, there is a large variation in the shear rate dependent of the gap size, as mentioned in **Chapter 5**. A steady shear flow (achieved with a concentric Couette geometry) did not lead to a decrease in GMP content and the size of glutenin particles. Under these conditions, the development was observed of an interconnected and continuous gluten network upon prolonged shearing. When the eccentricity of the Couette cell increased, longer processing led to a considerable reduction in GMP content and the size of glutenin particles. Micro-structural changes seen at the narrow gap position of the eccentric Couette cell occurred already at early stages of processing, leading to disintegration and thereby distribution of gluten aggregates. The results revealed that transient shear flow leads to elongation and thereby break up of gluten structures at a length scale relevant to microscopic study (a few hundred μm). **Chapter 5** also showed that this break up mechanism is a time-independent process. We therefore suggest that the mechanism of gluten break-up is strongly influenced by the local flow profile in a conventional mixer. The concept of "flow-induced deformation" can help to understand aggregation mechanism of the gluten. However, other mechanisms such as the different length scales as explained by the hyper-aggregation model must be considered to fully explain the aggregation mechanism of gluten.

This thesis proposes shear rate and thereby shear stress as relevant key process parameters in dough mixing studies, which opens up interesting possibilities for innovative mixer designs. The following specific topics were found to be of interest for future research:

- Further study into the role of hydration only process (non-frozen conditions in the ZD dough preparation) on gluten development and dough properties.
- More exhaustive studies on the properties of dough mixing resulting from a combined (adjustable) action of steady and transient shear flow should be carried out.
- More insight should be gained on understanding the different structural length scales in dough development, whose relevance is evident from the hyper-aggregation model.
- Considering bread as the most important bakery product, more studies on the role of shear-only processing on mechanical aeration during mixing which influence the final loaf volume are recommended.

Samenvatting

Bij het kneden van deeg vinden verschillende typen deformaties plaats, zoals afschuiving (shear) en rekstroming (elongation). Het is moeilijk om het kneden van deeg op een mechanistisch niveau te begrijpen omdat deze verschillende deformaties gelijktijdig plaatsvinden. De meeste studies gebruiken bekende parameters, zoals procestijd, temperatuur en energietoevoer (een combinatie van tijd en toerental) om het deegkneedproces te karakteriseren. Het aantal studies dat zich op meer fundamentele aspecten van deegkneden richt, zoals de verschillende deformaties, is beperkt. Als gevolg daarvan is het tot op heden nog niet duidelijk hoe het kneden deegeigenschappen beïnvloedt. Dit is dan ook de doelstelling van dit proefschrift. Hiertoe werd het effect van een goed gedefinieerde afschuifsnelheid op deegeigenschappen onderzocht. Ook werd gezocht naar relaties tussen relevante procesparameters en verandering in producteigenschappen.

Om dit doel te bereiken, diende een zogenaamd "zero-developed' (ZD) deeg gebruikt te worden als uitgangsmateriaal bij de experimenten. Dit ZD-deeg (tarwebloem gemengd met ijs zonder toevoeging van mengenergie) werd vergeleken met een bloem-water mengsel dat op de traditionele wijze gemengd werd in een labschaal deegkneder.

Hoofdstuk 1 laat zien dat tot de optimale mengtijd (time to peak, TTP) vergelijkbare deegeigenschappen gevonden werden voor het ZD-deeg en het traditionele deeg. Echter, verder mengen van het ZD-deeg liet een vermindering zien van het aggregatievermogen van de glutenine-deeltjes. Ook uit trektesten bleek dat deegeigenschappen negatief beïnvloed werden door het verder mengen. Het gebruik van ZD-deeg leidde tot een kortere optimale mengtijd (TTP). Als gevolg hiervan is er een extra risico voor overkneden bij het gebruik van ZD-deeg. Binnen randvoorwaarden mag er echter geconcludeerd worden dat ZD-deeg een geschikt uitgangsmateriaal is voor het bestuderen van de verschillende aspecten van deegkneden.

Hoofdstuk 2 laat het nut zien van het gebruik van goed gedefinieerde stromingsprofielen voor het bestuderen van deegontwikkeling en –eigenschappen onder condities, die relevant zijn voor de industrie. Met behulp van de "shear cell" (een zelf ontwikkeld apparaat, waarin materiaal vervormd wordt met behulp van een lineair afschuifprofiel) kon een deegproduct gemaakt worden met goede eigenschappen voor pasta doeleinden. De shear cell gaf zo de mogelijkheid om industrieel relevante condities systematisch te onderzoeken. De resultaten van dit hoofdstuk legden de basis voor verder onderzoek met afschuifsnelheid als belangrijke procesparameter.

De studie beschreven in **hoofdstuk 3** vergelijkt de effecten van lineaire afschuiving en traditioneel ("Z-blade") mengen op de fysische eigenschappen van GMP (Glutenine MacroPolymeer, de hoog geaggregeerde gluten fractie). Er kon worden geconcludeerd dat niet de mechanische energietoevoer bepalend is voor verandering in GMP, maar het type vervorming van het materiaal. In tegenstelling tot traditioneel mengen, leidde het afschuiven van het materiaal niet tot een afname van GMP en de deeltjesgrootte van het GMP. De hoeveelheid energie toegevoerd aan het materiaal was in beide processen vergelijkbaar.

De effecten van afschuiven versus traditioneel mengen op de deegmicrostructuur en de reologische eigenschappen is besproken in **hoofdstuk 4**. Het mengen gedurende een korte tijd leidde tot de vorming van eiwitgebiedjes, die voor een heterogene deegstructuur zorgden. Langer mengen leidde echter tot afbraak van deze eiwitgebiedjes, wat leidde tot een homogene structuur op lengteschalen van ongeveer 100 μm. Het langer mengen leidde ook tot een verlies aan GMP. Bovendien gingen de reologische eigenschappen gemeten in een uniaxiale trektest achteruit. In tegenstelling tot mengen, had langdurig afschuiven in de shear cell geen negatief effect op de hoeveelheid GMP en de reologische eigenschappen. De structuur van het materiaal vertoonde glutenstructuren die zowel parallel als loodrecht op de stromingsrichting stonden. Dit hoofdstuk bevestigde daarmee het belang van het onderscheiden van de verschillende typen vervormingen die op het materiaal worden aangebracht.

Een nieuw apparaat gebaseerd op een Couette-geometrie is geïntroduceerd in **hoofdstuk 5**. Het biedt de mogelijkheid om het effect van een veranderende afschuifsnelheid ("transient shear flow") te bestuderen. De afschuifsnelheid in het apparaat kon worden gevarieerd door de binnenste cilinder excentrisch te plaatsen.

In de centrische positie (leidend tot een constante afschuifsnelheid) werd geen afname van GMP gevonden. Na langdurig afschuiven leidde deze conditie tot een aaneengeschakeld glutennetwerk. Bij een vergrote excentriciteit van de binnen cilinder leidde langdurig bewerken tot een significante verlaging van GMP en de deeltjesgrootte van het GMP. Opmerkelijk was dat structurele veranderingen al waarneembaar waren bij kortstondig bewerken van het deeg. Desintegratie en verdeling van glutenaggregaten waren duidelijk zichtbaar in de vernauwing van het apparaat. Een veranderende afschuifsnelheid leidde tot uitrekking van het materiaal in het apparaat en afbraak van glutenstructuren. **Hoofdstuk 5** liet bovendien zien dat het opbreken van structuren via een tijdsonafhankelijk mechanisme plaatsvindt. Er kon verder worden geconcludeerd dat het concept van stromingsgeïnduceerde vervorming kan helpen om het aggregatiemechanisme van gluten te begrijpen. Echter, andere mechanismen zoals de verschillende lengteschalen in het hyperaggregatiemodel moeten worden meegenomen om een volledig beeld te krijgen van het aggregatiemechanisme van gluten.

Dit proefschrift stelt dat afschuifsnelheid en afschuifspanning moeten worden beschouwd als sleutelparameters in deegkneedstudies. Op deze wijze kunnen interessante, nieuwe ontwerpen voor deegkneders ontwikkeld worden. De volgende onderwerpen zijn van bijzonder belang voor verder onderzoek:

- Een verdere studie kan worden uitgevoerd naar de rol van het gluten hydratatieproces (in het bijzonder het produceren van ZD-deeg zonder gebruik te maken van ijs) op glutenontwikkeling en deegeigenschappen
- Een verdiepende studie kan zicht richten op de eigenschappen van deeg als gevolg van een gecombineerde (en instelbare) actie van een constante en veranderende afschuifsnelheid.
- Verder inzicht moet worden verkregen om de verschillende lengteschalen in het deegontwikkelingsproces, zoals voorgesteld in het hyperaggregatiemodel, beter te begrijpen
- Omdat brood het belangrijkste bakkerijproduct is, zou het wenselijk zijn om het effect van afschuiving te bestuderen op beluchting van het deeg. Dit is belangrijk in verband met de correlatie die bestaat tussen mate van beluchting in het deeg en het volume van het brood.

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I would like to express my profound gratitude and praises to the Almighty Allah for keeping me healthy and giving me the opportunity to finish this work. This thesis is by far the most significant accomplishment in my life. This achievement would never have been possible without people who supported me and believed in me. With no doubt, my lovely country "Iran" deserves all my appreciation for providing the fund for my PhD study in abroad.

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I am deeply indebted to my father and mother for their support and encouragement from an early age in my life. Their prayers certainly had a major role in my achievements. I sincerely appreciate my father-in-law and mother-in-law for their kind and supportive attention all over my PhD period. I thank them also for looking after my wife and my daughter for a while in Iran. I would like to express my appreciation to my brothers, sisters and brothers-in-law for their encouragements throughout my PhD study.

"A PhD-thesis is not only product of a single student brain, but also a group of hearts who are willing to help him". In this respect, I enjoyed the great support of a number of people:

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Seyed Hadi Peighambardoust

February 2006

Training and Supervision Plan (TSP)

VLAG Graduate School

Name: Seyed Hadi Peighambardoust
Group: Food and Bio-Process Engineering Group, WUR
Period: December 2001- February 2006
Promotors: Prof. dr. ir. Remko Boom, Prof. dr. Rob Hamer
Co-promotor: Dr. ir. Atze Jan van der Goot

	Year	CP♣
PhD Research Proposal	2001	4
VLAG PhD Courses		
VLAG PhD week, Bilthoven, the Netherlands	2002	0.8
Advanced Food Analysis, Wageningen, the Netherlands	2002	1
Industrial Proteins, Wageningen, the Netherlands	2003	1
Other PhD Courses		
PhD scientific trip to South Africa	2002	2
Brain storming week (section Proceskunde)	2003	1
Gluten structure, rheology and functionality, Harrogate, UK	2004	0.2
The Science of Baking, Reading, UK	2005	0.8
Language Courses		
Pre-intermediate English, Wageningen, CENTA	2002	1.2
Intermediate English, Wageningen, CENTA	2002	1.2
Academic Written, Wageningen, CENTA	2004	1.2
Scientific Writing, Wageningen, CENTA	2005	1.2
Presentations at Scientific Congresses		
2nd European Young Cereal Scientists and Technologists Workshop, Valencia, Spain (oral)	2003	1
Netherlands Process Technology Symposium (NPS3), Veldhoven, the Netherlands (Poster)	2003	0.6
Netherlands Process Technology Symposium (NPS4), Veldhoven, The Netherlands (Poster)	2004	0.6
12th ICC Cereal and Bread Congress, Harrogate, UK, (Poster)	2004	0.8
AACC Annual Meeting, San Diego, California, USA (Oral)	2004	1
4th European Young Cereal Scientists and Technologists Workshop, Vienna, Austria (Oral)	2005	1
AACC Annual Meeting, Orlando, Florida, USA (1 oral, 1 poster)	2005	1
Total		**21.6**

♣ cp= credit point; 1 cp = a study load of approximately 40 hours (1 week)

List of Publications

Peer-reviewed articles:

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

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

[3] S.H. Peighambardoust, A.J. van der Goot, T. van Vliet, R.J. Hamer and R.M. Boom, 2006. **Microstructure formation and rheological behaviour of dough under simple shear flow.** Journal of Cereal Science: In Press.

[4] S.H. Peighambardoust, A.J. van der Goot, R. M. Boom and R.J. Hamer. 2006. **Mixing behaviour of zero-developed dough compared to a flour–water mixture.** Journal of Cereal Science: Accepted for Publication.

[5] S.H. Peighambardoust, S. van Brenk, A.J. van der Goot, R.J. Hamer, and R.M. Boom. 2006. **Dough processing in a Couette-type device with varying eccentricity: effect on glutenin macro-polymer properties and dough micro-structure.** Journal of Cereal Science: Submitted for Publication.

Patent:

S.H. Peighambardoust, A.J. van der Goot, R.J. Hamer and R.M. Boom. 2005. **Process for the separation of gluten and starch.** EP 2005104257.0.

Farsi research papers:

[1] S.H. Peighambardoust and S.M. Seyyedin Ardabili. 1996. Studying the effect of different extraction rates of wheat flour on the quality of Iranian flat breads. Standard monthly journal, 28:13-23.

[2] S.H. Peighambardoust and M. Rahimi. 1997. Influence of incorporating pasta regrinds on the quality parameters of Spaghetti. Agricultural Science, 47:34-42

[3] S.H. Peighambardoust, A. OladeGhaffari, H. Gyaci and J. Hesari. 2005. Investigation of the effects of a high-temperature drying process on the quality of Spaghetti. Agricultural Science,

About the Author

Seyed Hadi Peighambardoust was born in 1971 in Marand, Eastern Azerbaijan, Iran. After accomplishing high school in biology science in 1989, he started his higher education studies in University of Tabriz (Tabriz, Iran) and received a BSc degree (with distinguished degree) in the filed of Food Science and Technology in 1994.

He continued his studies in Tarbiat Modarres University (Tehran, Iran) and accomplished his MSc (with distinguished degree) in the same field in 1996. The topic of his master thesis was on the "Effect of the Extraction Rate of Wheat Flour on the Quality of Iranian Flat Breads".

After completing the master studies, Hadi Peighambardoust was employed as a lecturer at the department of Food Science in University of Tabriz and worked there for 4 years.

In 2001, he was awarded a scholarship from the Ministry of Science, Research and Technology (MSRT) of Iran, allowing him to do a PhD in abroad. In December 2001, he joined the Food and Bio-Process Engineering Group of Wageningen University in the Netherlands to start his PhD study. A tough and unrelenting work in this group for 4 years and 3 months led to accomplishment of his PhD in February, 2006. This dissertation presents the results of his PhD study as five peer-reviewed articles in the scientific journals in the field of Cereal Science and Technology. His PhD study also resulted in one patent, which has been recently filed in European Patents.

From March 2006, he will continue his research in the field of Cereal Processing as a Postdoc at Food and Bio-Process Engineering Group of Wageningen University. After this research period, he will return to Iran to carry on his career as an assistant professor in University of Tabriz.

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که در آنها خطر از بین رفتن خواص مطلوب خمیر در حین فرآورش تا حد قابل ملاحظه ای کاهش یافته و فرآورش خمیر تا حدود زیادی تحت کنترل در می آید.

تحقیق انجام گرفته در این پایاین نامه اهمیت موضوعات ذیل برای مطالعات بیشتر را روشن می کند:

- مطالعات تکمیلی در مورد خصوصیات خمیرZD در رابطه با اثر تنها افزودن آب[1] (تحت شرایطی که از یخ استفاده نگردد) روی توسعه شبکه گلوتنی و خصوصیات خمیر
- مطالعات فراگیر بیشتر روی خصوصیات خمیردر یک مخلوی کن با قابلیت اعمال ترکیبی از نیروهای برشی یکنواخت و یا انتقالی در حالت قابل تنظیم
- مطالعات بیشتر در مورد اثرات فقط نیروهای برشی[2] روی هوادهی خمیردرطول مخلوط کردن و روی خواص کیفی (حجم) نان بعنوان محصول نهایی فرآیند

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[1] Hydration-only process
[2] Shear-only processing

موازی و هم در جهت عمود بر جریان برشی اعمال شده گردید. در نتیجه نتایج بدست آمده در این فصل اهمیت نوع جریان های تغییر شکلی در فرآورش خمیر را مورد تأیید قرار داد.

دستگاه جدید کوِت (Couette) معرفی شده در **فصل ۵** امکان مطالعه روشمند اثرات نیروهای برشی تحت شرایط یکنواخت[1] و گذرا (انتقالی) [2] را روی خصوصیات کیفی خمیر فراهم می سازد. زمانی که نیروی برشی یکنواخت اعمال می گردد، شدت برشی[3] در مناطق مختلف دستگاه ثابت باقی می ماند در حالیکه فرآورش تحت نیروهای برشی انتقالی منجر به ایجاد تغییرات زیادی در شدت این نیروها بسته به اندازه مجرا های دستگاه می گردد. نتایج مطالعه انجام گرفته در **فصل ۵** نشان داد که جریان برشی یکنواخت (در دستگاه کوِت از نوع هم مرکز[4]) باعث کاهش وزن GMP و اندازه ذرات گلوتنین نمی گردد. تحت این شرایط، فرآورش طولانی منجر به توسعه شبکه گلوتنی پیوسته در بافت خمیر می گردد. اما هنگامی که ناهم مرکزی[5] (غیر هم مرکز بودن) دستگاه افزایش می یابد، فرآورش طولانی منجر به افت قابل ملاحظه در وزن GMP و انداره ذرات گلوتنین می گردد. مطالعه بافت میکروسکپی خمیر در مجرا های تنگ دستگاه کوِت غیر هم مرکز نشان داد که فرآورش خمیر در این مناطق در همان ابتدای فرآیند منجر به جدا شدن و ریز شدن توده های بهم پیوسته گلوتن می گردد. این امر نشان دهنده این واقعیت است که جریان برشی انتقالی موجود در این نواحی دستگاه منجر به طویل شدن[6] و در نهایت پاره شدن[7] تودهای گلوتنی می گردد. **فصل ۵** نشان داد که مکانیسم این پاره شدن غیر وایسته به زمان می باشد و این پدیده در همان اثنای فرآورش رخ می دهد. در نتیجه پیشنهاد می شود که پاره شدن شبکه گلوتنی در اثر فرآیند مخلوط کردن خمیر قویا تحت تأثیر نمایه جریان های موضعی موجود در مناطق بین تیغه و جداره مخلوط‌کن می باشد. مطالعه تغییرات به عمل آمده در بافت خمیر تحت جریان های موضعی به درک مکانیسم توده شدن[8] و پاره شدن گلوتن کمک می کند. اما سایر مکانیسمها نیز از قبیل اثر شاخص های مرتبط با مقیاس های طولی مختلف موجود در مدل hyper-aggregation بایستی مدنظر قرار گیرند تا بتوان بطور کامل مکانیسم توده ای شده گلوتن را تشریح نمود.

در نهایت این پایان نامه اهمیت شاخص های نرخ برشی و تنش برشی بعنوان شاخص های کلیدی در فرآورش خمیر را نمایان می سازد. این امر امکان شایانی درطراحی میکسر های نوین نانوایی فراهم میسازد

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[1] Steady shear
[2] Transient shear
[3] Shear rate
[4] Concentric Couette geometry
[5] Eccentricity
[6] Elongation
[7] Break up
[8] Aggregation

فصل ۲ این پایان نامه نشان دهنده ارزش استفاده از جریان برشی در فرآورش خمیر جهت بررسی تأثیرات این جریان روی توسعه و خصوصیات کیفی خمیر به عمل آمده تحت شرایط وابسته به فرآورش صنعتی خمیر می باشد. نتایج آزمایشات انجام گرفته روی کیفیت پخت محصولات خمیری-مانند[1] بدست آمده از فرآیند برشی[2] نشان داد که دستگاه جدید طراحی شده برای اعمال نیروهای برشی دارای قابلیت بی نظیری برای مطالعه اثر این نیروها روی توسعه و خصوصیات خمیر تحت شرایطی که مقدار ا نرژی مکانیکی و تنش برشی[3] اعمال شده در فرآیند برشی مشابه مقادیر آنها در فرآیند صنعتی ورز دادن[4] (مخلوط کردن) و روزن رانی[5] خمیر می باشد. این نتایج نشان دهنده اهمیت نیروهای برشی بعنوان شاخص کلیدی در فرآورش خمیر و اهمیت آن در تعیین خصوصیات کیفی محصول نهایی می باشد.

مطالعه انجام گرفته در **فصل ۳** اثرات فرآورش خمیر با استفاده از نیروهای برشی در مقایسه با اثرات فرآورش خمیر با مخلوط کن z-تیغه[6] را روی خواص فیزیکی گلوتنین ماکرو پلیمر[7] (GMP) مورد بررسی قرار می دهد. نتایج بدست آمده نشان دادند که مقدار انرژی مکانیکی اعمال شده کمتر حائز اهمیت بوده و نوع نیروهای اعمال شده (جریان های برشی یا کششی) می باشند که نقش پراهمیتی دارند. فرآورش خمیر با استفاده از نیروهای برشی بر خلاف فرآورش آن با مخلوط کن z-تیغه، در سطوح قابل مقایسه انرژی اعمال شده، منجر به کاهش مقدار GMP یا کاهش اندازه ذرات گلوتنین نمی شود. این امر بیانگر مقاوم بودن ذرات گلوتنین در مقابل فرآورش با جریان برشی می باشد.

تأثیرات فرآورش با جریان برشی در مقایسه با فرآیند مخلوط کردن روی ساختار میکروسکپی و خواص رئولوژیکی خمیر در **فصل ۴** مورد بحث و بررسی قرار گرفت. مخلوط کردن خمیر در زمان کوتاه منجر به تشکیل بافت غیر همگن گلوتنی در خمیر با نواحی پروتئینی درشت گردید. در صورتیکه مخلوط کردن طولانی باعث تکه تکه شدن نواحی درشت پروتئینی در ساختار خمیر شده و در نهایت منجر به تشکیل یک بافت همگن در مقیاس با اندازه چندصد میکرون می گردد. مخلوط کردن طولانی خمیر همچنین باعث کاهش قابل توجهی در وزن مرطوب GMP و اندازه ذرات گلوتنین می شود. این امر با تحلیل رفتن خواص رئولوژیکی خمیر همراه می باشد. برخلاف فرآیند مخلوط کردن، فرآورش طولانی خمیر تحت جریان برشی وزن مرطوب GMP، اندازه ذرات گلوتنین و خصوصیات رئولوژیکی خمیر را بشکل معکوس تحت تأثیر قرار نداد. این فرآیند منجر به تشکیل بافت های گلوتنی بزرگ در ساختار خمیر هم در جهت

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[1] Pasta-like products
[2] Shear-only processing
[3] Shear stress
[4] Kneading
[5] Extrusion
[6] Z-blade mixer
[7] Glutenin macro-polymer

خلاصه

فرآیند مخلوط کردن خمیرآرد گندم در برگیرنده ترکیبی از جریان های برشی[1] و کششی[2] می‌باشد که به طور همزمان در تشکیل بافت خمیر و توسعه آن تأثیر می گذارند. ماهیت پیچیده فرآیند مخلوط کردن درک مکانیسم عمل این جریان ها را با مشکل مواجه می سازد. تحقیقات موجود در این زمینه بیشتر روی شاخص های شناخته شده کلی نظیر زمان فرآیند، دما و انرژی اعمال شده روی ماده (ترکیبی از اثر زمان و سرعت دورانی) تمرکز کرده اند. تنها تعداد کمی مقاله چاپ شده وجود دارند که بطور بنیادی و اساسی فرآیند مخلوط کردن خمیر را با در نظر گرفتن جریان های تغییر شکل[3] مختلف موجود در این فرآیند مورد بررسی قرار داده اند. بنابراین هنوز مشخص نیست که مخلوط کردن خمیر (که ترکیبی از جریان های برشی و کششی است) چگونه توسعه شبکه گلوتنی خمیر را تحت تأثیر قرار می دهد. لذا هدف این تحقیق بررسی اثرات یک جریان برشی مشخص و کنترل شده روی خواص خمیر آرد گندم جهت برقراری ارتباط بین شاخص های فرآیند و تغییرات ایجاد شده در خواص محصول می باشد.

جهت نیل به این هدف، یک خمیر توسعه یافته با مقدار انرژی مکانیکی صفر[4] (*خمیر صفر توسعه یافته یا خمیرZD* [5]) باید به عنوان ماده اولیه برای آزمایش های برشی مورد استفاده قرار می گرفت. نتایج ارائه شده در **فصل ۱** نشان داد که استفاده از خمیر ZD بجای مخلوط آرد-آب به شرطی که مخلوط کردن خمیر از نقطه TTP [6] تجاوز نکند منجر به نتایج مشابهی در خواص خمیر بدست آمده می گردد. اما مخلوط کردن خمیر از "خمیر ZD " بعنوان ماده اولیه اگر از حد TTP تجاوز کند منجر به تولید خمیری می گردد که در آن توانایی تجمع مجدد[7] ذرات گلوتنین بطور قابل ملاحظه ای کاهش یافته و خواص رئولوژیکی خمیر تحلیل می رود. زمانی که از خمیر ZD به عنوان ماده اولیه استفاده می شود، انرژی کمتری جهت رسیدن به نفطه TTP مورد نیاز می باشد. در نتیجه در چنین مواقعی باید دقت لازم مبذول گردد تا خمیر "بیش از حد مخلوط"[8] نشود. در مطالعاتی که از خمیر ZD بعنوان ماده اولیه استفاده میشود توصیه می گردد نیازمندی های مخلوط کردن چنین سیستمی در ابتدا بطور دقیق تعیین و مشخص گردد. علیرغم حساس بودن این سیستم و مشکلات ذکر شده، خمیر ZD یک مدل مناسب برای مطالعات فرآیند کردن خمیر، جائی که جنبه های مختلف مخلوط کردن مد نظر هستند ارائه می کند.

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[1] Shear flow
[2] Elongational flow
[3] Deformation flows
[4] "Zero mechanical energy" developed dough
[5] Zero-developed dough ; ZD dough
[6] Time To Peak
[7] Re-aggregation
[8] Over-mix

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و

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توسعه خمیرآرد گندم تحت جریان برشی

سید هادی پیغمبردوست

زمستان ۱۳۸۴

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گروه مهندسی فرآیندهای غذایی
دانشکده فناوری کشاورزی و صنایع غذایی
دانشگاه واگنینگن
هلند

بسم الله الرحمن الرحيم

"This is by the grace of my lord, Allah"

Design and lay out: by the author

Front page: a confocal scanning laser microscopic image of a sheared dough sample (higher magnification of the image presented in Fig. 12 C, Chapter 5).

Back page: five conclusions drawn form the five articles presented in this dissertation.

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