Large-Deformation Properties of Wheat Flour and Gluten Dough

Edward Sliwinski

Large-Deformation Properties of Wheat Flour and Gluten Dough

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Stellingen

1. Voor de mechanische eigenschappen van deeg van tarwebloem en gluten zijn orientatie-effecten van glutenine aggregaten van groot belang.
   hoofdstuk 3 en 4 van dit proefschrift

2. De toename van de breukvervorming van deeg van tarwebloem bij toenemende vervormingssnelheid wordt in sterke mate bepaald door de daarin aanwezige zetmeelkorrels. Des te hoger het zetmeelgehalte des te sterker het effect.
   dit proefschrift

3. Anders dan van Swieten aanneemt, is het onwaarschijnlijk dat de elasticiteit van deeg van tarwegluten veroorzaakt wordt door een ß-spiraal structuur van het repetitieve domein in de glutenine eiwitten.
   E. van Swieten, Structural characterization of the central repetitive domain of high molecular weight gluten proteins, proefschrift Universiteit Groningen, 2001.

4. Zoals Bloksma al in 1990 beweerde, is het belang van het verbreken van zwavelbruggen tussen gluteneiwitten tijdens kneden en het opnieuw vormen ervan tijdens rusten, in het deegonderzoek lang overschat. Desondanks zijn sommigen daarna nog lang van een dergelijk mechanisme uitgegaan.
   A.H. Bloksma (1990). Dough structure, dough rheology and baking quality. Cereal Foods World 35(2): 237-244.
   P.L. Weegels, R.J. Hamer, J.D. Schofield (1997). Depolymerisation and re-polymerisation of wheat glutenin during dough processing. II. Changes in composition. J. Cereal Science 35(2): 155-163.

5. De verklaring die Kulmyrzaev *et al.* geven voor het feit dat de aggregatie van met weieiwit gestabiliseerde olie-in-water emulsiedruppels afneemt met toenemende temperatuur bij verhitting gedurende een vastgestelde tijd boven 70°C, is onjuist.
   Kulmyrzaev, C. Bryant, D.J. McClements (2000). Influence of sucrose on the thermal denaturation, gelation and emulsion stabilization of whey proteins. J.Agric. Fd. Chem. 48: 1593-1597.
   E. L. Sliwinski, P.J. Roubos, F.D. Zoet, M.A.J.S. van Boekel, J.T.M. Wouters (2003). Effects of heat on physicochemical properties of whey protein-stabilised emulsions. Colloids and Surfaces B: Biointerfaces 31: 231-242.

6. Het is een groot onrecht dat de westerse landen per dag gezamenlijk ongeveer evenveel geld uitgeven aan landbouwsubsidies als in een heel jaar aan ontwikkelingshulp.

7. De aanpak van het Wereld Natuur Fonds waarbij grote natuurgebieden worden beschermd en toppredatoren als de Bruine beer, de Lynx en de Zeearend als symbool worden gekozen, heeft verre de voorkeur boven de in Nederland veelal toegepaste beheerstypen die gericht zijn op bescherming van één specifieke soort die lager in de voedselpyramide staat en waarbij natuurgebieden vastgezet worden in een bepaald successie-stadium.

8. In tegenstelling tot wat van Manen beweert is het niet aannemelijk dat wespendieven pas op relatief hoge leeftijd voor het eerst tot broeden komen.
   W. van Manen (2000). Reproductiestrategie van de Wespendief Pernis Apivorus in Noord-Nederland. Limosa 73:81-86.

Stellingen behorende bij het proefschrift
Large-deformation properties of wheat flour dough

Edward Lucian Sliwinski
Wageningen, 25 november 2003

Large-Deformation Properties of Wheat Flour and Gluten Dough

E.L. Sliwinski

Proefschrift
ter verkrijging van de graad van doctor
in de landbouw en milieu-wetenschappen
op gezag van de rector magnificus
van Wageningen Universiteit,
prof. dr. ir. L. Speelman,
in het openbaar te verdedigen
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voor mijn ouders

Abstract

Sliwinski, E.L. (2003). Large-deformation properties of wheat flour and gluten dough. Ph.D. thesis, Wageningen University, Wageningen, The Netherlands (194 pp., English and Dutch summaries).

Keywords: Wheat, gluten protein, bread, puff pastry, flour dough, gluten dough, rheology, uniaxial extension, biaxial extension, fracture.

Rheological and fracture properties of flour and gluten doughs from eight wheat cultivars were studied and related to gluten protein composition and baking performance in bread and puff pastry. For both uniaxial and biaxial extension flour dough showed a more than proportional increase of stress with increasing strain, a phenomenon called strain hardening. In uniaxial extension (i) stresses at a certain strain were higher and (ii) stress was less dependent on strain rate than in biaxial extension. Stress at a certain strain and strain hardening depended much stronger on the type of deformation for gluten than for flour dough. These findings are consistent with published data on birefringence of gluten and show that orientation of structure elements in elongational flow plays an important role in flour and gluten dough.

For flour dough fracture stress and strain increased with increasing strain rate. At higher strain rates and lower temperatures fracture strains hardly differed between different flour doughs no matter the protein content or composition. At lower strain rates and higher temperatures the smallest fracture strains were found for flour doughs with the lowest glutenin contents and/or the lowest protein contents. We concluded that the strain rate and temperature-dependency of the fracture strain is a very important factor to relate to protein composition. Fracture stresses were much higher for gluten than for flour dough, while fracture strains were in the same range or higher. Contrary to flour dough, the smallest fracture strains were found for glutens with the largest glutenin contents.

Puff pastry volume was positively correlated with strain hardening and negatively with the strain rate-dependency of the stress and the strain rate and temperature-dependency of the fracture stress and strain. For bread it were the doughs with intermediate dough strength that gave the highest loaf volume, while loaf volumes of flours with high dough strength (i.e. high stress-level and high strain hardening coefficient) gave intermediate loaf volumes. We concluded that a high internal stress limits the deformability of dough films between gas cells and with that of the loaf volume that can be obtained.

Voorwoord

Eindelijk is mijn proefschrift af. En dat ruim negen jaar nadat ik met het promotieonderzoek ben begonnen. Hoewel er voor mijn gevoel altijd nog zaken voor verbetering vatbaar zijn, is het echt de hoogste tijd om te stoppen met het maken van aanpassingen en deze klus definitief af te ronden. Het heeft nu lang genoeg geduurd. Op deze plaats wil ik graag iedereen bedanken die heeft bijgedragen aan de totstandkoming van mijn proefschrift.

Met de voltooiing van het proefschrift komt ook een eind aan een periode van zestien jaar waarin ik betrokken ben geweest bij de Wageningen Universiteit. Toen bleek dat de biologische tuinderij die ik in de omgeving van Oss had opgestart niet zo goed rendeerde, besloot ik in Wageningen te gaan studeren. Na in een razend tempo de deelcertificaten natuurkunde en scheikunde te hebben behaald, kon ik in september 1987 met mijn studie plantenziektekunde beginnen.

Het afstudeervak onder begeleiding van Willem Norde en Chip Haynes bij de vakgroep voor fysische en kolloidchemie beschouw ik als de kroon op mijn studie. Het onderwerp was de invloed van adsorptie op de structuurstabiliteit van globulaire eiwitten. Heel graag wilde ik op een dergelijk onderwerp promotieonderzoek doen. Bij fysische en kolloïdchemie was geen plaats, maar gelukkig kwam ik via Willem Norde in contact met Ton van Vliet. Hij was voor een onderzoek aan tarwegluten op zoek naar een promovendus die over zowel biochemische als fysisch-chemische kennis beschikte. Het zijn de bevindingen van dit onderzoek die u in dit proefschrift aantreft.

Als eerste wil ik graag mijn collega's bedanken. Albert Prins, u wil ik in de eerste plaats bedanken voor de geboden mogelijkheid om in uw groep promotieonderzoek te doen. Daarnaast ben ik u bijzonder dankbaar voor de wijze waarop u dat gedaan heeft. Uw filosofie om niet dominant aanwezig te zijn, maar medewerkers de vrijheid te geven, sprak mij bijzonder aan. Ook waardeer ik het zeer dat u ondanks dat de afronding van mijn proefschrift zo lang op zich heeft laten wachten, bereid was om als promotor te fungeren. Ton van Vliet, veel dank ben ik aan jouw verschuldigd. Grote bewondering heb ik voor de vasthoudendheid die je tot het eind toe in de begeleiding ten toon spreidde. Ook heb ik wat de reologie betreft erg veel van je geleerd. Peter Kolster, jij was vanuit ATO-DLO de initiatiefnemer van het onderzoeksproject dat de aanleiding is geweest voor de inhoud van dit proefschrift. Hartelijk dank, hiervoor. Ook wil ik je hartelijk bedanken voor de begeleiding die je mij vooral in de eerste jaren hebt geboden.

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Graag wil ik de leden van de begeleidingscommissie van het IOP bedanken voor de het nuttige commentaar tijdens de halfjaarlijkse bijeenkomsten: W.IJ. Aalbersberg, Jorrit Mellema, Toon van Hooijdonk. Daarnaast wil ik de vertegenwoordigers van TNO en de betrokken bedrijven bedanken voor de prettige samenwerking: Roel Orsel, Wim Lichtendonk, August Bekkers (TNO-voeding), Andre Delaporte, Marcel Feijs, Luc Aerts (Amylum), Aris Graveland, Peter Weegels, Arjen Bot (Unilever Research), Johan De Meester (Cargill), Nico de Jongh (Meneba), Henk Tigelaar (Latenstein) en Eva-Maria Düsterhoft (Sonneveld). Met name de samenwerking met de bakkers van Meneba heb ik erg op prijs gesteld.

Direct nadat mijn contract als assistent in opleiding afliep, werd ik aangesteld als toegevoegd docent zuiveltechnologie. Van de collega's waarmee ik in die periode heb samengewerkt wil ik met name Jan Wouters, Tom Geurts en Martin Alewijn noemen. Jan van jouw heb ik veel geleerd. Vooral de rustige en grondige wijze waarop je de werkbesprekingen voerde is me erg bijgebleven. Tom, de samenwerking met jouw heb ik altijd erg prettig gevonden. Martin, ik wens je veel succes bij de afronding van jouw proefschrift. Weet dat ik het een voorrecht heb gevonden om een aio, jij dus, te mogen begeleiden.

William, wij kennen elkaar al een tijdje. Bij jouw kon ik altijd terecht met praktische vragen op het gebied van de reologie. Vanaf jouw promotie zijn wij elkaar niet uit het oog verloren en hebben regelmatig contact gehouden. Het was voor zo'n druktemaker als ik heel prettig om een collega in de buurt te hebben die zoveel rust uitstraalde. Theo, jouw heb ik leren kennen als student toen ik als aio het practicum voortzetting levensmiddelennatuurkunde begeleidde. Daarna hebben we nog diverse malen samen opgetrokken: bij GLMT, je afstudeervak op het ATO en de begeleiding van het practicum natuurkunde voor eerstejaars. Het was vooral je vrolijkheid en gulle lach die ik zo waardeerde. Heel erg bedankt voor de prettige samenwerking en dat jullie mijn paranimfen willen zijn.

Lieve moeder en vader. Het is erg jammer dat jullie mijn promotie niet meer kunnen meemaken. Ik weet hoe trots jullie zouden zijn geweest.

Lieve Doret, in de periode waarin ik met het promotieonderzoek en het proefschrift bezig was, hebben wij samen veel meegemaakt. We hebben in die tijd allebei een ouder verloren. Maar wij zijn ook getrouwd en hebben samen twee kinderen gekregen, Arthur en Anna. Als er iemand geleden heeft onder mijn ambitie om te promoveren dan ben jij het wel. Toch heb je me altijd gesteund. Ik hoop dat we samen nog een mooie tijd tegemoet gaan.

Edward

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Chapter I

General Introduction

Wheat has been used as a food since early history. Flour of its kernels is well-suited for the production of light spongy products with a regular crumb structure, because wheat flour dough is able to retain gas during the baking process. All components of wheat flour have an effect on the final baked product but it is generally accepted that differences between flours in baking quality are mainly due to variation in gluten protein composition. The bread making process can be divided into three stages: mixing, fermentation and baking and can be seen as a combination of many chemical and physical sub-processes. During bread making the dough is being deformed in combinations of shear and uniaxial extension during mixing, sheeting, rolling and moulding and in biaxial extension during proofing. The rheological properties of the dough on deformation in shear and in uniaxial and biaxial extension therefore clearly affect these processing steps.

During mixing a large number of air cells are incorporated in the dough. Therefore from a physical point of view wheat flour dough can be considered as a foam. The stability of the gas cells in dough is strongly affected by two physical mechanisms: disproportionation and coalescence. The extent to which these mechanisms affect the final bread structure strongly depends on the mechanical properties of the dough films between the expanding gas cells. Consequently, the large-deformation and fracture properties of these dough films are of key importance for the quality of the final product, of which an even crumb structure and a high loaf volume are important quality attributes.

In this study we tried to relate the rheological behaviour of flour dough to gluten protein composition on the one hand and to baking performance of bread and puff pastry on the other hand. To that purpose the large-deformation and fracture properties of flour and gluten dough of a set of wheat cultivars were determined in uniaxial and biaxial extension as a function of deformation rate, temperature and dough composition.

1. WHEAT

1.1 THE WHEAT KERNEL

Bread wheat Triticum aestivum which is a member of the grass family (Graminae) produces one-seeded fruits, commonly called grains or kernels (Hoseney, 1994; Pommeranz, 1988). The size and hardness of the kernel varies among cultivars. The structure of the kernel is shown in figure 1. The kernel is constituted of the following tissues: bran, which is composed of several layers of cells, endosperm and embryo (germ). The endosperm cells are packed with starch granules embedded in a protein matrix. The germ comprises 2 to 3% of the kernel, the bran 13 to 17% and the endosperm the remainder. Protein is distributed unevenly throughout the wheat kernel. The embryo and the aleurone layer are the richest organs of the kernel in terms of protein concentration, consisting for about 30 and 20% of protein, respectively. The protein concentration in the endosperm is lower, but because it is by far the largest organ, it contains about 70% of the total protein of the kernel. Most of the proteins in the endosperm have a storage function, acting as a store of carbon, nitrogen and sulphur for the developing seedling, with the main evolutionary pressures being for efficient packaging for storage and mobilisation on germination of the developing plant (Shewry *et al.*, 2001).

1.2 WHEAT FLOUR COMPONENTS

1.2.1 CARBOHYDRATES: Starch

Starch is the major component of wheat flour, making up about 80% on dry weight. It is present in the kernel and flour of wheat as water-insoluble granules. The shape and size of the granules from different botanical sources vary considerably. In wheat starch two types of granules occur: lenticular ones with a diameter ranging from 10 to 45 μm (A granules) and polyhedral ones with a diameter up to 10 μm (B granules). The small granules make up about 26% of the total volume of wheat starch (Soulaka and Morrison, 1985). Wheat starch granules are mainly constituted of the biopolymers amylose and amylopectin. Amylose is an essentially linear molecule, whereas amylopectin is densely branched. With a molecular weight of around 10^8 amylopectin is one of the largest biopolymers known, while for amylose molecular weights of 10^5 to 10^6 have been reported. In wheat around 29% of the starch is amylose. In the starch granule amylose and amylopectin are organised into amorphous and crystalline regions, the crystallinity causing birefringence and X-ray diffraction.

At the wheat starch granule surface both proteins and lipids are present. The surface area of A-granules in wheat starch has been estimated to be about 0.25 m^2/g and that of B-granules, 0.7 m^2/g (Morrison and Scott, 1986). Thus, the total surface of all starch granules is large in concentrated starch systems, such as wheat flour dough. The starch granule surface is of importance for milling, because in this process hardness of endosperm is an important factor. During milling damage can be caused to a fraction of the starch granules, since the kernel will break at a weak point. In hard wheats the level of damaged starch is generally higher. This is probably due to a strong interaction between the starch granule and the surrounding protein matrix which prevents breakage of the kernel in areas close to the granule surface. In baking the water absorption of dough is strongly influenced by the amount of damaged starch (Tipples *et al.*, 1978).

Upon heating in water starch gelatinises. The gelatinisation process includes a number of changes: absorption of water and swelling of the granules, change in size and shape of the granules, loss of birefringence and X-ray diffraction pattern, leaching of amylose from the granules into the solvent and the formation of a paste or gel (Atwell *et al.*, 1988). At reduced water contents, such in dough, the changes resulting from gelatinisation are strongly dependent on the amount of water available (Eliasson, 1983). The increase in viscosity due to gelatinisation of starch has been suggested to restrict further expansion of gas cells during the baking process, and thus to contribute to the cessation of the increase in volume (Junge and Hoseney, 1981; Soulaka and Morrison, 1985). When gelatinised starch is cooled and stored a gel is formed of which the rigidity increases in time, a process which is called retrogradation. The initial gel-formation is attributed to crystallisation of amylose, whereas crystallisation of amylopectin within the granules dominates the increase in gel rigidity at longer ageing times (Miles *et al.*, 1985). Retrogradation influences the texture of starch-based food and is mainly responsible for the ageing of bread.

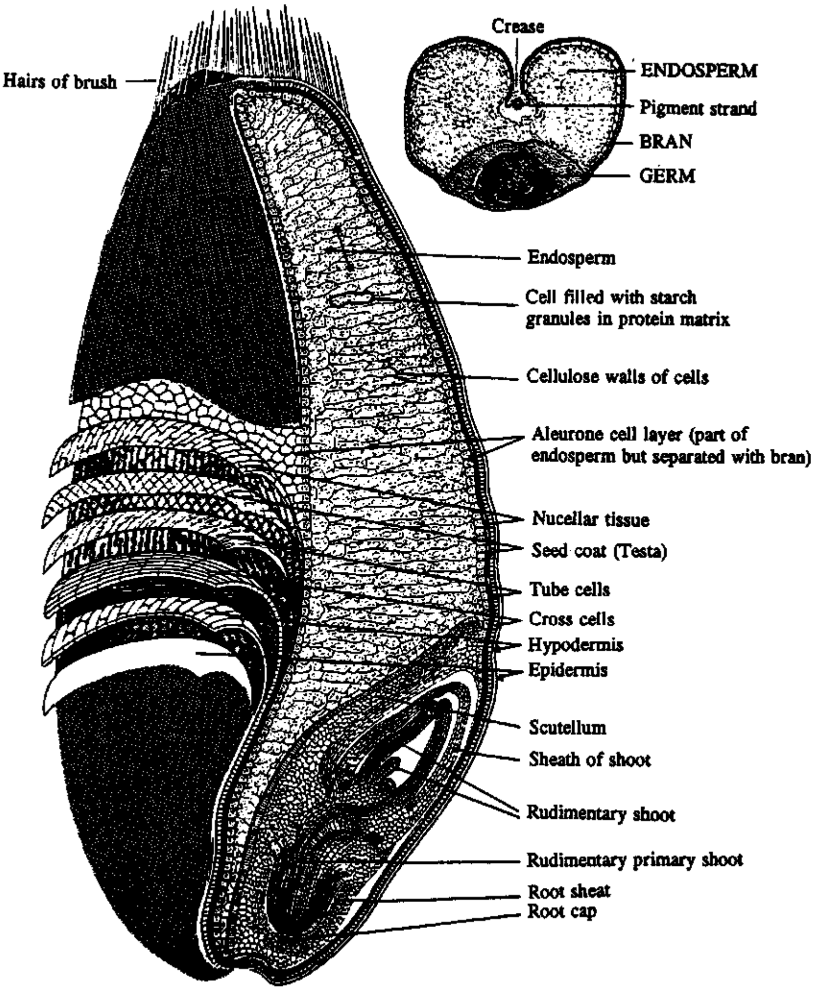

Figure 1. *The structure of the wheat kernel (from Hoseney, 1986).*

Non-starch polysaccharides

Wheat kernels and flour contain small amounts of polysaccharides other than starch. The major non-starch polysaccharide in wheat are pentosans making up about 2% on dry weight. Pentosans originate from the endosperm cell walls and can be divided into a water-soluble and a water-insoluble fraction. In wheat more than 60% of the pentosans are water-insoluble (Michniewicz *et al.*, 1990). The water-soluble pentosans of wheat have two interesting properties: they can give extremely viscous solutions (Udy, 1956; Izydorczyk *et al.*, 1991) and they can undergo oxidative gelation (Hoseney and Faubion, 1981). The functional properties of pentosans in dough and during baking can be related to their effect on the rheological properties, water holding capacity and surface activity (Michniewicz *et al.*, 1990; Shelton and D'Appolonia, 1985; Izydorczyk *et al.*, 1991). Contrary to barley and oats, in wheat only low amounts of β-glucans are present (Henry, 1987). The viscoelastic behaviour of β-glucans in solution was shown to be similar to that of guar gum, and typical for a polysaccharide solution that does not form a gel (Doublier, 1990).

1.2.2 PROTEINS: Wheat proteins

In wheat flour between 8 and 15% protein is found. Osborne (1907) has developed a classifica-tion of wheat flour protein based on the solubility in different solvents. Five fractions were distinguished: (i) albumin, soluble in water, (ii) globulin, soluble in salt solutions, (iii) gliadin, soluble in 70% ethanol, (iv) glutenin, soluble in dilute acid or alkali and (v) a residual fraction, which is frequently considered to be part of glutenin. The terms for the different groups of proteins are still used today. Most of the albumins and globulins are enzymes, such as α- and β-amylases, proteases, lipases, lipoxygenases and phosphatases, which may influence different stages of the bread making process. The three last fractions of the Osborne classification are considered to be the storage or gluten proteins. Each of the Osborne solubility classes, however, constitutes a heterogeneous mixture of proteins which may overlap considerably (Bietz and Wall, 1975). The composition of the solvent fractions is strongly dependent on the extraction conditions, such as extraction time, temperature and flour to solvent ratio (MacRitchie, 1985; Byers *et al.*, 1983). When washing a flour-water dough with water most of the soluble material like starch is removed and the remainder, gluten, consists for a large part of gliadin and glutenin. Depending on the thoroughness of washing, 75 to 85% of the gluten dry weight consists of protein, the remainder being carbohydrates (10 to 15%) and lipids (5 to 10%) (Roels, 1997).

Gluten proteins

Due to the inadequacy of extractability as a criterion for wheat protein classification an alternative nomenclature was proposed (Shewry *et al.*, 1986). In this system it was proposed to name all gluten proteins prolamins since both gliadins and glutenins contain high levels of proline and glutamine. Classification of the different proteins is then based on their molecular weight and sulphur content (see figure 2). Gliadins comprise a heterogeneous group of proteins. In a single wheat cultivar as much as 50 different gliadins were found. Gliadins are considered to be present as more or less globular protein structures stabilised by intramolecular disulphide bonds (when sulphur is present). The molecular weight of gliadin varies between 30 to 80 kDa (Schofield and Booth, 1983). They are classified as α, β, γ and ω-gliadins, according to their mobility on one-dimensional gel electrophoresis at low pH. The gliadins can also be divided into sulphur-rich (α,β,γ) and sulphur-poor (ω) proteins (Shewry *et al.*, 1984). The amino acid sequences of a number of gliadins have revealed the presence of repetitive sequences, rich in glutamine and proline. The N- and C-termini are non-repetitive. Among the various gliadin types the motif of these repeated stretches and the total length of the repetitive domain vary.

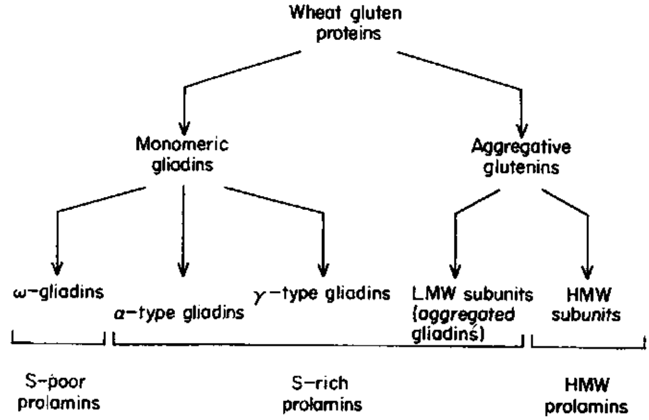

Figure 2. *Classification of the major endosperm proteins of wheat. HMW: high molecular weight; LMW: low molecular weight (from Shewry et al., 1984).*

In glutenin, the polypeptide chains are linked together covalently by inter-molecular disulphide bridges to form high-molecular-weight glutenin polymers (Kasarda, 1989; Shewry *et al.* , 1992; Giannibelli *et al.*, 2001). However, like the gliadins the glutenin polypeptides also contain intra-molecular disulphide bonds (Ewart, 1988). Similar to in other protein systems, non-covalent forces, such as hydrophobic interactions and hydrogen bonds are of considerable importance for the aggregation and stabilisation of the glutenin structure (Eliasson, 1990). Although gluten proteins contain a very low amount of charged amino acids, electrostatic interactions may also be of importance. Molecular weights ranging from a few hundreds of thousands up to several millions have been reported for glutenin (Huebner and Wall, 1976; Bietz and Simpson, 1992; Wahlund *et al.*, 1996). The glutenin polymers may be the largest polymers of protein in nature (Wrigley, 1996). The exact molecular weight and structure of glutenin polymers is, however, rather complicated to establish, due to the high molecular weight, the low charge and the high level of hydrogen bonding. Based on their mobility in SDS-PAGE two groups of subunits are distinguished, the low molecular weight (LMW) and the high molecular weight (HMW) glutenin subunits (Bietz and Wall, 1972). Glutenin may also contain medium molecular weight (MMW) proteins of non-gluten origin. It is believed that these proteins can act as chain terminators in glutenin polymer formation. The molecular weights of LMW glutenin subunits varies between 30 and 50 kDa, while the HMW glutenin subunits have molecular weights between 80 and 145 kDa. At least 20 different HMW glutenin subunits have been identified. Each cultivar possesses 3 to 5 of these HMW glutenin subunits and about 15 LMW glutenin subunits. The amino acid sequences of LMW and HMW glutenin subunits are very similar. The proteins have short non-repetitive N- and C-terminal domains in which cysteine residues are mainly located (see figure 3). The large central part is built up from short repeated motifs that are rich in glutamine, proline and glycine and poor in acid and basic amino acids.

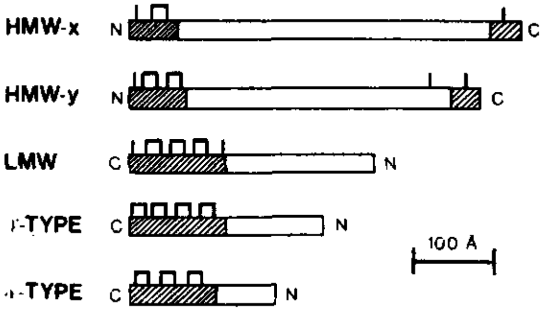

Figure 3. *Schematic representation of x- and y-type HMW glutenin subunits, LMW glutenin subunits, γ-gliadins and α-gliadins. Shaded areas represent unique sequences, white areas represent repetitive domains. Black bars represent potential interchain disulphide bonds. Linked black bars represent intrachain disulphide bonds. N: N-terminal region. C: C-terminal region (from Kasarda, 1989).*

It is still unclear how glutenin subunits are linked together to form polymers. Ewart (1972, 1977, 1979) proposed a model in which LMW and HMW glutenin subunits are linked together at random by disulphide bonds between the subunits thus forming linear concatenations. An alternative model was proposed by Graveland *et al.* (1985) consisting of a backbone of x-type and y-type HMW glutenin subunits that are alternatingly coupled head to tail and on which LMW glutenin subunits are inserted as small polymeric side-chains. LMW and HMW glutenin subunits have been shown to contain intra-molecular disulphide bonds and these are formed strictly directed and not randomly (Keck *et al.*, 1995; Keck-Gassenmeier and Wieser, 1996; Shimoni *et al.*, 1997). Using chromatographic methods on glutenin hydrolysates Keck-Gassenmeier and Wieser (1996) were able to show that besides the intramolecular disulfide bonds LMW glutenin subunits contain two additional cystein residues. These were found to be linked to different protein components: HMW glutenin subunits, LMW glutenin subunits and γ-gliadins and should thus be the reason for their intermolecular aggregation. They were also able to show that HMW glutenin subunits of the x- and y-type are polymerised linearly. These results support previous findings of dipeptide fragments being released by incomplete reduction of glutenin consisting of one x-type joined to one y-type HMW subunit (Lawrence and Payne, 1983; Werner *et al.*, 1992). Strong indications for a special interaction between x- and y-type 1D glutenin subunits come also from mixing studies involving the incorporation of HMW glutenin subunits (Bekes *et al.*, 1994; Gras *et al.*, 2001). Branching of glutenin polymers can not be excluded, for instance, because of the presence of a cysteine residue in the repetitive domain of HMW glutenin subunit 1Dx5 (Anderson *et al.*, 1989).

It is well-established that the glutenin protein fraction contributes to dough strength and bread making quality (Huebner and Wall, 1976; MacRitchie, 1987). Thorough reviews on this subject were published by Weegels *et al.* (1996) and more recently by Veraverbeke and Delcour (2002). Huebner and Wall (1976) found that wheat flours exhibiting long mixing times and strong doughs contain relatively large amounts of glutenin of large size. Glutenin fractions obtained by successive extraction of gluten proteins with dilute hydrochloric acid have been demonstrated to increase dough development time and loaf volume, whereas the gliadin fraction had the opposite effect (MacRitchie, 1987). Most studies indicate, however, that the most insoluble glutenin protein fraction is of less importance or even has detrimental effects on loaf volume than portions of the solubilised protein (Preston and Tipples, 1980; MacRitchie, 1987). Some of the detrimental effects can almost certainly be attributed to the much higher mixing requirements introduced by this fraction. The genes coding for HMW glutenin subunits have been ranked in relation to their effect on bread making quality (Payne *et al.*, 1981, 1987, Lukow *et al.*, 1989). Combinations of HMW glutenin subunits 5+10 and 7+8/9 were related to better bread making quality than the combinations of subunits 2+12 and 6+8, respectively. Also quantitative variation of HMW glutenin subunits (the total amount as well the amounts of individual subunits) was shown to be of importance for bread making quality (Huebner and Bietz, 1985; Kruger *et al.*, 1988; Wieser *et al.*, 1990). Kolster *et al.* (1992) suggested that a relation exists between the amounts of protein produced by HMW glutenin subunit genes and the contribution of HMW glutenin subunits to bread making quality.

1.2.3 LIPIDS

Generally one-third of the total lipid content of wheat flour is present as starch lipids, being almost exclusively monoacyl lipids (Morrison *et al.*, 1975). These form the starch inclusion complex and are not considered to contribute to the functional properties below the temperature at which starch gelatinisation is initiated (Eliasson and Larsson, 1993). The non-starch lipid fraction can be classified into a polar and a non-polar fraction. The non-polar lipids, which are mainly triglycerides (Morrison et al., 1975) are detrimental to loaf volume, whereas the polar lipids are beneficial (MacRitchie, 1978; Chung and Pomeranz, 1981). The function of lipids in baking can probably be related to their effects on the formation and stability of gas cells in dough.

2. BREAD MAKING

Bread is considered to be one of the basic food stuffs and it is eaten every day by billions of people all over the world. The appearance of bread and bread making procedures differ from country to country, depending on tradition and resources. Although wheat is the only cereal that can be used for making high-volume bread, various types of cereals such as barley, rye, oats and corn are also used. When wheat flour is mixed with water the special proteins which are present in wheat develop into gluten. It are these proteins that give the dough a coherent structure with the ability to retain gas and an extensibility that allows the dough to grow considerably in volume during fermentation and oven rise. It is well-known that it is not only the protein content but also the protein composition that is important for bread making quality. Flour in itself is a very complex biological material and the structure of gluten and the interactions between gluten, starch, lipids and other components are far from fully understood. Also regarding the relation between flour composition, dough rheology and baking performance many questions remain unanswered. Today, test baking is still the only really reliable method of determining bread making quality. The bread making process can be divided in three basic stages: mixing, fermentation and oven rise.

2.1 MIXING

The first stage in bread making is mixing: flour, water and other ingredients are mixed together under conditions where both shearing and extension are involved. Mixing has three important functions: (i) to blend the ingredients together into a macroscopically homogeneous mass, (ii) to develop the dough into a three-dimensional viscoelastic structure with gas-retaining properties and (iii) to include air which will form nuclei for the gas cells that grow during the fermentation of the dough (Bloksma, 1990a; Hoseney and Rogers, 1990). Both mixing intensity and mixing energy must be above a minimum critical level to develop the dough properly, the level varying with the flour and the mixer type (Kilborn and Tipples, 1972; MacRitchie, 1986). Recording dough mixers such as the mixograph give readings of the torque exerted by the dough on the pins of the mixer. During mixing the resistance against mechanical treatment increases until it reaches a maximum, which is commonly referred to as the optimum development of the dough. The time required for optimum dough development is positively correlated with the polymeric protein composition and the balance between polymers and monomers in the protein (MacRitchie, 1992). Frazier (1972) has argued that because the torque curve in fact reflects the changing viscosity of the dough, there is no reason why peak consistency in dough mixing provides any fundamental basis for optimum baking performance. He states that the only reliable way of establishing optimum conditions of mechanical development for baking is to test rheologically after a time interval comparable with that actually used in bread making. He found a positive correlation between dough strength as measured by stress relaxation time and loaf volume and showed that optimum dough development time clearly deviated from the position of peak consistency. The latter was confirmed by Roels *et al.* (1993) who studied the effect of variable water absorption levels and mixing times on loaf volume potential for six European wheat flours of constant protein level. An alternative means for developing doughs involves repeated passages through sheeting rolls (Moss, 1980; MacRitchie, 1986). Because dough is rotated after each fold, layers tend to become cross-hatched, emphasising the two-dimensional rather than unidirectional nature of the sheet-like structure being promoted within the dough (Kilborn and Tipples, 1974). Dough mixing using sheeting rolls is about 6 times as efficient than the use of the conventional pin mixer. Additionally with dough rollers bread with an exceptionally fine texture can be produced (Stenvert *et al.*, 1979).

2.2 FERMENTATION

During mixing air is included, forming small spherical cells dispersed throughout the dough. This is an important step since these cells will be sites for the growing gas cells during the fermentation of the dough. During fermentation the yeast produces carbon dioxide and ethanol by the fermentation of sugars (Bloksma and Bushuk, 1988). Initially, most of the carbon dioxide is dissolved in the liquid dough and the evaporation of gas and the growth of gas cells is low (Bloksma, 1990a). From the point where the carbon dioxide concentration reaches the saturation level, the rate of evaporation, the rate of diffusion into gas cells and accordingly the rate of the growth of the gas cells will gradually increase until these rates equal the rate of carbon dioxide production. From that moment on evaporation of carbon dioxide and growth of gas cells will keep pace with the production of carbon dioxide. During the growth of the gas cells the dough films surrounding the gas cells are continuously extended biaxially. Carbon dioxide is lost by evaporation due to diffusion of carbon dioxide to the external surface of the dough and through fracture of dough films at the surface (Bloksma and Bushuk, 1988). A fermenting dough has the appearance of a foam with gas cells of millimetre size incorporated into a continuous mass of dough. After mixing 10% of the total volume of the dough is occupied by gas cells of which between 10^2 to 10^5 are present per mm^3. During fermentation the number of gas cells can not increase. The gas cells however grow in size and at the end of the fermentation process the total volume has increased four to five times and about 75 to 80% of the total volume will be occupied by gas cells (Bloksma, 1990a). These cells were shown to be no longer spherical but polyhedral in shape by Sandstedt *et al.* (1954). Micrographs of cross-sections of dough membranes in a fermented dough showed nearly planar interfaces between the gas cells.

After mixing the dough is not only left to ferment, but it is also mechanically treated during sheeting, rolling, moulding and panning. Is the dough mainly deformed biaxially during fermentation, it is mainly deformed uniaxially and in shear during mechanical treatments like rolling and sheeting. With this respect it is important to bear in mind the time-dependency of dough behaviour, i.e. the possession of a rheological memory of what has gone before. During rolling large gas cells are destroyed in order to obtain an uniform crumb structure. Also the dough is deformed in a combination of shear and uniaxial extension. These deformations are performed at relatively high deformation rates (10^1-10^2 s^{-1}) compared to those during fermentation that occur at relatively low deformation rates (10^{-3}-10^{-4} s^{-1}). With this respect the deformation history of the dough is important, because dough films tend to become stronger in the direction of deformation and less stronger in the direction perpendicular to it. It gives that during successive fermentation steps dough will be mainly deformed in the direction perpendicular to the previous deformation, resulting in gas cells with a cigar-like shape.

2.3 OVEN RISE

During the first few minutes in the oven the dough rises quickly. This is called oven rise and is due to the following processes: (i) production of carbon dioxide by the yeast at an increasing rate until the yeast is inactivated at 50°C, (ii) increase of the saturation vapour pressure, (iii) evaporation of water into the gas cells and (iv) expansion of all gases following the temperature increase up to 100°C in the interior of the dough. At this stage of the baking process the thickness of the dough film has been reported to be of the magnitude of the diameter of a large starch granule, 30 µm, or even thinner (Sandstedt *et al.*, 1954; Gan *et al.*, 1990). The area of the gas-liquid interface must be quite large, which is why surface phenomena are assumed to be of great importance for the behaviour of dough films. To permit sufficient oven rise premature fracture of membranes between gas cells must be prevented and thus the extensibility must be maintained long enough (Bloksma, 1990a). During oven rise finally the dough structure changes from a foam into a sponge due to starch gelatinisation (Hoseney, 1994). At the beginning of starch gelatinisation, at 65°C, the loss in ability to retain gas is initiated (He and Hoseney, 1991). As the temperature in the oven increases above 100°C a crust is formed on the surface of the dough due to fast evaporation of water.

3. RHEOLOGY

Rheology is the science dealing with the relation between the deformation (strain) of a material, the stress applied and the time-scale. Regarding their mechanical properties materials can be divided into three groups, i.e. elastic, viscous and viscoelastic materials (Ferry, 1980; Barnes *et al.*, 1989; Meissner, 1997). A test piece can be deformed in various ways such as shear and elongation. In figure 4 simple shear is illustrated. A force F is applied parallel to surface A. The shear stress σ (= F/A) causes a deformation, the shear strain γ, which is by definition equal to tan α. The ratio between the shear stress and the strain is called the modulus. For an ideally elastic material the strain γ will be directly proportional to the applied stress σ, but will be independent of the deformation rate. For linear elastic materials holds:

$$\sigma = G\gamma \tag{1}$$

in which G ($N.m^{-2}$) is called the shear modulus. When a stress is applied ideally elastic materials store all the supplied strain energy. After the stress is taken away the material regains its original shape and the strain energy is totally released. On the contrary, ideally viscous materials immediately start to flow and dissipate all the strain energy supplied when a stress is applied. After the stress is taken away the material will not regain its original shape. The stress σ will be directly related to the resultant shear rate, $\dot{\gamma}$ = dy/dt, but will be independent of the deformation itself:

$$\sigma = \eta\, \dot{\gamma} \tag{2}$$

in which η (Pas) is the viscosity. As soon as a stress σ is applied the liquid will start to flow at a constant shear rate, $\dot{\gamma}$. When the stress is removed, there will be no change anymore in the shape of the test piece.

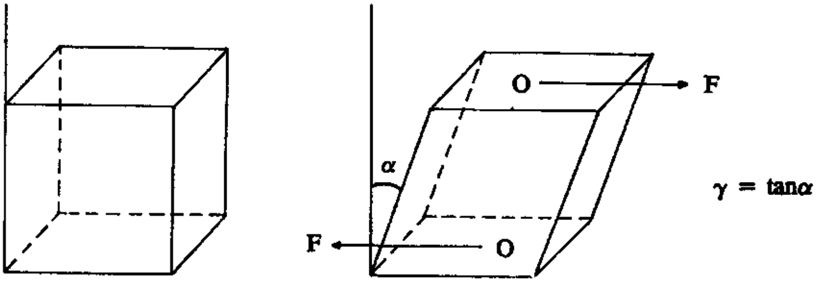

Figure 4. Illustration of simple shear (from Janssen, 1992). F(N) is the applied force.

Unfortunately, a lot of confusion exists over the terms elastic and viscous. In many cases materials are denoted elastic because of their high deformability/ extensibility. And a liquid is considered more viscous upon addition of a thickening agent, although after addition of a thickener the viscosity will have increased and officially often the material has become relatively more elastic. To make things even worse a material can be considered elastic or viscous depending on the time of observation. At intermediate time-scales, for instance, ice is considered an elastic material, but at very long time scales ice (think of glaciers) is known to flow and should thus be regarded viscous. On the contrary, liquid water is regarded viscous at intermediate time scales, but elastic at very small time scales ($t < 10^{-13}$ s). To be able to discriminate between elastic and viscous materials the Deborah number (De) was introduced. De is given by

$$De = \tau / T \qquad (3)$$

Where τ is the relaxation time of the bonds in the material and T is the time of observation. A high Deborah number denotes more elastic behaviour and a low value corresponds to a more viscous material (Barnes *et al.*, 1989). Materials of which the reaction on an applied stress is partly elastic and partly viscous are called viscoelastic. In general for a viscoelastic material it holds that the elastic contribution will dominate at relatively short time scales or high deformation rates and that at long time scales the dominating response is viscous. Wheat flour dough is a visco-elastic material.

3.1 RHEOLOGICAL MEASUREMENTS

3.1.1 Dynamic oscillation

In sinusoidal oscillation or dynamic measurements a sample is subjected to a sinusoidally varying strain ($\gamma = \gamma_0 \sin \omega t$) to which the material responds with a sinusoidally varying stress ($\sigma = \sigma_0 \sin \omega t$); its size depending on the properties of the material (Ferry, 1980). The strain is applied at a given angular frequency (ω) and σ_0 and γ_0 are the shear stress and strain amplitudes, respectively. For a viscoelastic material the stress will be out of phase with the strain by a phase angle δ due to the viscous properties of the material. The energy stored and the energy that is dissipated as heat can be separated into the storage modulus (G') and the loss modulus (G''), respectively. G' and G'' are given by:

$$G' = (\sigma_0 / \gamma_0) \,.\, \cos \delta \qquad (4)$$

$$G'' = (\sigma_0 / \gamma_0) \,.\, \sin \delta \qquad (5)$$

The phase angle δ gives information on the phase shift between the stress and strain in oscillation and so on the ratio of the viscous to the elastic properties of the material:

$$\tan \delta = G'' / G' \qquad (6)$$

Measurements should be done in the region where the modulus is independent of the strain, the so-called linear region, which means that the magnitude of the applied strain is not detrimental to the structure under investigation. G' and G'' can be determined over a range of time scales by varying the angular frequency. Nevertheless, the experimentally accessible frequency window will be limited for several reasons. In polymer rheology the frequency range can be extended by applying time-temperature superposition (Ferry, 1980). For biological and physical systems this application may be limited because of temperature-induced interactions.

3.1.2 Stress relaxation

When a viscoelastic material is exposed to a sudden strain, the response is an immediate rise in stress which gradually decreases to zero with time. The latter behaviour is called stress relaxation. The stress relaxation modulus $G(\mathrm{t})$ is obtained by dividing the shear stress σ as a function of time t by the applied strain γ:

$$G(\mathrm{t}) = \sigma(\mathrm{t}) / \gamma \tag{7}$$

With stress-relaxation measurements it is possible to investigate the rheological behaviour of a material over longer time scales, but it may be disadvantageous that the elastic and the viscous contribution can not be separated easily. Measurements at shorter time scales are limited by the finite strain rise time. The strain rise time has to be short compared with the relaxation time of the sample, and the strain has to be kept small to avoid structural changes induced by the measurement.

3.1.3 Creep tests

In a creep measurement a stress σ is applied for a certain period and then removed. It is possible to perform creep tests in shear and in elongation. The first response of an elastic or viscoelastic material to the applied stress is a momentary deformation, which is caused by elastic effects. For a viscoelastic material this is followed by a process of breaking and forming of bonds in the material resulting in a gradual increase of the strain. After removal of the stress these bonds partly return to the original situation via a reversed process. From the strain versus time curve the elastic recoil, the delayed recoil and the lasting deformation can be determined. From the slope of the viscous part of the curve the apparent shear viscosity η_{app} can be calculated:

$$\eta_{\mathrm{app}} = \sigma / \gamma \tag{8}$$

3.1.4 Biaxial extension

In a biaxial extension test a cylindrical test piece is compressed between two parallel plates due to a lowering of the upper plate at a constant speed v (Chatraei *et al.*, 1981; see figure 5). Other set ups have been developed by Dobraszczyk (1997) and Morgenstern *et al.* (1996). In uniaxial compression the stress is calculated from

$$\sigma = F_t / A = F_t / \pi r^2 \tag{9}$$

where F_t is the force on the moving disc at time t, A is the cross sectional area of the disc and r is the radius. The strain is referred to as the Hencky strain

$$\varepsilon_h = \ln (h_t / h_o) \qquad (10)$$

where h_o is the original height of the test sample and h_t is the height at time t. The biaxial strain ε_B is defined as:

$$\varepsilon_B = \varepsilon_h / 2 \qquad (11)$$

During compression at a constant test speed v the relative rate of deformation ε increases because the height of the test piece decreases:

$$\dot{\varepsilon} = d\varepsilon_h / dt = 1 / h_t \ (dh / dt) = v / h_t \qquad (12)$$

The biaxial extensional strain rate is defined as:

$$\dot{\varepsilon}_B = - \dot{\varepsilon} / 2 \qquad (13)$$

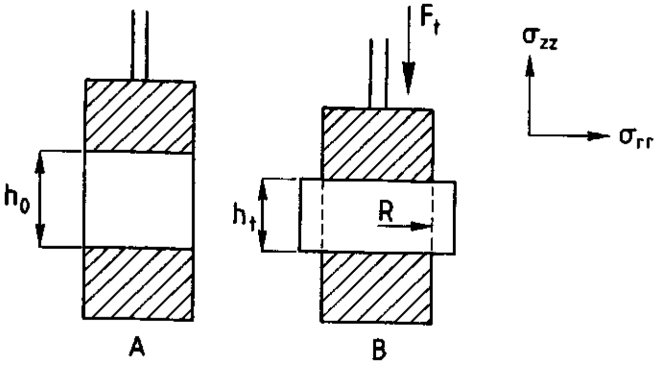

Figure 5. *Schematic view of the geometry for lubricated uniaxial compression tests. A: situation at the beginning of the experiment; B: situation during compression.* F_t *is the force at time t,* h_t *the height of the test piece at time t, R the radius of the compression plate,* σ_{zz} *and* σ_{rr} *are the normal and the radial stresses respectively (from Janssen, 1992).*

With this type of test the fracture behaviour cannot be determined. To compare extensional behaviour with shear behaviour it is convenient to study the Trouton ratio:

$$\eta_{EB}(\varepsilon) / \eta\ (\gamma) \qquad (14)$$

where η_{EB} is the biaxial extension viscosity. For ideal Newtonian liquids the Trouton ratio is:

$$\eta_{EB} = 6\ \eta \qquad (15)$$

but can be much higher for elastic liquids.

3.1.5 Uniaxial extension

In an uniaxial extension measurement a test sample with a rectangular shape is clamped at both ends and stretched in one direction up to fracture (Lenk, 1978). Perhaps the best equipment to perform such tests is the Meissner-type rheometer equipped with the rotary clamp technique (Meissner and Hostettler, 1994). To avoid premature fracture at the grips of the testing machine sometimes dumbbell-shaped samples were used as was done by Schofield and Blair (1933), Gluklich and Shelef (1962), de Bruijne *et al.* (1990). A method which involved the extension of circular dough rings was described by Tschoegl *et al.* (1970a). In uniaxial extension the stress is calculated from

$$\sigma = F_t / A \qquad (16)$$

where F_t is the force on the moving clamp at time t and A is the cross sectional area of the test piece. The strain is referred to as the Hencky strain

$$\varepsilon_h = \ln\ (l_t / l_o) \qquad (17)$$

where l_o is the original length of the test sample and l_t is the length at time t. In the Meissner-type elongational rheometer is is possible to perform tests at constant strain rates. In order to do so the deformation rate of the clamp should increase during the extension test. In the case where uniaxial extension tests are performed at a constant test speed v the relative rate of deformation $\dot{\varepsilon}$ decreases:

$$\dot{\varepsilon} = d\varepsilon_h / dt = 1 / l_t\ (dl / dt) = v / l_t \qquad (18)$$

Contrary to lubricated uniaxial compression with uniaxial extension it is possible to determine the fracture properties. These properties are generally expressed in the stress at fracture, σ_f and the strain at fracture, ε_f, which are both parameters that can be obtained from the stress-strain curve. For uniaxial extension the Trouton ratio is 3.

4. DOUGH RHEOLOGY

4.1 DYNAMIC OSCILLATION

For flour-water dough a very small linear region, if any, has been found (Smith et al., 1970). The storage modulus was reported to be strain-independent below strain values between 0.0002 (Smith *et al.*, 1970) and 0.002 (Hibberd and Wallace, 1966). The storage modulus of gluten dough is less dependent on the strain. Strain values of 0.03 to even 0.1 were reported to be in the linear region for freeze-dried and hydrated gluten doughs (Uthayakumaran et al., 2002; Cornec *et al.*, 1994, Tsiami *et al.*, 1997; Wang and Kokini, 1995). Apparently, the magnitude of the strain dependence of the storage modulus is determined primarily by the starch content in dough (Smith *et al.*, 1970; Larsson and Eliasson, 1997; Uthayakumaran *et al.*, 2002). This is consistent with the effect of a rigid filler like starch in a polymer matrix (Nielsen, 1974). It has, however, been reported by Dhanasekharan *et al.* (2001) that the onset of non-linear viscoelasticity for gluten dough is a function of the testing frequency. At low testing frequencies the linear viscoelastic strain limit was 0.1, while at high testing frequencies the strain limit shifted to 0.0001.

Generally, both for flour and gluten dough the storage and the loss modulus increase with increasing frequency. The frequency-dependence of the storage modulus decreases with increasing protein content. For gluten doughs both the frequency-dependence of the storage modulus and value for tan δ were found to decrease with increasing glutenin to gliadin ratio (Janssen *et al.*, 1996) and with an increasing amount of glutenin of high molecular weight (Cornec *et al.*, 1994; Popineau *et al.*, 1994). For both flour and gluten doughs the storage modulus was found to decrease with increasing water content, while tan δ stayed relatively constant at all frequencies (Smith *et al.*, 1970; Kokelaar *et al.*, 1996; Uthayakumaran *et al.*, 2002). Flour and gluten dough show temperature softening. In the temperature range between 20°C and 55°C the storage modulus decreases with increasing temperature (Kokelaar *et al.*, 1996; Dhanasekharan *et al.*, 2001). At a further temperature increase the storage modulus was found to increase due to starch gelatinisation and a higher cross-link density of gluten proteins.

4.2 STRESS RELAXATION AND CREEP RECOVERY

The rheological behaviour of wheat flour dough at longer times may be more efficiently determined in stress-relaxation measurements. When wheat flour and gluten dough are subjected to a sudden stress, stress relaxation shows the terminal zone (Carlson, 1981). This implies that the flowing elements giving stress relaxation are not permanently cross-linked. That the flowing elements in dough are not bound permanently by cross-links can also be concluded from creep-recovery experiments, where a steady state viscosity is observed after recovery from the elastic deformation (Smith and Tschoegl, 1970). Transient junctions such as entanglements and non-covalent interactions are thus consistent with the rheological behaviour as shown by wheat flour and gluten dough (Larsson et al., 1997). This is, however, not in conflict with the fact that permanent cross-links, such as disulphide cross-links, are important for protein structure.

4.3 ELONGATIONAL FLOW

Schofield and Scott-Blair (1933) were the first who performed simple uniaxial elongational flow measurements on dough by stretching cylindrical dough samples. They reported a phenomenon they called "work hardening", similar to hardening of metals during working. According to Lenk (1978) at a place of weakness in the test sample during elongation always a neck will form. When a neck has formed one of two things can happen on further elongation of the test piece (see figure 6). The sample can become progressively thinner at the location of the neck and eventually will fracture at relatively low strains. It is also possible that the neck may stabilise itself at a constant cross-sectional area as the shoulders travel along the test piece. In that case premature fracture is prevented by the occurrence of strain hardening, which is defined as a more than proportional increase of the stress with increasing strain. If strain hardening occurs depends if the proportionally limit is exceeded or not. For uniaxal extension the relative increase of the stress with a relative increase of the strain should be higher than 1 (Lenk, 1978).

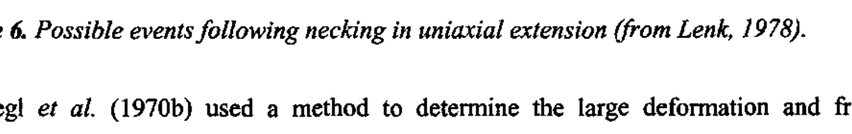

Figure 6. *Possible events following necking in uniaxial extension (from Lenk, 1978).*

Tschoegl *et al.* (1970b) used a method to determine the large deformation and fracture properties of flour dough in uniaxial extension whereby dough rings were submerged in a liquid of matching density. They found that at a constant strain rate the true stress increased continuously with the strain, indicating that the elastic retractive force increases throughout the test and no trend towards steady state flow develops. They also found that flour type, water content, temperature and strain rate have a drastic effect on the stress-strain behaviour. Using the same method Rinde *et al.* (1970) studied the large-deformation properties of gluten dough. They reported that gluten was stronger, more elastic and less extensible than flour dough and showed that fracture stress and strain depended on the extension rate. Kieffer *et al.* (1981)

developed a method to determine the large deformation and fracture properties of small samples of flour and gluten dough in uniaxial extension called the micro-extensograph. Using this method Kim *et al.* (1988) found that for gluten dough the resistance to extension decreased and the extensibility increased with increasing gliadin to glutenin ratio. Schweizer and Conde-Petit (1997) studied the large-deformation and fracture properties of flour dough in uniaxial extension using the elongational rheometer developed by Meissner and Hostettler (1994) which is based on the rotary clamp technique. They found that the stress was higher if the sample was elongated parallel to the last sheeting direction than perpendicular. It followed from their results that in uniaxial extension flour dough shows a well-developed strain hardening behaviour. Uthayakumaran *et al.* (2002) subjected flour and gluten dough to uniaxial elongation and found that gluten doughs possessed larger elongational viscosities than flour doughs, the differences being larger at lower strain rates.

The relevant type of deformation around an expanding gas bubble is biaxial extension (van Vliet *et al.*, 1992). Lubricated uniaxial extension experiments in which this type of deformation is applied on flour dough have been performed by several researchers (Bagley et al., 1988; Huang and Kokini, 1993; Janssen *et al.*, 1996; Kokelaar *et al.*, 1996). Other experimental set-ups have been developed by Morgenstern *et al.* (1996) and Dobraszczyk (1997), which have the possibility to determine fracture properties in biaxial extension. An important result was that flour and gluten doughs also show strain hardening behaviour in biaxial extension (Janssen *et al.*, 1996; Kokelaar *et al.*, 1996). It was found that the rheological behaviour of flour dough is influenced by flour type, strain rate and temperature. The main factors that determined the rheological behaviour of gluten dough in biaxial extension were found to be the glutenin to gliadin ratio and the source from which the glutenin originated. In biaxial extension the stress was found to be higher for gluten than for flour dough and less dependent on the strain rate. Gluten dough exhibited stronger strain hardening than flour dough. An increase of the temperature from 20°C to 55°C resulted for gluten dough in a decrease of the stress, of the strain rate-dependency of the stress and of strain hardening.

4.4 THE GLUTEN NETWORK IN DOUGH

In the 1930's a freshly mixed flour dough was described by Swanson (1938) as having a colloidal structure constituted of a continuous water phase surrounding the starch granules and gluten strands. Some molecules of the solutes are apparently adsorbed on the starch granules and the gluten strands. The gluten proteins, forming a three-dimensional network, also form a continuous phase. Swanson (1938) considered it likely that the gluten protein particles or molecules have a structure like that of a nucleus from which extend several "streamers" which easily would become entangled in each other and thus form thread-like strands. The elastic properties of the dough, although imperfect, are contributed by the peculiar structure of the gluten protein particles. According to Swanson (1938) the mechanical behaviour is as that the gluten strands are composed of short-length filaments. Each filament is elastic by itself, but they can slide along each other due to the water films which are adsorbed on the starch granules and the other gluten filaments.

In the 1960's and 1970's dough development was explained by the formation of a continuous network with covalent disulphide cross-links between separate protein molecules formed by thiol-disulphide interchange reactions. In this model the gluten network was considered as large as the piece of dough examined. Meredith (1964) called it the "giant protein molecule". Muller (1969) applied the statistical theory of rubber elasticity to wheat gluten and concluded that the concept of gluten quality is essentially one of cross-linkage. Later Tatham *et al.* (1984); supposed these elastic properties to be due to the presence of particular repetitive amino acid sequences in the beta-turn conformation, which glutenin has in common with elastin, an elastic protein occurring in animal ligaments. However, based on the solubility of gluten proteins and the effect of temperature on the modulus of flour and gluten dough, it was concluded that the model of Meredith could not be true (Bloksma, 1990b). An alternative hypothesis assuming un-branched glutenin molecules with disulphide cross-links within the chains, which are mutually connected only by non-covalent cross-links and which are locally aligned was proposed by Ewart (1968, 1979). This model easily explains viscous deformation. Glutenin chains slide one along another by breaking only non-covalent cross-links. Breaking of cross-links, sliding, and formation of new cross-links at new positions can occur simultaneously; the last step restores the original strength of the material. Wall (1979) took an intermediate position and suggested that the glutenins soluble in dilute acetic acid have the structure as proposed by Ewart and that part of the residue protein is present in the form of isolated bodies containing a cross-linked network. In this model, non-gluten proteins, gliadins, and part of the residue protein are dispersed in the soluble protein.

Wrigley *et al.*, (2001) depicted a model for gluten protein interactions based on the general property of all polymer systems that above a critical molecular size, physical properties such as tensile strength show a much steeper rate of increase with increasing molecular size. This is believed to be due to frictional resistances at widely spaced points in the polymer chain, referred to as entanglements. An entanglement network appears to be a good model for the continuous protein structure of a developed dough (Singh and MacRitchie, 2001). The entanglements act as transient cross-links, i.e. they provide a resistance when dough is subjected to a stress, but unlike true covalent cross-links, are potentially able to slip free as the stress is maintained. In addition, non-covalent bonds between the chains can be seen to determine slippage of these chains along one another, thus also affecting dough properties. According to Singh and MacRitchie (2001), dough mixing properties, dough strength and extensibility therefore can be understood in terms of the extension of large glutenin molecules giving rise to rubber elasticity and to the presence of molecular entanglements which contribute strength and extensibility to dough systems. These authors also argued that the low solubility of gluten proteins compared to many other proteins that have been studied evidently arises from the low entropy of mixing of the largest size glutenins and that the relatively high value for the Flory-Higgins interaction parameter is mainly due to a low concentration of ionisable groups.

Based on a thorough analysis of the mechanical spectra of gluten Cornec *et al.* (1994) concluded that the connection density in the network is governed primarily by the amount of the largest glutenin aggregates, whereby the temporary cross-links connecting the glutenin aggregates have more to do with low-energy bonds than with entanglements (i.e. topological constraints in flexible chain systems). In their view gluten can be considered as suspensions of colloidal flocs (floc = large glutenin aggregate) with neighbouring flocs connected in space by non-covalent interactions (Lefebvre *et al.*, 1994).

The structure property relationship of gluten has been described in terms of a highly amorphous, multi-polymer system, water-plasticisable but water-insoluble, and capable of forming continuous multi-dimensional viscoelastic films and networks. Using polarisation light microscopy Slade *et al.* (1989) demonstrated that stretched gluten films have positive birefringence, which is typical for biopolymer fibres as gelatin, starch and cellulose, all having a fringed micelle structure with the chain direction in the microcrystalline regions of the fibre parallel to the long axis of the fibre. The photomicrographs of stretched wheat gluten showed oriented macrofibrils with a diameter of about 0.5 µm and embedded in a viscous, amorphous matrix. The micrographs also showed that fibrillation occurred as a result of stretching. Detailed experiments allowed the identification of the macrofibrils as glutenin and the matrix as gliadin. Fibrillation of gluten can be minimised by applying biaxial extension of folded, sheeted doughs like in blow-moulding or spherical extension of air cells during fermentation of yeasted doughs (Slade *et al.*, 1989).

A prerequisite for phenomena as birefringence is that the polymers show anisotropy. Using a combination of viscometry and light scattering Sreeramulu *et al.*, (1999) were the first to show that glutenin aggregates that were sequentially fractionated using dilute hydrochloric acid indeed are anisotropic. Data are shown in table 1. Molecular weights of glutenins were reported to range from 11 to 40 million Dalton. For glutenin fractions of both cultivars the ratio between the radius of gyration and the hydrodynamic radius ranged from 5 to 10, which, according to Tanford (1961) indicates high axial ratios and thus anisotropy of these biopolymers. Intrinsic viscosities increased with increasing fraction number for glutenin fractions of both cultivars. Intrinsic viscosities were higher for Hereward than for Glenlea, which, according to Flory (1953) indicates longer chains with a higher axial ratio for Hereward glutenins.

Table 1. *Hydrodynamic radius, R_h, radius of gyration, R_g, size, M_w, and intrinsic viscosity, $[\eta_o]$, of glutenin fractions that were sequentially fractionated in dilute hydrochloric acid from gluten of the European wheat cultivar Hereward and the Canadian wheat cultivar Glenlea (from Sreeramulu, 1999).*

Fraction number	**Glenlea**				**Hereward**			
	R_h (nm)	R_g (nm)	M_w (10^6 Da)	$[\eta_o]$ (cc.g^{-1})	R_h (nm)	R_g (nm)	M_w (10^6 Da)	$[\eta_o]$ (cc.g^{-1})
7	88	823	11.3	72	99.7	580	14	146
8	94	936	14.6	76	129.4	632	19	200
9	114	836	23.3	91	126.3	831	23	235
10	159	1053	37.0	72	154.2	1040	40	267

It was originally believed that the repetitive region of glutenin subunits has a β-spiral structure which exhibits intrinsic elasticity and thereby contributes elasticity to the gluten network (Tatham *et al.*, 1984). This hypothesis strikes against the fact that, contrary to rubber and elastin, the resistance of dough to an elastic deformation decreases with increasing temperature. Recently it was shown that upon hydration glutenin proteins form β-sheet structures (Belton *et al.*, 1995). This reduction in force with increasing temperature therefore should result from inter and intramolecular hydrogen bonds being broken and replaced by protein-water interactions. (Tatham *et al.*, 2001). In order to explain this behaviour the loop and train model, originally developed for the adsorption of polymers at interfaces (Fleer and Scheutjens, 1982) was applied to gluten aggregation by Belton (1999). The model is depicted in figure 7. He proposed that on stretching the network will first deform by deformation of the loops and then by the trains being pulled apart. The structure will relax by returning to the equilibrium of loops and trains. According to Belton (1999), the restoring force will consist of an entropic term associated with the conformational entropy of the loops and with the enthalpy of hydrogen bond formation in the trains. The entropy loss resulting from inter-chain hydrogen bond formation will, in part, be compensated by the increased entropy of the hydrogen water released. Since the repetitive regions of gluten proteins are rather apolar, the hydrophobic effect will also be of importance for the temperature-dependence of structure formation. It was concluded that the loop and train model therefore is able to explain (i) the spectroscopic properties of HMW glutenin subunits, (ii) that esterification of glutenine residues decreases the coherence and resistance to extension in doughs, (iii) that deuteration increases dough strength, the elastic component of gluten dough and that water plasticises dough (Shewry *et al.*, 2001).

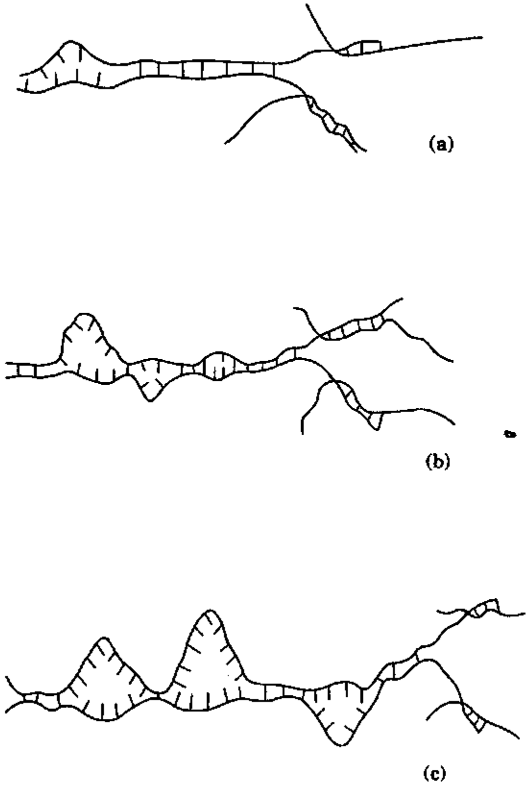

Figure 7. *The effects of hydration on the loop and train behaviour of HMW glutenin subunits. For simplicity, interactions between two chains are shown. In reality many chains may interact. (a) Low levels of hydration - hydrogen bonds are mainly interchain. (b) Intermediate levels of hydration - some loops are formed. (c) High levels of hydration - the system contains many loops but sufficient interchain bonds exist to maintain interchain contacts (from Belton, 1999).*

4.5 DOUGH RHEOLOGY AND BREAD MAKING

Differences in baking quality can not be correlated to only one rheological parameter, rather a combination of three criteria is required (Bloksma, 1990a; van Vliet *et al.*, 1992; Janssen *et al.*, 1996; Kokelaar *et al.*, 1996; van Vliet, 1999). It is proposed that for the production of a bread with a high loaf volume and a fine crumb structure the following bulk rheological properties are required: (i) resistance to extension within an optimum range, (ii) extensibility in extension above a minimum value and (iii) strain hardening in extension above a minimum value. Rheological tests on dough can reliably predict its behaviour in a bakery only if the rates and extents of deformation in these tests are in the same range as those during dough processing (Tschoegl *et al.*, 1970a; Bloksma, 1990b; van Vliet *et al.*, 1992; Dobraszczyk and Roberts, 1994; Morgenstern *et al.*, 1997). In fact, little information is available of studies in which useful rheological data are linked to results from baking tests. In most cases rheological data are used that are obtained at irrelevant conditions.

Many authors consider those flours of good bread making performance that require longer mixing times and exhibit higher dough strength. It was for instance shown by Tschoegl et al (1970b) who performed uniaxial extension tests on doughs of two flours that differed in bread making performance that the weaker dough dissipated the elastic strain energy faster than the stronger, better baking dough. Rao *et al.* (2000) compared rheological properties and bread making performance of a set of medium strong (MS) and extra strong (ES) wheat flours. MS cultivars proved to be better suited for bread making, although for ES cultivars longer mixing times and longer relaxation times were found. The fact that the resistance to deformation should be within an optimum range is supported by the observations of Uthayakumaran *et al.* (2002) who studied the rheological properties of flour and gluten dough at small and large strains in uniaxial extension and found lower elongational viscosities for the bakers dough than for the stronger dough.

To permit sufficient oven rise extensibility of dough films should be maintained long enough (Bloksma, 1990b). Preferably fracture properties of dough films are determined under conditions such as experienced by the dough during baking. Tschoegl *et al.* (1970b) reported that fracture strains were smaller at lower extension rates and higher temperatures for the weaker than for the stronger dough, while at the lowest temperature and the higher extension rate the converse was true. Similar observations were made by Dobraszczyk and Roberts (1994) who found that for low rates of inflation and high temperatures the weaker dough failed at much lower strains. A positive correlation has been observed between bread making performance of a flour and the strain hardening coefficient of a dough in biaxial extension (van Vliet *et al.*, 1992; Dobraszczyk and Roberts, 1994; Janssen *et al.*, 1996; Kokelaar *et al.*, 1996). It was also found that the strain hardening coefficient of the better baking flour depended to a lesser extent on deformation rate and temperature.

5 AIM AND OUTLINE OF THIS THESIS

The aim of the investigations described in this thesis is to increase our knowledge on the relationship between dough rheology, flour composition and baking quality. During the fermentation stage bread dough is mainly deformed in biaxial extension at relatively low deformation rates as a result of the growth of gas cells. However, during mixing, sheeting and moulding treatments bread dough is deformed at large strains in uniaxial extension at relatively high deformation rates. These treatments can vary depending on the bakery process and product. Since flour dough is a viscoelastic material, the deformation history of the material affects its rheological behaviour. To improve our insight we therefore studied the rheological behaviour of wheat flour and gluten dough in uniaxial and biaxial extension at large-deformations. Important variables were flour type, deformation rate, temperature and dough composition. Flours of six European and two Canadian wheat cultivars, which performed differently in cereal products were used. Results found were related to gluten protein composition and to baking quality for bread and puff pastry.

In chapter 2 a thorough analysis of the Kieffer Extensibility Rig is presented. Also the formula's are given that are required to recalculate force-displacement curves into stress-strain curves. In chapter 3 a comparison is made between the large-deformation properties of flour dough in uniaxial and biaxial extension. Besides doughs were tested in small angle sinusoidal oscillation. In chapter 4 the large-deformation properties of gluten doughs were determined in uniaxial and biaxial extension and compared with those of the parental flour doughs. In chapter 5 the gluten protein composition of the set of flours was determined and related to the rheological and fracture properties of optimally mixed flour doughs tested in uniaxial extension. In chapter 6 the baking performance of the flours for bread and puff pastry was determined and related to the rheological properties of flour doughs in uniaxial extension. The effect of temperature and of the addition of a mix of additives was included in the tests.

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Chapter II

The Kieffer Dough and Gluten Extensibility Rig - an experimental evaluation[1]

SUMMARY

Load-extension tests on flour dough are widely used by plant breeders, millers and bakers. The 'Kieffer dough and gluten extensibility rig' is a small-scale version of the Brabender extensograph, in which test pieces of about 0.4 g are extended. With the Kieffer rig lower strain rates can be applied than in the Brabender extensograph and the experimental data can be expressed in terms of stress and strain. In this paper the performance of the Kieffer rig is illustrated by measurements on a weak and a strong dough. Formulas are given for the calculation of fundamental rheological parameters from the results of measurements with the Kieffer rig. Sagging and bending of the test pieces before measurements could be started, caused difficulties in the determination of the exact starting point of extension. The deformation was not purely uniaxial extension, because also a shear component was observed. The amount of dough that is extended did not increase throughout the test. Probably due to the occurrence of a shear component fracture occurred mainly near the hook. A relatively large variation in stress and strain at fracture was observed. The maximum in stress represents the strain at which the sample fractures macroscopically better than the maximum in force. Variation in deformation history and volume of the test pieces have a negative effect on the reproducibility.

INTRODUCTION

Currently wheat is one of the most abundant crops in the world (Hoseney and Rogers, 1990). From wheat flour a large variety of food products can be made, like breakfast cereals, breads, crackers, cakes, biscuits and pasta. By mixing, flour and water are transformed into a cohesive dough with viscoelastic properties (Schofield and Blair, 1932). The rheological properties of wheat flour dough affect its behaviour during processing and consequently the quality of the finished loaf of bread (Bloksma, 1990b). This conviction has led to the design of instruments with which rheological properties can be determined. Such measurements are widely used for the selection of new cultivars in breeding, for quality control in mills and bakeries and to study the effects of ingredients and adaptations of processes in the milling and baking industry.

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[1] B.Dunnewind, E.L. Sliwinski, K. Grolle, T. van Vliet. Journal of Texture Studies. Submitted for publication.

Fracture of dough membranes during fermentation and baking restricts the bread volume that can be obtained. Therefore tests determining large-deformation and fracture properties are relevant.

Information on the resistance to extension and the extensibility of dough can be obtained by load-extension instruments like the Brabender extensograph (Bloksma, 1990a). In this apparatus a cylindrical piece of dough is deformed in uniaxial extension by a hook which travels downwards at a constant rate. The required force to deform the dough is expressed in Brabender Units (BU) as a function of extension. From the extensograph load-extension curve several parameters can be derived, such as maximum resistance (R_{max}), extensibility to maximum resistance (E_{max}), total extensibility (E_{tot}) and the total area under the curve (A_{tot}) as a measure of applied energy (Muller *et al.* 1961). It has been used to predict baking performance of a flour based on these parameters (Bloksma and Bushuk 1988; Walker and Hazelton 1996). Although Bloksma (1962) has related the data obtained by this test to more fundamental parameters, the test still remains very empirical. The force and extension are not expressed in Newtons and strain, respectively. Major practical disadvantages of this test are that the position of the cradle (the clamp) depends on the force and that the amount of dough deformed increases with extension (Bloksma and Bushuk 1988). The conversion of extensograph load-extension curves into stress-strain curves is therefore seriously hampered. Under fermentation conditions, the rate of deformation of dough is three orders of magnitude smaller than the maximum rate of deformation of the test-piece in load-extension instruments like the Brabender extensograph (Bloksma 1990a). Therefore, the applied rates of deformation may be relevant for mixing and for the shock resistance of dough during processing, but the relevance of the data obtained at this high rate for baking performance can be questioned. Moreover, the dominant type of deformation during fermentation and oven rise is biaxial extension and not uniaxial (van Vliet *et al.* 1992). Finally, a large amount of flour (300 g) is needed to perform the test.

An apparatus similar to the Brabender extensograph was developed by Kieffer: the 'Kieffer dough and gluten extensibility rig', also called a micro-extensograph (Kieffer *et al.*, 1981a and 1981b). In this apparatus a test can be performed with only about 0.4 g of dough and the apparatus can be fitted on any materials testing machine, which gives us the possibility to measure the force in Newtons, to adjust the test speed and temperature and to store the data in computer files for further calculations. Kieffer *et al.* (1981b and 1998) have compared the test with the Brabender extensograph and also related it to bread making performance. At a speed of about 410 mm/min the strain rate is about the same as that of the Brabender extensograph. Recently, Grausgruber *et al.* (2002) comparing both instruments, concluded that the micro-extensograph method is valuable in early-generation selection for wheat quality where the amount of available sample does not allow testing by the standard extensograph. The test method itself, however, has not been described extensively and formulas to calculate the stress and relative deformation rate are not given. Also only very little has been reported about the reproducibility of the test. To test this uniaxial extension instrument we studied the rheological properties of a weak and a strong dough and paid special attention to the correct determination of the type of deformation, the starting point of the actual extension and the reproducibility of the test.

THEORY

In Figure 1A the extensibility rig is drawn schematically. Test pieces of dough are formed to 5 cm long pieces with a trapezium like cross-section (3/5 x 4 mm) in a teflon mould. After a resting period a dough piece is clamped between two plates by means of springs under the lower plate and extended in the upper direction with a hook (diameter 1.20 mm). As a small amount of the dough is squeezed between the plates, the dough volume increases somewhat and due to gravity the sample will bend to a certain extent. This means that the point where the actual extension starts, lies above the surface of the lower plate. So the deformation is zero up to that specific point. In Figure 1A, the distance which the hook has to travel from the surface of the lower plate to that point, is represented by y_0.

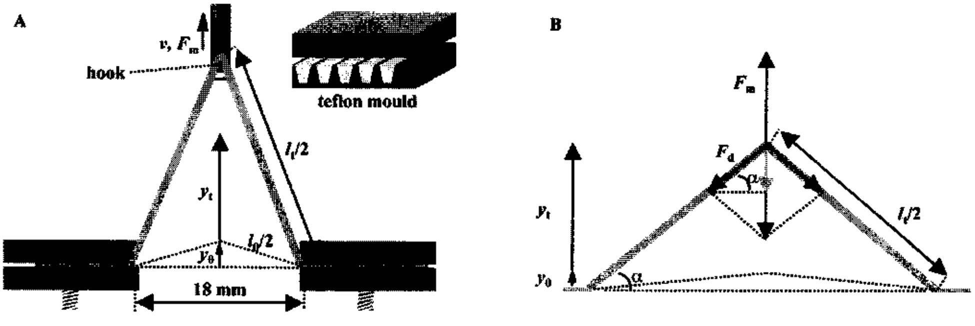


Figure 1. 1A: Schematic drawing of the Kieffer extensibility rig with its teflon mould. 1B: Schematic drawing of the forces acting on the dough piece (equation 5).

The formulas for the calculation of fundamental rheological parameters can be derived from geometry. The initial length of the sample l_0 and the length l_t at time t are:

$$l_0 = 2 \cdot \sqrt{9^2 + y_0^2} \qquad [1]$$

$$l_t = 2 \cdot \sqrt{9^2 + (y_t + y_0)^2} \qquad [2]$$

in which y_t is the displacement of the hook from the point at which the actual extension starts, and 9 is half the width of the gap in the lower plate through which the hook passes (Fig. 1A). The relative deformation or Hencky strain and the strain rate can then be written as:

$$\varepsilon_H = \ln\left(\frac{l_t}{l_0}\right) = \ln\left(\frac{\sqrt{9^2 + (y_t + y_0)^2}}{\sqrt{9^2 + y_0^2}}\right) \qquad [3]$$

$$\dot{\varepsilon} = \frac{d\varepsilon_H}{dt} = \frac{dl}{l_t\,dt} = \frac{1}{l_t} \cdot \frac{2(y_t + y_0)}{\sqrt{9^2 + (y_t + y_0)^2}} \cdot \frac{dy_t}{dt} = \frac{4 \cdot (y_t + y_0) \cdot v}{l_t^2} \qquad [4]$$

in which v is the speed of the hook. The maximum in the extension rate curves for the micro-extensograph occurs when $y_0 + y_t$ is 9 (half the gap size) and the dough is at an angle of 45° to the plane of the clamps. In a Brabender extensograph l_0 is 37 mm and the speed is fixed at 840 mm/min (Bloksma 1962). As a result of the smaller initial length of the sample the strain at a certain hook displacement is higher in the micro-extensograph (Fig. 2A). The relative strain rate is very high in the Brabender extensograph (Fig. 2B). At a speed of about 410 mm/min the strain rate is about the same as that of the Brabender extensograph (Kieffer *et al.*, 1981b).

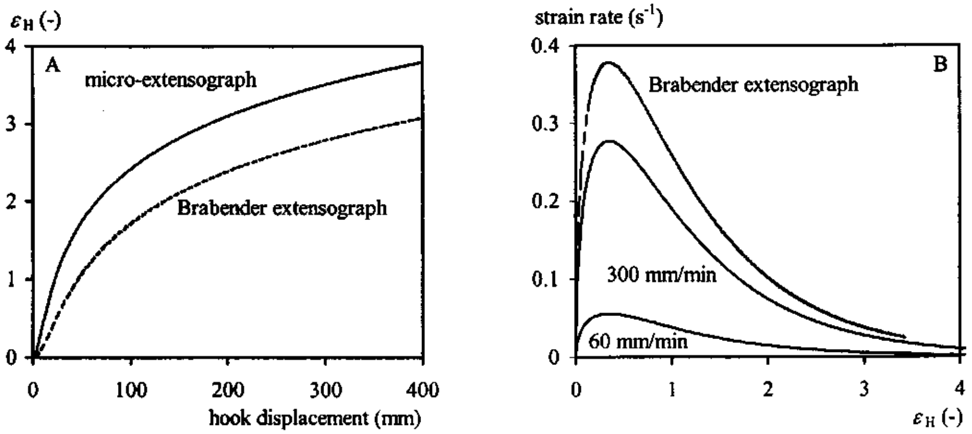


Figure 2. *Comparison of micro-extensograph (solid lines) with Brabender extensograph (broken lines). 2A: Hencky strain ε_h as a function of the displacement of the hook. 2B: Strain rate as a function of Hencky strain ε_h; hook displacement speed for the micro-extensograph indicated.*

The measured force F_m is not the force acting on the dough F_d. Assuming that the hook passes exactly through the centre of the gap, F_m is divided equally over both stretches of dough at each side of the hook. Sinα can therefore be expressed in forces as well as in lengths (Fig. 1B):

$$\sin\alpha = \frac{F_m/2}{F_d} = \frac{y_t + y_0}{l_t/2} \qquad [5]$$

$$F_d = \frac{F_m l_t}{4 \cdot (y_t + y_0)} \qquad [6]$$

Assuming that the dough piece has the same cross-section over its whole length, the surface over which the force is acting is V/l_t, V being the volume of the dough piece that is extended. The stress σ can then be calculated according to:

$$\sigma = \frac{F_d}{V/l_t} \qquad [7]$$

and the apparent extensional viscosity η_E as:

$$\eta_E = \frac{\sigma}{\dot{\varepsilon}} \qquad [8]$$

An uniaxial strain hardening parameter and strain rate thinning parameter can be calculated in a way similar to that in biaxial extension (Kokelaar *et al.*, 1996).

EXPERIMENTAL

Materials

Two types of flour were used. One was the commercial, cookie-type mixture Kolibri with 15.0% moisture and 10.5% protein, obtained from Meneba. Inherently, a cookie-type flour has a poor bread making quality. The other was Vivant with 14.4% moisture and 10.3% protein, also obtained from Meneba and also having poor baking quality. Vivant gives a weak dough, while Kolibri, in combination with glucose oxidase, formed a strong dough.
NaCl (analytical grade) was from Merck, Germany. Glucose oxidase was Oxygo L5 of Genencor, Finland (5364 glucose oxidase units/ml). Water was deionised.

Methods

Dough preparation was as described for Kolibri (Dunnewind *et al.*, 2003) and for Vivant (Sliwinski *et al.*, 2003). Water addition was 60% (T = 20°C) for Kolibri and 63% (T = 8°C) for Vivant (on flour basis). In the case of Kolibri 6 ml water contained 0.2 g NaCl (2% on flour basis) and 20 μl glucose oxidase. The doughs were mixed in a mixograph (National Mfg. Co., Lincoln) for 7 (Vivant) and 4 (Kolibri) min.

For test piece preparation the dough was made into a roll, put on the lubricated (paraffin oil) lower plate of the teflon mould and compressed with the lubricated top plate (Fig. 1A). The doughs were left resting at 25 ± 1°C for 60 min (Vivant) and at 30 ± 1°C for 45 min (Kolibri + glucose oxidase).

Three methods for taking the test pieces out of the teflon mould were compared. In the first one the upper plate of the mould was slided away until a test piece was accessible. The test piece was then scooped out with a spatula. The second method made use of the fact that the ends of the test pieces are exposed at the side of the teflon mould. This makes it possible to press the test pieces out of the mould with a stick. To prevent the dough from flowing past the stick, first a small pellet of paper was put on one exposed end of the dough piece. When almost out of the mould the test piece was grabbed at the top with a pair of tweezers. For the third method the plastic strips belonging with the mould were used. They were laid in the grooves before the dough was put in it. After opening the mould the dough pieces were taken out simply by lifting the strip.

Extension tests were performed with the Kieffer rig fitted on a Zwick materials testing machine equipped with a 50 N load cell and a plexiglas container and a heater to maintain the temperature. Tests were performed at 25 ± 1°C and 30 ± 1°C for Vivant and Kolibri dough, respectively. The plates and hook were lubricated with paraffin oil. Just before the start of the test both ends of the sample were clamped between the two plates of the Kieffer rig, which resulted in some sagging of the test piece. During the test the middle of the free hanging cylindrically shaped sample was pulled upwards by the hook until fracture occurred. The force required to do so and the displacement of the hook were recorded as a function of time and used for further calculations. Drying out of the dough during testing was prevented by lubrication of the test samples with paraffin oil and by preventing the relative humidity from becoming too low. The latter was done by placing a dish with demineralised water in the plexiglas container.

The sagging of the test piece after clamping in the extensibility rig, as a result of the squeezing of dough from between the plates, was measured by a kathetometer. The distance between the bottom of the dough piece and the top of the lower plate was determined, which should be equal to y_0.

For determining the weight of the part of the dough piece that is extended, test pieces were clamped in the extensibility rig and the part of the test piece in the gap was cut off and weighed on an analytical balance.

By drawing lines on the surface of the dough pieces with an overhead marker, the types of deformation playing a role during clamping and extension of the test piece were made visible. The types of deformation were also determined for a thicker hook (diameter 4.55 mm).

RESULTS AND DISCUSSION

Taking test pieces from the mould

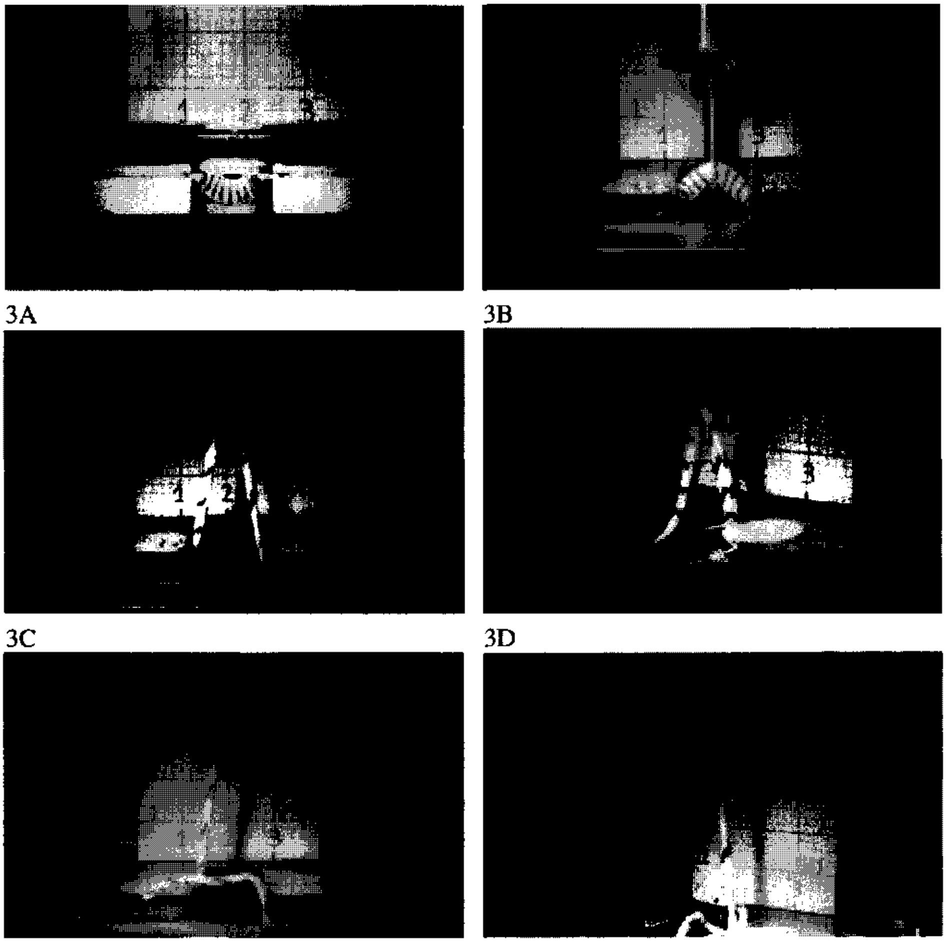
After mixing the test pieces are put in the lubricated mould. Opening the mould and scooping the test piece out with a spatula, requires much experience. The test piece is easily deformed locally to a large extent and therefore this method was considered as not well suited. Using the strips supplied with the mould also required much skill and the strips were hard to detach from flour doughs, leading again to unwanted deformations. Pressing the dough piece out of the mould using a stick required only a very small force as the surfaces of the mould were lubricated with paraffin oil. As a test piece is hanging down from the pair of tweezers gravity will deform it, but the force involved is small. This method is very easy and fast, requires no special training, and does not involve local damage of the dough piece. 'Using strip' and 'pressing out', test pieces were compared in extension tests at 3 different speeds (12, 60 and 300 mm/min). No differences in extensional properties were found (results not shown). The 'pressing out' method was used for all further tests.

Weight and volume of test piece

The weight of the dough piece in the gap of the apparatus was 0.345 g (±0.01 st.dev.) for the weak dough (Vivant) and 0.395 g (±0.018 st.dev.) for the strong dough (Kolibri with glucose oxidase). So Kolibri + glucose oxidase test pieces had a 14.5% higher weight than Vivant. This may be caused by the weaker dough being more compressed between the plates, thereby flowing in all directions and causing more friction, which retards the flow towards the gap. Assuming 1.24 g/cm^3 as the specific weight of both doughs (Baker and Mize, 1941), the dough volume that is actually extended was about 0.278 and 0.319 cm^3 for Vivant and Kolibri, respectively. A varying volume has consequences for calculations that are based on volume. The influence of variation in the mass on the calculated stress was already discussed by Muller *et al.* (1961) in great detail for the extensograph.

Clamping of the test pieces

During clamping the dough is compressed between the plates whereby part of the test piece is squeezed. This results in an increase of dough volume and sagging of the test piece in the area of the gap between the clamps (see Fig. 3A). After clamping the distance from the top of the lower plate to the bottom of the dough piece was on average 2.82 (±0.42 st.dev.) and 1.32 (±0.46 st.dev.) mm for Vivant and Kolibri, respectively. This corresponds to relative deformations of 0.05 and 0.01 for Vivant and Kolibri, respectively. It is likely that Vivant sagged more by its own weight due to its lower resistance to deformation. In several cases clamping led to changes in the test piece which complicated a correct analysis of the test. Clamping of test pieces of the stronger dough sometimes resulted in bending of the dough in a horizontal plane (Fig. 4A). In such a case the relative deformation at a certain hook



3A 3B

3C 3D

3E 3F

Figure 3. *Some typical situations at different stages of extension of a flour dough sample in the Kieffer Extensibility Rig. Lines are drawn on the test sample for the determination of the type of deformation during extension. The numbers on the board refer to centimetres. 3A: Front view of the test piece after clamping before the start of the extension test. Note the sagging of the test piece. 3B: Side view of the test piece at an early stage of extension. 3C: Front view of the test piece at a further stage of extension. Note the tilted lines drawn on the test piece. 3D: Side view of the test piece at a further stage of extension. 3E: Front view of the extended test piece after fracture. Note the inhomogeneous deformation. 3F: Side view of the extended test piece after fracture.*

displacement will be higher than assumed in the calculations. Sometimes after clamping the test piece was unevenly distributed between the plates at each side of the hook (Fig. 4B). This will result in an unequal deformation at each side of the hook.

These deformations shortly prior to testing will result in differences in deformation history between test pieces and therefore will have negative effects on the quality of the tests and the reproducibility of the results. Several other methods to determine the large deformation and fracture properties of wheat flour dough have been described in literature (Tschoegl *et al.*, 1970; de Bruijne *et al.*, 1990; Schweizer and Conde-Petit, 1997; Uthayakumaran *et al.*, 2002). In these cases sagging of the test-piece was prevented by submerging the sample in a liquid of matching density (Tschoegl *et al.*, 1970), by extending the dough on a mercury bath (de Bruijne *et al.*, 1990), by flowing carrier gas between the sample and the sample table (Schweizer and Conde-Petit, 1997) and by pulling apart a cylindrical dough piece that was adhered between a lower and upper grip using super-glue (Uthayakumaran *et al.*, 2002).

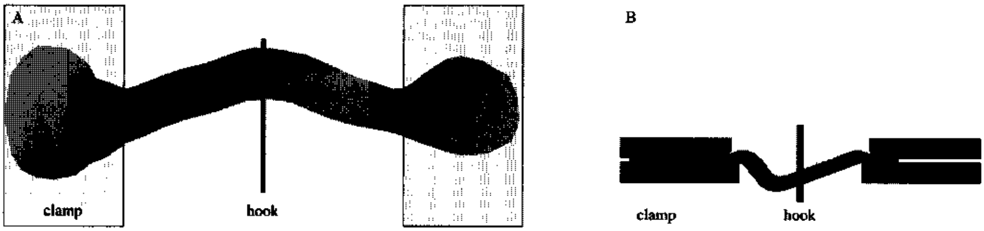


Figure 4. *Deformation of the test piece in the gap during clamping next to sagging. 4A: bending in a horizontal plane (top view). 4B: unequal distribution of dough over the gap as a result of unequal amounts of dough between the plates at each side of the gap (front view).*

Type of deformation

As described above, the dough sample is deformed shortly before the start of the extension test due to the events resulting from the clamping of the test piece. During extension the dough just beside the clamps and on the hook became thinner in vertical direction and between 2 and 3 times as wide in horizontal direction as the rest of the sample (Fig. 5A and 5B at the points 1, 3, 4 and 6, and photograph in Fig. 3C). This unwanted deformation could be reduced by using a thicker hook (diameter 4.55 mm).

The type of deformation during clamping and extension was further investigated by marking test pieces with an overhead marker. Figure 3 shows typical pictures taken at different stages of a test. In figure 3A the sagging of the sample is very clear. Figure 3B shows the early stage of extension. The marks at the side and on the top of the sample are connected and perpendicular to the sides of the test piece. At further extension (Fig. 3C) the lines on the side of the test piece became tilted. The direction and extent of tilt of the lines varied along the sample. It implies a shear component in the x-y-direction. This complicates the strain calculation. In the calculation of the stress-strain curves we will neglect the contribution of the shear component to the total strain. During mixing dough is deformed in combinations of shear and uniaxial elongation at high rates (MacRitchie, 1986). According to Gras *et al.* (2000) dough mixing by a pin mixer can be viewed as a series of uniaxial extension tests. The Kieffer test therefore is related to mixing.

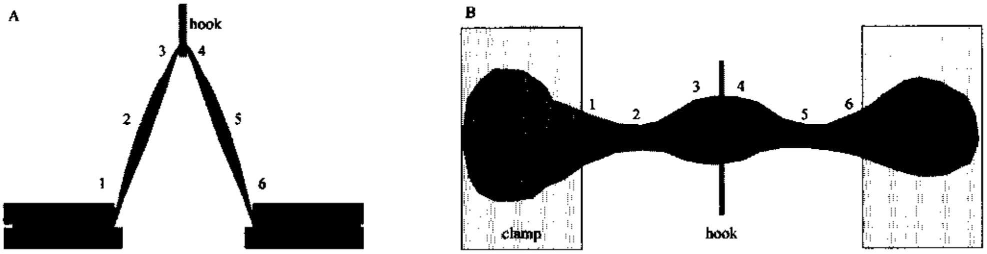


Figure 5. *The deformation of the test piece in the micro-extensograph. 5A: front view. 5B: top view. The numbers are referred to in the text.*

Photograph 3D shows that the marks at the top were shifted compared to the lines on the side. Sometimes a shear component in the x-z-direction was noticed (not visible on the photographs). A tortuous sample or inhomogeneities in the dough may be the cause. Pictures 3E and 3F show very inhomogeneous deformation; some parts were more extended than others and therefore fractured earlier. Contrary to what was observed for the extensograph (Bloksma and Bushuk, 1988) and the mercury bath method (de Bruijne *et al.*, 1990) the amount of dough that is extended remains more or less constant throughout the test with the Kieffer rig. Inhomogeneous deformation of dough samples during uniaxial extension was also described for the extensograph by Muller *et al.* (1961), for the stretching of dough rings by Tschoegl *et al.* (1970) and was very obvious in the extension of a cylindrical dough piece that was adhered using super-glue (Uthuyakumaran *et al.*, 2000). Maybe in theory the best piece of equipment for this purpose is the Meissner caterpillar-type rheometer (Meissner and Hostettler, 1994) However, more recently, Schweizer (2000) described a large collection of experimental difficulties associated with the measurement of uniaxial extension properties in this apparatus. Besides it was shown that to obtain a homogeneous deformation in the Meissner caterpillar-type rheometer very careful sample preparation is of outmost importance (Schweizer and Conde-Petit, 1997).

Force and stress

Initially, the actual force exerted on a dough is higher than the measured force (Fig. 6). At the end of the measurement, however, it will be about half of the measured force. The latter is due to the change in angle under which the force is applied (equations 5 and 6). Because the deformation is never completely homogeneous and the force acts locally on an area smaller than the average one (equation 7), the calculated stress will always be somewhat lower than the maximum real stress in the test piece. In Figure 6 it can be seen that the calculated stress, assuming a homogeneous deformation, reaches its maximum at a higher deformation than the force. The difference is the largest in case of the higher displacement speed, where fracture occurs at a high deformation. While the length of the dough cylinder increases steadily, the area on which the force acts decreases. If the force decrease is less than the decrease in cross section of the test piece the stress will still increase. The maximum in stress better represents the strain at which the sample fractures macroscopically than the maximum force. An increase of the force (and thus stress) was observed with increasing hook displacement speed (Fig. 6). Similar trends were reported for wheat flour dough in uniaxial extension tests (Tschoegl *et al.*, 1970) and in biaxial extension tests (Janssen *et al.*, 1996; Kokelaar *et al.*, 1996).

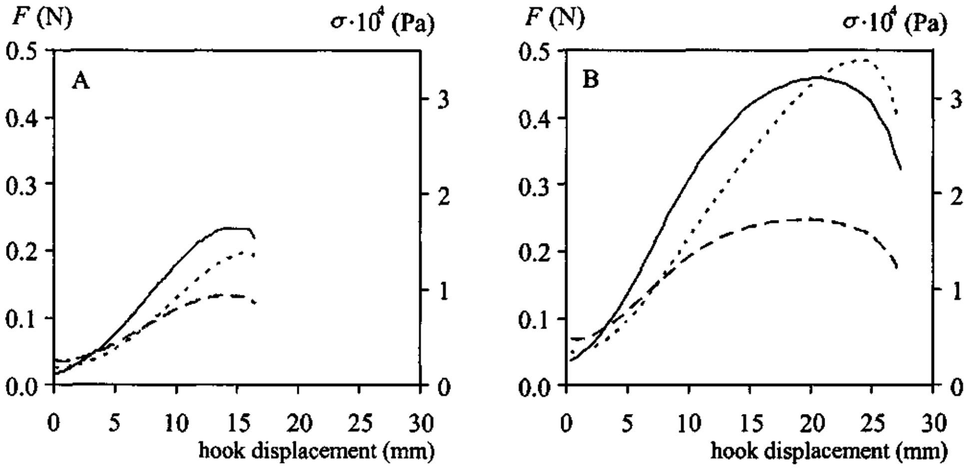


Figure 6. *An average (n = 10) curve of measured force (F_m, solid line) and calculated force (F_d, broken line) and stress (σ, dotted line) in Kolibri + glucose oxidase dough for a displacement speed of 12 (A) and 300 (B) mm/min. Position of 2 mm above the surface of the lower plate was chosen as the start of extension.*

Starting point

Squeezing the dough out of the clamps results in a dough piece which is longer than the width of the gap (Fig. 3A). The starting point of the extension has to be defined as the hook position above the lower plate were the dough piece has the same length as before the hook touched it. This means that the hook has to travel to a point y_0 above the lower plate before it really starts to extend the test piece (Fig. 1A). For correct calculations of strain, strain rate and stress, this point has to be known exactly.

To determine this point for every separate test piece it can be reasoned that the extension actually starts when the force has passed the force that gravity exerts on it. At that moment y_t is set to zero. Assuming a mass of 0.4 g for the sample the force exerted by gravity is $0.4.10^{-3} * 9.81 = 0.004$ N. However, it was observed that at any speed v tested the force of 0.004 N was already exceeded when the hook was still below the surface of the upper plate (see Fig. 7). This shows that a pre-load force can not be used for determining the starting point of this extension test. The resistance against bending of the dough, while it is lifted by the hook, will be responsible for this phenomenon. Due to bending some parts of the test piece will be compressed and other parts somewhat extended. The higher the speed, the higher the bending rate and the higher the required force.

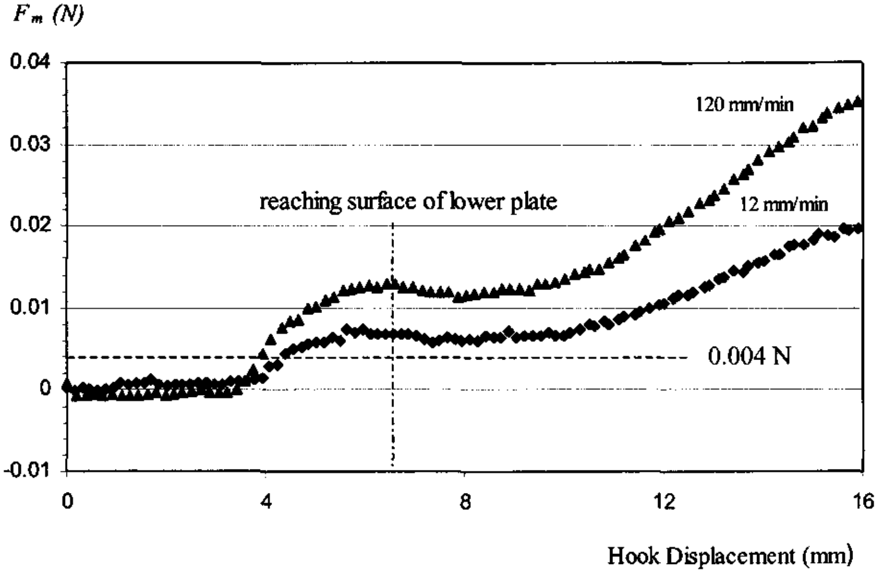


Figure 7. *Measured force F_m as a function of the hook displacement for Vivant dough. The dotted vertical line indicates the displacement when the hook reaches the upper surface of the lower plate at the given speed (indicated in mm/min). The broken horizontal line indicates the displacement when a force of 0.004 N was reached.*

Another possible way to determine the exact starting point of the test is by measuring the distance the hook moves between the point where it touches the dough piece, i.e. where the force starts to deviate from zero, and the surface of the lower plate. This difference should be equal to y_0. However, the point at which the force starts to deviate from zero was difficult to estimate accurately enough from the data, especially at lower speeds. So, when using a force to determine the starting point an error may be introduced.

Figure 7 shows that in the first part of the force-hook displacement curve three stages can be distinguished: an initial rise of the force, a part where the force increases less steeply with hook displacement or even decreases and a part where the force increases at an increasing rate. It can be argued that at y_0 the force will start to increase faster through actual extension of the dough, so one can also choose the inflection point in the force-displacement curve as starting point of the extension. As the inflection point in the force-displacement curve is hard to estimate, especially at high speeds, it would be better to use a method that is independent of the force-displacement curve.

Perhaps the best method to calculate the starting point is to combine the measured distance between the top of the lower plate and the bottom of the dough piece after clamping and the displacement of the hook where it has reached the surface of the lower plate. The starting point is then defined as the point where the hook has lifted the dough as much as it had sagged. This point should be determined for each type of dough again as sagging varies with cultivar, amount of water added, mixing time etc.

Fracture

Photographs 3E and 3F show the fractured dough. The location at which the dough fractured, has been scored for 33 samples at strongly different test speeds v (30 up to 300 mm/min). The score was 1, 0, 14, 16, 0 and 2 for the positions 1 to 6 indicated in figure 5. The test speed and type of dough had no influence. The bending over the hook, involving an important shear deformation above the tensile deformation, is probably the cause for the fracture occurring mainly at positions 3 and 4. When using a thicker hook (4.55 mm) fracture of Kolibri doughs occurred more often at positions 1 and 6, probably also caused by the relative stronger bending of the sample at these points.

It was noticed that just before fracture extension was concentrated in a certain part of the test piece. At that position the dough piece became increasingly thinner and finally fractured. It depended on dough properties how fracture occurred. The weaker dough showed the strongest necking effect at the position where it was going to fracture. The stronger dough fractured more suddenly and faster. Sometimes holes were formed in the thinner regions close to the plates and the hook, in some cases leading to fracture.

As already mentioned in the paragraph force and stress, the maximum in stress is probably the best choice for the strain at macroscopic fracture. It is likely that fracture is initiated already before the strain where the force is highest. However, due to low crack speeds, as a consequence of extensive energy dissipation, it will take some time (depending on the dough) before the crack has extended over the whole cross-section of the test piece (slow fracture propagation) (van Vliet *et al.*, 1993).

The deformation at fracture increased with increasing speed (Fig. 7 and Fig. 8). This is in accordance with measurements on flour doughs (Tschoegl *et al.*, 1970) and gluten doughs (Rinde *et al.*, 1970). However, it is opposite the effect usually found for viscoelastic materials (van Vliet *et al.*, 1993). For flour doughs this might be caused by the abundance of starch granules and gluten "particles" in the doughs. Much energy will be dissipated due to friction between them. The large energy dissipation gives that crack growth in dough only proceeds slowly. During the time the crack that ultimately will cause fracture grows the dough is further deformed. The higher the speed the larger this deformation during crack growth. This results in a larger measured fracture strain at higher speeds (van Vliet *et al.*, 1993).

Reproducibility

Figure 8 gives an example of a series of measurements at different speeds. No special precautions were taken to standardise the measuring procedure. At every speed the graph shows a large variation in force at certain values of extension and in force and deformation at fracture.

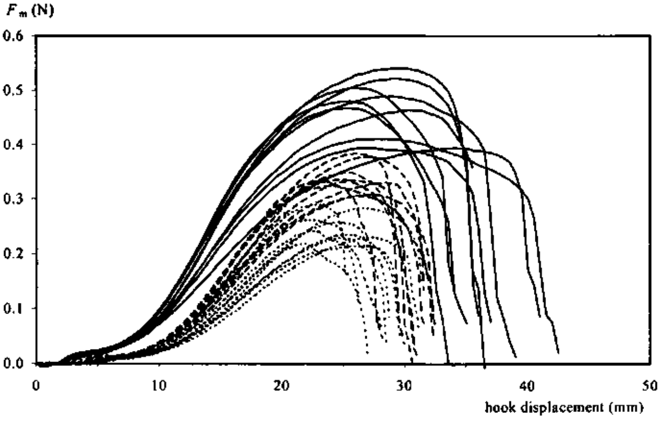


Figure 8. *Measured force F_m as a function of hook displacement at three different speeds measured on Kolibri + glucose oxidase. Solid lines 300, broken lines 60 and dotted lines 12 mm/min.*

Reproducibility of fracture stress and strain was determined for 44 samples of both Vivant and Kolibri dough at a displacement speed of the hook of 60 mm/min. Results are shown in table 1. Both fracture stress and strain varied relatively strong. Presumably the large variation partly originates from the relative small sample size and the different preparations of small doughs in the mixograph. Furthermore variations in test piece volume and deformation history, as described above, will have their influence on the reproducibility of the test.

Table 1. *Fracture stress,* σ*, and fracture strain,* ε_h*, determined in uniaxial extension at a displacement speeds of the hook of 60 mm/min for flour dough of Vivant and Kolibri. Data are averages of 44 measurements. T = 25°C and 30°C for Vivant and Kolibri, respectively.*

dough	**σ (Pa)**		**ε_h (-)**	
	average	**st.dev.**	**average**	**st.dev.**
Vivant	7090	1322	1.82	0.235
Kolibri	28750	2916	0.96	0.134

However by using well standardised procedures much less variation between different determinations can be obtained (Kieffer *et al.*, 1998; Verbruggen and Delcour, 2003; Sliwinski *et al.*, 2003). It is nevertheless preferable to apply large differences in speeds to obtain clear (significant) effects of deformation speed on the stress-strain curve.

CONCLUSIONS

The 'Kieffer dough and gluten extensibility rig' is a load-extension instrument that can be considered as a micro-extensograph. Contrary to the extensograph, with the 'Kieffer dough and gluten extensibility rig' smaller dough samples can be tested, lower and therefore more relevant strain rates can be applied and experimental data can be expressed in terms of stress and strain. Test pieces are easiest taken from the mould by pressing them out of the mould using a stick. Clamping of the test pieces shortly before the start of experiments caused sagging of the dough cylinders resulting in a deformation of the test sample shortly before the start of the measurement and difficulties in the determination of the exact starting point of extension. To determine this point two methods are recommended. A hook position above the lower plate could be taken, which is based on the measured distance between the top of the lower plate and the bottom of the dough piece after clamping, but it is also possible to estimate the inflection point in the force-hook displacement curve. Deformation was not purely uniaxial extension. A shear component was also observed, which probably caused fracture to occur mainly near the hook. No additional material is drawn out of the clamps throughout the test. The maximum in stress represents better the strain at which the sample fractures macroscopically than the maximum in the force. A relatively large variation in stress and strain at fracture was observed. Variation in the deformation history and the volume of the test pieces have a negative effect on the reproducibility. Therefore a well-standardised procedure has to be followed.

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Chapter III

Large-Deformation Properties of Wheat Dough in Uni- and Biaxial Extension. Part I: Flour Dough[1]

SUMMARY

Rheological and fracture properties of optimally mixed flour doughs from three wheat cultivars which perform differently in cereal products were studied in uniaxial and biaxial extension. Besides doughs were tested in small angle sinusoidal oscillation. In accordance with previously published results the linear region was found to be very small. The rheological properties at small deformations hardly depended on the cultivar. A higher water content of the dough resulted in a lower value for the storage modulus and a slightly higher value for tan δ. For both uniaxial and biaxial extension a more than proportional increase in stress was found with increasing strain, a phenomenon called strain hardening. In uniaxial extension (i) stresses at a certain strain were much higher and (ii) the stress was less dependent on the strain rate than in biaxial extension. This indicates that in elongational flow orientational effects are of large importance for the mechanical properties of flour dough. This conclusion is consistent with published data on birefringence of stretched gluten. Fracture stress and strain increased with increasing deformation rate. The observed time-dependency of fracture properties can best be explained by inefficient transport of energy to the crack tip. Presumably, this is caused by energy dissipation due to inhomogeneous deformation because of friction between structural elements, e.g. between dispersed particles and the network. Differences in the rheological properties at large deformations between the cultivars were observed with respect to (i) stress, (ii) strain hardening, (iii) strain rate dependency of the stress, (iv) fracture properties and (v) the stress difference between uniaxial and biaxial extension.

INTRODUCTION

To be able to explain properly the behaviour of flour dough during processing it is necessary to test dough under conditions that are relevant for the bread making process (van Vliet *et al.*, 1992). During all stages of the bread making process (i.e. mixing, proofing, moulding and baking) bread dough undergoes large deformations including fracture (Bloksma, 1990). The speed at which dough is deformed and the type of deformation dough undergoes vary strongly between the different stages of the bread making process.

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[1] E.L. Sliwinski, P. Kolster, T. van Vliet. Rheologica Acta. In press.

During fermentation the carbon dioxide produced by the growing yeast cells leads to expansion of the gas bubbles that deform the dough essentially in biaxial extension at relatively small deformation rates up to strains of 300% (Bloksma, 1990; van Vliet *et al.*, 1992; van Vliet, 1999). During baking it is the raise in temperature that results in expansion of gas bubbles and a concomitant deformation of bread dough in biaxial extension. In the oven deformation rates are somewhat larger than during fermentation. During baking the raise in temperature also results in other changes in the dough like the gelatinisation of starch that affect the mechanical properties (Hoseney and Rogers, 1990).

During the mixing process the deformation of dough is a combination of shear and uniaxial elongation which differ with the type of mixer (MacRitchie, 1986; Gras *et al.*, 2000; Zheng *et al.*, 2000). Generally during mixing large deformations are imposed on the dough at deformation rates that are some orders of magnitude higher than the ones applied during fermentation (Bloksma, 1990). Gras *et al.* (2000) stated that at least in the National Mixograph dough mixing can be viewed as a series of uniaxial extension tests. During moulding and shaping deformation of the dough is also a combination of shear and uniaxial elongation at deformation rates that are generally lower than those during mixing. To summarise, during bread making flour dough is subjected to large deformations (including fracture) alternatingly (i) in uniaxial extension at intermediate to large deformation rates and (ii) in biaxial extension at small to intermediate deformation rates.

Tschoegl *et al.* (1970a) determined the large deformation and fracture properties of flour dough in uniaxial extension using a method whereby dough rings were submerged in a liquid of matching density and were stretched at constant rates of extension until fracture occurred. They found that at a constant strain rate the true stress increased continuously with the strain, indicating that the elastic retractive force increases throughout the test and no trend towards steady-state flow develops. They also found that flour type, water content, temperature and strain rate have a drastic effect on the stress-strain behaviour (Tschoegl *et al.*, 1970a). Visual observations indicated that fracture of flour dough is less well-defined than it is for most elastomeric materials that fracture quite suddenly at reasonably well-defined values of stress and strain. They observed that shortly before dough fracture either a neck or small tears formed in various portions of the specimen.

Schweizer and Conde-Petit (1997) have studied the large deformation and fracture properties of flour dough in uniaxial extension using the elongational rheometer RME based on the rotary clamp technique. This apparatus was developed by Meissner (Meissner and Hostettler, 1994). It appeared that with dough careful preparation of samples (including mixing, sheeting and resting) is extremely important. They found that the stress depended on the sheeting direction: the stress was higher if the sample was elongated parallel to the sheeting direction than perpendicular to it. It followed from their results that in uniaxial extension flour dough shows a well-developed strain hardening behaviour. They also noticed that after a steady increase of the deformation the force suddenly decreased at a certain strain. At this elongation the sample started to neck or even showed fracture.

Kieffer *et al.* (1981) have developed a micro-method for extension tests with flour doughs the Kieffer Extensibility Rig, that can be used in combination with most types of mechanical testing machines. With this method, that can be considered as a miniature edition of the Brabender extensograph, a cylindrically shaped dough sample that is clamped at both ends, is extended uniaxially by a hook that moves upwards. With the Kieffer Extensibility rig it is possible to obtain results that can be expressed in SI-units and to apply a range of deformation rates (Dunnewind *et al.*, 2003).

The relevant type of deformation around an expanding gas bubble is biaxial extension (van Vliet *et al.*, 1992). Experiments in which this type of deformation is applied have been performed on wheat flour doughs by several researchers (Bagley *et al.*, 1988; Huang and Kokini, 1993; Janssen *et al.*, 1996, Kokelaar *et al.*, 1996). With lubricated uniaxial compression a cylindrically shaped dough piece is compressed uniaxially on the y-axis and at the same time biaxially extended on the x- and the z-axes. Other experimental set-ups to perform biaxial extension have been developed by Morgenstern *et al.* (1996) and by Dobraszczyk (1997). With these two set-ups also fracture properties can be determined in biaxial extension. An important result was that gluten and flour doughs in biaxial extension show strain hardening behaviour (Janssen *et al.*, 1996). Kokelaar *et al.* (1996) found that the rheological behaviour of flour dough in biaxial extension is influenced by flour type, water content, strain rate and temperature.

During bread making wheat flour dough undergoes a concatenation of processing steps during which dough is deformed uniaxially as well as biaxially at large deformations at a range of deformation rates. As discussed above for being able to predict dough behaviour during processing it is of key importance to have information about the large deformation properties of flour dough in uniaxial and biaxial extension. Dhanasekharan *et al.* (1999) have attempted to compare observed rheological properties of flour dough with predictions based on three non-linear differential viscoelastic models. The results they obtained in uniaxial extension were, however, hard to compare with those obtained in biaxial extension. In this paper results are presented for flour doughs of three wheat cultivars performing differently in cereal products. The rheological properties of these flour doughs were determined using the Kieffer Extensibility Rig for uniaxial extension and lubricated uniaxial compression for biaxial extension. Because flour dough is often tested in dynamic measurements at small deformations results from such tests are also presented.

THEORY

For the production of bakery products like bread and puff pastry the ability of wheat flour dough to retain gas is of key importance. The variation in potential for gas retention among wheat flour doughs is mainly due to differences in the large-deformation properties of dough films (van Vliet *et al.*, 1992). Therefore flour doughs were tested in uniaxial and biaxial extension. Calculations from force-displacement curves into stress-strain curves were performed as described previously for uniaxial extension by Dunnewind *et al.* (2003) and for biaxial extension by Kokelaar *et al.* (1996). Here mathematical formulas of the various stresses and strains only will be presented in short.

The relative deformation in uniaxial extension of a cylindrically shaped test piece can be described as the Hencky strain ε_h which will be simply called ε:

$$\varepsilon = \ln (l_t/l_o) \qquad (1)$$

where l_o is the original length of the test piece and l_t the length at time t.

The relative rate of deformation is given by:

$$\dot{\varepsilon} = d\varepsilon / dt \qquad (2)$$

Assuming that the cross-section of the test piece is constant over its whole length and that its volume remains constant throughout the test, the radius of the test piece will decrease proportionally with the increase of its length. Since the force F acts on the dough in the direction parallel to the length of the test piece the stress σ becomes:

$$\sigma = F_t / \pi R^2 \qquad (3)$$

In compression the relative deformation of the test piece the Hencky strain ε_h is calculated as follows:

$$\varepsilon_h = \ln (h_t/h_o) \qquad (4)$$

where h_o is the original height of the test piece and h_t the height at time t.

Since in uniaxial lubricated compression the test piece is extended biaxially in the directions perpendicular to the compression direction, the biaxial strain will be:

$$\varepsilon_B = \varepsilon_h / 2 \qquad (5)$$

Combining formulas 2 and 5 the biaxial extension rate is than defined as:

$$\dot{\varepsilon}_B = -\dot{\varepsilon}_h / 2 \qquad (6)$$

Since the radius of the part of deformed material on which the force F acts is constant, the difference between the normal stress σ_{zz} and the radial stress σ_{rr} becomes:

$$\sigma_{zz} - \sigma_{rr} = F_t / \pi R^2 \qquad (7)$$

on the condition that edge effects can be neglected because of the absence of friction.

Fracture of dough films will lead to coalescence of gas cells. Its extent depends on how the material reacts on the development of weak spots during extension (Lenk, 1978; van Vliet *et al.*, 1992). These will always arise because of the presence of inhomogeneities in the material. At these weak spots the specimen will become locally thinner than the rest of the material. When such a thinner spot (a so-called neck) is formed two things can happen on further extension: (i) either the neck becomes progressively thinner and eventually the specimen breaks; (ii) or the neck may become stabilised. The specimen will be stable against fracture if the resistance to further thinning of the thinner part is larger than that of a less extended and proportionally thicker part. For uniaxial extension this condition was described mathematically by Lenk (1978) as follows:

$$d\ln\sigma / d\varepsilon > 1 \qquad (8)$$

A relative increase in length implies a similar relative decrease in thickness for a film (or in the cross-section for a thread). Thus the thinner part of an uniaxially extended dough film has a larger resistance to further thinning than a thicker part if the ratio of the relative increase of the stress over the increase of the strain is larger than 1 (Lenk, 1978). If the stress increases less with increasing strain, the force for extending a part of a dough film decreases with decreasing thickness and thus with increasing deformation. It causes that a thinner part of a dough film will be extended more than its surroundings. It will become progressively thinner and the film will fracture eventually. Van Vliet *et al.* (1992) have shown that in biaxial extension of a dough film the ratio of the relative increase of the stress to the accompanying increase of the strain should be larger than 2. So, the criterion is different in biaxial than in uniaxial extension. The phenomenon where the stress increases more than proportionally with the strain is called strain hardening. Strain hardening of dough is considered an important criterion for the extensibility of dough films between gas cells and thereby for gas retention.

EXPERIMENTAL

Materials

For this study, three European wheats, Vivant, A6/13 and Hereward were selected, which show large differences in baking performance (see table 1). In table 1 also relevant analytical data of the flours are presented. Both protein content and loaf volume increase in the order

Vivant > Hereward > A6/13. Vivant and Hereward were pure cultivars, while A6/13 was a mixture of two unknown cultivars. Samples of 400 kg each were milled to a milling degree of approximately 70% in the experimental mill of Meneba BV, Rotterdam, The Netherlands. The flour was stored at –20°C.

Table 1. *Analytical and baking data for flours.*

	Moisture Content %	**Protein Content** %	**Damaged Starch** %	**Mixing Requirements**[1] (min)	**Baking Performance**[1] (cm^3/ g flour)
Vivant	14.4	10.3	10	5.5	6.6
A6/13	14.0	12.0	12	7.5	7.5
Hereward	15.3	11.0	5	10.0	7.2

[1] *Data are from baking tests in which a large mixer was used (Sliwinski et al., 2003b).*

Chemical characterisation

Moisture and damaged starch content were determined according to AACC method 44-19 and 76-30A, respectively (AACC, 1983). Protein content was determined by the Kjeldahl method (N x 5.7) using an auto-analyser system.

Preparation of flour doughs

Flour-water doughs were mixed in a 10 g National Mixograph with a target dough temperature of 27°C. Water additions were based on baking absorption values (Sliwinski *et al.*, 2003b). Water additions were chosen as high as possible, but should be within the working area of a flour and should not cause problems with dough stickiness. Mixing times were based on results from biaxial extension tests. Doughs were considered optimally mixed when the stress at a certain strain as a function of mixing time was the highest on the condition that doughs were still manageable with respect to stickiness. Manageability of the doughs was determined manually. Applied water additions and mixing times are presented in table 2.

Sinusoidal oscillation tests

Sinusoidal oscillation tests were performed in a Bohlin VOR constant shear Rheometer essentially as described by Kokelaar *et al.* (1996). The instrument was equipped with a plate-plate geometry (radius 1.5 cm). In all cases the plates were covered with emery-paper. This was done since it has been demonstrated that for flour dough plate roughening does eliminate slip between the sample and the plate (Kokelaar *et al.*, 1996; Phan-Tien *et al.*, 1997). The gap between the plates was set at 3 mm. The storage modulus (G') and the loss modulus (G'') were determined as a function of the strain at a frequency of 1 Hz. The flour doughs were placed between the plates at maximum 1 hour after mixing and the measurements started after a resting period of 30 minutes, allowing the stress in the test-sample to relax before the start of the measurement. To prevent drying out of the test-piece, the rim of the sample was coated with grease. G' and G'' were also determined as a function of frequency at a strain of 1.10^{-3} at 25°C. Results were means of 4 experiments on at least three different flour dough preparations (average coefficients of variation CV = ±4%, ±4% and ±2% for G', G" and tan δ, respectively).

Biaxial extension tests

Biaxial extension tests were performed by lubricated uniaxial compression in a Zwick mechanical testing apparatus LBR 2000 equipped with a 50 N load cell essentially as described by Kokelaar *et al.* (1996). The test pieces were compressed between two cylindrical teflon plates with a diameter of 20 mm. To allow relaxation of the stress in the samples the flour doughs were stored after mixing for approximately 1 hour in a cylindrical teflon cover after lubrication with paraffin oil at the inside. To prevent drying out of the upper and lower part of the test piece the doughs were covered with a teflon cover with the same diameter as the test piece. The initial height of the test pieces was 20 mm and they were compressed to a final height of 1 mm. This test piece height was chosen to avoid problems caused by compression of test pieces by gravity and with test pieces slipping out between the test plates during the measurement. To prevent friction between the test plates and drying out of the test pieces they were lubricated with paraffin oil. The force required to compress the dough samples and the displacement of the upper plate were recorded as a function of time and used for further calculations. Displacement speeds were 5, 12 and 120 $\mathrm{mm.min^{-1}}$. Results shown are mean values of four measurements on at least three different dough preparations (average coefficient of variation CV = ±3% and ±4% for biaxial stress and strain hardening, respectively). Calculation of stress and strain as described above allowed also the calculation of a relative increase of the stress with a relative increase of the biaxial strain rate $\mathrm{d}\ln\sigma_B/\mathrm{d}\ln\dot{\varepsilon}_B$ which will be called the strain rate dependence of the stress in biaxial extension.

Uniaxial extension tests

Uniaxial extension tests were also performed using a Zwick mechanical testing apparatus equipped with a 50 N load cell and a Kieffer Extensibility Rig (Kieffer, 1981) essentially as described previously (Dunnewind *et al.*, 2003). Directly after mixing the flour doughs were shaped in a stencil and cut into 5 cm long pieces with a trapezium like cross-section (3/5 x 4 mm). The dough samples were stored in the stencil for approximately 1 hour to allow the stress in the samples to relax. The stencil was lubricated with paraffin oil to avoid drying out of the samples. Just before the start of the test both ends of the sample were clamped between the two plates of the Kieffer Rig, which resulted in some sagging of the test piece. After clamping the shape of the elongating dough sample can be approximated as a cylinder. For the start of the measurement was chosen the inflection point of the force-hook displacement curve, where the hook started to change the length of the test piece. During the test the middle of the free hanging cylindrically shaped sample was pulled upwards by the hook until fracture occurred. The force required to do so and the displacement of the hook were recorded as a function of time and used for further calculations. Displacement speeds were 12, 48 and 120 mm.min^{-1}. Results shown are mean values of at least five measurements on at least four different dough preparations (average coefficient of variation CV = ±3%, ±4%, ±4% and ±6% for uniaxial stress, strain hardening, fracture strain and fracture stress, respectively). Calculation of stress and strain as described above allowed also the calculation of a relative increase of the stress with a relative increase of the strain rate $d\ln\sigma/d\ln\dot{\varepsilon}$ which will be called the strain rate dependence of the stress in uniaxial extension.

RESULTS AND DISCUSSION

Small deformations

The dependence of the storage modulus (G'), loss modulus (G'') and loss tangent (tan δ) of the strain is shown in figure 1 for Vivant and Hereward flour doughs. Experiments were performed at a frequency of 1 Hz and at 25°C. At very low deformations some scattering was observed in the results, probably caused by inaccuracy of the measuring device. The storage modulus became clearly strain dependent at a strain of about 0.0005, while G'' became only strain dependent at strains an order of magnitude larger (figure 1A). As a consequence for both flour doughs an increase of tan δ with increasing strain was observed (figure 1B). Wheat flour dough is known to be very strain-sensitive; a linear region if any is only observed at small strains. More or less linear behaviour for flour dough has been reported up to strains of 0.0002 (Smith *et al.*, 1970; Kokelaar *et al.*, 1996; Larsson and Eliasson, 1997). The main factor responsible for the strain-dependency of G' is the starch present in dough (Smith *et al.*, 1970; Larsson and Eliasson, 1997, Uthayakumaran *et al.*, 2002). Moduli of Vivant and Hereward flour doughs differ only slightly. The observed dependence of G' and tan δ on the strain at already small strains indicate a particle-like network structure for flour doughs. In such systems the mechanical properties depend on the interactions between the particles and the deformability of the particles.

In figure 2 G', G'' and tan δ are shown as a function of frequency for flour doughs of Vivant, A6/13 and Hereward. Measurements were performed at a strain of 1.10^{-3}. This strain is somewhat outside the linear region of flour dough, but measuring at this strain offers the possibility to perform measurements over a relatively large range of frequencies. On a log-log-scale a linear increase was observed for G' and G'' as a function of frequency for all doughs (figure 2A). No differences were observed between the cultivars regarding the dependence of G' on frequency. Values for G' and G'' were slightly lower and values for tan δ somewhat higher for A6/13 than for Vivant and Hereward flour dough. Presumably this is caused by the higher water content of A6/13 flour dough, which according to Kokelaar *et al.* (1996) is a main factor determining *G'* and *G"*. For all flour doughs tan δ was independent of frequency, except the somewhat higher values at low frequencies (figure 2B).

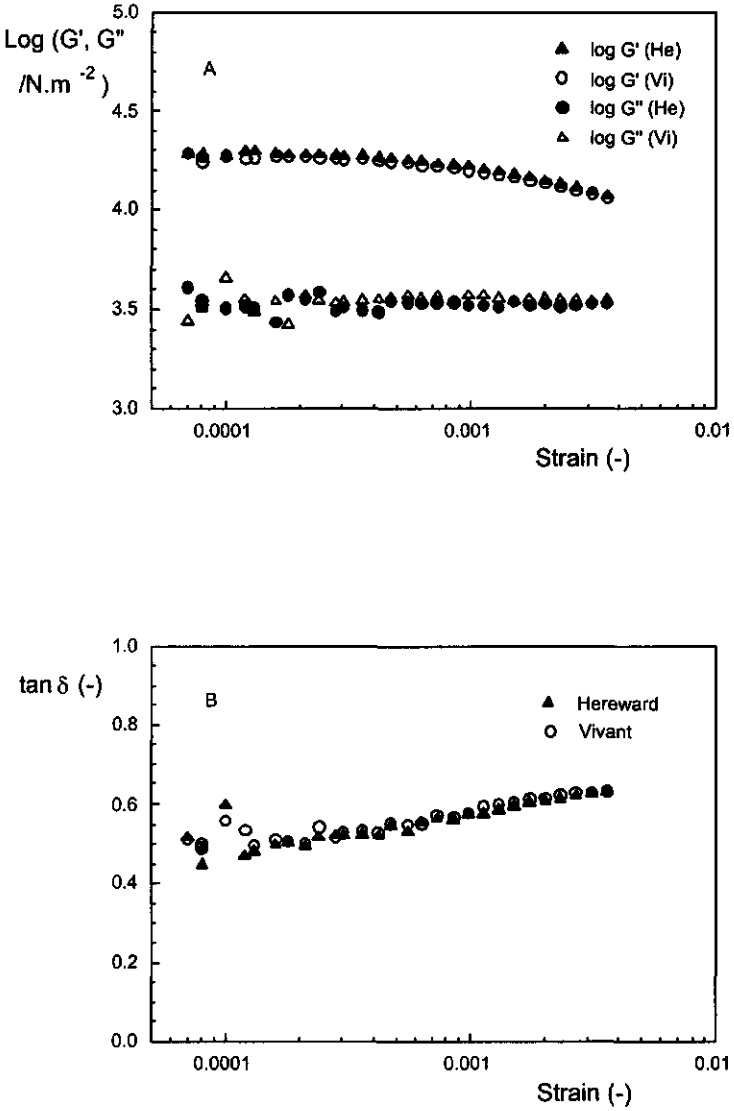

Figure 1. *Storage modulus and loss modulus (1A) and loss tangent (1B) as a function of applied strain for Hereward and Vivant flour doughs. Data are at 1 Hz and at 25°C.*

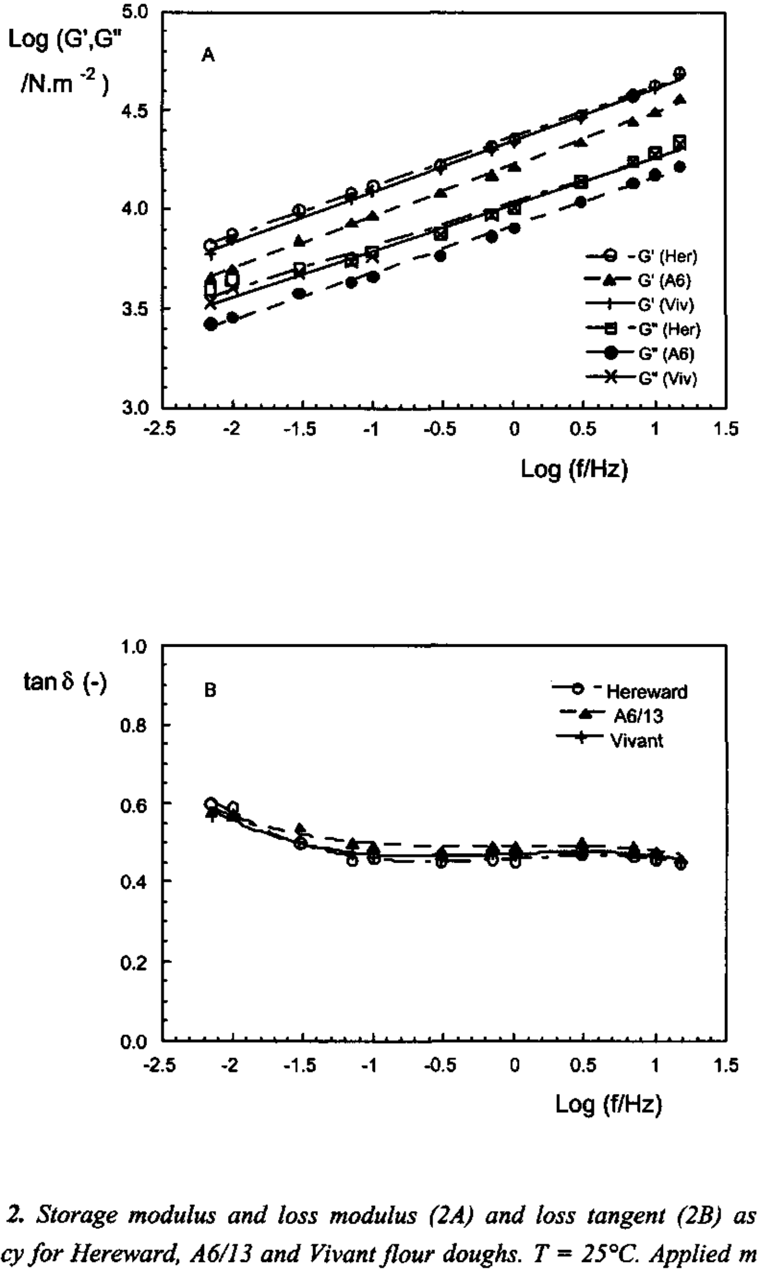

Figure 2. *Storage modulus and loss modulus (2A) and loss tangent (2B) as a function of frequency for Hereward, A6/13 and Vivant flour doughs. T = 25°C. Applied maximum strain was 0.001.*

Large deformations: biaxial extension

Force-displacement curves were obtained in lubricated uniaxial compression for flour-water-doughs of Vivant, A6/13 and Hereward. Examples of force-displacement curves for Hereward dough are shown in figure 3A. From force-displacement curves stress-strain curves were calculated, taking into account the changing dimensions of the dough sample and assuming a constant dough volume throughout the experiment. In figure 3B examples of such stress-strain curves are shown for Hereward flour dough.

The stress-strain curves were recalculated into curves showing the biaxial stress σ_B as a function of the strain rate at constant strains. Results are presented for Hereward and Vivant in figure 4A and 4B, respectively. For these graphs data of experiments at three different displacement speeds were combined. Recalculation was required because the strain rate during a test was not constant but increased by an order of magnitude. This is caused by the fact that the dimensions of the dough sample change during the experiment, while the plunger moves at a constant speed. For all flours the strain rate dependency of σ_B, which was calculated from the slopes of the lines connecting σ_B at a certain strain as a function of strain rate, was rather independent of the strain. It was somewhat larger for Vivant than for Hereward flour dough.

From the curves presented in figure 4 stress-strain curves were calculated at a constant strain rate. Typical examples for optimally mixed Vivant, A6/13 and Hereward flour dough at a constant strain rate of 0.01 s^{-1} are shown in figure 5. The stress level increased going from A6/13, to Vivant and Hereward dough. For the latter σ_B is two times higher than for A6/13 dough. The lower stress level of A6/13 dough can be explained partly by the higher water content (see table 1); the differences between Vivant en Hereward, however, can not. This indicates that there must be more factors that determine σ_B as is well-known. Strain hardening was calculated from the slopes of the curves in figure 5B. It was highest for Hereward dough.

The influence of variables as water addition, mixing time and resting time was studied in biaxial extension for Vivant and Hereward flour dough. Doughs were tested at water additions of 60% and 63% for Vivant and of 63% and 66% for Hereward. In all cases an increase in water addition led to a decrease of σ_B while strain hardening was hardly affected (results not shown). A decrease in stress with increasing water content has also been reported by others (Tschoegl *et al.*, 1970; Kokelaar *et al.*, 1996). The influence of dough resting time on the rheological properties was investigated for resting times of 30, 60 en 90 minutes. This variation in resting time appeared to have only a small effect on σ_B (results not shown). No trend could be observed for the effect of resting time on the value for strain hardening.

The effect of mixing time on σ_B as a function of strain is shown in figure 6 for Hereward (6A) and Vivant (6B). For both flour doughs a significant increase of σ_B was observed with increasing mixing time. Values for strain hardening also increased with mixing time (results not shown). With the test used no information was obtained on fracture properties. For uniaxial extension measurements Tschoegl *et al.* (1970) also observed an increase in stress level at a certain strain with increasing mixing time.

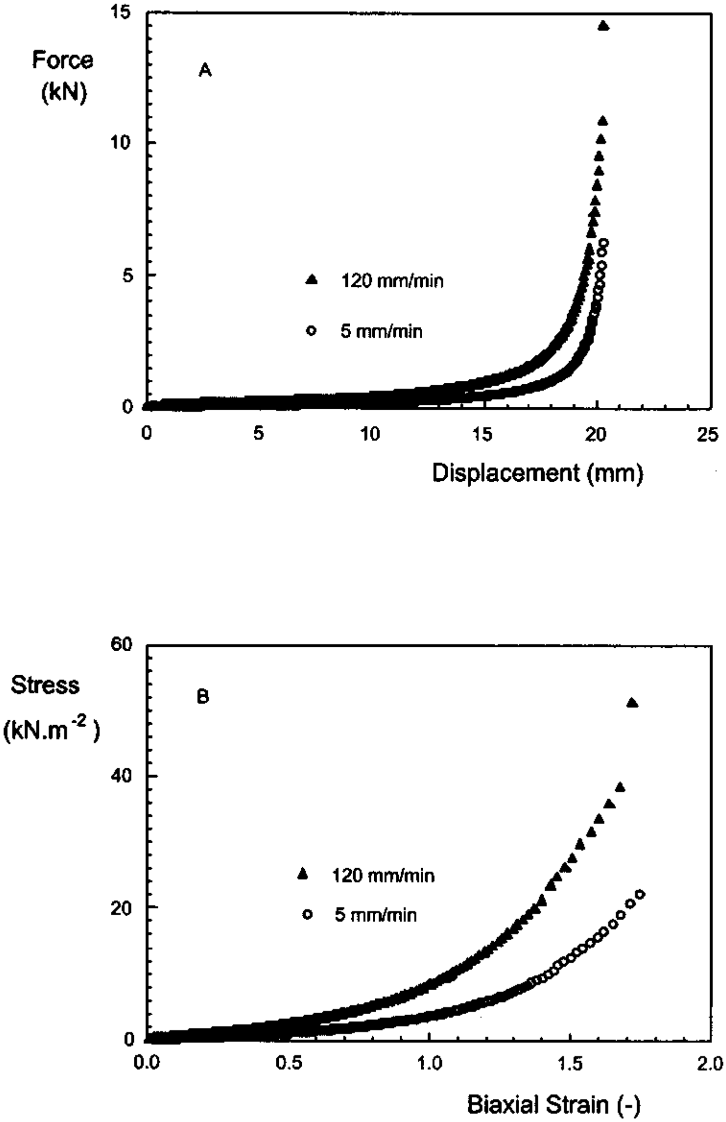

Figure 3. *Results of biaxial extension tests on Hereward flour dough at two compression speeds. 3A: Force as a function of displacement of the plunjer. 3B: Stress as a function of biaxial strain. T = 25°C.*

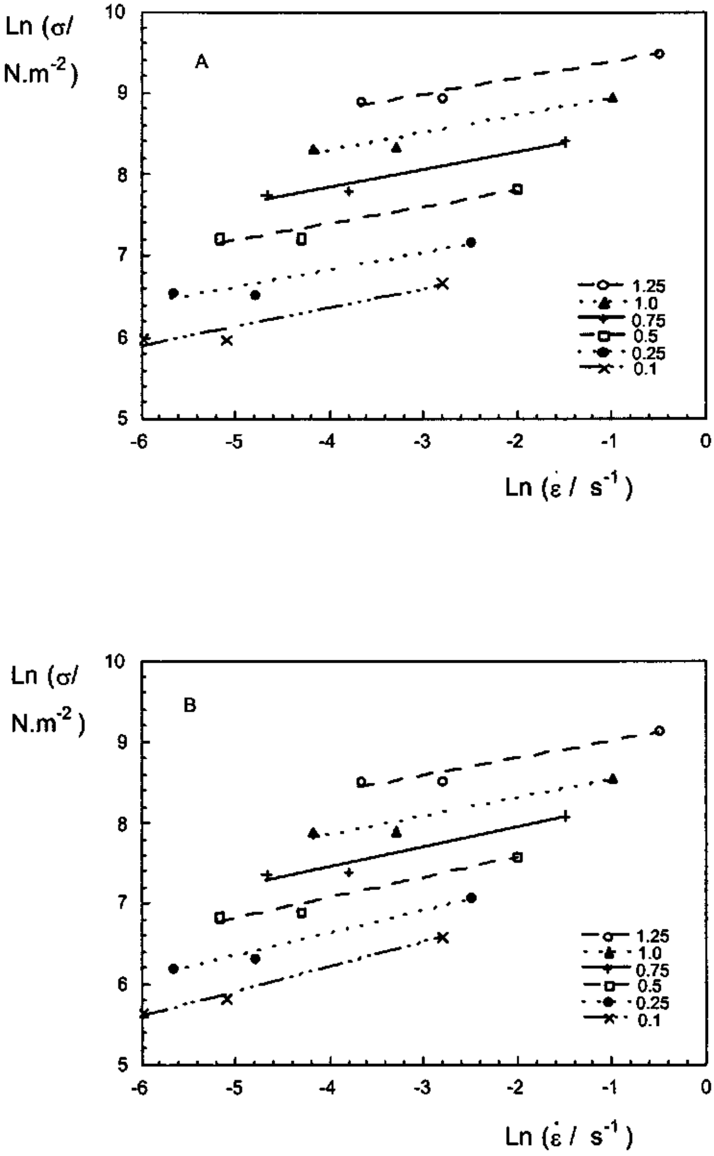

Figure 4. *Biaxial stress as a function of the biaxial strain rate,* $\dot{\varepsilon}_B$, *and the strain (indicated) for Hereward (4A) and Vivant (4B) flour doughs. T = 25°C.*

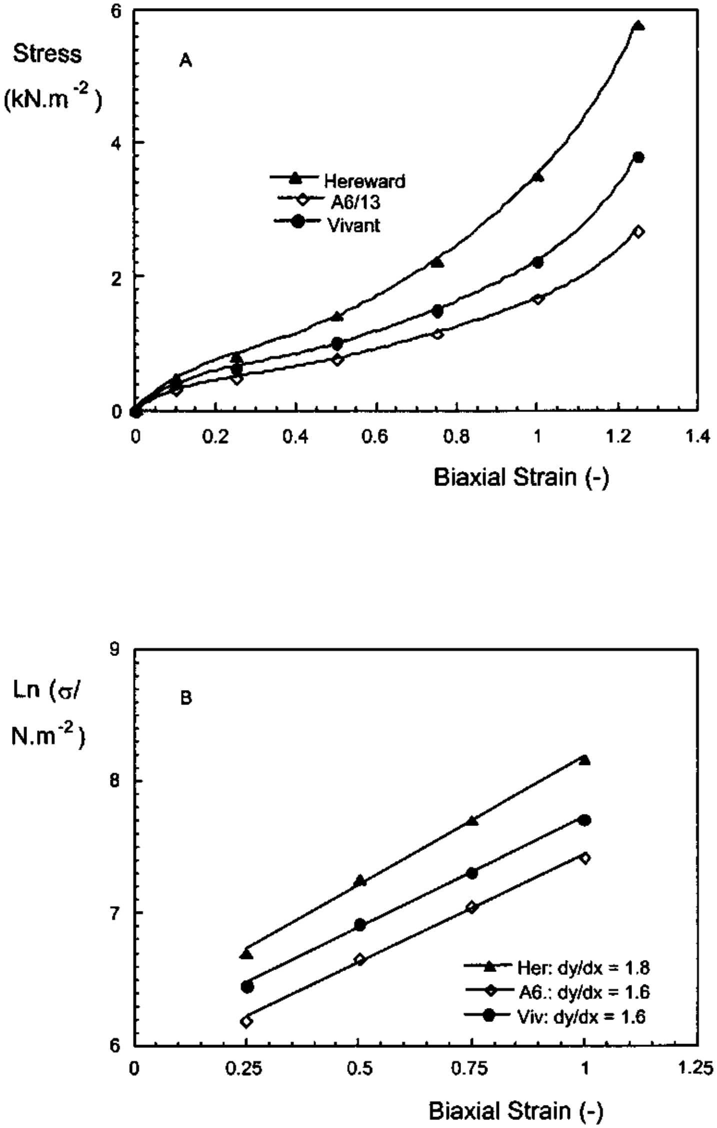

Figure 5. *Biaxial stress as a function of strain at a constant strain rate of 0.01* s^{-1} *for Hereward, A6/13 and Hereward flour doughs. Stress on normal scale (5A) and on log scale (5B). T = 25°C.*

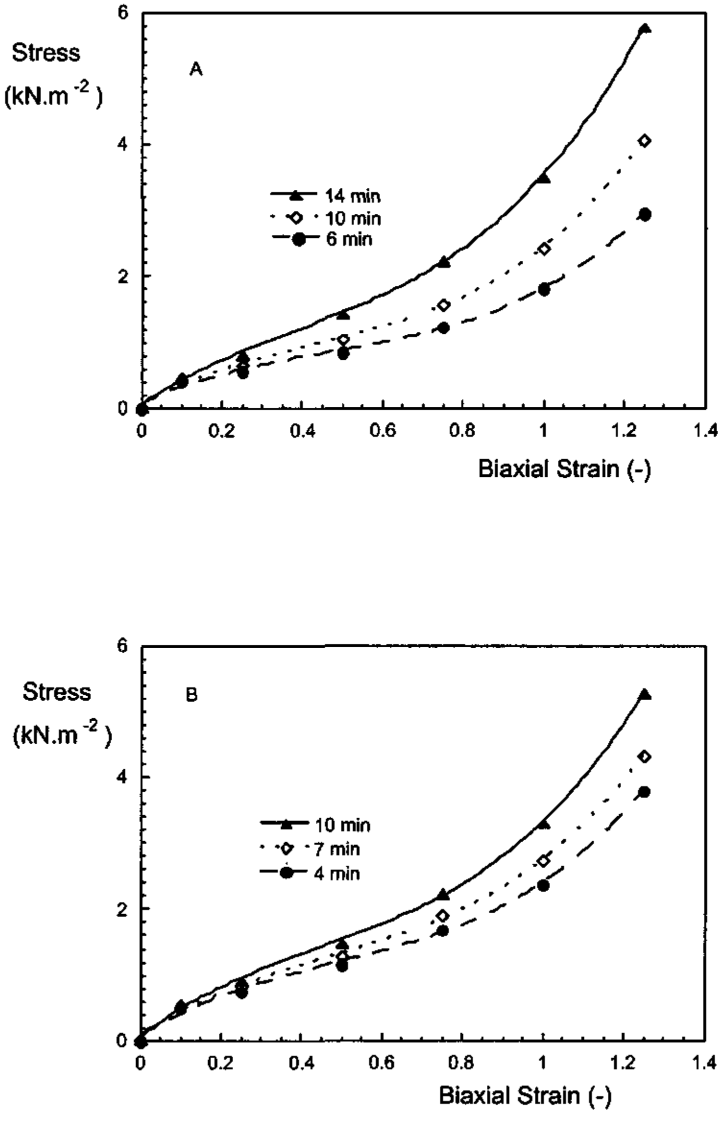

Figure 6. *Biaxial stress as a function of strain at a constant strain rate of 0.01* s^{-1} *for different mixing times for Hereward (6A) and Vivant (6B) flour doughs.* $T = 25°C$.

They also reported a decrease of the fracture strain with increasing mixing time, while the fracture stress went through a maximum. When mixed under nitrogen they observed a smaller increase of σ with increasing mixing time, which led them to the conclusion that during mixing oxygen is incorporated in the dough, leading through redox-reactions to an increase of the stress level. Gras *et al.* (2000) also found that the fracture stress passes through a maximum as a function of mixing time and observed a strong positive correlation between Mixograph resistance and fracture stress in uniaxial extension as a function of mixing time. In addition Kieffer and Stein (1999) observed that after repetitive uniaxial elongational tests in the Extensograph the resistance to deformation increased. They considered the hypothesis that cross-linking of gluten proteins is the reason for the increase in resistance is highly improbable, because of the clear reversibility of the effect. We also considered it much more logic that the observed increase of σ with increasing mixing time and/or the uniaxial extension of dough has a physical cause. Two processes could be responsible for the increase of the stress: (i) stretching of chains and/or aggregates of large size or (ii) orientational changes as a result of the deformational processes. In fact this is the same question as why flour dough shows strain hardening. This question will be dealt with in another part of this chapter.

Large deformations: uniaxial extension

Flour-water doughs of Vivant, A6/13 and Hereward were tested in uniaxial extension using the Kieffer Extensibility Rig. In figure 7 measured force-displacement curves are shown for Hereward and Vivant flour dough at two displacement speeds. For Hereward flour dough the force kept increasing with increasing displacement of the hook for both speeds. Similar curves have been reported for doughs of other strong wheat cultivars (Kieffer *et al.*, 1981). Generally these curves resemble the curves from extensograph measurements. It is striking that with increasing displacement speed of the hook both the recorded force as the displacement at which fracture occurred increased (Dunnewind *et al.*, 2003).

The curves recorded for Vivant flour dough follow a different pattern. At a low displacement speed of the hook the force increased initially, after which the force remained more or less constant on a kind of plateau-value. Such a pattern is also known from extensograph measurements. At a displacement speed of 120 mm.min^{-1} the force-displacement curve follows even a different trend. At small displacements the force increases up to a maximum, after which a minimum follows and the curve increases again until fracture occurs. Apparently the rheological behaviour of Vivant dough in uniaxial extension is strongly dependent on the deformation rate. A curve like this is not known from extensograph measurements. However, Tschoegl *et al.* (1970) always got similar curves performing uniaxial extension tests. They stretched the samples in a polymer solution with the same density as dough.

Force-displacement curves were recalculated into stress-strain curves taking into account the changing dimensions of the sample and assuming a constant volume. In figure 8 such curves are shown for Hereward and Vivant dough. In all cases σ increased strongly with increasing strain. The higher the deformation rate the higher σ.

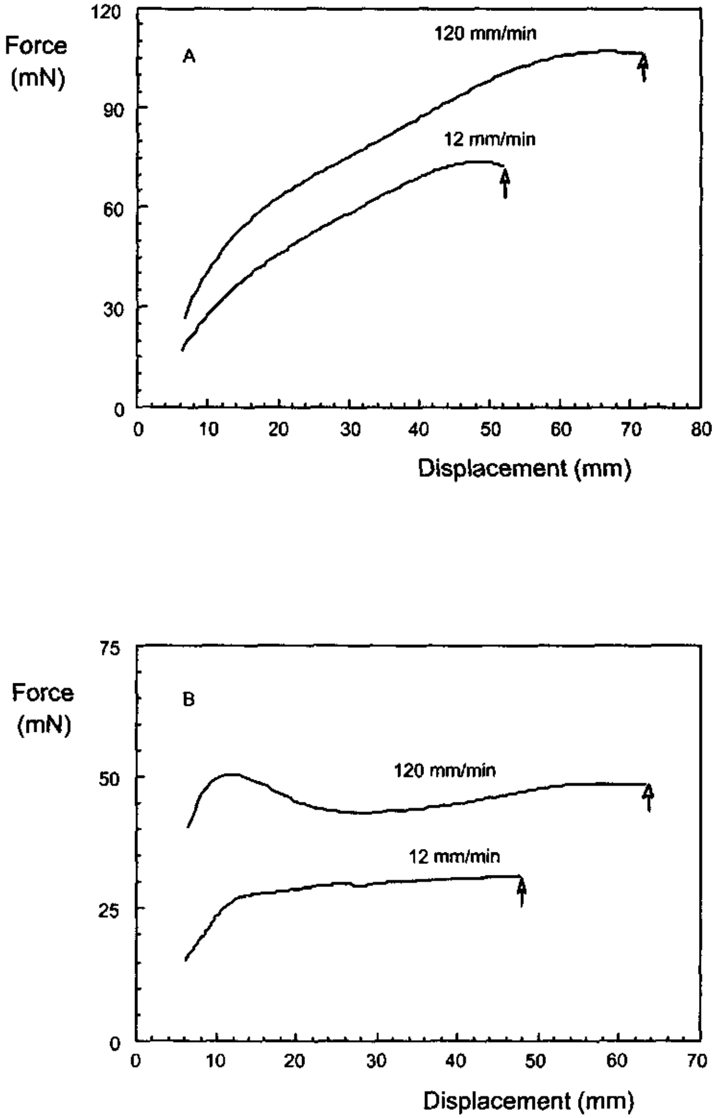

***Figure** 7. Force in uniaxial extension as a function of displacement of the hook at two displacement speeds for Hereward (7A) and Vivant (7B) flour dough. T = 25°C.*

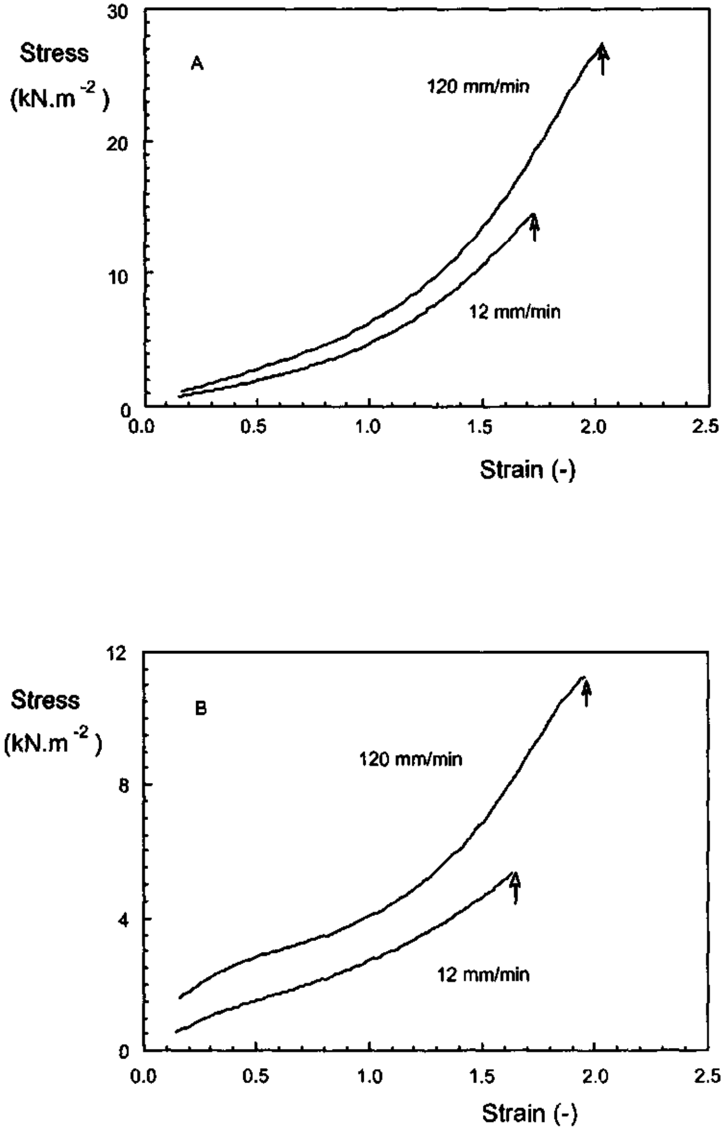

Figure 8. *Stress as a function of strain at two displacement speeds of the hook in uniaxial extension for Hereward (8A) and Vivant (8B) flour dough. T = 25°C.*

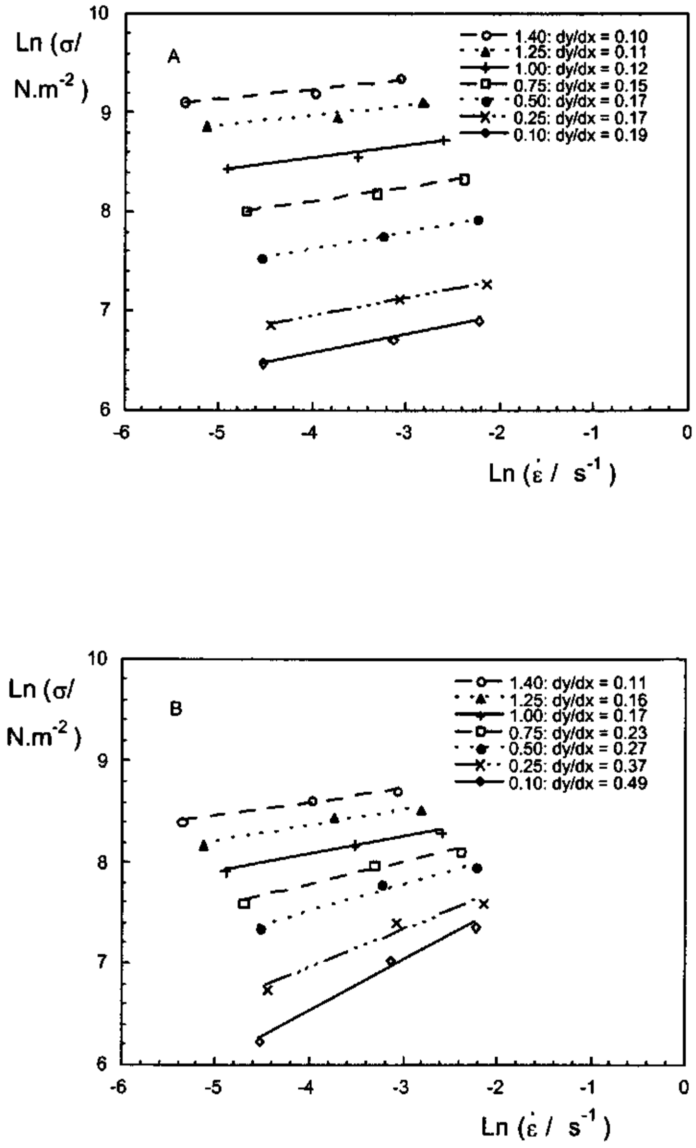

Figure 9. *Uniaxial stress as a function of the strain rate,* $\dot{\varepsilon}$*, for various strains (indicated) for Hereward (9A) and Vivant (9B) flour doughs. T = 25°C.*

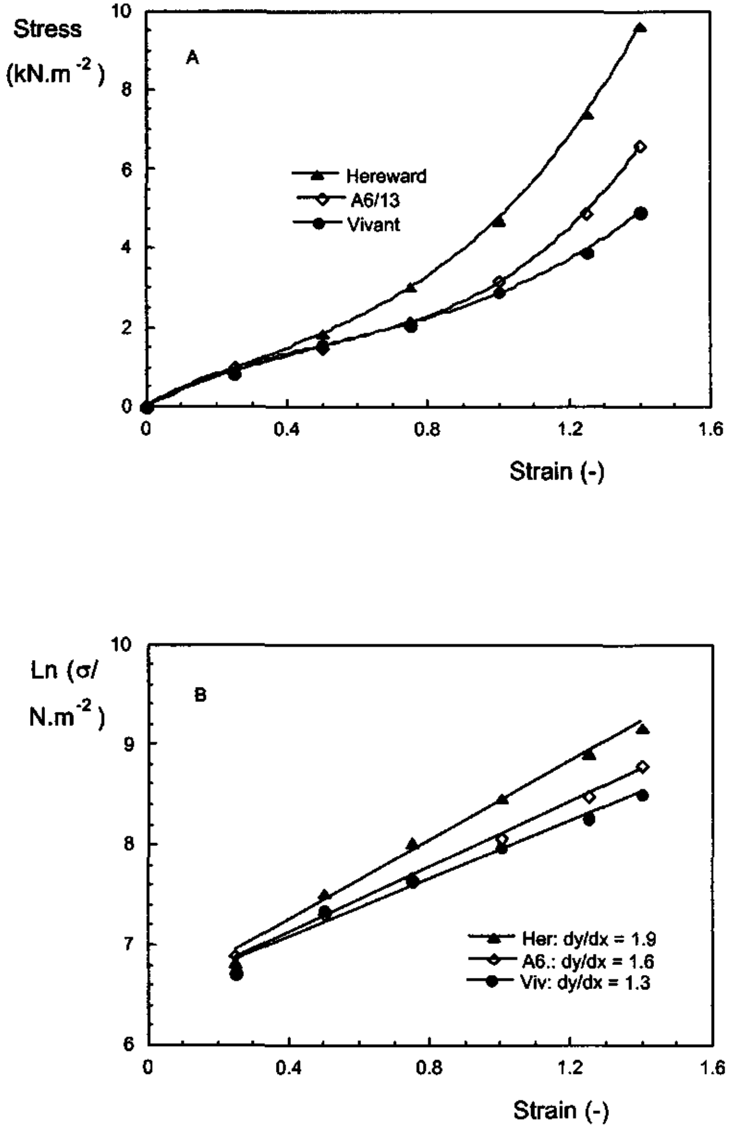

Figure 10. *Uniaxial stress as a function of strain at a constant strain rate of 0.01 s^{-1} for Vivant, A6/13 and Hereward flour doughs. Stress on normal scale (10A) and on log scale (10B). T = 25°C.*

The difference in σ between the displacement speeds was larger for Vivant than for Hereward flour dough, which indicates that the strain rate dependency of σ is larger for Vivant than for Hereward. Fracture strains for Hereward and Vivant flour dough were roughly similar.

During a measurement the hook displaced at a constant speed, but because the dimensions of the dough sample change, the strain rate was not constant. Therefore just as for the biaxial results data were recalculated to σ as a function of the strain rate for various strains (figure 9). Results show that in this test the strain rate as a function of the strain passes through a maximum. For both flours the strain rate dependency of σ was the largest at small strains and decreased with increasing strain. It was stronger for Vivant flour dough, so this cultivar is the most viscous one.

The data presented in figure 9 were recalculated into a stress-strain curve at a constant strain rate. In figure 10A stress-strain curves are shown for Vivant, A6/13 and Hereward flour dough for a strain rate of 0.01 s^{-1}. For all cultivars a similar pattern was observed; an increase of σ with increasing strain. Similar trends were reported by Tschoegl *et al.* (1970b) and Meissner (1997). At larger strains σ increases going from Vivant to A6/13 and Hereward. At a strain of 1.4 σ is twice as high for Hereward than for Vivant flour dough. At small strains similar stress-values were observed for A6/13 and Vivant flour doughs, but at larger strains σ increased more strongly for A6/13 than for Vivant. In figure 10B σ is given on a logarithmic scale. From this graph it can be concluded that at larger strains the value for strain hardening increases in the same order as the stress-level (Vivant < A6/13 < Hereward).

In table 2 the fracture properties of the doughs of the three cultivars are shown. Values for fracture stress were lowest for Vivant. Fracture strains for Vivant and Hereward flour doughs were very similar. They were largest for A6/13 doughs. For all cultivars an increase in fracture stress and strain was observed with increasing deformation rate. Similar trends were reported for wheat flour dough by Tschoegl *et al.* (1970a) and Dunnewind *et al.* (2003). An increase of fracture stress and strain with increasing deformation rate has also been found for Leicester cheese (Dickinson and Goulding, 1980), for 9-month-old Gouda cheese (Luyten *et al.*, 1991) and for heated concentrated starch systems (Keetels *et al.*, 1996). According to van Vliet *et al.* (1995) the observed time-dependency of fracture properties can best be explained by inefficient transport of energy to the crack tip during crack propagation. Both cheese and flour dough consist of various structural elements with different mechanical properties. At larger deformations and deformation rates energy dissipation occurs to a larger extent due to friction between these structural elements. This will clearly retard the speed of crack propagation. So, it is likely that for flour dough crack speed will not increase proportionally with the deformation rate, resulting in the observed dependence of fracture stress and strain on strain rate. For flour dough this behaviour is probably due to the presence of starch granules in a gluten matrix. However, Rinde *et al.* (1970) have observed the same behaviour for gluten doughs. So, maybe there is something in the gluten itself that causes friction. We come back on this point in a forthcoming paper (Sliwinski *et al.*, 2003a). Fracture primarily occurred near the hook or close to the place where the samples were clamped. At these places the deformation is not purely elongational, but contains a clear shear component (Dunnewind *et al.*, 2003). Contrary to the findings of Dobraszczyk and Roberts (1994) and the expectations of Uthayakumaran *et al.* (2000) no positive correlation was found between the value for strain hardening and fracture strain.

Table 2. *Water addition and mixing time applied to flour-water doughs unless otherwise stated. Stress and strain at fracture in uniaxial extension at two hook displacement speeds for Vivant, A6/13 and Hereward flour doughs. T = 25°C.*

Cultivars	**Water addition** (% flour)	**Mixing time**[1] (min)	**Fracture Strain** (-)		**Fracture Stress** ($kN.m^{-2}$)	
			d.p.[2] = 12 $mm.min^{-1}$	d.p. = 120 $mm.min^{-1}$	d.p. = 12 $mm.min^{-1}$	d.p. = 120 $mm.min^{-1}$
Flour dough[3]						
Vivant	63	7	1.6	2.0	5	11
A6/13	67	11	1.9	2.1	11	24
Hereward	64	14	1.7	2.0	13	27

[1] *In 10 g Mixograph.*

[2] *Displacement speed of the hook.*

[3] *Water contents were based on baking tests and chosen as high as possible (Sliwinski et al., 2003b), while mixing times were defined as those giving the highest stress at a chosen strain at a constant strain rate in biaxial extension on the condition that doughs were manageable with respect to stickiness (see section materials and methods of this paper).*

Large deformations: uniaxial versus biaxial extension

In figure 11 stress-strain curves are shown for Hereward flour dough in uniaxial and biaxial extension both calculated at a strain rate of 0.01 s^{-1}. The stress at a certain strain was higher in uniaxial than in biaxial extension and the difference in stress-level became larger with increasing strain. In table 3 some key rheological values are presented for Vivant, A6/13 and Hereward flour doughs. For all flour doughs σ is higher in uniaxial than in biaxial extension. For A6/13 the difference was the largest. This is contrary to the estimation of de Bruijne *et al.* (1990) that uniaxial extension yields lower stress-values than those obtained in biaxial extension, but in agreement with the findings reported for synthetic polymers by Meissner (1997). Striking differences were observed between the cultivars with respect to strain hardening coefficients in uniaxial and biaxial extension. For Vivant strain hardening is stronger in biaxial than in uniaxial extension, for A6/13 there is no difference, while for

Hereward strain hardening was slightly stronger in uniaxial than in biaxial extension. The strain rate dependency of σ for A6/13 and Hereward was higher in uniaxial than in biaxial extension, while for Vivant it was about the same.

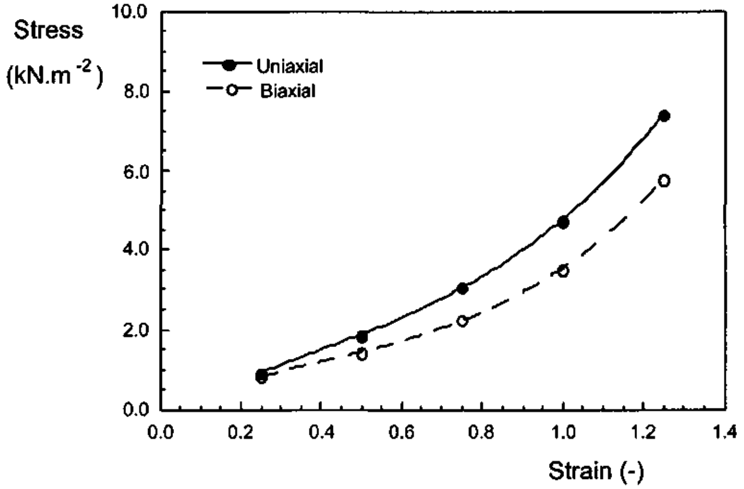

Figure 11. *Stress as a function of strain in uniaxial and biaxial extension at a constant strain rate of 0.01* s^{-1} *for Hereward flour dough.* $T = 25°C$.

Using results from creep tests in shear and lubricated uniaxial compression tests Rouillé *et al.* (2002) compared the rheological behaviour in shear and in biaxial extension through calculating the Trouton ratio. This ratio equals six for a Newtonian fluid, but in concentrated suspensions of non-spherical particles it may be much higher (Giesekus, 1983). Rouillé *et al.* (2002) reported values varying between 6 and 26 depending on the flour composition and the strain rate, thereby confirming earlier findings of Huang and Kokini (1993). According to theory one would expect that biaxial viscosity is about a factor two higher than uniaxial viscosity. However, in our data we observed the contrary. It is therefore to be expected for flour dough that for uniaxial extension Trouton ratios should be in the same range or higher than for biaxial extension.

As mentioned for flour dough σ is higher in uniaxial than in biaxial extension and increases more than proportionally with the strain. It indicates that flour dough not only becomes stronger when stretched, but that it becomes specifically stronger in the direction in which it is stretched. This conclusion is supported by the findings of others. Schweizer and Conde-Petit (1997) studying flour dough in uniaxial elongational flow using the equipment of Meissner found that stress-levels were higher if dough after mixing had been reshaped in the direction of flow than if dough had been reshaped perpendicularly to the direction of flow. Kieffer and

Stein (1999) reported a remarkable increase of σ if a dough piece was first extended in uniaxial extension, reshaped and extended again. Originally this phenomenon was attributed to oxidative processes occurring in the gluten network or to a better entanglement of glutenin polymers permitting physical interactions between them (Bloksma and Hlynka, 1960). Because of the clear reversibility of the effect this hypothesis is considered highly improbable. Another more likely explanation is that orientation of structure elements plays an important role during extension of flour dough.

Table 3. *The stress, the strain-dependency of the stress and the strain rate-dependency of the stress determined in uniaxial and biaxial extension for Vivant, A6/13 and Hereward optimally mixed flour doughs. T = 25°C.*

Cultivar	**Biaxial Extension**			**Uniaxial Extension**		
	σ_B $(N.m^{-2})$ ε=0.75; $\dot{\varepsilon}$=0.01 s^{-1}	$d\ln\sigma_B/d\varepsilon_B$ (-) $\dot{\varepsilon}$=0.01 s^{-1}	$d\ln\sigma_B/d\ln\dot{\varepsilon}_B$ (-) ε=0.75	σ $(N.m^{-2})$ ε=0.75, $\dot{\varepsilon}$=0.01 s^{-1}	$d\ln\sigma/d\varepsilon$ (-) $\dot{\varepsilon}$=0.01 s^{-1}	$d\ln\sigma/d\ln\dot{\varepsilon}$ (-) ε=0.75
Vivant	1400	1.6	0.24	2000	1.3	0.23
A6/13	1100	1.6	0.33	2200	1.6	0.24
Hereward	2000	1.8	0.22	3000	1.9	0.15

The phenomenon of strain hardening and the dependence of the stress of the type of deformation were also reported for synthetic polymers by Meissner (1997). They also reported higher stresses in uniaxial than in biaxial extension. It has, however, to be realised that in uniaxial extension the stress is maximum in the direction of extension and minimum in the direction perpendicular to the direction of extension (Levine and Slade, 1990; Meissner, 1997). In the production of plastic bottles the technological solution for the anisotropy of films produced by uniaxial extension is blow-moulding (Levine and Slade, 1990). By applying biaxial extension isotropic orientation of partially crystalline macrofibrils is allowed with a concomitant isotropy of film strength. In the baking industry an alternative method for the production of cookies, pizza, bread and pastry doughs is sheeting and laminating (Levine and Drew, 1990). With regard to energy consumption dough development with sheeting rolls is very efficient (Kilborn and Tipples, 1974). In this method the development of a biaxial structure in the dough rather than an unidirectional one is promoted by combining the sheeting action of the rollers with rotation of the axis of the dough between successive passages (MacRitchie, 1986).

For several synthetic polymer systems in uniaxial extension birefringence is observed due to orientation of structural elements. In addition for some materials elongation not only leads to enhancement of birefringence, but also to fibrillation resulting from lateral separation between macrofibrils (Slade *et al.*, 1989). In relation to this Bloksma and Isings (1957) observed that birefringence appears after stretching of gluten and disappears in time similar to the relaxation of stress in gluten and dough. This birefingence, which was also observed by Huang *et al.* (1989), showed that orientation effects occur in dough during deformation and that the disorder in the arrangement of the structure elements responsible for its disappearance increased during relaxation. The development of tears in films of wheat gluten, parallel to the direction of stretching, which interrupt the continuity of the perpendicularly weakened matrix, show that in gluten also fibrillation takes place (Slade *et al.*, 1989). According to them, two-dimensional isotropic orientation of gluten macrofibrils can be provided by biaxial extension of folded, sheeted doughs or spherical extension of gas cells during fermentation.

It is unlikely that we in the case of flour and gluten dough are dealing with a type of network similar to that of synthetic polymers. According to Cornec *et al.* (1994) the large-scale structure of gluten dough has more in common with that of a colloidal non-covalent gel rather than that of an entangled polymer system. Nevertheless the effect of orientation of polymers and/ or aggregates during extension has been shown undeniably for flour dough. The flour doughs of Vivant, A6/13 and Hereward differed considerably in the extent to which the rheological properties between uniaxial and biaxial extension varied. Until now the cause for the observed differences is not known. Variation in water content, protein content and protein composition will be of importance and in the case of a particle network the anisometry of the particles and the type and strength of the interaction forces between them. These factors are known to influence the rheological properties of flour dough at large deformations. Examination of the behaviour of a larger set of cultivars than in this study is required before more conclusions can be drawn. Well-known is that the rheological behaviour of flour dough at large deformations is dominated to a large extent by the gluten fraction (Rinde *et al.*, 1970; Kokelaar *et al.*, 1996; Uthayakumaran *et al.*, 2002). To be able to explain better the observed differences between uniaxial and biaxial extension for flour doughs it is desired to expand this study to the rheological properties at large deformations of the glutens isolated from the flours. Results of that study will be described in a forthcoming paper (Sliwinski *et al.*, 2003a).

CONCLUSIONS

The small deformation properties of optimally mixed flour doughs hardly varied between wheat cultivars which perform differently in cereal products. In contrast large differences were observed between cultivars in measurements at large deformations. Fracture stress and strain increased with increased deformation rate, a behaviour which is common for many food materials of which the fracture properties greatly depend on the speed of deformation. Since the underlying cause is friction between structural elements, e.g. between dispersed particles and the network, it is likely that the starch granules present in dough have a strong impact on its fracture behaviour.

For all flour doughs the uniaxial and biaxial stress increased more than proportionally with the strain, a phenomenon which is called strain hardening. The strain hardening behaviour together with the birefingence reported before indicates that orientational processes of structure elements in the direction of flow play an important role in dough. This conclusion is supported by the fact that, in line with the findings of Meissner for synthetic polymers, for flour dough σ is higher in uniaxial than in biaxial extension.

In uniaxial extension the stress is maximum in the direction of extension and minimum in the direction perpendicular to the direction of extension (Levine and Slade, 1990; Meissner, 1997). In biaxial extension the stress is the same in the two directions in the plane of deformation. To prevent anisotropy of dough films in the baking process it is preferable to promote a biaxial structure in the dough. This can be achieved by sheeting the dough in combination with rotation of the axis of the dough between successive passages or by spherical extension of gas cells during fermentation (Levine and Drew, 1990).

From the above summarised dough characteristics a picture has emerged of flour dough as a composite material of which the rheological properties strongly depend on the deformation history, the type of deformation and the speed of deformation and in which orientational processes play an important role. It is to be expected that the gluten protein fraction has a strong influence on this behaviour at large deformations It is therefore strongly desired to expand this study to the rheological properties at large deformations of the glutens isolated from the flours.

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Chapter IV

Large-Deformation Properties of Wheat Dough in Uni- and Biaxial Extension. Part II: Gluten Dough[1]

SUMMARY

Glutens were isolated from flour of three European wheat cultivars which perform differently in cereal products. The rheological and fracture properties of gluten-water doughs were determined in uniaxial and biaxial extension at large deformations and small angle sinusoidal oscillation tests and compared with the mechanical properties of the parental flour doughs. At 25°C the linear region was in the same range as that of flour dough, while at a higher temperature (45°C) the linear region was more than an order of magnitude higher. At 45°C the storage modulus and tan δ were lower than at 25°C. Variation in moduli between cultivars was much more pronounced for gluten than for flour doughs.

Similarly to flour dough in both uniaxial and biaxial extension the stress (σ) increased more than proportionally with the strain, a phenomenon called strain hardening. The stress at a set strain and strain hardening depended much stronger on the type of deformation for gluten than for flour dough: σ was higher in biaxial extension for gluten than for flour dough, but was much higher in uniaxial extension. This indicates that orientational effects in elongational flow are of even larger importance for the mechanical properties of gluten than of flour dough. It is likely that it is the glutenin fraction that because of its large size confers these direction dependent properties to gluten and flour doughs. Fracture stresses were much higher for gluten than for flour dough, while fracture strains were in the same range or higher. For gluten dough fracture strains increased less strongly with increasing strain rate than for flour dough. Glutens exhibiting a higher stress at a certain strain had a smaller fracture strain.

Our findings confirm the conviction that the large deformation properties of flour dough are mainly governed by the gluten fraction. However, there are also differences. Compared to flour dough gluten dough exhibits (i) a stronger strain hardening, (ii) a larger difference in σ between uniaxial and biaxial extension and (iii) a smaller strain rate dependency of the fracture strain.

INTRODUCTION

From wheat flour light, spongy products with a regular crumb structure can be made, because flour dough has the special ability to retain gas (Hoseney and Rogers, 1990). In order to

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[1] E.L. Sliwinski, F. van der Hoef, P.Kolster, T. van Vliet. Rheologica Acta. In press.

produce high quality loaves dough must remain extensible for a long enough time to avoid premature fracture of dough films (Bloksma, 1990). During the bread making process flour dough is deformed at large deformations (including fracture) alternatingly (i) in uniaxial extension at intermediate to large deformation rates and (ii) in biaxial extension at small to intermediate deformation rates (Sliwinski *et al.*, 2003a). An essential property in this respect is that wheat flour dough in extension shows strain hardening, a more than proportional increase of the stress with increasing strain (van Vliet *et al.*, 1992). The large deformation and fracture properties of flour dough in extension were found to depend further on flour type, water content, mixing time, temperature and strain rate (Tschoegl *et al.* 1970; Kokelaar *et al.*, 1996; Zheng *et al.*, 2000; Uthayakumaran *et al.*, 2002). Sliwinski *et al.* (2003a) found that σ was much higher and less dependent on strain rate in uniaxial than in biaxial extension. They concluded that in elongational flow orientational effects are of large importance for the mechanical properties of flour dough.

The ability of wheat flour dough to form highly extensible films with gas holding capacity is primarily due to the large deformation properties of the gluten protein fraction (Schofield and Scott-Blair, 1937). Moreover differences in bread making performance between cultivars are mainly caused by variation in gluten quality (Gras *et al.*, 2001). The gluten proteins of wheat can be considered as having a bimodal distribution of monomeric gliadins and polymeric glutenin proteins. The polymeric glutenin is in itself polydisperse (i.e. is made up of polymers of different molecular weight) and contains very large insoluble polymer molecules (Wrigley, 1996; Wahlund *et al.*, 1996; Sliwinski *et al.*, 2003c). From flour by aqueous washing procedures wheat gluten can be isolated that generally contains 80% protein (dry basis) apart from residual starch and other minor components.

Rinde *et al.* (1970) studied the large deformation and fracture properties of gluten-water mixtures (further to be called gluten dough) in simple tension. They reported that wheat gluten dough was stronger, more elastic and less extensible than flour dough and showed that stress and strain at fracture depended on extension rate. Kim *et al.* (1988) reported the effect of glutenin to gliadin ratio on the mechanical properties of gluten dough in uniaxial extension tests. They found that with increasing gliadin content the resistance to extension decreased and the extensibility increased. Uthayakumaran *et al.* (2002) subjected gluten dough to uniaxial elongation and found that fracture strain increased with strain rate. For a strong wheat variety fracture strain was larger for flour than for gluten dough, while for a good baking wheat the opposite was observed. Janssen *et al.* (1996a,b) reported that gluten dough shows strain hardening in biaxial extension. The main factors that determined their rheological behaviour were found to be the glutenin to gliadin ratio and the source from which the glutenin originated. The rheological properties of flour and gluten doughs of different origin were compared in biaxial extension by Kokelaar *et al.* (1996). They reported that in biaxial extension σ was higher and less dependent on strain rate for gluten than for flour dough and that gluten dough exhibited stronger strain hardening behaviour. They also found that the difference in stress level during deformation was more pronounced for gluten than for flour doughs of different origin. For gluten dough an increase of the temperature from 20°C to 55°C resulted in a decrease of σ, strain hardening and the strain rate dependency of σ.

Contrary to flour dough small deformation measurements have proven to be very useful to study the structure of gluten doughs and to discriminate between glutens of different origin. Kokelaar *et al.* (1996) showed that differences in moduli were much more pronounced for gluten doughs of different origin than for flour doughs. They also reported a decrease of G' and tan δ on increasing the temperature from 20°C to 55°C. The composition of the gluten dough was found to be important. Janssen *et al.* (1996a,b) reported a higher G' and a lower tan δ at higher glutenin content. Also the source of the glutenin affected the rheological properties at small deformations. Based on a thorough analysis of the mechanical spectra of gluten Cornec et al. (1994) concluded that the cross-link density in the network is governed primarily by the amount of the largest glutenin aggregates, whereby the cross-links with low relaxation times connecting glutenin aggregates are more likely low-energy bonds, than entanglements (i.e. topological constraints in flexible chain systems). In their view gluten can be considered as suspensions of colloidal flocs (floc = large glutenin aggregate) with neighbouring flocs connected in space by physical interactions (Lefebvre *et al.*, 1994). According to Tsiami *et al.* (1997a) gluten proteins are associated through disulphide bonds, hydrogen bonds and other non-covalent interactions forming large aggregates. They concluded that because of the presence of non-covalent associations the degree of cross-linking depends on temperature resulting in a decrease of the modulus with increasing temperature. It was observed that the ratio of high to low molecular weight fractions was very important for the rheological properties of the network (Tsiami *et al.*, 1997b). The higher the amount of high molecular weight glutenin the higher the storage modulus and the lower the tan δ values were. Their data suggest that in gluten (locally) relatively compact objects are present of which the compactness can be increased by adding salt or increasing the glutenin concentration or the glutenin size.

As discussed above the large deformation properties of wheat flour dough in uniaxial and biaxial extension are important in bread making. Data for flour dough are discussed by us in a former paper (Sliwinski *et al.*, 2003a). It appeared that for flour dough in uniaxial extension σ was much higher and less dependent on strain rate than in biaxial extension. This led us to the conclusion that for the mechanical properties of flour dough in elongational flow orientational effects are of large importance. Since the large deformation properties of flour dough are mainly governed by the gluten fraction, we expect that in gluten orientational effects are even larger. Therefore we studied the large deformation properties in uniaxial and biaxial extension of gluten doughs of three wheat flours of which we had studied the rheological behaviour. In addition small deformation measurements were performed in dynamic oscillation.

MATERIALS AND METHODS

Materials

Three European wheats, Vivant, A6/13 and Hereward were selected, which show large differences in baking performance (see table 1). In table 1 also relevant analytical data of the parental flours and the glutens isolated thereof are presented. All selected wheat samples were more or less pure cultivars, except A6/13 which was a mixture of two undefined cultivars. Samples of 400 kg each were milled to a milling degree of approximately 70% in the experimental mill of Meneba BV, Rotterdam, The Netherlands. The flour was stored at –20°C.

Table 1. *Analytical and baking data for parental flours and glutens isolated thereof.*

Cultivar	**Flour Protein Content** (% dry basis)	**Gluten Protein Content** (% dry basis)	**Mixing Requirements**[1] (min)	**Baking Performance**[1] (cm^3/g flour)
Vivant	10.3	86	5.5	6.6
A6/13	12.0	85	7.5	7.5
Hereward	11.0	83	10.0	7.2

[1] *Data are from baking tests in which a large mixer was used (Sliwinski et al., 2003b).*

Chemical characterisation

Moisture content was determined according to AACC method 44-19 (AACC, 1983). Protein content was determined by the Kjeldahl method (N x 5.7) using an auto-analyser system.

Gluten isolation

Glutens were isolated from flour according to the method of Shogren *et al.* (1969) with some modifications. A batter of 1 kg flour with 1.2 L demineralised water of 40°C was mixed for 2 minutes using a Janke & Kunkel RW20 mixer. The batter was directly centrifuged for 15 minutes at 2500 rpm (< 1000 g) at 20°C. After centrifugation from top to bottom four separate layers could be observed: an aqueous layer, a gel layer, a gluten layer and a starch pellet as described previously (Larsson and Eliasson, 1996). After removal of the supernatant and the gel layer, the gluten layer was separated from the starch pellet and further washed by hand using demineralised water. The wet gluten was frozen using liquid nitrogen and freeze-dried. Freeze-dried gluten were milled in a Retsch Grinder using a 150 μm sieve. For every flour the gluten of six extractions was mixed to get uniform material for all tests. It is possible that because of the use of demineralised water gliadins have been washed out and that therefore the gluten preparations contain a relatively higher percentage of glutenin compared with the original material. From the flours between 60 to 75 grams of gluten per kg flour were isolated. Gluten were stored at –20°C.

Preparation of gluten doughs

Gluten doughs were prepared by mixing 2.5 g gluten with the appropriate amount of water in a 10 g National Mixograph. Water additions were defined for each gluten individually as the amount of water required to assure complete hydration on the condition that after 10 minutes of mixing all the water was absorbed by the gluten. Both properties were determined by visual observation. To determine the mixing requirements for each gluten doughs were tested in uniaxial extension as a function of mixing time. Mixing times applied were 6, 10, 14 min for Vivant, 6, 10, 14, 18 min for A6/13 and 10 14, 18 min for Hereward. Optimum mixing times were defined for each gluten individually as those giving the largest fracture strain and the strongest strain hardening in uniaxial extension. Water contents and mixing times used are given in table 2.

Sinusoidal oscillation tests

Sinusoidal oscillation tests were performed in a Bohlin VOR constant shear Rheometer essentially as described previously (Kokelaar *et al.*, 1996; Sliwinski *et al.*, 2003a). The instrument was equipped with a plate-plate geometry (radius 1.5 cm). In all cases the plates were covered with emery-paper to avoid slip. Gluten doughs were placed between the plates at maximum 1 hour after mixing and the measurements started after a resting period of 30 minutes. To prevent drying out of the test-piece, the rim of the sample was coated with grease. Coating with mineral oil did not prevent drying. G' and G'' were determined as a function of frequency at a strain of 1.10^{-3} at 25°C and at 45°C. Their strain dependency was determined at a frequency of 1 Hz at both temperatures also. Results were means of 4 experiments on at least three different flour dough preparations (average coefficients of variation CV = ±4%, ±4% and ±2% for G', G" and tan δ, respectively).

Biaxial extension tests

Biaxial extension tests were performed using a Zwick mechanical testing apparatus equipped with a 50 N load cell essentially as described previously (Kokelaar *et al.*, 1996; Sliwinski *et al.*, 2003a). The initial height of the test pieces was 10 mm and they were compressed to a final height of 1 mm. This test piece height has been chosen to avoid problems with compression of test pieces by the gravity force and with test pieces slipping out of the test plates during the measurement. To prevent friction between the test plates and drying out of the sample test pieces were lubricated with paraffin oil. The force required to compress the gluten sample and the displacement of the upper plate were recorded as a function of time and used for further calculations. Displacement speeds were 5, 12 and 120 mm.min^{-1}. Results shown are mean values of four measurements on at least three different dough preparations (average coefficient of variation CV = ±3% and ±4% for biaxial stress and strain hardening, respectively). The force-displacement curves were recalculated into stress-strain curves as described previously (Kokelaar *et al.*, 1996; Sliwinski *et al.*, 2003a). Calculation of stress and strain allowed also the calculation of a relative increase of the stress with a relative increase of the biaxial strain $\mathrm{dln}\sigma_B/\mathrm{dln}\varepsilon_B$ which will be called strain hardening in biaxial extension and a relative increase of the stress with a relative increase of the biaxial strain rate $\mathrm{dln}\sigma_B/\mathrm{dln}\dot{\varepsilon}_B$ which will be called the strain rate dependence of the stress in biaxial extension.

Uniaxial extension tests

Uniaxial extension tests were performed using a Zwick mechanical testing apparatus equipped with a 50 N load cell essentially as described previously (Dunnewind *et al.*, 2003; Sliwinski *et al.*, 2003a). The Zwick was equipped with the Kieffer Extensibility Rig (Kieffer *et al.*, 1981). Directly after mixing the gluten doughs were shaped in a stencil and cut into 5 cm long pieces with a trapezium like cross-section (3/5 x 4 mm). They were stored in the stencil for approximately 1 hour whereby the stencil was lubricated with paraffin oil to avoid drying out of the samples. Just before the start of the test both ends of the sample were clamped between the two plates of the Kieffer Rig. The beginning of the measurement was chosen at the inflection point of the force-hook displacement curve, where the hook started to change the length of the test piece. The force required to extend the free hanging cylindrically shaped sample and the displacement of the hook were recorded as a function of time. Displacement speeds were 12, 48 and 120 $\mathrm{mm.min^{-1}}$. Results shown are mean values of at least five measurements on at least four different dough preparations (average coefficient of variation CV = ±3%, ±4%, ±4% and ±6% for uniaxial stress, strain hardening, fracture strain and fracture stress, respectively). The force-displacement curves were recalculated into stress-strain curves as described previously (Dunnewind et al., 2003; Sliwinski et al., 2003a). Calculation of stress and strain allowed also the calculation of a relative increase of the stress with a relative increase of the strain $\mathrm{dln}\sigma/\mathrm{dln}\varepsilon$ which will further be called strain hardening in uniaxial extension and a relative increase of the stress with a relative increase of the strain rate $\mathrm{dln}\sigma/\mathrm{dln}\dot{\varepsilon}$ which will be called the strain rate dependence of the stress in uniaxial extension.

RESULTS

Gluten dough preparation

Optimum relative amounts of added water on total dough weight for our glutens varied between 62 and 64%, while these values for the parental flour doughs amount to 40% (see table 2). The resulting dough water contents are in the same range as those reported by Uthuyakumaran *et al.* (2002) who calculated these values for flour dough and for gluten-starch mixtures using the standard method (American Association of Cereal Chemists, 2000). Using phase separation of wheat flour dough by ultracentrifugation Larsson and Eliasson (1996) apart from an aqueous phase were able to separate three distinct phases. Water contents of the three separated phases were about 80%, 50–60% and 30%, for the gel, the gluten and the starch phase, respectively. Generally, the solids of wheat gluten comprise about 80% of protein (of which about 8% might be non-gluten proteins), around 10% starch, between 5-10% lipids and also minerals and non-starch polysaccharides (Roels, 1997). If we assume that the solids of our glutens comprise 5, 85 and 10%, for lipids and pentosans, proteins and starch, respectively, and that after ultracentrifugation of gluten dough water contents of the three corresponding phases and the volume of the aqueous phase are similar to those found by Larsson and Eliasson (1996), a required water addition of 61% can be estimated for our glutens. It seems therefore that at the water levels that were applied in this work similar amounts of water go into the gluten phase of flour and of gluten dough.

Small deformations

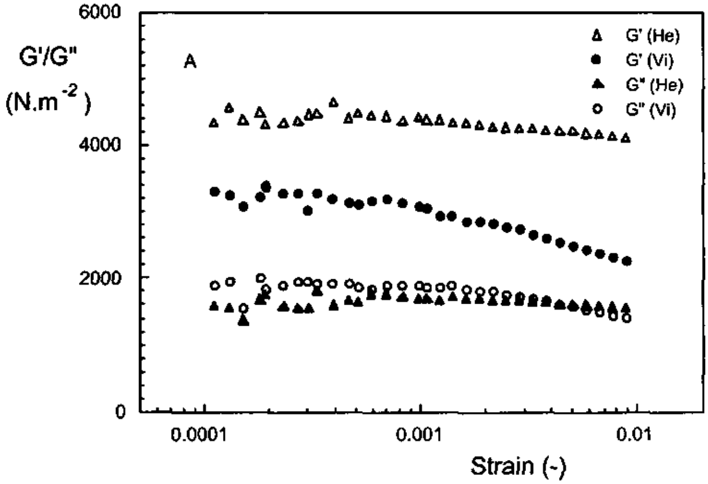
In figure 1 the storage modulus (*G'*), the loss modulus (*G''*) and the loss tangent (tan δ) are shown as a function of strain for Hereward and Vivant optimally mixed gluten dough measured at 25°C. For both glutens *G'* was roughly independent of the strain up to a strain of 8.10^{-4}. At larger strains *G'* decreased slightly with increasing strain. The decrease was larger for Vivant than for Hereward gluten dough. For both glutens tan δ was roughly independent of strain up to a strain of 0.01. At 45°C *G'* and tan δ were independent of the strain up to a strain of 0.01 for Hereward and gluten dough (figure 2). Values for *G'* and tan δ were much lower at 45°C than at 25°C for both glutens. Both at 25°C and at 45°C *G'* was higher and tan δ was lower for Hereward than for Vivant gluten dough the difference being larger at the higher temperature.

At 25°C *G'* was independent of the strain up to a strain of 8.10^{-4}, while at 45°C all gluten doughs showed linear behaviour up to a strain of 0.01. The results obtained with gluten at 25°C differ slightly from the results observed for the flour doughs of these wheat cultivars (Sliwinski *et al.*, 2003a). For flour dough *G'* started to decrease at a strain larger than 2.10^{-4} and decreased more strongly than for gluten dough.

For gluten dough lower values for *G'* were found which is probably caused by the higher starch and lower water content of flour dough. A small linear region for flour doughs and higher values for the *G'* for flour doughs than for gluten doughs have been reported by numerous authors (Smith *et al.*, 1970; Kokelaar *et al.*, 1996; Uthuyakumaran *et al.*, 2002). For gluten dough in most cases a much longer linear region has been reported than for flour dough (Cornec *et al.*, 1994; Wang and Kokini, 1995; Khatkar *et al.*, 1995). However, data from Lindborg *et al.* (1997) indicate a similar linear viscoelastic strain limit for gluten dough as for flour dough (Phan-Thien *et al.*, 1997). Recently, Dhanasekharan *et al.* (2001) have shown that the strain limit of gluten doughs depends on the applied frequency and gap width.

The linear region could range from 1.10^{-4} to 0.1, the larger linear strains at smaller frequency and higher gap width. Uthayakumaran *et al.* (2002) reported a decrease of the linear strain limit by an order of magnitude with an increase of gluten dough water content from 40% to

60%. This shows that the linear strain limit of gluten dough not only decreases with increasing starch content, but also with increasing water content.



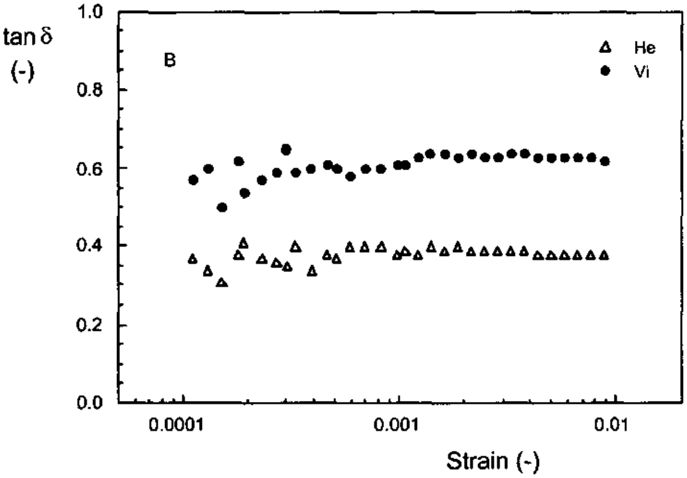


***Figure 1**. Storage modulus and loss modulus (1A) and loss tangent (1B) as a function of applied strain for Hereward and Vivant optimally mixed gluten doughs. Data are at 1 Hz and at 25°C.*

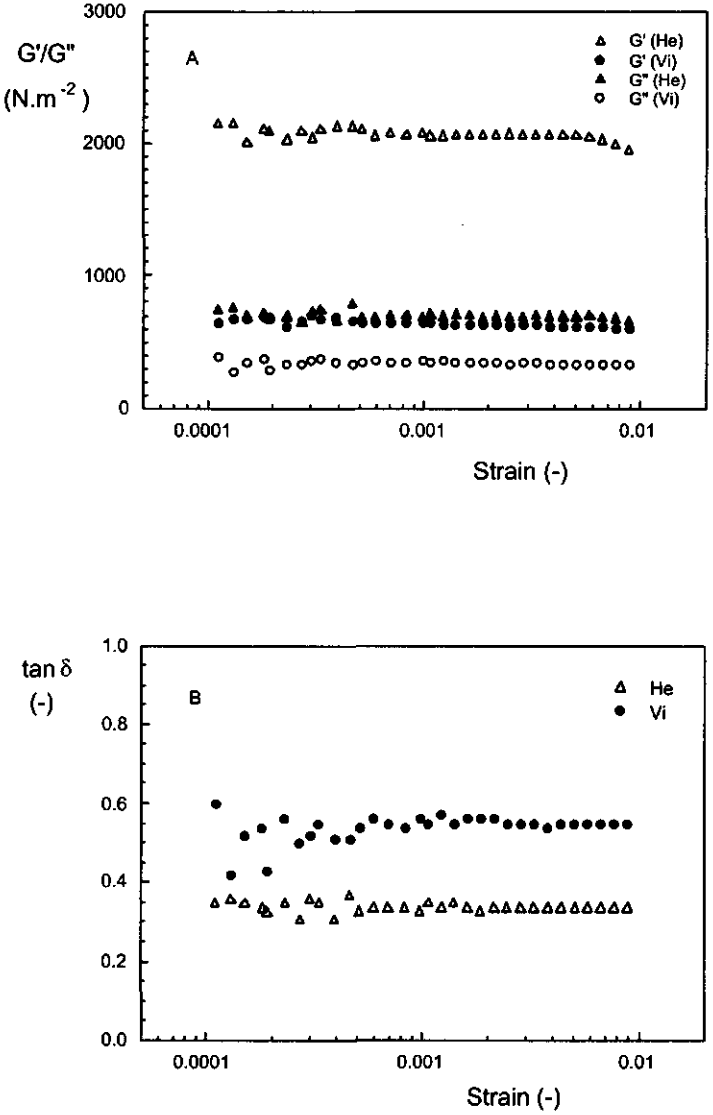


Figure 2. *Storage modulus and loss modulus (2A) and loss tangent (2B) as a function of applied strain for Hereward and Vivant gluten doughs. Data are at 1 Hz and at 45°C.*

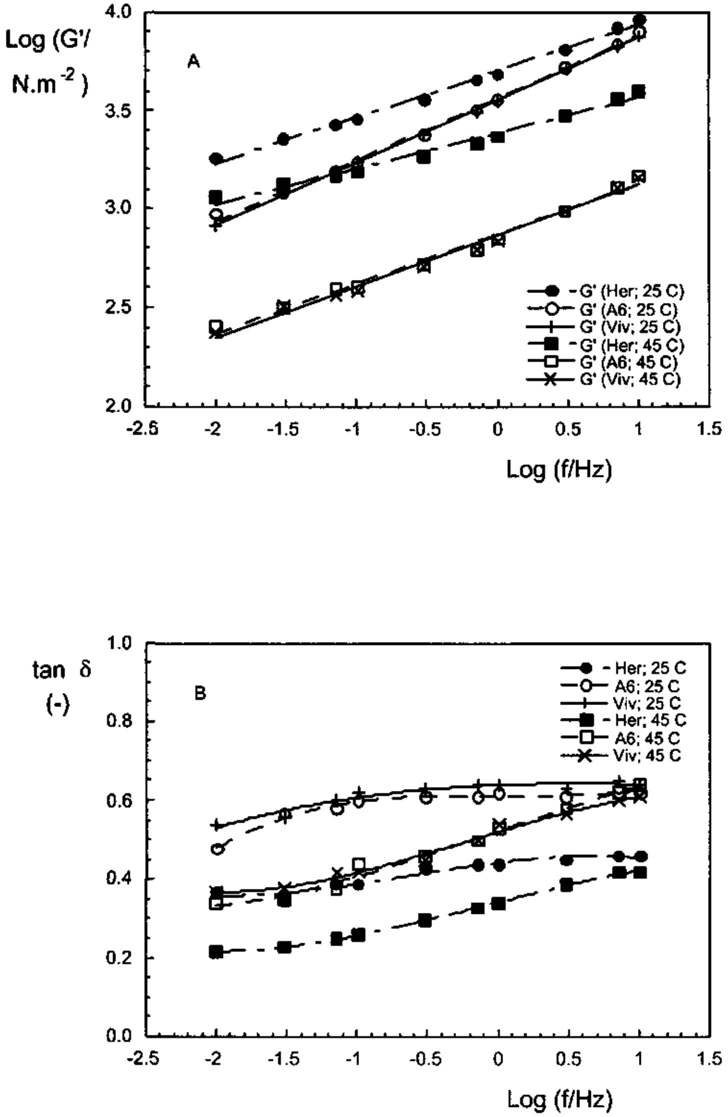


Figure 3. *Storage modulus (3A) and loss tangent (3B) as a function of frequency for Hereward, A6/13 and Vivant gluten doughs. Data are at 25°C and at 45°C. Applied maximum strain was 0.001.*

Our data showed an increase of the linear strain limit, a decrease of G' and a decrease of tan δ with increasing temperature. For all glutens G' was lower and less dependent on the frequency at 45°C than at 25°C in agreement with data reported by Lefebvre *et al.* (1994), Kokelaar *et al.*, (1996) and Tsiami *et al.* (1997a). According to Lefebvre *et al.* (1994) temperature will affect primarily gluten viscoelasticity through its effect on the cross-links responsible for network connectivity. Depending on their nature the non-covalent bonds are either strengthened or weakened by temperature. Tsiami *et al.* (1997a) explained the observed changes to shifting of the dissociation-association equilibria of hydrogen bonds with increasing temperature.

In figure 3 G' and tan δ are shown as a function of frequency for Hereward, A6/13 and Vivant gluten dough measured at 25°C and 45°C. In all cases G' increased with increasing frequency. At 25°C a small increase of tan δ with increasing frequency was found. These findings are in line with results found by others (Janssen *et al.*, 1996a,b; Kokelaar *et al.*, 1996), although they applied a higher strain. For all glutens G' increased less and tan δ increased more with increasing frequency at 45°C than at 25°C. These findings are in agreement with data reported by Kokelaar *et al.* (1996). For Hereward gluten dough G' was less dependent on the frequency and tan δ was lower than for A6/13 and Vivant gluten dough. At 45°C G' was less dependent on the frequency and tan δ was lower for Hereward than for A6/13 and Vivant gluten dough.

Generally G' was higher and tan δ was smaller for Hereward than for A6/13 and Vivant gluten dough, indicating that Hereward gluten is more elastic. Comparable differences between gluten samples of different origin have been found by others (Cornec *et al.*, 1994; Janssen *et al.*, 1996a,b; Tsiami *et al.*, 1997b). The most probable reason for these differences lies in the protein composition of the gluten samples and more specifically the gliadin to glutenin ratio and the size distribution of the glutenin fraction. The differences in moduli observed between the gluten doughs in this paper are much more pronounced than those between the parental flour doughs which were presented before by Sliwinski *et al.* (2003a).

Large deformations: biaxial extension

Gluten doughs were extended biaxially by lubricated uniaxial compression at three displacement speeds of the plunger. In figure 4 results are presented of measurements on Vivant and Hereward gluten. Due to the way the experiments were performed, the strain rate was not constant during compression experiments, but increased by more than two orders of magnitude (Kokelaar *et al.*, 1996; Sliwinski *et al.*, 2003a). For both cultivars an increase of the stress (σ_B) was observed with strain and strain rate. As can be seen in figure 4 the slopes of the lines connecting measurement points at the same strain, but at different strain rate are about independent of the strain, indicating that the strain does not have much influence on the strain rate dependency of σ_B. The latter was higher for Vivant than for Hereward, indicating a more viscous behaviour for Vivant gluten. This conclusion is in line with the observed values for tan δ for both gluten doughs. Large differences were observed in σ_B between Hereward and Vivant gluten doughs. At the same strain stress-levels were much higher for Hereward gluten doughs than for Vivant.

The data presented in figure 4 were used to calculate stress-strain curves at a constant strain rate of 0.01 s^{-1} for Vivant, A6/13 and Hereward gluten doughs (figure 5). Stress-levels increased going from Vivant to A6/13 and Hereward. At the same strain σ_B of Hereward gluten was more than four times higher than that of Vivant (figure 5A). In biaxial extension all gluten doughs studied showed strain hardening (figure 5B). Strain hardening was stronger for Hereward than for Vivant and A6/13 gluten dough. Janssen *et al.* (1996a) and Kokelaar *et al.* (1996) presented results comparable with ours in many respects. However, they found higher σ values and strain hardening coefficients varying between 2.2 to 3.0. Probably these differences are related to the different water content of the gluten doughs. The water content of the gluten doughs varied between 62 - 64% in our case, while Janssen and Kokelaar used water contents of around 54%. It was however not possible to hydrate our glutens sufficiently at the water contents applied by Janssen and Kokelaar. This difference in water requirement between our glutens and theirs might well be caused by differences in the isolation procedures like the use of a salt solution during washing of the flour batters.

Large deformations; uniaxial extension

Gluten doughs were extended uniaxially using the Kieffer Extensibility Rig at three displacement speeds of the hook. In figure 6 data are presented for Vivant and Hereward gluten. During one extension test the strain rate passes through a maximum which is reached when the hook makes an angle of 45° with the geometry (Dunnewind *et al.*, 2003). For both cultivars an increase of σ was observed with increasing strain and strain rate. The slopes of the lines connecting measurement points at the same strain give the strain rate dependency of σ. In contrast with biaxial extension measurements the strain rate dependency of σ was largest at small strains for both gluten samples. At strains of 0.75 or higher the strain rate dependency of σ was rather independent of the strain. Just as for biaxial extension tests at the same strain σ was much higher for Hereward than for Vivant gluten dough while the strain rate dependency of σ was higher for Vivant, indicating a more viscous behaviour of Vivant gluten.

Using the data presented in figure 6 stress-strain curves were calculated at a constant strain rate of 0.01 s^{-1} for Vivant, A6/13 and Hereward gluten doughs (figure 7). The stress-level increases in the order Vivant, A6/13 and Hereward, although the difference between A6/13 and Vivant is very small. The stress-level of Hereward is more than three times higher than that of Vivant and A6/13 (figure 7A). In uniaxial extension all gluten doughs studied showed strain hardening to a similar extent (figure 7B). The values for the strain hardening coefficients, $d\ln\sigma/d\varepsilon$, found in uniaxial extension are much higher than those obtained in biaxial extension.

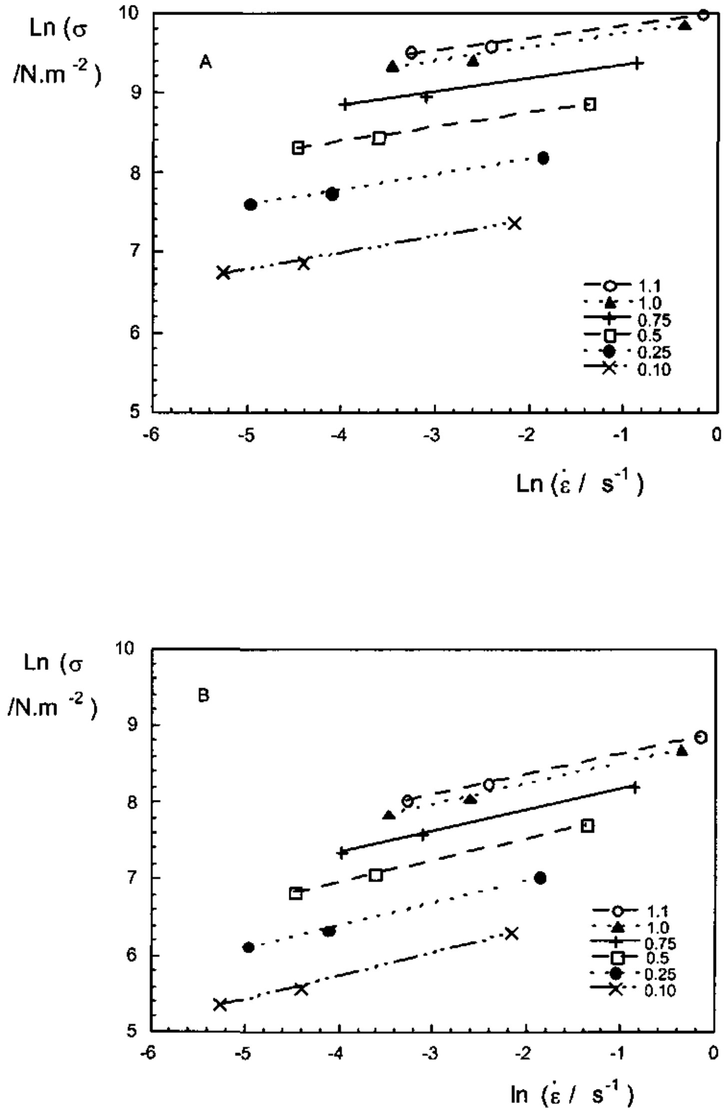


Figure 4. *Biaxial stress as a function of the strain rate, $\dot{\varepsilon}_B$, and the strain (indicated) for Hereward (4A) and Vivant (4B) gluten doughs. T = 25°C.*

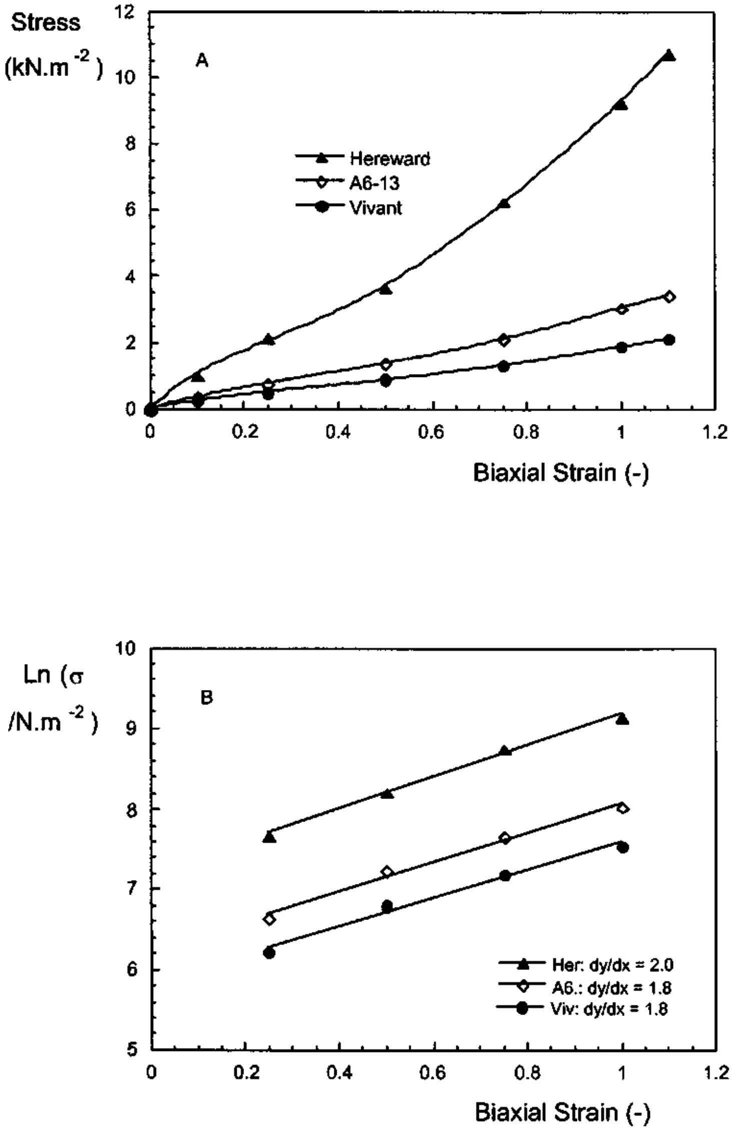


Figure 5. *Biaxial stress as a function of strain at a constant strain rate of 0.01* s^{-1} *for Hereward, A6/13 and Hereward gluten doughs. Stress on normal scale (5A) and on log scale (5B). T = 25°C.*

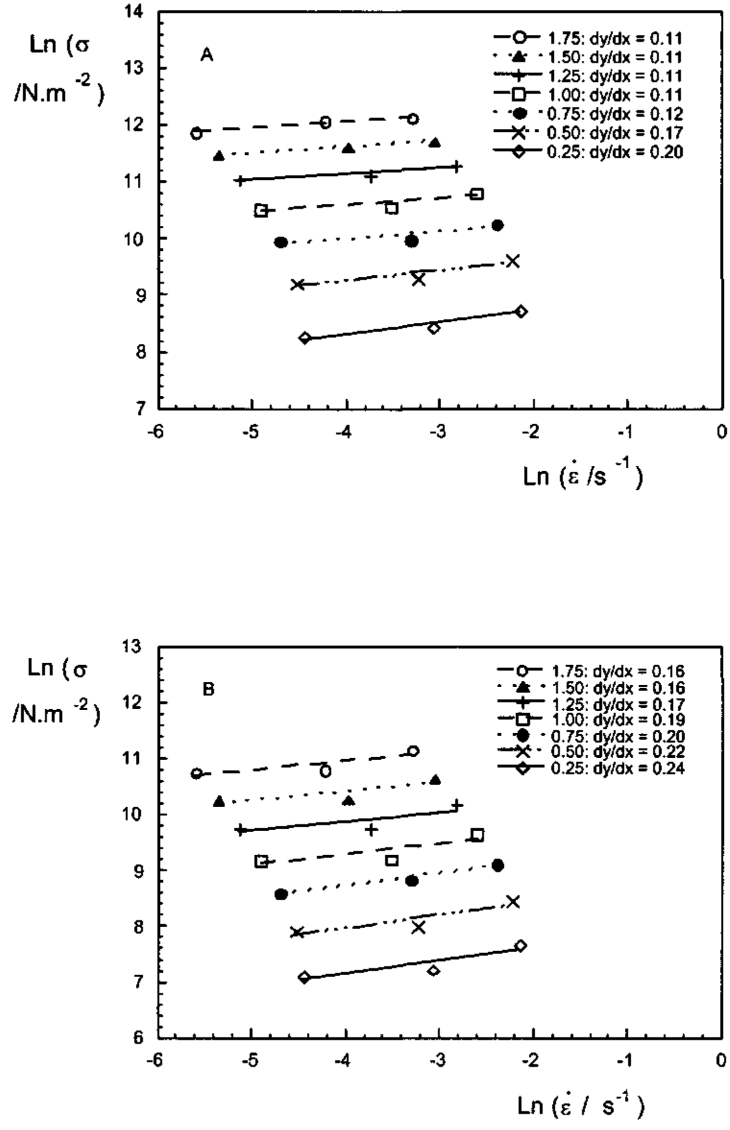


Figure 6. *Uniaxial stress as a function of the strain rate,* $\dot{\varepsilon}$ *, and the strain (indicated) for Hereward (6A) and Vivant (6B) gluten doughs. T = 25°C.*

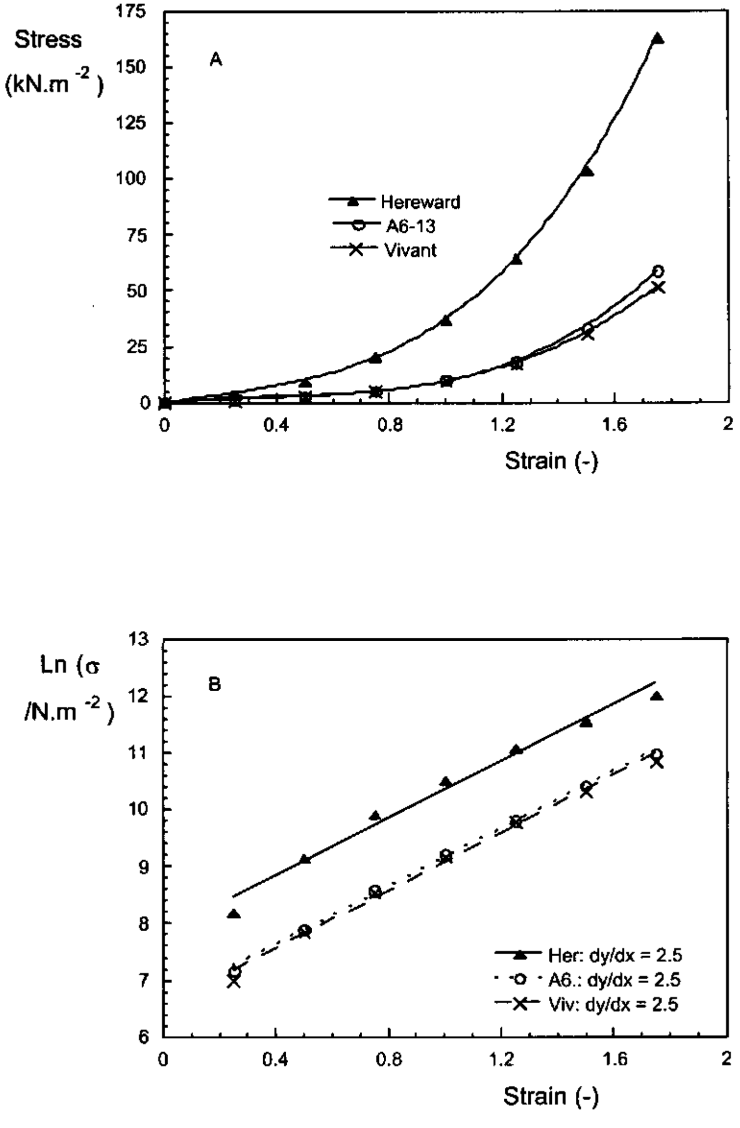


***Figure** 7. Uniaxial stress as a function of strain at a constant strain rate of 0.01 s^{-1} for Hereward, A6/13 and Vivant gluten doughs. Stress on normal scale (7A) and on log scale (7B). T = 25°C.*

Table 2. *Water content and mixing time applied to flour and gluten doughs. Stress and strain at fracture in uniaxial extension at two hook displacement speeds for Vivant, A6/13 and Hereward flour and gluten doughs. T = 25°C.*

Cultivars	**Water Content**[1] (%)	**Mixing Time**[2] (min)	**Fracture Strain** (-)		**Fracture Stress** ($kN.m^{-2}$)	
			d.p.[3]= 12 $mm.min^{-1}$	d.p. = 120 $mm.min^{-1}$	d.p. = 12 $mm.min^{-1}$	d.p. = 120 $mm.min^{-1}$
Flour dough[4]						
Vivant	39	7	1.6	2.0	5	11
A6/13	40	11	1.9	2.1	11	24
Hereward	39	14	1.7	2.0	13	27
Gluten dough[5]						
Vivant	64	10	2.2	2.3	90	165
A6/13	62	10	2.2	2.3	100	190
Hereward	62	14	1.8	1.9	150	220

[1] *Percentage of added water on total dough weight.*

[2] *In 10 g Mixograph.*

[3] *Displacement speed of the hook.*

[4] *For flour dough water contents were chosen as high as possible, while mixing times were defined as those giving the highest stress at a chosen strain at a constant strain rate in biaxial extension on the condition that doughs were still manageable with respect to stickiness (Sliwinski et al., 2003a).*

[5] *For gluten dough water additions were defined as the amount of water required to assure complete hydration, while mixing times were defined as those giving the largest fracture strain and the strongest strain hardening in uniaxial extension (see section materials and methods of this paper).*

The fracture properties of gluten doughs of the three cultivars are presented in table 2. In all cases a larger fracture strain and stress were observed at a higher strain rate. For Hereward gluten dough the highest fracture stress and the lowest fracture strain were found. Fracture strains for Vivant and A6/13 were similar; of the two A6/13 showed the highest fracture stress. Rinde *et al.* (1970) testing a commercial gluten sample reported also an increase in fracture strain and stress with increasing strain rate. From our results it appeared that fracture strain depended less on strain rate for gluten than for flour dough. According to van Vliet *et al.* (1995) for complex materials containing different structural elements energy dissipation will occur at larger deformations due to friction between structural elements. This energy dissipation will clearly retard the speed of crack propagation. Because it increases strongly with deformation rate, it can result in a less than proportional increase of the crack speed with increasing deformation rate and so in an increase of the observed fracture strain with strain rate. For flour dough the dependence of the fracture stress and strain on strain rate was related to the presence of starch granules in a gluten matrix. For gluten dough we observed similar trends as for flour dough although to a smaller extent. For gluten dough the dependence of the fracture strain of the strain rate could among others be due to the residual starch granules present. However, for a more decisive conclusion we should know more about the dependence of fracture properties on the strain rate as a function of starch content. In principle the strain rate dependence of fracture strain can also be due to friction between (large) glutenin aggregates during deformation. This aspect deserves further study.

Based among others on the strain rate dependency we concluded that Hereward is more elastic than A6/13 and Vivant gluten doughs. It appears that elastic behaviour in gluten doughs is positively correlated with σ and negatively with fracture strain. Strain hardening coefficients in uniaxial extension did not vary between the cultivars. As a consequence no correlation, positive or negative, could be observed between the value for strain hardening and stress-level or fracture strain.

Discussion

In figure 8 σ is shown as a function of strain at a constant strain rate for Hereward flour and gluten dough in biaxial (8A) and uniaxial extension (8B). In uniaxial extension σ is higher than in biaxial extension, the larger the strain the larger the difference. For Vivant and A6/13 similar differences were observed. Some key characteristics calculated from the stress-strain curves in uniaxial and biaxial extension for Hereward, A6/13 and Vivant flour and gluten doughs are presented in table 3. For all glutens σ at a certain strain and strain hardening coefficients were higher in uniaxial than in biaxial extension. Also for all cultivars the strain rate dependency of σ was higher in biaxial than in uniaxial extension. Apparently gluten doughs behave more elastic in uniaxial than in biaxial extension. The relative difference in σ between uniaxial and biaxial extension was largest for Vivant gluten and smallest for A6/13, while the difference in strain hardening between uniaxial and biaxial extension was smallest for Hereward gluten. A comparison of the characteristics of the mechanical properties of flour and gluten dough shows the following: (i) in uniaxial and biaxial extension both dough types show strain hardening, (ii) both dough types show higher values for σ and strain hardening coefficients in uniaxial than in biaxial extension, (iii) in biaxial extension values for σ and strain hardening coefficients were higher for gluten than for flour dough and (iv) but in uniaxial extension values for σ and strain hardening coefficients were much stronger for gluten than for flour dough. Thus the effect of the type of deformation on σ and strain hardening was much stronger for gluten than for flour dough. This is a strong indication that it is the gluten fraction that is responsible for the strain hardening behaviour of flour dough. The relative differences in σ between gluten and flour dough differ between the cultivars. At a biaxial strain of 0.75 σ had about the same value for Vivant, while it was nearly 2 times higher for A6/13 gluten and more than 3 times higher for Hereward gluten. At the same strain in uniaxial extension σ was 2.5 times higher for A6/13 and Vivant gluten and nearly 7 times higher for Hereward gluten.

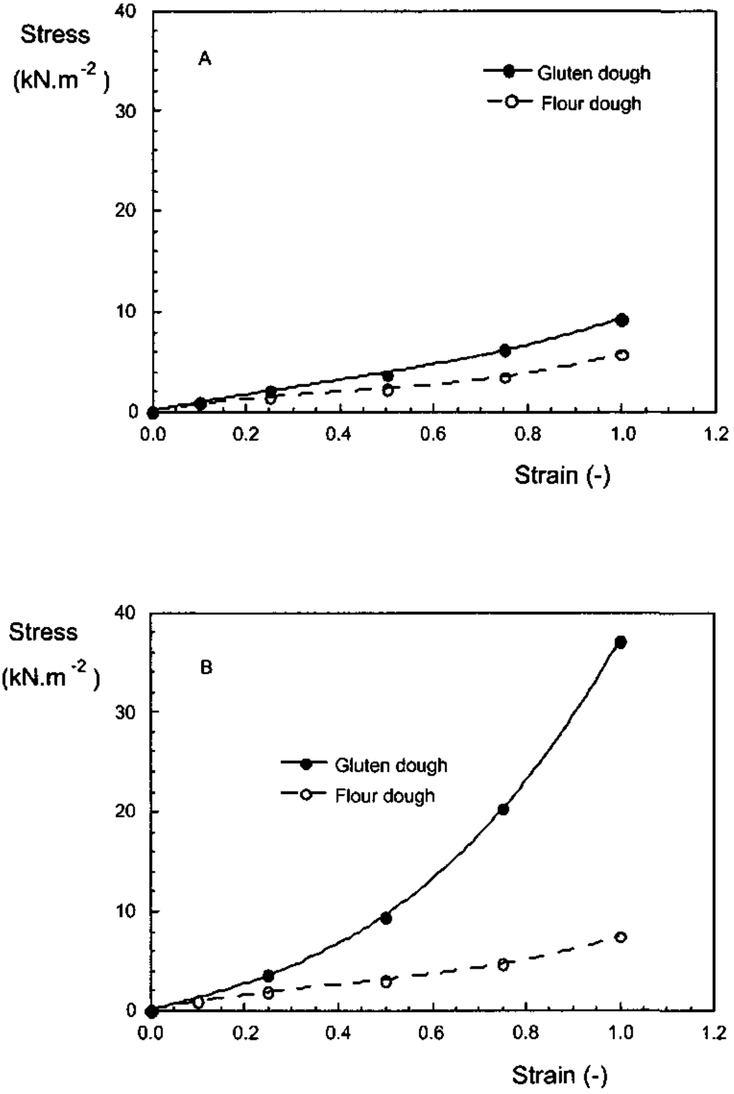


Figure 8. *Stress as a function of strain for Hereward flour and gluten dough at a constant strain rate of 0.01* s^{-1} *in biaxial (8A) and uniaxial (8B) extension.* $T = 25°C$.

Table 3. *The stress, the strain-dependency of the stress and the strain rate-dependency of the stress determined in uniaxial and biaxial extension for Vivant, A6/13 and Hereward optimally mixed flour and gluten doughs. T = 25°C.*

	Biaxial Extension			**Uniaxial Extension**		
	σ_B (N.m^{-2}) ε=0.75; $\dot{\varepsilon}$=0.01 s^{-1}	$d\ln\sigma_B/d\varepsilon_B$ (-) $\dot{\varepsilon}$=0.01 s^{-1}	$d\ln\sigma_B/d\ln\dot{\varepsilon}_B$ (-) ε=0.75	σ (N.m^{-2}) ε=0.75, $\dot{\varepsilon}$=0.01 s^{-1}	$d\ln\sigma/d\varepsilon$ (-) $\dot{\varepsilon}$=0.01 s^{-1}	$d\ln\sigma/d\ln\dot{\varepsilon}$ (-) ε=0.75
Flour dough						
Vivant	1400	1.6	0.24	2000	1.3	0.23
A6-13	1100	1.6	0.33	2200	1.6	0.24
Hereward	2000	1.8	0.22	3000	1.9	0.15
Gluten dough						
Vivant	1300	1.8	0.27	5000	2.5	0.20
A6-13	2100	1.8	0.26	5250	2.5	0.16
Hereward	6250	2.0	0.18	20000	2.5	0.11

Values for fracture stress were considerably higher for gluten than for flour dough. For Vivant and A6/13 fracture strains were larger for gluten than for flour dough, while for Hereward they hardly differed. Fracture strain and stress depended less on strain rate for gluten than for flour dough, which may be related to the lower starch content of gluten.

Based on the above presented similarities between flour and gluten dough we conclude that the large deformation properties of wheat flour dough are mainly determined by the gluten fraction. However, also differences in the mechanical behaviour between flour and gluten doughs were observed. These differences presumably originate from the differences in water, starch and gluten protein content between flour and gluten doughs. Because it is not possible to duplicate exactly the rheological properties of flour doughs by gluten-starch mixtures, it is likely that other flour components such as pentosans, lipids and water soluble proteins also influence dough rheology (Uthayakumaran *et al.*, 2002; Rouillé *et al.*, 2002).

Using results from creep tests in shear and lubricated uniaxial compression tests Janssen *et al.* (1996a) calculated the Trouton ratio for gluten dough, which equals six for Newtonian fluids. The values they reported did not differ much from six, which might be caused by the fact that the creep tests were applied at rather small strains. At larger strains the ratios are expected to be higher. According to theory one should expect that biaxial viscosity is about a factor two higher than uniaxial viscosity. However, in our data we observed the contrary. In line with the results obtained for flour dough (Sliwinski *et al.*, 2003a), for gluten dough Trouton ratios are probably in the same range or even higher for uniaxial than for biaxial extension.

The results presented show that in elongational flow orientation of structure elements in flour and gluten dough plays an important role. It has been demonstrated by several authors that wheat gluten dough shows birefringence when uniaxially stretched (Bloksma and Isings, 1957; Slade *et al.*, 1989). Detailed experiments with stretched samples of various origin, isolated gliadins and isolated glutenins observed by polarisation microscopy have allowed the identification of glutenin as the orientable aggregates and gliadin as the amorphous matrix in gluten films (Slade *et al.*, 1989). Using a combination of static light scattering and viscometry Sliwinski *et al.* (2003c) showed that glutenin polymers can be very large in size and show anisotropy. So, it is very likely that it is the glutenin fraction that confers the stretch direction dependent properties to gluten and flour dough. The difference in stress-level originating from direction-dependent deformation is then probably caused by (i) an increase in orientation of structure elements in the direction of flow, (ii) stretching of structure elements in the direction of flow and (iii) the formation of additional bonds as a result of the process of orientation.

An explanation in terms of a network structure is less straightforward. Nowadays it is generally agreed that gluten proteins apart from containing permanent (i.e. disulphide) cross-links are held together by transient junctions such as hydrogen bonds. In literature there is no common opinion whether we are dealing with protein chains that strongly associate, but behave essentially like synthetic polymers (Ewart, 1977; Singh and MacRitchie, 2001) or that we are dealing with protein aggregates that associate and hardly become intermixed (Lefebvre *et al.*, 1994). In the first case stretching of gluten will induce stretching and orientation of protein chains and therefore a stress-increase. In the other case we are dealing with protein aggregates that become orientated (and stretched). For wheat gluten subunits a mechanism was proposed in which the viscoelasticity is explained by the balance between protein residues involved in intrachain hydrogen bonds through β-sheet formation and those which are hydrated (Belton, 1999; Shewry et al., 2001). This would lead to a model consisting of domains in which gluten molecules are aggregated and connected by more flexible chains. In the first domain protein-protein interactions dominate and in the second region protein-solvent interactions dominate. Recent thermoelasticity studies have indicated that in contrast with the behaviour of the hydrophobic repeats in elastin elasticity in wheat gluten subunits indeed may be associated with extensive hydrogen bonding (Tatham *et al.*, 2001).

Probably the most important conclusion of this work is that gluten dough not only becomes stronger when stretched, but that it becomes specifically stronger in the direction in which it is stretched. The phenomenon of strain hardening and the dependence of the stress-level on the direction of deformation in elongational flow have been reported before for wheat flour dough by us (Sliwinski *et al.*, 2003a), but also for synthetic polymers (Meissner, 1997). Using the equipment of Meissner for uniaxial elongation Schweizer and Conde-Petit (1997) observed a specific direction effect after wheat flour dough was pre-stretched. If dough was elongated in the same direction as in which it had been stretched during preparation, σ was higher than if the dough was elongated in the direction perpendicular to it. The observation that in elongational flow stress-levels of flour and gluten dough depend on the direction in which they are stretched has large technological consequences. It proves that the method of dough preparation influences the mechanical behaviour of dough during proofing and baking. And it offers possibilities to improve dough preparation methods.

CONCLUSIONS

The storage modulus G' started to decrease at a somewhat larger strain and decreased less strongly with increasing strain for gluten than for flour dough. It is likely that these differences are due to the higher starch content of flour dough. At 45°C the linear region was longer, while G' and tan δ were lower than at 25°C. So, at 45°C wheat gluten is less strong and more elastic than at 25°C. Likely these differences are due to a weakening of secondary interactions between gluten proteins/aggregates at a higher temperature. Differences in dynamic parameters between cultivars were larger for gluten than for flour dough.

Similarly to flour dough gluten dough not only becomes stronger when it is stretched, but it specifically becomes stronger in the direction in which it is stretched. This conclusion is based on two observations: (i) both in uniaxial and biaxial extension the stress increased more than proportionally with the strain, a phenomenon called strain hardening and (ii) values for stress and for strain hardening were higher in uniaxial than in biaxial extension. The conclusion that orientational effects play an important role is supported by the fact that wheat gluten in uniaxial extension shows birefringence and that glutenin aggregates can be of very large size and show anisotropy when uniaxially extended (Slade *et al.*, 1989).

Three phenomena that largely determine large deformation behaviour of wheat flour and gluten dough are: (i) the further dough is being deformed the higher the resistance to deformation, (ii) in uniaxial extension σ is higher than in biaxial extension and (iii) stress and strain at fracture increase with increasing deformation rate. The clear similarity between the results for gluten and flour dough indicate that the large deformation properties of the latter are largely determined by the gluten fraction. Despite the similarities in rheological behaviour also differences were observed flour and gluten dough.

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Chapter V

Rheological Properties of Wheat Flour and Gluten Dough: Effect of Gluten Protein Composition and Temperature[1]

ABSTRACT

Six European and two Canadian wheat cultivars were selected that perform differently in cereal products. The gluten protein composition of the respective flours was studied and related to the rheological and fracture properties of optimally mixed flour doughs tested in uniaxial extension. Water addition required for optimum dough development was positively correlated with gluten protein content, indicating that all glutens required similar amounts of water for proper hydration. Both water addition and gluten protein content were positively correlated with the fracture strain. Mixing time required for optimum dough development was correlated with several stress-related dough properties: positively with the stress at a large strain and strain hardening and negatively with the strain rate-dependency of the stress. These stress-related dough properties were correlated with differences in the amount and the size-distribution of the gluten proteins. A positive correlation was found between the stress at large strain and the percentage of polymeric protein of large size (UEP+P1) and a negative one between the strain rate-dependency of the stress and the percentage of HMWgs on total protein. These findings are consistent with the known strong dependence of rheological properties on molecular weight and molecular weight distribution for polymers in general.

The effect of temperature on the large-deformation properties of flour and gluten dough was studied for three cultivars that were considered representative for the whole set. The fracture properties of flour dough strongly depended on strain rate and temperature. At higher strain rates and lower temperatures fracture strains hardly differed between the flour doughs no matter the protein content or composition. On the other hand at lower strain rates and higher temperatures the smallest fracture strain was found for dough of flour with the lowest glutenin content and/or the lowest protein content. Contrary to flour dough, for gluten-water mixtures (further to be called gluten dough) the fracture strain was largely independent of strain rate and temperature. The smallest fracture strain was found for the gluten dough with the highest glutenin content. So, regarding the effect of protein composition, strain rate and temperature on the fracture strain the behaviour of flour and gluten dough clearly differs. The observed differences in large deformation and fracture properties are most likely due to the large differences in starch and gluten protein content between flour and gluten dough.

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INTRODUCTION

Despite a relatively low protein content (usually 8-15%) and a deficiency of some essential amino acids (mainly lysine), wheat is the greatest source of protein in the human diet [1]. The most important use of wheat flour is in bread making, with lesser quantities being used for the production of cookies, pastry, pasta products and breakfast cereals [2,3]. Based on an analysis of the bread making process Bloksma proposed two rheological properties that are essential for producing high quality loaves: (i) the dough must have a sufficiently large viscosity to prevent the ascent of gas cells and (ii) it must remain extensible for a long enough time during baking to avoid premature fracture of membranes between gas cells [4]. Later van Vliet *et al.* have proposed a third rheological criterion which fulfilment is essential for the extensibility of dough films between gas cells and thereby for gas retention [5]. It comes down to the requirement of strain hardening in extension exceeding a specific limit. Strain hardening is defined as the phenomenon whereby the stress increases more than proportionally with the strain (at constant strain rate). Studying the bulk rheological properties of doughs of wheat flour samples with pronounced differences in baking performance Kokelaar *et al.* have found that good baking flours indeed exhibited stronger resistance to extension, greater strain hardening and greater extensibility [6]. During bread making flour dough is subjected to large deformations (including fracture) alternatingly in uniaxial and in biaxial extension. Sliwinski *et al.* have reported that in uniaxial extension (i) stresses were much higher and (ii) less dependent on the strain rate than in biaxial extension, which led them to the conclusion that orientational effects have a large impact on the mechanical properties of flour dough [7].

The rheological behaviour of flour dough at large deformations is dominated to a large extent by the gluten fraction [6,8,9,10,11]. Similarly to flour dough for gluten-water mixtures (further to be called gluten dough) the stress was found to increase more than proportionally with the strain in both uniaxial and biaxial extension [6,8,11]. However, the stress at a set deformation and strain hardening coefficients were higher for gluten than for flour dough [11]. Presumably, orientational effects in elongational flow are even more important in gluten than in flour dough. Another difference is that for gluten dough the fracture strain increases less with increasing strain rate than for flour dough [11]. Finally fracture stresses for gluten dough are generally much higher, while fracture strains are found to be lower, in the same range or higher for gluten than for flour dough [8,9,11].

The variation in dough rheology and bread making performance between wheat cultivars is largely determined by differences in protein quantity and composition [1,3]. Classically, the proteins of wheat are subdivided in the following classes: water-soluble albumins, salt-soluble globulins, 70% ethanol-soluble gliadins, and acid- or alkali-soluble glutenins; all protein fractions being heterogeneous [12]. Together gliadins and glutenins make up the storage or gluten proteins. Glutenins are present as large complexes formed by subunits linked together by disulphide bonds. These complexes are extremely polydisperse with respect to molecular size; molecular masses range from a few hundred thousands to several millions Da. The two major groups of subunits are the low molecular weight glutenin subunits (LMWgs) and the high molecular weight glutenin subunits (HMWgs). Particularly important are the HMWgs which have been ranked in order of their influence on bread making quality [13]. Evidence for the special role of the HMWgs with respect to dough properties and bread making quality was provided by examination of near-isogenic lines [14,15]. Flours without HMWgs, obtained by breeding in null genes in all three genomes, resulted in doughs without 'any strength'.

Several reports were published on the relation between dough rheological properties and gluten protein composition. Huebner and Wall found that wheat flours exhibiting long mixing times and strong doughs contain relatively large amounts of glutenins of large size [16]. A strong correlation was found between dough strength and the total amount of glutenin, but an even stronger correlation was found with glutenin fraction of largest size, i.e. the insoluble fraction plus the fraction eluting in the void volume. Similarly, a very strong correlation was observed by Cornec *et al.* between the shear modulus of hydrated gluten and the proportion of the largest glutenin polymers (excluded SE-HPLC peak) [17]. They concluded that gluten viscoelasticity is primarily determined by the interactions of large concatenations formed by HMWgs and LMWgs. Gupta *et al.* used wheat isogenic lines lacking HMWgs and LMWgs from one, two or three loci to study the effects of these polypeptides on glutenin polymer formation and on dough and gluten properties [15]. Changes in the size distribution of polymeric protein could account for all differences in dough strength. When both HMWgs and LMWgs were present, the amount of polymers of large size was much higher than when only one group was present, suggesting a positive interaction between these two groups of subunits on polymer formation. Loss of the genes coding for HMWgs reduced the proportion of polymeric protein and the strength of flour and gluten dough to a much greater extent than the loss of all the genes coding for LMWgs.

The glutenin to gliadin ratio clearly affects the mechanical properties of gluten dough in uniaxial extension tests [18]. With increasing gliadin content the resistance to extension decreased and the extensibility increased. From measurements on glutens reconstituted at various glutenin/gliadin ratios, Janssen *et al.* found that at constant protein content the main factor determining the rheological behaviour of hydrated gluten is the glutenin to gliadin ratio [19]. Gluten of a cultivar giving good bread flour had a higher modulus and a lower loss tangent in dynamic measurements and higher values for the stress and for strain hardening in biaxial extension tests. By interchanging the gliadin and glutenin fractions of the two glutens, it was shown that the source from which the fractions originated, particularly that of the glutenin fraction, was also important.

The objective of this paper is to relate differences in gluten composition to the mechanical properties of wheat flour dough. To this purpose six European and two Canadian wheat cultivars were selected, that perform differently in cereal products. Flour doughs were tested in uniaxial extension at different deformation rates. Three representative cultivars were more intensively studied. Information about the gluten protein composition of the set of flours was gathered by studying the gliadin to glutenin ratio, the relative amounts of HMW glutenin subunits and the size distribution of glutenin polymers.

EXPERIMENTAL

Materials

Grain samples (400 kg) of six European and two Canadian wheat cultivars were milled to flour to a milling degree of approximately 70% and an ash content of about 0.47 in the experimental mill of Meneba BV, Rotterdam, The Netherlands. Ash content varied between 0.45 and 0.50% with an average of 0.47%. Protein content varied between 10.3 and 13.4% with an average of 11.6% (see Table 1). All selected wheat samples were pure cultivars, except A6/13 which was a mixture of at least two undefined cultivars. The flour was stored at –20°C.

Methods

Flour protein was quantitatively separated in four classes following a procedure described before by others [20,21]. Flour samples (2 g) were sequentially extracted at room temperature with four solvents (30 ml) in the following order: (i) distilled water, (ii) 0.5 M NaCl+0.05 mM EDTA, (iii) 70% (v/v) ethanol and (iv) alkaline solvent containing 0.0125 M $Na_2B_4O_7$, 0.043 M NaOH, 0.5% (w/v) SDS, and 1% (v/v) β-mercapto-ethanol of pH 8.0. After two hours the dispersion in one of the solvents was centrifuged for 10 min at 6000 g. The supernatant was kept for analysis and the pellet was redispersed in the next solvent. Protein content in each class was determined using Kjeldahl (N X 5.7). Protein extractions and Kjeldahl determinations were both carried out in duplicate (average coefficient of variation CV = ±2%). Results are presented as averages. The percentage of total protein extracted varied between 96.8 and 103.3% with an average of 99.3%. The percentage of protein or other N-containing components that remained in the final pellet varied between 0.6 and 3.5% with an average of 2.3%.

Protein composition of the extracts was analysed under reducing conditions by SDS-PAGE on a 12.5% (w/v) acrylamide separating gel and a 5% (w/v) stacking gel. Electrophoresis was done at a constant current of 35 mA per gel for 5 hours at 4°C. Gels were stained overnight using Coomassie Brilliant Blue R250, according to the method developed by Neuhoff [22]. Stained gels were rinsed with 0.3% (w/v) Brij 35 and stored in plastic bags in 20% Ammoniumsulfate. To identify and quantify the protein bands gels were scanned with a laser LKB Bromma 2202 Ultroscan densitometer (absorption at 633 nm) using Quantity One software. No corrections were made for differences in specific absorbance between different proteins. Two independent protein extracts were on the gel each in duplicate (average coefficient of variation CV = ±4%). Results are presented as averages.

High Molecular Weight glutenin subunits (HMWgs) were extracted from wheat flour, analysed by SDS-PAGE and quantified using densitometric analysis as described by Kolster *et al.* [23]. Flour (50 mg) was vortexed with SDS-PAGE sample buffer (0.6 ml) for 10 s in a 2.5 ml Eppendorf tube. After 2 hrs the sample was centrifuged for 5 min at 16.000 g in an Eppendorf centrifuge. The pellet was resuspended in sample buffer (0.3 ml) and re-extracted as before. After centrifugation 0.15 ml of supernatant was added to 0.3 ml supernatant of the first extraction. The extract was stored at 4°C. SDS-PAGE was carried out to analyse the protein composition of the extracts using a 8.3% (w/v) acrylamide separating gel and a 5% (w/v) stacking gel. Electrophoresis was done at a constant current of 30 mA per gel for 8 hours at 4°C. Staining and densitometry were as described above. The conversion of absorbance values to absolute amounts was as described previously by Kolster and van Gelder [24]. The specific absorbance used was 0.18 AU/μg for the subunits 8 and 9 and 0.24 AU/μg for the other subunits. Protein extraction was carried out twice. Each extract was applied on two lanes of two different gels (average coefficient of variation CV = ±6%). Results are presented as averages.

Gluten was isolated from flour according to the method of Shogren *et al.* [25] with some modifications. A batter of 1 kg flour with 1.2 L demineralised water of 40°C was mixed for 2 minutes using a Janke & Kunkel RW20 mixer. The batter was directly centrifuged for 15 minutes at 2500 rpm at 20°C. After centrifugation from top to bottom four separate layers could be observed: an aqueous layer, a gel layer, a gluten layer and a starch pellet as described previously [26]. After removal of the supernatant and the gel layer, the gluten layer was separated from the starch pellet and further washed by hand using demineralised water. The wet gluten was frozen using liquid nitrogen and freeze-dried. Freeze-dried gluten was milled in a Retsch Grinder using a 150 μm sieve. Gluten was stored at –20°C. Small scale gluten isolations (50 g flour) using demineralised water for the eight cultivars gave gluten yields between 10.6 and 11.4% with an average of 11.0% and gluten protein recoveries between 76.7 and 83.5% with an average of 80.2%. For large scale gluten isolations (6x1kg flour) moisture content varied between 6.5 and 7.5% with an average of 7% and protein content varied between 76.3 and 83.4% with an average of 80%. The use of salt during gluten isolation was avoided since there are indications that it may have significant effects on its functionality [27].

Gluten for protein extraction and gel filtration were isolated as described above, but from defatted flour. To remove the lipids wheat flour (100 g) was extracted with petroleumether (200 g) for 30 min at room temperature and the slurry was filtered [28]. This procedure was repeated twice. Residual solvent was evaporated by air drying overnight. Total unreduced protein of freeze-dried gluten powder was extracted with 2% (w/v) SDS, 0.05 M Tris/HCl buffer of pH 8.0 at a solvent to solid ratio of 100:1 according to methods described [15,29]. Extractions were carried out at room temperature in 50 ml centrifuge tubes which were moved gently for 24 h. The dispersions were centrifuged for 10 minutes at 10.000 g at 25°C after which the supernatant was decanted and the residue was dispersed in 1% (w/v) DTT, 2% (w/v) SDS, 0.1 M Tris/HCl buffer of pH 8.0. The dispersions were moved gently for 2 h at room temperature and next centrifuged during 20 min at 17.000 g at 25°C. The clear supernatant was designated the SDS-Unextractable Polymeric (UEP) protein fraction. The percentage of protein or N-containing components that remained in the final residue was less than 1%.

Gel filtration chromatography was carried out at room temperature using a Superose-6 column (25 x 410 mm) from Pharmacia that was equilibrated with 0.1% (w/v) SDS, 0.05 M Tris-HCl buffer of pH 8.0. Samples of 100 μl of gluten extracts (containing 15-20 mg of protein) were applied to the column at a flow rate of 0.2 ml/min. Of the UEP protein fraction 25 μl was applied to the column. Proteins of known molecular weight were run in a separate run to calibrate the column. Chromatograms were divided into five zones: the excluded volume, P1 ($M>500kDa$), P2 ($100kDa < M < 500kDa$), P3 ($50kDa < M < 100kDa$), P4 ($25kDa < M < 50kDa$), P5 ($1kDa < M < 25kDa$). Protein concentration in the effluent was monitored continuously at 214 nm with an LKB UV-monitor, Uvicord II. Protein extraction was carried out twice and each extract was analysed two times (average coefficient of variation CV = ±3%). Results are presented as averages. Following gel filtration samples were collected and the protein composition thereof was analysed by SDS-PAGE as described above.

Flour and gluten doughs for rheological measurements were prepared by mixing in a 10 g National Mixograph with a target dough temperature of 27°C. Considerable attention was paid to the determination of optimum water additions and mixing times for each flour and gluten individually. Flour doughs were prepared as described previously [7]. Water additions were derived from baking tests [30]. Water additions were chosen as high as possible, but should be within the working area of a flour and should cause no problems with dough stickiness. To determine the mixing requirements doughs were tested in biaxial extension as a function of mixing time. Optimum mixing times were defined as those giving the highest stress at a chosen strain at a constant strain rate on the condition that doughs were still manageable with respect to stickiness. Manageability was determined manually. Used water contents and mixing times for flour doughs are given in table 5. Gluten doughs were prepared by mixing 2.5 g gluten with the appropriate amount of water as described previously [11]. Water additions were defined for each gluten as the amount of water required to assure complete hydration on the condition that after 10 minutes of mixing all the water was absorbed by the gluten. Both properties were determined by visual observation. To determine the mixing requirements of the glutens, doughs were tested in uniaxial extension as a function of mixing time. Optimum mixing times were defined for each gluten individually as those giving the largest fracture strain and the strongest strain hardening in uniaxial extension. Used water contents and mixing times for gluten doughs are given in table 7. The use of salt in dough preparation was avoided since it may influence dough rheological properties and with that the comparison with biochemical parameters.

Uniaxial extension tests were performed using a Zwick mechanical testing apparatus equipped with the Kieffer Extensibility Rig as described previously [7,11,31]. After mixing the doughs were stored in a stencil at the test temperature for 60 minutes. Test samples were coated with paraffin oil to avoid evaporation of water. Dough pieces were primarily uniaxially extended by an upward moving hook, while the cylindrically shaped dough pieces were clamped at both ends. Force-displacement curves were recalculated into stress-strain curves and next were calculated the strain and strain rate dependence of the stress as well as the stress and strain at fracture as described previously [7,11,31]. The measuring device was equipped with a plastic container and a heater to maintain the temperature. Tests were performed at 25°C and at 45°C. The latter temperature was chosen to avoid changes in dough rheological properties due to starch gelatinisation that can occur above 45°C [2,4,6]. Displacement speeds of the hook were 12, 48 and 120 $mm.min^{-1}$. Results shown are mean values of at least five measurements on at least four different dough preparations (average coefficient of variation CV = ±3%, ±4%, ±4%, ±6% for uniaxial stress, strain hardening, fracture strain and fracture stress, respectively).

RESULTS AND DISCUSSION

First results for the whole set of eight cultivars will be presented and discussed and after that the results of the more intensively studied three cultivars.

Part 1: eight wheat cultivars

1.1 Protein composition

Using a modified Osborne sequential extraction method, the protein of the flour of six European and two Canadian wheat cultivars was separated into four fractions: albumins, globulins, gliadins and glutenins. The protein composition of the fractions was further analysed using SDS-PAGE (results not shown). The total amount of protein and the fraction soluble in 70% ethanol (further to be called gliadin) and in the alkaline solvent (further to be called glutenin are shown in table 1. We choose to use the terms gliadin for the fraction soluble in ethanol and glutenin for the fraction soluble in the alkaline solvent since the use of these terms is wide-spread. It is important to realise that amounts and types of protein extracted differ with the extraction procedure and that the gliadin and glutenin fraction, here defined as a solubility fraction, are not pure gliadin or glutenin fraction as defined on protein composition [12,20]. The distinction between proteins that are soluble in pure water and water containing also some NaCl is not further discussed.

The protein content of the flours varied from 10.3 to 13.4%. Those of Vivant and Soissons flour were lowest, while for the Canadian cultivars Glenlea and Katepwa the highest protein contents were found. For most cultivars total protein consisted for about 69 - 72% of gluten protein. A high gluten protein content was found for Glenlea. Gluten protein content of Apollo was exceptionally low (63%), which is due to the 1B/1R wheat/rye translocation [32], and is expressed in a low gliadin content (24%). For the other cultivars the gliadin content was higher and amounted to an average of 30% of total protein. Percentages of glutenin on total protein for Hereward, Soissons, Minaret and Glenlea were significantly higher (42.5%) than for the other four cultivars (39%). This difference is expressed in the gliadin to glutenin ratio that is highest for A6/13, Katepwa and Vivant. Gliadin to glutenin ratios found are in the same range as those reported by others [12,19,33].

Table 1. *Relative amounts of proteins based on solubility in distinct buffers*

Cultivar	**Protein** (% D.M.)	**Gluten** (% D.M.)	**Gliadin** (% D.M.)	**Glutenin** (% D.M.)	**Gli/Glu** (-)
Glenlea (Gl)	13.3	9.8	3.9	5.9	0.66
Minaret (Mi)	11.4	8.1	3.4	4.7	0.72
Soissons (So)	10.4	7.5	3.0	4.5	0.67
Hereward (He)	11.0	7.6	3.0	4.6	0.65
Katepwa (Ka)	13.4	9.5	4.2	5.3	0.79
A6/13 (A6)	12.0	8.4	3.7	4.7	0.79
Vivant (Vi)	10.3	7.2	3.2	4.0	0.80
Apollo (Ap)	11.1	7.0	2.7	4.3	0.63

Protein composition of the glutenin fraction was determined by SDS-PAGE in combination with laser scanning densitometry. Three fractions were distinguished: HMWgs, Medium Molecular Weight (MMW) proteins and LMWgs (Table 2). MMW proteins are no glutenin subunits, but other proteins like α-amylases and gliadins that possibly can act as chain terminators and therefore could have a negative effect on the size distribution of the glutenins [34]. Relatively low contents of HMWgs and relatively high contents of LMWgs were found for A6/13, Vivant and Apollo, resulting in significantly higher LMWgs to HMWgs ratios. Soissons and Glenlea contained lowest percentages of MMW proteins in the glutenin fraction, while for Apollo, Vivant and Minaret highest amounts were found. In literature for wheat glutenin LMW to HMW ratios varying between 1.4 and 5.6 have been reported [35]. Our data are well within this range.

The amounts of types of HMWgs were determined using SDS-PAGE and densitometry. Results are shown in table 3. The cultivars Vivant and Apollo have the lowest total amount of HMWgs. This is caused by the fact that these cultivars contain only four different HMWgs of which subunit 2 and 6 are present in relatively low amounts compared to subunit 5 and 7, respectively. The HMWgs composition of A6/13 illustrates that this wheat is not a pure cultivar, but a mixture of two. The total amount of HMWgs found for this wheat was also rather low. Large amounts of HMWgs were found in Glenlea, which is due to a combination of the presence of 5 different HMWgs and of extremely high amounts of HMWgs 7. The latter is caused by the fact that Glenlea contains an extra gene for this subunit [36]. Although the cultivar Hereward contains only four different HMWgs, the total amount is rather high. Similar total amounts were found for the cultivars Minaret, Soissons and Katepwa, which contain five different HMWgs and have the HMWgs 7, 5 and 10 in common. The amounts of the HMWgs are in the same range as those reported by Kolster *et al.* [23]. From the data presented in table 1 and 2 also the total amount of HMWgs can be calculated. When corrected for the moisture content of the flours these values are close to those presented in table 3.

Table 2. *Relative amounts of main protein fractions in the different glutenins fraction as determined with SDS-PAGE and laser scanning densitometry.*

Cultivar	**HMW-GS** (% glutenin)	**MMW**[1] (% glutenin)	**LMW-GS** (% glutenin)	**LMW/HMW** (-)
Glenlea	25.0	8.7	66.3	2.7
Minaret	24.2	12.7	63.1	2.6
Soissons	26.1	7.1	66.8	2.6
Hereward	26.2	10.2	63.6	2.4
Katepwa	23.7	10.4	65.9	2.8
A6/13	20.5	9.9	69.6	3.4
Vivant	18.1	12.6	69.3	3.8
Apollo	20.4	11.2	68.4	3.4

[1] *MMW proteins are proteins with a molecular weight of approximately 70-80 kDa like α-amylases and ω-gliadins which can make part of the glutenin fraction [29,30].*

The size distribution of the glutenin fraction of glutens isolated from flour of the eight wheat cultivars under study was studied by extraction in a SDS-buffer and gel filtration. Three different glutenin fractions were identified: the SDS-unextractable fraction (UEP), a polymeric protein fraction of very high molecular weight eluting at the void volume (P1) and a polymeric protein fraction of some lower molecular weight eluting as a broad peak directly after the void volume and before monomeric proteins elute (P2). In figure 1 gel filtration patterns are shown of the SDS-extractable fraction (Fig. 1A) and the SDS-unextractable protein fraction after reduction with DTT (Fig. 1B) for gluten of the cultivar Glenlea. The chromatograms are in agreement with those reported by others [16,17]. The protein composition of the fractions was analysed using SDS-PAGE (results not shown). Areas under the curves were calculated to determine the amount of protein of the protein fractions searched for. The results are presented in table 4 as relative amounts of protein fractions on total protein.

Table 3. *Type and amounts of HMW glutenin subunits (in mg protein/g flour) of six European and two Canadian wheat flour samples.*

Cultivar	**Glu-1Ax**		**Glu-1Bx**		**Glu-1By**			**Glu-1Dx**			**Glu-1Dy**		**Total**
	1	2*	6	7	8	9	17/18	2	3	5	10	12	
Glenlea		1.3		4.5	1.8					2.4	2.6		12.6
Minaret	1.1			2.7		1.4				1.9	2.9		10.1
Soissons		1.1		3.0	1.1					1.7	3.0		9.9
Hereward				3.5		2.7			2.7			1.9	10.8
Katepwa		1.2		3.1		1.4				2.4	2.7		10.8
A6/13				1.9			1.5	0.7		1.2	2.1	0.9	8.3
Vivant			0.8		1.5			1.3				2.5	6.2
Apollo			0.9		1.9			1.5				3.2	7.6

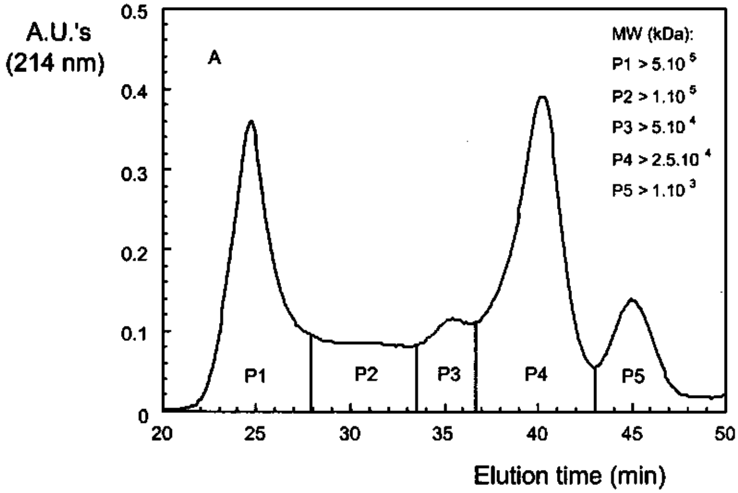
Low percentages of protein unextractable in SDS (10-13%) were found for Glenlea, A6/13, Vivant and Apollo, while high UEP percentages (25-26%) were found for Minaret and Hereward. Similar numbers were reported for flour, but with a slightly different extraction procedure by Gupta *et al.* [15]. For Glenlea the P1 polymeric protein fraction (29%) was by far the largest, while it was relatively low for Hereward, Minaret and Katepwa. Using sonication it has been shown that the SDS-unextractable fraction consists mainly of glutenins of large size [15,17]. So, added together the unextractable and the P1 fraction yield the percentage of polymeric protein of large size. This percentage was relatively low for A6/13, Vivant and Apollo (31-33%), while for Glenlea, Minaret and Hereward this percentage was relatively high (39-43%). Percentages of extractable polymeric protein of relatively small size (P2) vary from 5% for Minaret and Katepwa to 12% for Apollo. For Katepwa the lowest percentage of total polymeric protein (40%) was found, while Glenlea, Minaret and Hereward contained the highest relative amounts (47-49%).

From the data presented in table 1 the percentage of glutenin on total protein can be calculated for the flours. These values were compared with the percentages for total polymeric protein obtained for the glutens (table 4). It is to be expected that during gluten isolation the percentage of non-gluten protein in wet gluten decreases and the percentage of gluten proteins and thus also glutenin increases. In agreement with this, percentages of total polymeric protein for gluten were on average 10% higher than flour glutenin percentages. Moreover differences between the samples are still present. This shows that the use of demineralised water in gluten isolation did not result in a specific loss of gliadins. We concluded therefore that the gluten isolation procedure we applied did not significantly change the differences between the cultivars regarding glutenin content.

We concluded that the flours involved in this study differ considerably regarding protein content, gluten protein content and gluten protein composition. The cultivar Apollo is, because of the 1B/1R wheat/rye translocation, an outlier in the set of flour samples. Its protein composition is characterised by a relatively low gliadin content resulting in a low gluten protein content and an exceptionally low gliadin to glutenin ratio. The total amount of HMWgs of the flours was strongly associated with protein characteristics that are commonly used to describe differences in gluten protein composition as the gliadin to glutenin ratio, glutenin content, the LMWgs to HMWgs ratio and glutenin size distribution. This is no surprise, since an increase in HMWgs content should lead to an increase of the amount of glutenin and to a decrease of the LMWgs to HMWgs ratio and the gliadin to glutenin ratio, assuming that the amounts of other protein categories remain unchanged.

Table 4 *Percentages of SDS-Unextractable Polymeric Protein (UEP), P1 Polymeric Protein and P2 Polymeric Protein on Total Polymeric Protein for glutens of eight wheat cultivars. Fractions were obtained after protein extraction in SDS-buffer and subsequent gelfiltration on a Superose-6 column.*

Cultivar	**UEP Polymeric Protein**	**P1 Polymeric Protein**	**P2 Polymeric Protein**	**UEP+P1 Polymeric Protein**	**Total Polymeric Protein**
	(% Total Protein)	(% Total Protein)	(% Total Protein)	(% Total Protein)	(% Total Protein)
Glenlea	10	29	8	39	47
Minaret	26	17	5	43	48
Soissons	17	20	8	37	45
Hereward	25	14	10	39	49
Katepwa	17	18	5	35	40
A6/13	13	20	11	33	44
Vivant	12	21	11	33	44
Apollo	10	21	12	31	43



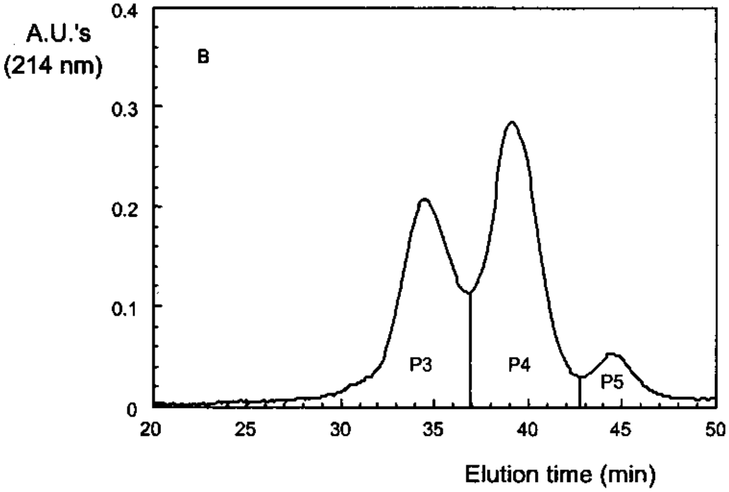


Figure 1. *FPLC chromatograms of cultivar Glenlea gluten fractions separated on a Pharmacia Superose 6 column in 0.1% (w/v) SDS, 0.05 M Tris-HCl buffer of pH 8.0. Chromatogram A is for SDS-extractable protein and chromatogram B is for SDS-unextractable protein dispersed in 1% (w/v) DTT, 2% (w/v) SDS, 0.1 M Tris/HCl buffer of pH 8.0. Chromatograms were divided into five zones: the excluded volume, P1 (M>500kDa), P2 (100kDa < M <500kDa), P3 (50kDa < M <100kDa), P4 (25kDa < M < 50kDa), P5 (1kDa < M < 25kDa).*

Therefore the HMWgs content is an important parameter for flour quality and as we shall see later for the large-deformation properties of wheat flour and gluten dough.

However, it is still a question in how far subunits differ in their contribution to the size distribution of gluten protein and thus technological dough properties. It has been shown that x-y pairs of subunits 5+10 and 2+12 resulted in a much stronger increase in mixing time than expected from their calculated molecular weights [37]. The observed synergistic effect was maximum when equimolar ratios of x- and y-type 1D subunits were used. It seems therefore that the effect of an increase of HMWgs on gluten protein composition and functional properties like mixing time is the largest when similar amounts of 1D x- and y-type subunits are present. Until now the exact reason for this synergistic effect remains unclear. In our data set the content of y-type HMWgs was fairly constant for all flours. On the contrary large differences were found in the content of x-type HMWgs. Therefore the variation in x-type HMWgs is responsible for most variation in HMWgs content between the flours. In agreement with published data [15] for our set of samples a higher percentage of HMWgs in flour was associated with a higher percentage of polymeric protein of large size (UEP+P1) in gluten isolated from the parental flours. An exception was Glenlea for which the ratio of UEP to P1 polymeric protein strongly deviated from the values found for the other cultivars and for which also high HMWgs and glutenin contents were found. Since for Glenlea the ratio of subunit 5 and 10 is close to 1 the low UEP to P1 ratio can not be explained by the amounts of these subunits. Perhaps the extremely high amounts of subunit 7 of Glenlea have a negative influence on glutenin polymerisation. However, it is also possible that the low UEP to P1 ratio of Glenlea is caused by environmental effects as differences in growth conditions during grain maturation [35]. The relationship between protein composition and size distribution of Glenlea and Hereward gluten will be further discussed in a forthcoming paper [38].

1.2 Dough rheology

Optimum water additions and mixing times were determined for each flour individually. The resulting water additions and mixing times are shown in table 5. Lowest amounts of water had to be added to Apollo flour, while the Canadian flours required the highest water additions. Optimum water addition was positively correlated with protein content (r = 0.87) and with gluten protein content (r = 0.97; Fig. 2). This finding is in agreement with literature results that protein content is an important factor in determining the water uptake of flour [39]. Optimum development of flour-water doughs of Vivant and Apollo required short mixing times, while for A6/13 and Katepwa intermediate mixing times were required and long mixing times had to be applied for Minaret, Soissons, Hereward and Glenlea. Mixing time was correlated with several biochemical parameters: positively with the percentage of glutenin protein on total protein in flour (r = 0.87; Fig. 3), the percentage of HMWgs on total flour (r = 0.82) and the percentage of polymeric protein of large size (UEP +P1) in gluten (r = 0.79) and negatively with the LMWgs to HMWgs ratio in flour (r = 0.83). Apparently, flours that contain relatively more glutenin protein (of large size) require longer mixing times. This conclusion is in line with earlier reports [15,16,37,40].

The rheological and fracture properties of the optimally mixed flour doughs were determined in uniaxial extension using the Kieffer Extensibility Rig. In figure 4 the stress (σ) calculated at a constant strain rate ($\dot{\varepsilon} = 0.01\ s^{-1}$) as a function of the relative deformation (ε) is shown on a normal scale (fig. 4A) and on a logarithmic scale (fig. 4B). Further results are shown in table 5. All cultivars displayed a more than proportional increase of the stress with increasing strain, a phenomenon called strain hardening. Considerable differences were observed in stress-level between the respective flours. The set of flours could be divided in three categories. For Apollo and Vivant low stress-levels and low strain hardening coefficients were observed in combination with a high strain rate-dependency of the stress. A6/13, Katepwa and Glenlea combined intermediate stress-levels with intermediate strain hardening coefficients and intermediate to high strain rate-dependency of the stress. High stress-levels, intermediate to high strain hardening coefficients and a low to intermediate strain rate-dependency of the stress were found for the wheat cultivars Minaret, Hereward and Soissons.

In figure 5 the stress-strain curves determined in uniaxial extension are shown for flour doughs of Glenlea (5A) and Apollo (5B) at two different displacement speeds of the hook. In the stress-strain curves the point of fracture of the dough samples is indicated. For both cultvars an increase in stress level, fracture stress and fracture strain was observed with increasing displacement speed of the hook. These results are in line with previous observations [7,41]. The increase in fracture stress with increasing displacement speed of the hook is larger for Apollo flour dough than for Glenlea, indicating a stronger strain rate-dependency of the fracture properties for Apollo, which is in line with the difference in strain rate-dependency of the stress observed for these cultivars. In table 6 the fracture stress and strain determined in uniaxial extension at two displacement speeds of the hook are shown for the doughs of all wheat cultivars. Fracture stresses are determined by three factors: stress-level, strain hardening and fracture strain. For Vivant and Apollo low values for fracture stress were observed that are caused by the low stress-level, low strain hardening and the small to intermediate fracture strains. Intermediate to high values for fracture stress were found for A6/13, Katepwa and Glenlea flour dough due to the intermediate stress-level and large fracture strains. The high stress-level, the intermediate to high strain hardening coefficients and the intermediate fracture strains resulted in intermediate to high values for fracture stress for Minaret, Soissons and Hereward.

Mixing time was correlated with several stress-related dough properties of the set of flours under study: positively with the stress at a large strain ($r = 0.86$), strain hardening ($r = 0.83$) and the stress at fracture ($r = 0.85$ and 0.74) and negatively with the strain rate-dependence of the stress ($r = 0.87$; Fig. 6). These findings are in line with the observed association between mixing time and dough strength reported by others [16].

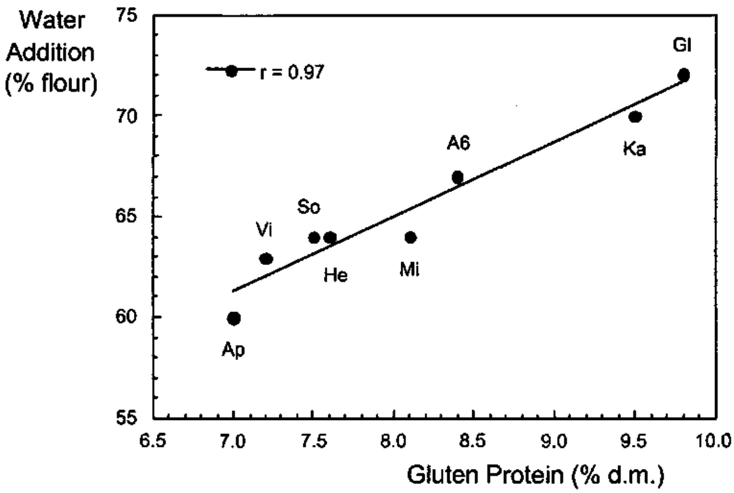


Figure 2. Correlation between the water addition applied for preparation of flour-water doughs and gluten protein content (in % dry matter) for flours of six European and two Canadian wheat cultivars. (For explanation of abbreviations see table 1).

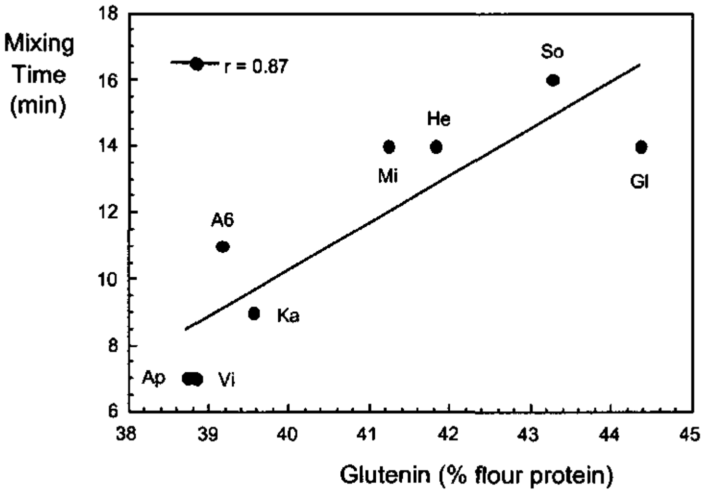


Figure 3. Correlation between the mixing time applied for preparation of flour-water doughs and the percentage of glutenin on total protein for flours of six European and two Canadian wheat cultivars.

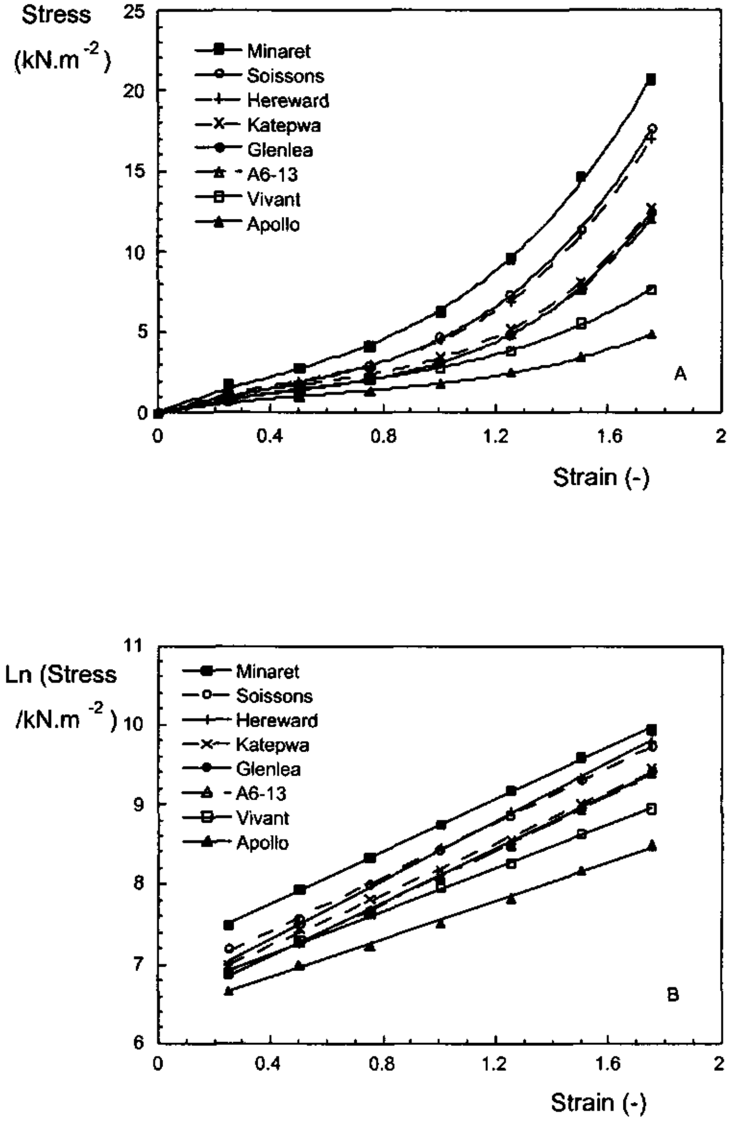
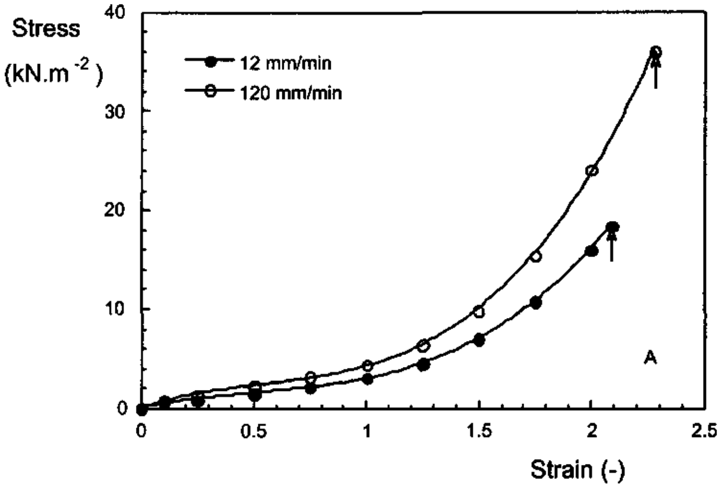


Figure 4. *Uniaxial tensile stress as a function of strain at a constant relative deformation rate of 0.01* s^{-1} *for flour doughs of six European and two Canadian wheat cultivars (indicated) presented on a normal scale (A) and on a logarithmic scale (B). Data are at 25°C.*



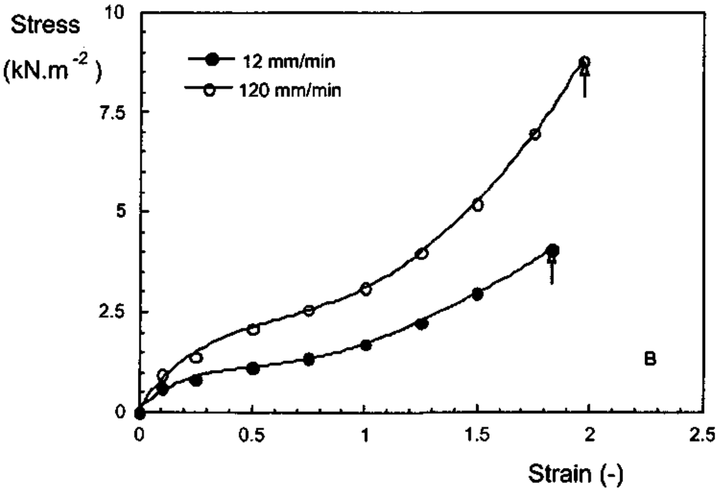


Figure 5. *Uniaxial tensile stress as a function of strain at displacement speeds of the hook of 12 mm.min*$^{-1}$ *and 120 mm.min*$^{-1}$ *for flour doughs of the wheat cultivars Glenlea (A) and Apollo (B). Data are at 25°C. The point of fracture is indicated with an arrow.*

That the mentioned properties are correlated is no surprise, but consistent with what should be expected for this kind of viscoelastic materials. Flour dough has a very complex composition (starch, proteins, carbohydrates), most for network formation essential gluten proteins are present in aggregates with a wide size distribution, and the structure elements in dough are connected by several types of bonds. This makes that stress relaxation in wheat flour and gluten dough occurs over a spectrum of relaxation times [42]. It is to be expected that a relatively long relaxation time will be found for a flour dough with a larger number of bonds exhibiting longer relaxation times. If such a material is deformed at a chosen strain rate the stress will increase to an extent which depends on the spectrum of relaxation times. The opposite, a relatively short relaxation time, will than be found for a flour dough with a smaller number of bonds exhibiting longer relaxation times. If such a dough is deformed at the same strain rate the stress will increase less, because more stress relaxed already during the test.

One of the main aims of mixing is the formation of a three-dimensional viscoelastic structure with gas-retaining properties. From the discussion above follows that to reach a certain stage of dough development higher energy inputs and thus longer mixing times are required for doughs that contain a larger number of bonds exhibiting longer relaxation times. And thus that variations in stress as a result of deformation between different doughs will be related to differences in the amount and the size-distribution of the gluten proteins that provide the structure elements of the doughs. In agreement with the description given above we found that such variables were correlated: a positive correlation between the stress at large strain and the percentage of polymeric protein of large size (UEP+P1) ($r = 0.89$) and a negative one between the strain rate-dependency of the stress and the percentage of HMWgs on total protein ($r = 0.91$; Fig. 7). These findings are in line with the observation reported by others that if a flour sample contains more protein and/or more protein of large size the overall relaxation time of the dough will increase [42,43].

The fracture strain is an essential property for bread making, because during baking dough must remain extensible up to large strains to avoid premature fracture of membranes between gas cells [4,5,6]. For all cultivars an increase in fracture stress and strain was observed with increasing deformation rate in line with earlier reports [7,10,31,41,44]. The observed rate-dependency of fracture properties can best be explained by inefficient transport of energy to the crack tip [45]. At larger deformations energy dissipation will occur due to friction between the different structural elements present in dough. This energy dissipation will clearly retard the speed of crack propagation and is stronger at higher deformation rates. This causes that for flour dough crack speed will not increase proportionally with the deformation rate, resulting in the observed strain rate-dependency of fracture stress and strain. The fracture stress of flour dough was positively correlated with the amount of HMWgs in flour (Fig. 8A), indicating an influence of gluten protein composition on fracture stress. This is in line with the explanation given earlier that apart from the fracture strain the stress-level of dough and strain hardening contribute each to fracture stress.

Table 5. *Water addition, mixing time, stress, relative change in stress over a relative change in strain ($d\ln\sigma/d\varepsilon$) and relative change in stress over a relative change in strain rate ($d\ln\sigma/d\ln\dot{\varepsilon}$) determined in uniaxial and biaxial extension for flour doughs of six European and two Canadian wheat cultivars. T = 25°C.*

Cultivar	Water Addition	Mixing Time	σ (N.m^{-2})	$d\ln\sigma/d\varepsilon$ (-)	$d\ln\sigma/d\ln\dot{\varepsilon}$ (-)
	(% flour)	(min)	(ε=0.75, $\dot{\varepsilon}$=0.01 s^{-1})	($\dot{\varepsilon}$=0.01 s^{-1})	(ε=0.75)
Glenlea	72	14	2200	1.6	0.15
Minaret	64	14	4200	1.6	0.16
Soissons	64	16	3000	1.7	0.13
Hereward	64	14	3000	1.9	0.15
Katepwa	70	9	2500	1.5	0.18
A6/13	67	11	2200	1.6	0.24
Vivant	63	7	2100	1.3	0.23
Apollo	60	7	1400	1.1	0.27

Table 6. *Fracture stress (σf) and strain (εf) determined in uniaxial extension at two displacement speeds of the hook for optimally mixed flour-water doughs of six European and two Canadian wheat cultivars. T = 25°C.*

Cultivar	12 mm.min^{-1}		120 mm.min^{-1}	
	σf (N.m^{-2})	εf (-)	σf (N.m^{-2})	εf (-)
Glenlea	18000	2.1	36000	2.3
Minaret	18000	1.7	26000	1.8
Soissons	16000	1.9	26000	2.0
Hereward	13000	1.7	27000	2.0
Katepwa	14000	2.0	29000	2.2
A6/13	11000	1.9	24000	2.1
Vivant	5000	1.6	11000	2.0
Apollo	4000	1.8	9000	2.0

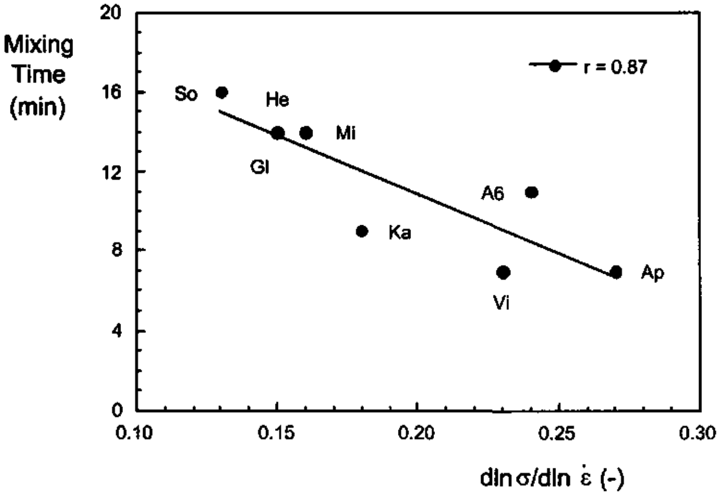


Figure 6. Correlation between the applied mixing time and the strain rate-dependency of the stress in uniaxial extension for flour-water doughs of six European and two Canadian wheat cultivars. Data are at 25°C.

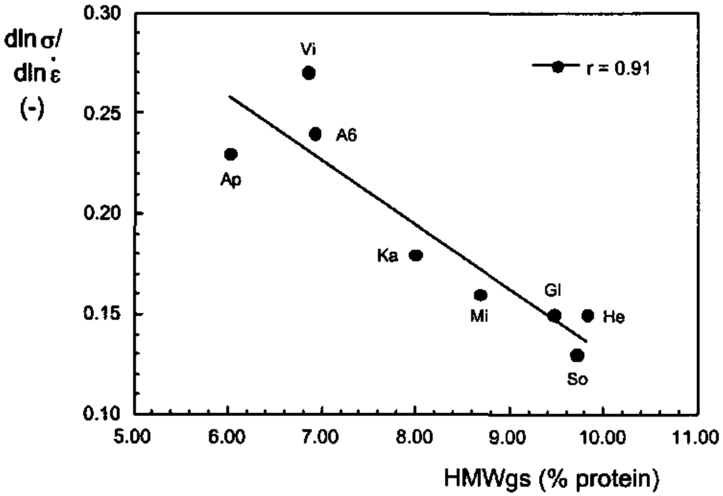


Figure 7. Correlation between rate-dependency of the stress in uniaxial extension and the percentage HMW glutenin subunits on total protein for flour doughs of six European and two Canadian wheat cultivars. Data are at 25°C.

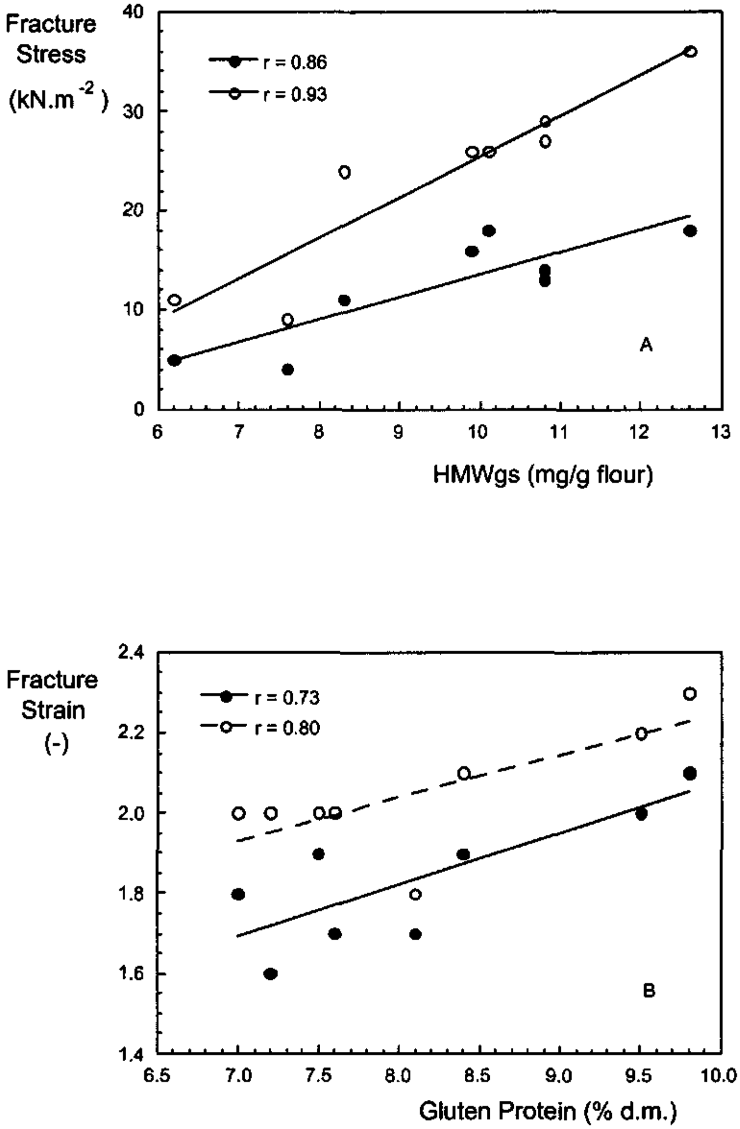


Figure 8. *Correlation between the fracture stress in uniaxial extension and the amount of HMWgs (in mg/g flour) (A) and the fracture strain in uniaxial extension and gluten protein content (% dry matter) (B) at a displacement speed of the hook of 12 (O) and 120 (●) mm.min^{-1} and for flour doughs of six European and two Canadian wheat cultivars. Data are at 25°C.*

Strain hardening offers a protection mechanism against premature fracture of dough films [5,46]. For our set of data no correlation, positive or negative, was observed between fracture strain and strain hardening behaviour. This is no surprise, since the strain hardening coefficients in uniaxial extension of all flour doughs are larger than 1. Because the basic criterion to prevent fracture is fulfilled other factors than strain hardening should therefore be responsible for the differences in fracture behaviour between the respective doughs. The fracture strain was positively correlated with the gluten protein content of the flours (Fig. 8B). A positive correlation was also found between the gluten protein content of flour and the water addition for dough (see figure 2) and therefore the mentioned positive correlation could also indicate a relation between the water content of a dough and the extent to which a dough sample can be extended. The correlation between fracture strain and gluten protein content was less strong at the lower displacement speed. Instead correlations between the fracture strain and factors related to gluten protein composition like the amount of HMWgs were stronger at the lower displacement speed, indicating that under those conditions stronger doughs fracture at larger strains than weaker doughs. Our results are therefore not in line with those reported by others who found a positive correlation between the gliadin to glutenin ratio and fracture strain [47,48]. To get more clarity on the factors that govern the fracture strain, some additional data were gathered on flour and gluten dough for three flours that were considered representative for the whole set. This will be discussed next.

Part 2: three wheat cultivars

2.1 Rheology of flour dough

The large-deformation properties of flour and gluten doughs of the cultivars Vivant, A6/13 and Hereward were studied in uniaxial extension at 25°C and at 45°C. Considerable attention was paid to determine optimum conditions for the preparation of both dough types. The protein and glutenin contents of the parental flours and the glutens isolated thereof and also the water addition and mixing times applied in dough preparation are given in table 7. Differences in protein contents between the glutens were smaller than between the flours. The remaining solids in the gluten are most likely starch, lipids and non-starch polysaccharides. Water additions were higher for gluten than for flour dough. The amounts added by us are in the same range as those applied by Uthayakumaran *et al.* who determined the required water additions using standard methods of the AACC for flour dough and for gluten-starch mixtures [10]. We estimated that with the water additions applied by us, similar amounts of water go into the gluten phase of flour and gluten dough and thus that sufficient water is added to fully hydrate and develope the gluten phase [11]. Differences in glutenin content between flours and glutens remained intact. The glutens should therefore be regarded representative for the parental flours. Mixing times for flour and gluten doughs were in the same range. Contrary to what was found for flour dough mixing times for gluten dough for A6/13 and Vivant were similar. This suggests that the difference in mixing requirements for flour dough originates from the difference in protein content between these two flours, because the percentage of glutenin on total protein was similar.

***Table** 7. Protein and glutenin content of flours and glutens. Water content and mixing time applied to flour and gluten doughs.*

Cultivar	**Protein** (% dry solids)	**Glutenin** (% protein)[1]	**Water Content** (% dough)[2]	**Mixing Time** (min)
flour				
Hereward	11.0	42	39	14
A6/13	12.0	39	40	11
Vivant	10.3	39	39	7
gluten				
Hereward	86	49	62	14
A6/13	85	44	62	10
Vivant	83	44	64	10

[1] For flour from sequential protein extraction and Kjeldahl; for gluten from protein extraction and FPLC.

[2] Percentage of added water on total dough weight.

The results from the rheological tests on flour and gluten dough are shown in tables 8A and 8B. At first the effect of temperature on the rheological properties will be discussed for flour dough. For Vivant and A6/13 similar trends were observed. The stress, the strain hardening coefficient, the strain rate-dependency of the stress, the fracture stress and the fracture strain decreased with increasing temperature. For Hereward a decrease in the strain hardening coefficient and thus also of the stress at larger strains was observed with increasing temperature, while the other values hardly changed. The most drastic effects were noted for Vivant: a decrease of the strain hardening coefficient from 1.3 to 1.0 and a decrease of the fracture strain from 1.6 to 1.3 and from 2.0 to 1.6 at displacement speeds of the hook of 12 and 120 $\mathrm{mm.min^{-1}}$, respectively. These results are in agreement with those from Tschoegl *et al.* who also reported a decrease of the stress for flour dough in uniaxial extension with increasing temperature from 25°C to 45°C [41].

The combined effect of strain rate and temperature on the fracture strain differed between cultivars. At lower deformation rates and higher temperatures the fracture strain was much larger for A6/13 than for Vivant dough, while at 25°C and the higher extension rate the difference was only small (figure 9A). Payne *et al.* studying the mechanical properties in simple tension of flour doughs of three isogenic lines containing only one HMWgs, reported similar differences in the temperature-dependency of the fracture strain [49]. In figure 9B the fracture stresses of the doughs are compared. This figure shows that regardless of the temperature and extension rate, the fracture stress for A6/13 dough is more than twofold greater than that of Vivant dough.

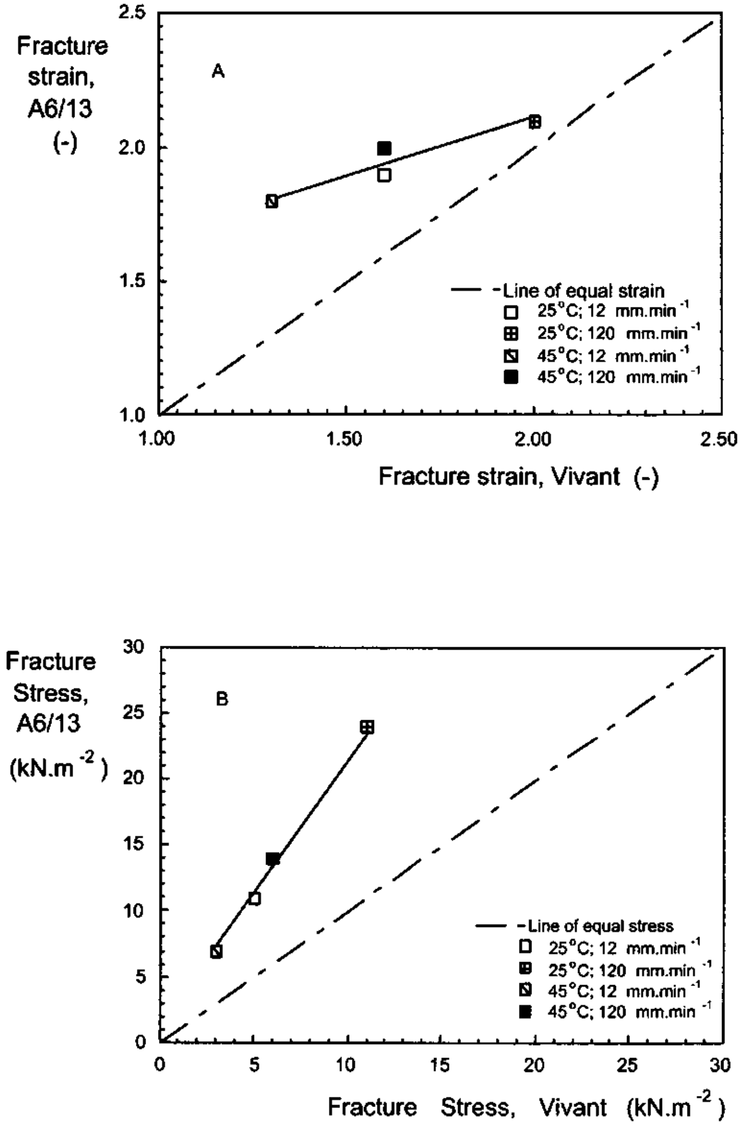


Figure 9. *Fracture strain (A) and fracture stress (B) for A6/13 flour dough plotted against fracture strain and fracture stress, respectively, for Vivant flour dough determined in uniaxial extension at two displacement speeds of the hook and two temperatures.*

Tschoegl *et al.* tested doughs of two flours which differed in protein content and composition in uniaxial extension [41]. They reported similar findings regarding the ratio of the fracture strain and fracture stress as a function of temperature and deformation rate. Dobraszczyk and Roberts measured the fracture properties of bread dough of two wheat flours in biaxial extension at a range of temperatures and strain rates using a modified Chopin Alveograph [44]. They also observed that doughs of the weaker flour were more sensitive to rate than doughs of the stronger flour.

Apparently there is a relationship between the stress-level and the degree of strain hardening of a flour dough in uniaxial extension and the extent to which the fracture strain depends on the strain rate and temperature. For a flour dough with low stress-level and small strain hardening coefficients the fracture strain apparently depends to a larger extent on the strain rate and the temperature. Conditions can be chosen so that compared to A6/13 and Hereward, Vivant exhibits the smallest fracture strain, but also so that Vivant exhibits the largest fracture strain. In view of this result any calculation of correlations between fracture strains determined at one condition and factors that are related to dough strength has to be treated with great care. Instead it is much more meaningful to study correlations between such factors and the strain rate- and temperature-dependency of the fracture strain.

As stated above dough films are protected against premature fracture by the mechanism of strain hardening. When the basic criterion to prevent fracture is fulfilled (i.e. the strain hardening coefficient in uniaxial extension is larger than 1.0) other factors than strain hardening should be responsible for the fracture behaviour of dough films. When strain hardening coefficients come close to the criterion value, as was found for Vivant flour dough, it is to be expected that fracture will occur at much smaller strains. This was indeed observed. To prevent premature fracture of biaxially extended dough films strain hardening coefficients should be higher than 2.0 [5]. Since in literature values for biaxial strain hardening are reported of 2.0 or lower [6,8], for flour dough in biaxial extension the specific power limit is never exceeded. Therefore one may expect that fracture of dough films will occur at much smaller strains in biaxial than in uniaxial extension. However, the criterion for strain hardening in biaxial extension does only count when several conditions are fulfilled [5]. Firstly variations in strain rate with the strain should be taken into account, which causes the criterion to be less strict during baking [5]. Secondly, for a dough film that is thinner than about 50 μm wheat flour dough may not be considered as a homogenous material, since such a film consists of gluten films interspersed with large starch granules [4]. Finally for gas volume fractions below 0.8 the dough is still a polyspheric foam implying that the dough films between gas cells are not plan parallel but curved going from the point of closest approach to the outside. It is therefore to be expected that in practice the criterion for strain hardening in biaxial extension will be less strict than 2.0. This aspect deserves further study.

Of the three flour doughs studied Vivant had the lowest fracture strain at 45°C and at the lower displacement speed of the hook. For A6/13 the fracture strain decreased slightly with an increase of temperature from 25°C to 45°C, while the fracture strain for Hereward flour dough remained relatively unchanged. In a previous paper we have shown that for flour dough a strong influence of the filler (starch) on the rate-dependency of the fracture strain was found [7]. Dobraszczyk and Roberts reported that at lower temperatures and higher deformation rates fracture of dough films in biaxial extension occurred at a thickness of about 30 μm [43]. Wheat flour consists for about 80% of starch. The starch granules present in wheat flour occur in two types: 'A' granules with a diameter of 10-45 μm and 'B' granules with a diameter up to 10 μm [50]. Apparently fracture of dough films occurs at a thickness of the film that is at minimum equal to the diameter of one starch granule. It is to be expected that the effect of protein composition will be more clear at lower strain rates when the influence of the filler on the fracture strain is smaller [45]. Therefore the observed differences between the flour doughs of the three tested cultivars at lower strain rates and at higher strain temperatures might well be related to differences in large-deformation properties of the gluten phase. And than it seems that under those conditions doughs from flours which have lower protein and/or glutenin contents fracture at smaller strains than doughs from flours with high protein and/or glutenin contents (see table 8).

Table 8A. *Stress, relative change in stress over a relative change in strain ($d\ln\sigma/d\varepsilon$) and relative change in stress over a relative change in strain rate ($d\ln\sigma/d\ln\dot{\varepsilon}$) for flour and gluten doughs of three European wheat cultivars. Data are at 25°C and at 45°C.*

Cultivar	**Dough type**	**Temp**	**σ ($kN.m^{-2}$)**	**$d\ln\sigma/d\varepsilon$ (-)**	**$d\ln\sigma/d\ln\dot{\varepsilon}$ (-)**
			(ε=1.50, $\dot{\varepsilon}$=0.01 s^{-1})	($\dot{\varepsilon}$=0.01 s^{-1})	(ε=0.75)
Hereward	Flour	25°C	11.6	1.9	0.15
	Flour	45°C	11.3	1.7	0.17
	Gluten	25°C	20.0	2.5	0.11
	Gluten	45°C	20.3	2.5	0.11
A6/13	Flour	25°C	7.7	1.6	0.24
	Flour	45°C	5.7	1.4	0.13
	Gluten	25°C	5.3	2.5	0.16
	Gluten	45°C	3.9	2.4	0.13
Vivant	Flour	25°C	5.4	1.3	0.23
	Flour	45°C	4.6	1.0	0.15
	Gluten	25°C	5.0	2.5	0.20
	Gluten	45°C	4.1	2.5	0.17

2.2 Rheology of gluten dough

The large-deformation properties of the gluten doughs of the three cultivars were studied in uniaxial extension at 25°C and at 45°C. Results are shown in table 8. At 25°C the stress-level of Hereward was more than three times higher than that of Vivant and A6/13, the difference between Vivant and A6/13 being very small. At an intermediate strain ($\varepsilon = 0.75$) the stress-level of Hereward gluten was higher than the stress-level of its flour dough, while the stress-level of A6/13 and Vivant gluten was lower than that of their flour doughs. Strain hardening coefficients did not vary between the cultivars and were much higher for gluten than for flour dough. The strain rate-dependency of the stress increased in the order Hereward < A6/13 < Vivant. The higher stress-level and the lower strain rate-dependency of the stress indicate that Hereward has longer relaxation times and thus is more elastic than A6/13 and Vivant gluten dough. A similar conclusion was drawn based on the results of small deformation oscillation tests in which the storage modulus G' was higher and tan δ was lower for Hereward than for A6/13 and Vivant gluten dough. At 25°C the strain rate-dependency of the stress was lower and fracture stresses were much higher for gluten than for flour dough. The higher strain hardening coefficients and the lower strain rate-dependency of the stress indicate that from a physical point of view gluten dough is more elastic than flour dough. Based on results from biaxial extension tests Kokelaar *et al.* arrived at the same conclusion [6].

For Hereward gluten the stress-level stayed about the same with increasing temperature, while the stress-level for Vivant and A6/13 gluten decreased. Strain hardening coefficients were similar no matter gluten type and temperature. The strain rate-dependency of the stress decreased with increasing temperature for A6/13 and Vivant, while it remained unchanged for Hereward gluten dough. In small deformation measurements for all glutens G' was lower and less dependent on the frequency at 45°C than at 25°C [11], which indicates that at the higher temperature gluten dough is less stiff and more elastic. Since in this temperature range disulphide bonds are not affected, the observed changes in stress and modulus are presumably due to a decrease of the number and/or strength of non-covalent interactions with increasing temperature [11,51,52]. With increasing temperature fracture stresses increased slightly for Hereward gluten, decreased slightly for Vivant gluten and decreased strongly for A6/13 gluten, while fracture strains remained constant or increased slightly for all glutens. Fracture strains were considerably larger for cultivars with the lowest glutenin content, A6/13 and Vivant, than for the cultivar with the highest glutenin content, Hereward. This result is in line with the findings of others who reported a positive relationship between the gliadin to glutenin ratio and fracture strain of wheat gluten [10,18,47]. It is however, contrary to the results for flour dough, for at lower strain rates and higher temperatures dough from flour with the lowest glutenin content gave the smallest fracture strain. Remarks about a relationship between the degree of strain hardening and the fracture strain are difficult to make, because the effect of temperature on both parameters was rather small.

***Table 8B**. Fracture stress and fracture strain determined in uniaxial extension at two displacement speeds of the hook for flour and gluten doughs of three European wheat cultivars. Data are at 25°C and at 45°C.*

Cultivar	**Dough type**	**Temp**	**σ_f (kN.m^{-2})**		**ε_f (-)**	
			(v=12 mm. min^{-1})	(v=120 mm. min^{-1})	(v=12 mm. min^{-1})	(v=120 mm. min^{-1})
Hereward	Flour	25°C	13	27	1.7	2.0
	Flour	45°C	17	24	1.8	1.9
	Gluten	25°C	150	195	1.8	1.9
	Gluten	45°C	220	237	1.9	1.9
A6/13	Flour	25°C	11	24	1.9	2.1
	Flour	45°C	7	14	1.8	2.0
	Gluten	25°C	100	190	2.2	2.3
	Gluten	45°C	79	124	2.3	2.4
Vivant	Flour	25°C	5	11	1.6	2.0
	Flour	45°C	3	6	1.3	1.6
	Gluten	25°C	90	165	2.2	2.3
	Gluten	45°C	86	136	2.3	2.3

From the results on dough from flour and gluten several conclusions can be drawn. The effect of temperature on the stress-level of flour and gluten dough was similar for each cultivar. This suggests that for flour dough the effect of temperature on the stress is mainly determined by the gluten fraction. This trend is also reflected in the fracture stress. Contrary to what was observed for flour dough, strain hardening coefficients and fracture strains for gluten dough did not decrease with increasing temperature. Another difference is that for flour dough the smallest fracture strain was found for the cultivar with the lowest glutenin content (Vivant), while for gluten dough the smallest fracture strain was found for the cultivar with the highest glutenin content (Hereward). Regarding these aspects the behaviour of flour and gluten dough clearly differs.

With regard to their composition flour and gluten mainly differ from each other in two respects: flour has a much higher starch content and a much lower protein content than gluten. Additionally water content applied by us is higher for gluten than for flour dough. The starch granules present in flour dough act as a filler. As reported before it is presumably the filler phase that causes the differences between flour and gluten dough in the dependence of the storage modulus G' on the strain at small deformations [10,11,53] and in the rate dependence of the fracture strain [11].

As mentioned above flour and gluten dough also differ with respect to the effect of temperature on strain hardening and fracture strain. Strain hardening and fracture strain of gluten are much less sensitive to changes in strain rate and temperature than those of flour dough. Strain hardening and the strain rate and temperature-dependency of the fracture strain for flour dough depend on the amount and/or size distribution of gluten protein. The higher the protein and/or glutenin content of flour, the higher the strain hardening coefficient of its dough and the smaller the strain rate and temperature dependency of the fracture strain. Since glutenin content for gluten is much higher than for flour, it is to be expected that the strain hardening coefficient is higher and the strain rate and temperature-dependency of the fracture strain are smaller for gluten than for flour dough.

For flour dough the smallest fracture strain was found for the cultivar with the lowest glutenin content (Vivant), while for gluten dough the smallest fracture strain was found for the cultivar with the highest glutenin content (Hereward). This difference in the effect of protein composition on the fracture strain between flour and gluten dough requires another explanation. Based on small deformation measurements Cornec *et al.* concluded that it are the glutenin polymers of large size that provide the matrix in gluten dough, while the monomeric gliadins provide the viscous component and thus essentially act as a plasticiser [17]. They found a linear relation between G' and the proportion of glutenin polymers of large size. Their results showed that in gluten a minimum concentration of 2% of glutenins of large size is required for the formation of a gluten network. It can be seen in table 1 that for our set of flours the percentage of glutenin is 4.0, 4.7 and 4.6% for Vivant, A6/13 and Hereward, respectively. By combining these data with the ones shown in table 4 we estimated percentages of glutenin of large size (UEP + P1) of 3.0, 3.5 and 3.7% for Vivant, A6/13 and Hereward, respectively. These values are rather close to the critical limit found by Cornec *et al.* [17]. If the content of glutenin polymers of large size comes more close to the minimum proportion limit, it is to be expected that the effective number of bonds exhibiting relatively longer relaxation times will decrease stronger than proportional resulting in a decrease of network connectivity and eventually lower fracture strains. So, differences in fracture strains between flour doughs might well be caused by variation in the amount of glutenin polymers of large size in relation to the criticial limit. In gluten, the monomeric gliadin proteins act essentially as a plasticiser. It is easily understood that at the percentages of large-sized glutenin polymers present in all gluten doughs an increase of the percentage of plasticiser, i.e. the relative amount of gliadin, results in an increase of the viscous component in gluten dough and with that of the flow properties during the deformation process and thus in an increase of the fracture strain. We concluded therefore that the difference in the effect of protein composition on the fracture strain between flour and gluten dough might well be caused by the fact that in flour dough the amount of glutenins of large size is limiting, while in gluten dough it is the amount of gliadin that can be too low. It shows that to reach maximum fracture strains the content of glutenin polymers of large size in flour and gluten dough should be not too low, but also not too high.

CONCLUSIONS

The total amount of HMWgs of the flours was strongly associated with protein characteristics that are commonly used to describe differences in gluten protein composition as the gliadin to glutenin ratio, glutenin content, the LMWgs to HMWgs ratio and glutenin size distribution. We concluded therefore that HMWgs content is an important parameter for flour quality. In our data set differences in the amount of x-type HMWgs were responsible for most of the variation in total HMWgs content between the flours.

We concluded that the observation that several stress-related dough properties of the set of flours under study were correlated with mixing time is consistent with what should be expected for the time-dependency of the stress for this kind of viscoelastic materials. In line with the known strong dependence of the rheological properties on molecular weight and molecular weight distribution for polymers in general we have found a positive correlation between the stress at a chosen strain and the percentage of polymeric protein of large size and a negative one between the strain rate-dependency of the stress and the percentage of HMWgs on total protein.

For gluten dough the fracture strain was largely independent of strain rate and temperature, while for flour dough the fracture strain decreased with decreasing strain rate and increasing temperature. At the lowest strain rate and the higher temperature the smallest fracture strain was found for the flour dough with the lowest glutenin content and/or the lowest protein content. On the contrary, for gluten dough the smallest fracture strain was found for the gluten with the highest glutenin content. We concluded therefore that flour and gluten dough show a clearly different behaviour regarding the combined effect of protein composition, strain rate and temperature on the fracture strain in uniaxial extension. The observed differences in large deformation and fracture properties are most likely due to the large differences in starch and gluten protein content between flour and gluten dough.

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Chapter VI

On the Relationship between Large-Deformation Properties of Wheat Flour Dough and Baking Quality[1]

SUMMARY

Baking performance for bread and puff pastry was tested for Six European and two Canadian wheat cultivars and related to the rheological and fracture properties in uniaxial extension of optimally mixed flour-water doughs and doughs to which a mix of bakery additives was added. Extensive baking tests were performed as a function of water addition for puff pastry and as a function of water addition and mixing time for bread. For optimum baking performance puff pastry doughs required lower water additions than bread doughs. Baking performance of the flours differed for the two products. For puff pastry higher volumes were obtained per gram of flour than for bread. Puff pastry volume was positively correlated with optimum bread dough mixing time, while bread volume was not. Instead, bread volume was positively correlated with gluten protein content.

All doughs exhibited strain hardening, a more than proportional increase of the stress with the strain. For all doughs fracture stress and strain increased with increasing displacement speed of the hook and decreasing temperature. Large differences were observed between the cultivars regarding stress, strain hardening, strain rate-dependency of the stress, fracture stress and fracture strain. At both 25°C and 45°C addition of a mix of bakery additives resulted in a decrease of the stress at relatively small strains and a significant increase of the strain hardening coefficient. Fracture strains remained the same or increased as a result of addition of the mix. Differences between flours regarding the strain rate and temperature-dependency of the fracture strain remained. The weaker the dough the stronger the strain rate and temperature-dependency of the fracture strain.

Puff pastry volume was positively correlated with strain hardening and negatively with the strain rate-dependency of the stress. In short, the stronger the dough the higher the puff pastry volume. For bread it were not the strongest doughs that gave the highest loaf volumes, but those with intermediate dough strength. Low volumes for puff pastry and bread were found for doughs having a low fracture stress and low strain hardening coefficients. Loaf volumes of flours with high dough strength (i.e. high stress-level and high strain hardening) gave intermediate loaf volumes. We concluded that a high stress can hamper the extensibility of dough films between gas cells, thus limiting the expansion of gas cells during fermentation and baking and with that the loaf volume that can be obtained.

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INTRODUCTION

Wheat is one of the three most important crops in the world, together with maize and rice [1]. Approximately 600 million tonnes are harvested annually. Important factors that have contributed to its success are that it may give high yields under a range of conditions and the ability of wheat flour dough to retain gas which is essential for the production of light leavened products, like bread and puff pastry [2,3]. To obtain a bread crumb with a light and even texture (i.e. with a large specific volume and cells of apparent uniform size) after mixing a sufficient number of gas cells has to be incorporated in the dough [4]. This is due to the fact that the carbon dioxide produced during fermentation can make existing gas cells grow, but is not capable of forming new gas cells [5]. Not all carbon dioxide is involved in the expansion of gas cells, part of it will evaporate. The higher the gas production and the thinner the dough membranes the higher the rate of evaporation [4,6]. As fermentation proceeds, gas cells become larger and the distance between them decreases. During the latter part of pan proof and during baking the foam structure of dough is characterised by a large area of the gas-liquid interface, a consequence of the small thickness of the dough membrane. Nevertheless, at this stage of the baking process surface phenomena are of relatively little importance [4,7]. The relevant types of physical instabilities in bread dough are Ostwald ripening (disproportionation) and coalescence of gas cells [4,6]. The mechanism behind disproportionation is diffusion of gas through the dough from small to large cells caused by a difference in gas pressure between gas cells of different size [7]. The result is the growth of large gas cells at the expense of small ones. Coalescence of gas cells is initiated by fracture of the dough film between them [6]. In the dough it results in a coarse bread crumb, while fracture of dough films between gas cells and the surrounding air results in a loss of gas and a small loaf volume.

The variation in the potential for gas retention among wheat flour doughs is largely due to variation in bulk rheological properties [6,8,9]. An earlier analysis of the bread making process revealed two requirements for a satisfactory performance of wheat flour dough that can be expressed in rheological terms [4]. First the viscosity has to be high enough to prevent the ascent of gas cells. This condition is met by virtually all doughs. Further dough must be extensible in order to prevent coalescence and thus premature fracture of membranes between gas cells. The extensibility must be maintained long enough under baking conditions to permit sufficient oven rise. An additional new rheological criterion was proposed by van Vliet *et al.* (1992) for the extensibility of dough films between gas cells (and thereby for gas retention) that states that strain hardening of the dough in biaxial extension has to exceed a specific power limit [6]. Another factor is that during processing dough is not only deformed biaxially, but also uniaxially [10]. For instance, during moulding and shaping the dough is deformed in a combination of shear and uniaxial elongation at relatively high deformation rates. Interestingly for flour dough the criterion for strain hardening is much easier fulfilled in uniaxial than in biaxial extension [10,11].

Rheological tests on dough can reliably predict its behaviour in a bakery only if the rates and extents of deformation in these tests are in the same range as those during dough processing (4,6,12,13,14). In fact, only a few studies are available in which large deformation rheological data in uniaxial and biaxial extension at low strain rates are linked to results from baking tests [9]. Mostly rheological data are used that are obtained at small deformations and/or at high strain rates.

Tschoegl *et al.* (1970b) performed uniaxial extension tests on doughs of two flours that differed in bread making performance [15]. They found that for both flours the predominant response in uniaxial extension was delayed elastic and not viscous flow. However, the weaker dough dissipated the elastic strain energy faster than the stronger dough. Fracture strains were larger for the stronger dough at the higher temperatures, while at the lower temperature and higher extension rate the converse was true. Regardless of temperature and extension rate the fracture stress of the stronger dough was more than fourfold higher than that of the weaker dough. Dobraszczyk and Roberts (1994) who measured dough rheological properties in biaxial extension also found that fracture strain depended on flour type, deformation rate and temperature [13]. For low rates of inflation and high temperatures the weaker dough failed at much lower strains.

Janssen *et al.* (1996) studied dough rheological properties in biaxial extension for several flours and discussed the results in relation to bread making performance [8]. Their study suggested that in order to obtain a high loaf volume and a fine crumb structure, wheat flour dough has to exhibit biaxial strain hardening and an extensibility exceeding a minimum level and that the resistance to deformation may vary within a certain range. Kokelaar *et al.* (1996) found that glutens from wheat cultivars with good baking quality had higher values for the storage modulus, G', and lower values for the loss tangent [9]. Results from biaxial extension tests and Extensograms and Alveograms showed that in general good baking flours exhibited stronger resistance to extension and a greater extensibility. Uthayakumaran *et al.* (2002) studied the rheological properties of flour and gluten dough at small and large strains of two commercial wheat flours [16]. They found that in uniaxial extension elongational viscosities were highly dependent on the strain rate. Higher elongational viscosities were found for flour dough of the wheat cultivar with the higher protein content, while fracture strains were less dependent on the strain rate for the flour with the higher glutenin to gliadin ratio.

The objective of this paper is to relate differences in baking performance to some dough rheological properties that are considered to be relevant for baking. These properties were determined for six European and two Canadian wheat cultivars. Extensive bread and puff pastry baking tests were carried out. Large-deformation and fracture properties of flour doughs were determined in uniaxial extension at different deformation rates. The effect of temperature and of the addition of a mix of additives on the large-deformation and fracture properties of flour dough was studied for three representative cultivars.

EXPERIMENTAL

Materials

For this research project six European wheat cultivars, Apollo, Vivant, A6/13, Hereward, Soissons and Minaret and two Canadian wheat cultivars, Katepwa and Glenlea, were selected. All selected wheat samples were pure cultivars, except A6/13 which was a mixture of at least two undefined cultivars. Samples of 400 kg each were milled to a milling degree of approximately 70% and an ash content of about 0.47 in the experimental mill of Meneba BV, Rotterdam, The Netherlands. Ash content varied between 0.45 and 0.50% with an average of 0.47%. Protein content varied between 10.3 and 13.4% with an average of 11.6% (see Table 1). Flour was stored at –20°C. All reagents were either of guaranteed reagent grade or of the best grade available.

Methods

Moisture Content was determined according to AACC method 44-19, damaged starch according to AACC method 76-30A and Hagberg falling numbers according to AACC method 56-81B for all flours[17]. Protein content was analysed using a Kjeldahl procedure (N x 5.7). Data are presented in table 1.

White tin bread baking tests were performed using a recipe and process that was constant for every flour except water addition and mixing time. The standard recipe comprised flour (3.5 kg), yeast (2%), salt (2%), bakery fat (1%), sugar (0.5%), ascorbic acid (20 ppm) and malt. Malt was added in different amounts to the flours dependent on the falling number. Following mixing of the dry ingredients with water for 3 min at the lower speed in a Kemper mixer (Kemper, The Netherlands), doughs were mixed at the higher speed in the same mixer for times between 2-15 min. During mixing energy-input and dough temperature were monitored. Water and flour temperature were chosen such that the dough temperature at the end of the mixing stage was around 27°C. After mixing the dough underwent a bulk-fermentation of 1 hour at 30°C. The doughs were cut into six pieces of which the weight depended on the water content of the dough (approx. 600 g flour), punched and rolled and fermented for 90 minutes at 30°C. Following sheeting, rolling and moulding the doughs were proved for 90 minutes at 30°C and baked for 45 minutes in an oven of 240°C. Of six loaves the two extremes with regard to volume and exterior were discarded. Loaf volumes were measured 2 hrs after baking by rape seed displacement (average coefficient of variation CV = ±1%). Dough properties, loaf appearance and crumb structure were evaluated visually by an experienced baker. Grading of loaf appearance and crumb structure was expressed as a number on a scale from 1 to 5 on which 1 means poor and 5 means good.

Puff pastry baking tests were performed using the French method [18] with some minor modifications. The amount of water to be added to the flour was calculated taking into account the moisture content of the flour and the farinograph water absorption. To 100 g flour was added 2% salt, 10 g fat and the calculated amount of water. A dough was mixed for 2 minutes with a Hobart Mixer. Directly after mixing 76 g fat was folded into the dough in one piece and the dough was sheeted up to a thickness of 7 mm and refolded in threefold. This procedure was repeated two times after which the dough was left to relax for 30 minutes. Than the dough was sheeted and refolded in threefold for two more times after which the dough was left again to relax for 30 minutes. After this resting period square-shaped dough pieces were cut out and weighed and puff pastry was baked in an oven of 200°C for 20 minutes. Puff pastry volume was determined by rape-seed displacement (average coefficient of variation CV = ±2%). Changes in dough piece size as a result of baking were determined by measuring length and width of dough pieces and final products (average coefficient of variation CV = ±4%). Dough piece changes were defined as shrinkage in the direction parallel to the last sheeting action (S.P.) and as shrinkage in the direction anti-parallel to the last sheeting action (S.A.).

Flour doughs for rheological measurements were prepared by mixing in a 10 g National Mixograph with a target dough temperature of 27°C as described previously [10,11]. Considerable attention was paid to the determination of optimum water additions and mixing times for each flour individually. Water additions were based on results of baking tests. Water addition was chosen as high as possible, but should be within the working area of a flour (see figure 1) and should not cause problems with dough stickiness. Mixing times were based on results from biaxial extension tests. Doughs were considered optimally mixed when the stress at a certain strain as a function of mixing time was the highest on the condition that doughs were still manageable with respect to stickiness. Manageability was determined manually. Water contents and mixing times used are given in table 3. Optimum mixing times obtained from mixograph mixing curves correlated more strongly with the mixing times required for rheological tests than with the ones derived from the bread making tests.

Table 1. *Analytical data for flours.*

Cultivar	**Moisture Content** (% D.M.)	**Protein Content** (% D.M.)	**Damaged Starch** (% D.M.)	**Falling Number** (s)
Glenlea (Gl)	13.3	13.3	12	372
Minaret (Mi)	14.3	11.4	5	312
Soissons (So)	13.0	10.4	8	336
Hereward (He)	15.3	11.0	5	344
Katepwa (Ka)	13.5	13.5	12	324
A6/13 (A6)	13.2	12.0	11	331
Vivant (Vi)	14.4	10.3	10	362
Apollo (Ap)	13.0	11.1	5	359

Apart from flour-water doughs also doughs were prepared to which a mix of bakery additives was added. The mix of additives comprised: 2 % (w/w) NaCl, 1% (w/w) bakery fat, 0.5% (w/w) sugar, 0.5% (w/w) CSL (calcium stearoyl-2-lactylate), 50 ppm ascorbic acid, 100 ppm fungal α-amylase (Bioferm P, Quest International, The Netherlands) and 100 ppm xylanase (Grindamyl H121, Danisco Ingredients, Denmark). All ingredients were added prior to mixing.

Uniaxial extension tests were performed using a Zwick mechanical testing apparatus equipped with the Kieffer Extensibiliy Rig as described previously [10,19,20]. After mixing the doughs were stored in a stencil at the test temperature for 60 minutes. Test samples were coated with paraffin oil to avoid evaporation of water. Before the start of the test the cylindrically shaped dough pieces were clamped at both ends. At the start of the test dough pieces were primarily uniaxially extended by an upward moving hook. The force-displacement curves were recalculated into stress-strain curves which allowed also the calculation of the strain and strain rate dependence of the stress as well as the stress and strain at fracture [10,11,20]. The measuring device was equipped with a plastic container and a heater to maintain the temperature. Tests were performed at 25°C and at 45°C. The latter temperature was chosen to avoid changes in dough rheological properties due to starch gelatinisation that can occur above 45°C [3,4,7]. Displacement speeds were 12, 48 and 120 $mm.min^{-1}$. Results shown are mean values of at least five measurements on at least four different dough preparations (average coefficient of variation CV = ±3%, ±4%, ±4% and ±6% for stress, strain hardening, fracture strain and fracture stress, respectively).

RESULTS AND DISCUSSION

In the first part results for the whole set of eight cultivars will be presented and discussed and after that the results of the more intensively studied three cultivars.

Part 1: eight wheat cultivars

1.1 Baking performance for bread

For all cultivars bread making tests were performed as a function of water addition and mixing time. As an example results of bread making tests for Vivant and Hereward flour are shown in table 2. With prolonged mixing dough properties developed from undermixing (short, slack), to good (smooth, elastic, body), to overmixing (slack, sticky). At the lower water addition loaf volume went through a maximum as a function of mixing time. For both flours at the higher water addition optimum dough properties and loaf quality were obtained after shorter mixing. Also for both flours loaf volume increased and crumb structure grew worse with increasing water addition. Vivant flour required lower water additions and shorter mixing times than Hereward. With Hereward higher volumes were obtained than with Vivant flour.

***Table 2.** Results from bread making tests as a function of water addition and mixing time for Vivant and Hereward flour. Mixing was done in a Kemper mixer.*

Cultivar	**Water addition**	**Mixing Time**	**Dough properties**[1]	**Loaf volume**	**Crumb structure**[1,2]
	(% flour)	(min)	(-)	(cm^3/g flour)	(-)
Vivant	60	5.0	short	6.4	3
	60	**5.5**	**smooth**	**6.5**	**5**
	60	7.0	slack	6.3	2
	63	**5.0**	**elastic**	**6.6**	**4**
	63	5.5	slack	6.5	2
	63	6.5	sticky	6.2	2
Hereward	64	8.0	slack	6.4	4
	64	**10.0**	**body**	**7.2**	**4**
	64	11.5	sticky	7.1	3
	66	**8.0**	**elastic**	**7.3**	**3**
	66	9.5	slack	7.3	3
	66	11.0	sticky	7.2	3

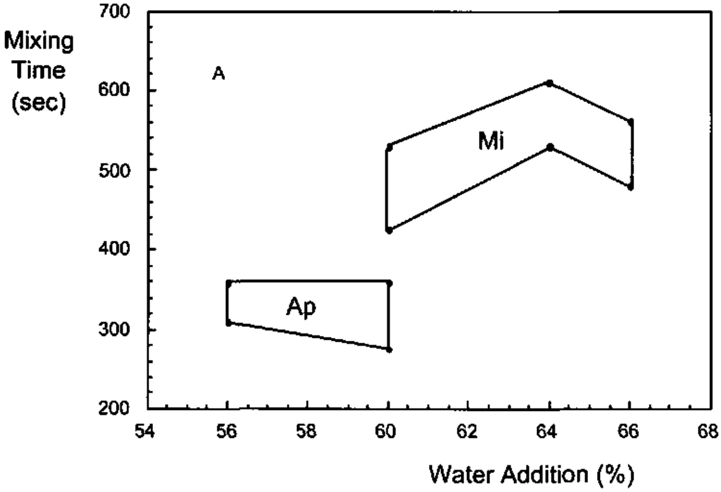
Optimum dough preparation conditions in bold.

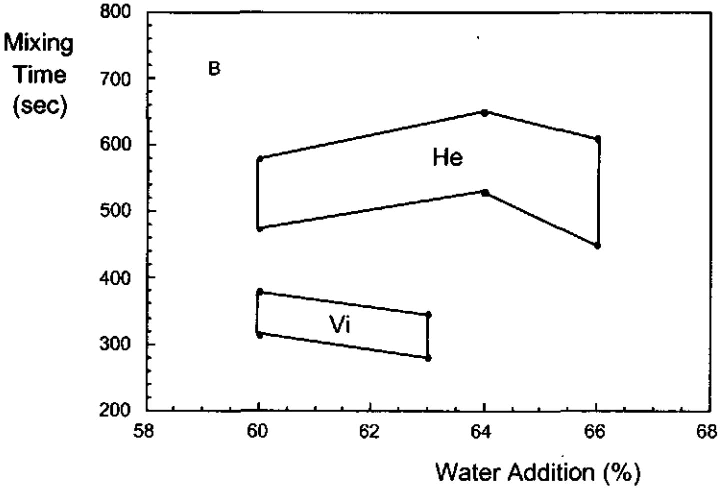
[1] *As determined by an experienced baker.*

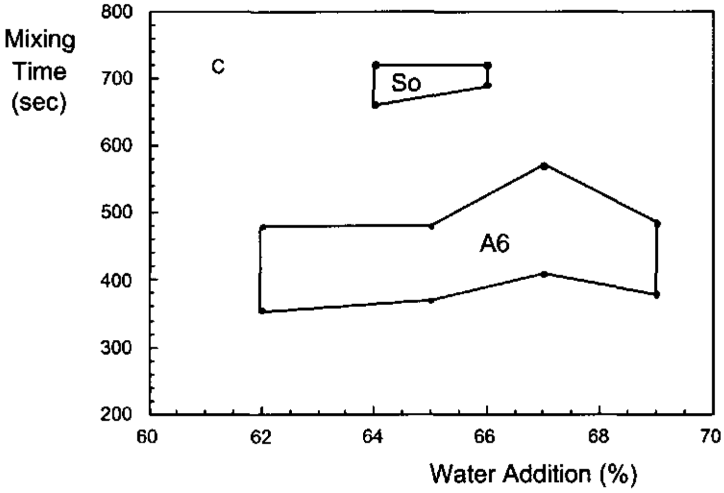
[2] *On a scale from 1 to 5 on which 1 means poor and 5 means good.*

The results for all six European and two Canadian wheat cultivars are presented in figure 1 as working areas for every flour individually (loaf volumes not included). Combinations of water addition and mixing time within the working area of a flour resulted in an acceptable to good dough manageability and product quality, while combinations outside the working area led to inferior dough manageability and/or product quality. Similar trends were found for all flours. Too short mixing times and/or too low water additions resulted in stiff/short doughs and low loaf volumes, while too long mixing times and/or too high water additions resulted in weak, sticky doughs, low loaf volumes and a deterioration of crumb structure. This is in line with earlier findings of Kilborn and Tipples [21].

Large differences were found between the cultivars in the size and place of the working areas within which good dough properties and baking quality could be expected. Similar findings were previously reported by Roels *et al.* [22]. For A6/13 the largest working area was observed, while for Apollo and Vivant a very small working area was found. In the case of Soissons and Glenlea it was almost impossible to apply the concept of a working area. For these cultivars good dough and loaf properties were obtained at very long mixing times using ice-water. Longer mixing times could not be applied, because dough temperature after mixing would become too high. To shorten mixing times of these two flours probably a higher mixing intensity is required.







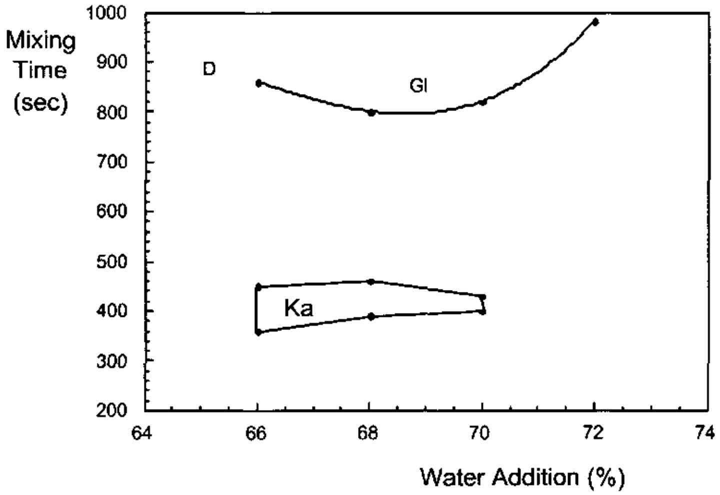


Figure 1. *Working areas that give good quality bread for Apollo (Ap) and Minaret (Mi) flour (1A), Vivant (Vi) and Hereward (He) flour (1B), A6/13 (A6) and Soissons (So) flour (1C) and Katepwa (Ka) and Glenlea (Gl) flour (1D). Variables are water addition and mixing time.*

Considerable differences were observed between the cultivars regarding the amount of water to be added to a flour. Water additions resulting in acceptable dough and loaf properties varied from 56% to 60% for Apollo to 62% to 69% for A6/13 flour. Large differences between cultivars were also observed regarding the required mixing time. At a water addition of 60% Apollo flour required a mixing time of between 275 to 360 s, while for Glenlea flour in all cases more than 700 s mixing was needed. The cultivars also differed considerably in the range of mixing times which could be used. From Vivant flour an acceptable loaf could be prepared at a water addition of 60% with mixing between 315-380 sec, while for A6/13 flour at a water addition of 67% mixing times varying from 410-570 sec resulted in acceptable dough and loaf properties. In other words the resistance to overmixing is higher for dough from A6/13 than from Vivant flour.

Table 3. *Water addition, mixing time, energy input, loaf volume, crumb structure and loaf exterior for bread of flour of six European and two Canadian wheat cultivars produced at optimum dough water contents and mixing times.*

	Water addition	**Mixing Time**	**Energy Input**[1]	**Loaf Volume**	**Crumb**[2] **Structure**	**Loaf**[2] **Exterior**
	(% flour)	(min)	(kJ)	(cm^3)		
Glenlea	68	15.0	360	4600	2	4
Minaret	64	9.5	200	4300	2	4
Soissons	64	12.0	260	3950	2	3
Hereward	64	10.0	215	4125	4	4
Katepwa	68	7.0	140	4350	2	4
A6/13	67	7.5	180	4250	4	5
Vivant	63	5.0	70	3800	4	3
Apollo	60	5.0	80	3850	3	2

[1] *Energy input in kJ for doughs prepared from 3.5 kg flour.*

[2] *On a scale from 1 to 5 on which 1 means poor and 5 means good.*

As explained before within the working area of a flour combinations of water addition and mixing time could be found resulting in optimum end-product quality. We defined this as the highest obtainable loaf volume in combination with reasonable crumb structure and loaf exterior. For most flour types the optimum conditions were situated in the top-right side of the working area, i.e. using a rather high water addition and a relatively long mixing time. In general within the working areas it was possible to obtain a better crumb structure, but this led to a lower loaf volume. Results are shown in table 3. Apollo and Vivant combined low water additions, short mixing times, small working areas and low loaf volumes and are therefore considered less suitable for bread making. For A6/13 and Katepwa high water additions and relatively short mixing times were found in combination with relatively high loaf volumes and good loaf appearance. The working area of Katepwa was much smaller than that of A6/13. To produce intermediate to high loaf volumes Hereward and Minaret required moderate water additions and relatively long mixing times. Soissons and Glenlea both required very long mixing times. With Glenlea flour the highest loaf volume of all cultivars was obtained, while with Soissons flour rather low loaf volumes were obtained. For Glenlea the best loaf was obtained at a water addition of 72%, but its volume was lower than that at a water addition of 68%. For Soissons a higher loaf volume was obtained at a water addition of 66%, but dough properties were on the edge of manageability. We concluded therefore that for these two flours the given water additions, mixing times and loaf volumes also represent optima. Katepwa and A6/13 flour fit best the requirements of the baker. With relatively small mixing times relatively high loaf volumes of reasonable quality could be obtained.

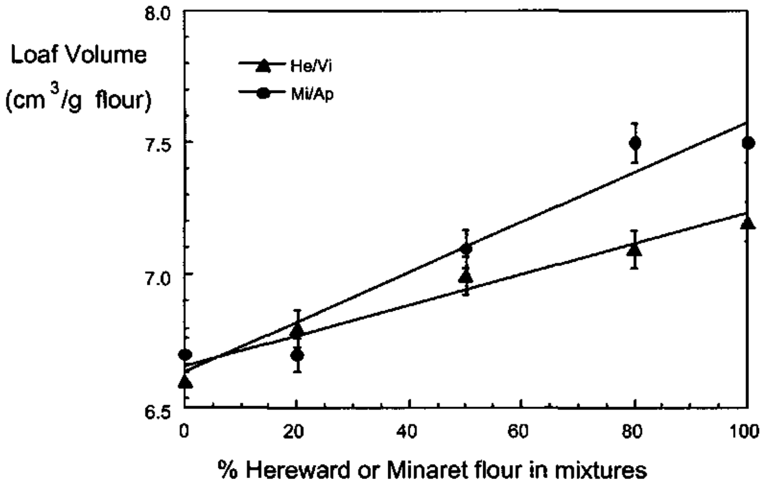


Figure 2. *Loaf volumes for bread of mixtures of Hereward and Vivant flour and of Minaret and Apollo flour. Optimum conditions for dough preparation of flour mixtures (malt addition, water addition and mixing time) were calculated from the optimum conditions of the individual flours.*

Additional baking tests were performed with mixtures of flours with different water and mixing requirements. Based on the working requirements of the individual flours the required water addition and mixing time of the mixtures were calculated as well as the required malt addition. The loaf volume to be expected of al mixtures was calculated using only two variables: the percentage of flour in a mixture and the optimum loaf volume of an individual flour. The results of the baking tests are shown in figure 2. In all cases loaf volumes obtained were close to the ones calculated from the volumes for the individual flours. It shows that the determination of the bread making potential of mixtures of flours does not have to yield unexplicable results once the bread making potential and the required working conditions of the individual flours are known and taken into account.

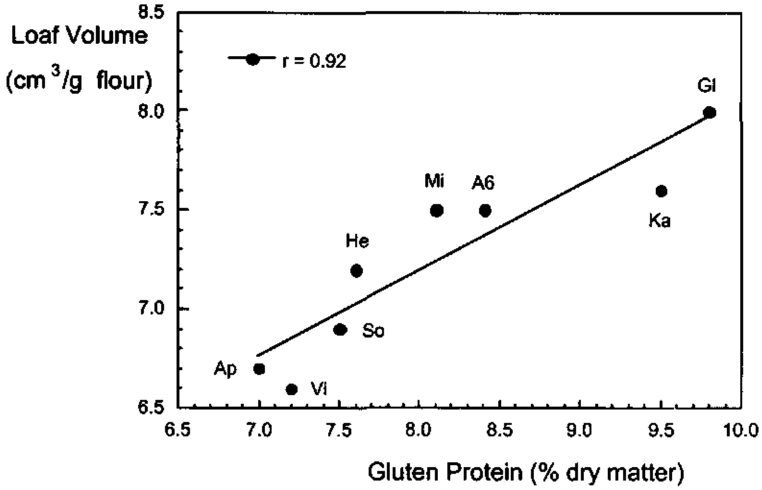


Figure 3. *Correlation between bread volume and flour glutenin content for six European and two Canadian wheat flours. Data on glutenin content from Sliwinski et al. [11]. For abbreviations see table 1.*

In the present data set the correlation between loaf volume and dough mixing time was weak (r = 0.61). Similar observations were made by Finney and Barmore [25], Wieser and Kieffer [26], Rao et al. [27] and Uthuyakumaran *et al.* [28]. We concluded therefore that long mixing flours do not necessarily give higher loaf volumes than short mixing flours. In an earlier paper we have reported that flours that contain relatively more glutenin protein and/or glutenin protein of large size require longer mixing times [11]. This is in line with the findings of others [22,23,24]. It suggests that the mixing requirements of a flour are strongly influenced by its gluten protein composition. For our data set we observed a positive correlation between loaf volume and gluten protein content (r = 0.92; Fig. 3), which suggests that for optimally developed bread doughs the loaf volume that can be obtained depends to a high extent on gluten protein content and to a lesser extent on gluten protein composition. For hearth bread which is baked without the side wall support of a pan opposite trends were observed. With flours that require longer mixing times (and thus contain relatively more glutenin) higher loaf volumes and higher ratios of loaf height to loaf width could be obtained than with weaker flours on the condition doughs were mixed and proved at optimum conditions [30,31]. This difference between pan and hearth bread was attributed to the fact that for hearth bread a higher dough strength is required to retain a proper shape during the baking process.

1.2 Baking performance for puff pastry

For all cultivars puff pastry baking tests were performed with varying water addition, but with constant mixing time and sheeting procedure. The results of the puff pastry baking tests for Vivant and Hereward flour are shown in table 4. Vivant flour required higher water additions than Hereward. For both flours dough weight per dough piece area after sheeting was highest at the intermediate water addition. Highest volumes were obtained at the intermediate water addition for Hereward and at the lowest water addition for Vivant flour. Higher volumes were obtained with Hereward than with Vivant flour. Hereward puff pastry showed more shrinkage than those of Vivant. The higher puff pastry volume, the higher puff pastry height and the higher shrinkage percentage. The association between puff pastry volume and shrinkage percentage is not unexpected. In puff pastry dough preparation it is important that the dough phase and the fat phase stay separated during sheeting and baking [14,32]. Fracture of dough films as a result of extension during sheeting and expansion during baking will lead to a decrease of the number of intact layers and a concomitant decrease of puff pastry volume. Fracture of dough films will also lead to a decrease of the stress that is built up in the material as a result of sheeting and with that probably of the shrinkage percentage. This explanation is supported by the observation of Morgenstern *et al.* [14] that undesirable shrinkage occurs when pastry dough is not fully relaxed.

***Table 4**. Water addition, dough weight, pastry height and shrinkage in the direction parallel to the last sheeting action (S.P.) as a function of water content for Vivant and Hereward flour.*

Cultivar	**Water addition**	**Dough weight**	**Pastry height**	**Pastry height**	**S.P.**
	(% flour)	(g)	(cm/ 100 g dough)	(cm)	(%)
Vivant	52	476	7.4	35.0	19.1
	55	520	6.6	34.5	15.7
	58	474	5.9	28.0	14.7
Hereward	46	531	9.4	50.0	17.4
	50	558	9.7	54.0	21.7
	53	533	8.8	47.0	20.7

In table 5 for all cultivars the required water addition and properties of optimum puff pastry end-product quality are shown. Water additions varied between 46% for Apollo to 57% for Glenlea with an average of 52% and were weakly positively correlated with protein content (r = 0.68). Likely this weak correlation is at least partly due to the variation in damaged starch content between the cultivars (Table 1 and see below). Lowest puff pastry volumes were found for Apollo, Vivant and A6/13 flour. Highest volumes were obtained with Hereward, Soissons and Glenlea flour. For all cultivars the percentage of shrinkage parallel to the direction of the last sheeting action (S.P.) was higher than the shrinkage percentage in the anti-parallel direction (S.A.). On average shrinkage percentage was lower for low volume flours than for flours with which high puff pastry volumes could be obtained. The correlation between puff pastry volume and shrinkage percentage was however weak (r = 0.53). For instance for Glenlea a high puff pastry volume was found in combination with relatively small shrinkage percentages. For the baker a good puff pastry flour should combine a relatively high volume with a relatively small shrinkage percentages. Because Apollo, Vivant and A6/13 gave low volumes these flours are not regarded suitable for commercial puff pastry production. The ideal puff pastry flour was Glenlea, which combined a high volume and little shrinkage. Katepwa, Minaret and Hereward flour also fit the requirements. The volume of Soissons flour is above the critical limit, but it suffers from a high shrinkage percentage. This indicates that Soissons dough was not fully relaxed during resting [14]. It might therefore be advantageous for strong doughs to use longer resting times to decrease the percentage of shrinkage.

Table 5*. Water addition, volume, height, shrinkage in the direction parallel to the last sheeting action (S.P.) and shrinkage in the direction anti-parallel to the last sheeting action (S.A.) for puff pastry of flour of six European and two Canadian wheat cultivars produced at optimum dough water contents.*

Cultivar	**Water addition**	**Volume**	**Height**	**S.P.**	**S.A.**
	(%/ g flour)	(cm3/g dough)	(cm/100 g dough)	(%)	(%)
Glenlea	57	5.0	8.9	17.5	12.0
Minaret	49	4.9	9.2	19.4	15.0
Soissons	52	5.1	9.6	25.0	17.2
Hereward	50	5.2	9.7	21.7	16.7
Katepwa	56	4.6	8.5	19.1	14.9
A6/13	55	4.2	7.4	18.2	14.0
Vivant	52	4.2	7.4	19.1	13.4
Apollo	46	3.8	7.2	18.6	15.6

Water additions required for optimum dough development were lower for puff pastry than for bread dough, which is presumably due to differences in dough preparation between the two products. Puff pastry dough is regularly sheeted in order to form alternating layers of fat and dough. This probably makes that it is of more importance to avoid dough stickyness in the production of puff pastry than of bread. In figure 4 optimum water content for puff pastry is plotted as a function of optimum water content for bread making for all cultivars. Water content for puff pastry is lower for flour of the cultivars Apollo, Minaret and Hereward than for the other flours. These three flours contain relatively low amounts of damaged starch (Table 1). This indicates that the amount of damaged starch is relatively more important in determining the required water addition in puff pastry production than it is in bread making. Above results are in agreement with the known fact that the main factors that affect the water uptake of flours are (gluten) protein content and the degree of damaged starch [32,33]. The correlation between dough water addition and gluten protein content was the highest for bread dough (r = 0.91), while the correlation between water addition and the content of damaged starch was the highest for pastry dough (r = 0.93).

Table 6*. Bread dough mixing time, bread volume, puff pastry volume, puff pastry height and puff pastry shrinkage in the direction parallel to the direction of the last sheeting action (S.P.) for optimally prepared doughs of flours of six European and two Canadian wheat cultivars.*

Cultivar	**Mixing time for bread**	**Volume bread**	**Volume puff pastry**	**Height puff pastry**	**S.P.**
	(min)	(cm3/g flour)	(cm3/g flour)	(cm/g flour)	(%)
Glenlea	15.0	8.0	11.1	11.0	17.5
Minaret	9.5	7.5	10.4	10.7	19.4
Soissons	12.0	6.9	11.1	11.1	25.0
Hereward	10.0	7.2	11.2	12.3	21.7
Katepwa	7.0	7.6	10.2	9.8	19.1
A6/13	7.5	7.5	9.6	9.6	18.2
Vivant	5.0	6.6	9.0	8.6	19.1
Apollo	5.0	6.7	7.9	8.3	18.6

Puff pastry volumes per g flour were in the same range as those reported by Hay [32] (Table 6). For all cultivars higher volumes were obtained per gram of flour with the puff pastry baking test than with the bread making test. This difference was smallest for Apollo and largest for Hereward and Soissons. Apollo and Vivant that require short mixing times gave low volumes for both bakery products. A6/13 and Katepwa gave a good bread, but a low puff pastry volume.

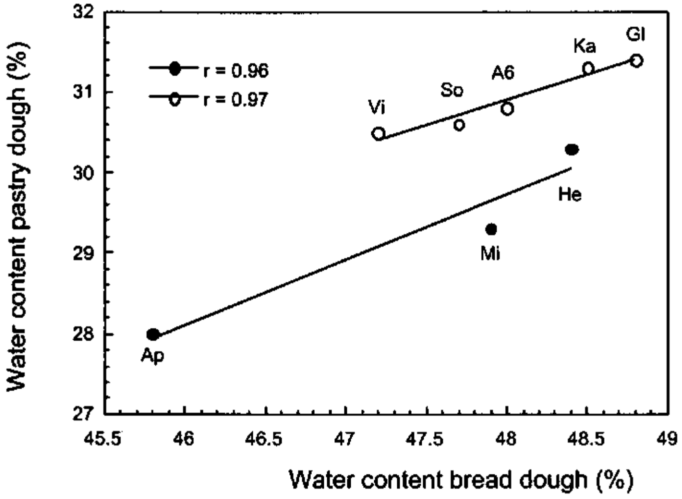


Figure 4. Correlation between bread dough water content and puff pastry dough water content for six European and two Canadian wheat flours.

Minaret, Hereward and Soissons combine long mixing times with intermediate loaf volumes and high puff pastry volumes. In this respect Glenlea is exceptional, because for this flour the longest mixing times, the highest loaf volumes and the best puff pastry products were found. As shown earlier with bread making a positive correlation was found between loaf volume and gluten protein content (r = 0.92; Fig. 3). With pastry production no significant correlation was observed between volume and gluten protein content (r = 0.32). Instead, puff pastry volume was positively correlated with the optimum mixing time for bread production (r = 0.82) as is shown in figure 5. This correlation suggests that these properties have a similar basis. During mixing dough is deformed in a combination of uniaxial extension and shear at relatively high deformation rates [2,34]. There is a suggestion from Gras *et al.* [35] that at least in a mixograph, dough mixing can be viewed as a series of extension tests. During rolling and sheeting dough is also deformed in a combination of uniaxial extension and shear at relatively high deformation rates [2,36]. It is therefore to be expected that doughs react similarly on the deformations applied in both processes. According to Davies *et al.* high pastry volumes can be obtained with flours that form thin dough laminates dough during processing [37]. They found that lift during baking and thus pastry height and volume are mainly governed by the mechanical properties of these thin dough layers, which led them to the conclusion that the rheological properties of the dough before baking have a strong influence on the potential volume-increase of the product. In fact the same holds for bread, the difference being the fermentation stage in bread making in which dough is deformed in biaxial extension at relatively low deformation rates. To produce high puff pastry volumes apparently flours are required that contain relatively high contents of glutenin protein (of large size) since this factor is positively correlated with mixing time [11]. In agreement with these observations Hay reported that flours that contain relatively high glutenin to gliadin ratios gave higher puff pastry heights and volumes [32]. Interestingly, for hearth bread a positive correlation was found between mixing time and loaf volume as well [30]. This suggests that for puff pastry also the absence of a side-wall support could be of importance for baking performance.

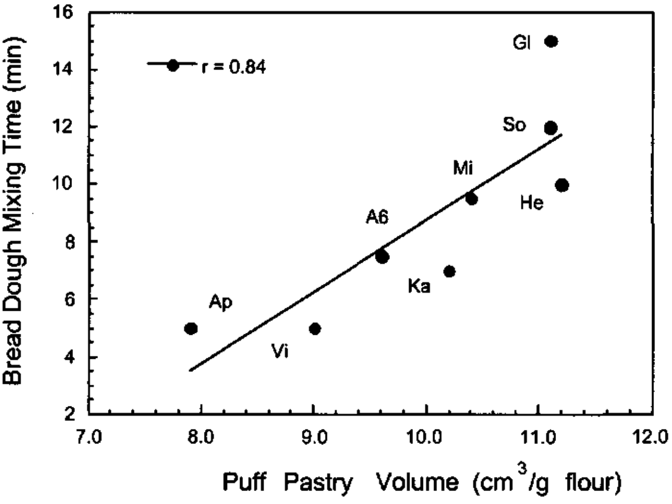


__Figure 5.__ Correlation between bread dough mixing time and puff pastry volume for six European and two Canadian wheat flours.

1.3 Dough rheology

The rheological and fracture properties of the flour doughs of the eight wheat cultivars were determined in uniaxial extension using the Kieffer Extensibility Rig. These results were already presented in a previous paper in which the relationship between protein composition and dough rheology was discussed [11]. Here we will present only a summary of the most essential characteristics in tables 7A and 7B. Considerable differences were observed in stress-level between the respective flours. Higher stress-levels were found for the wheat cultivars Minaret, Hereward and Soissons, intermediate ones for A6/13, Katepwa and Glenlea, while for Apollo and Vivant low stress-levels were observed. Besides for all cultivars an increase in stress was observed with increasing displacement speed of the hook. Also fracture stress and strain increased with increasing hook displacement speed (Table 7B). An increase of fracture stress and strain with increasing deformation rate is typical for viscoelastic materials which consist of various structural elements with different mechanical properties [38]. For flour doughs the rate-dependency of the fracture properties is likely to be caused by the abundance of starch granules [10,38]. All cultivars displayed a more than proportional increase of the stress with increasing strain. The strain rate-dependency of the stress was the highest for the flour doughs that displayed the lowest stress-levels and the lowest strain hardening coefficients. Fracture strain and strain hardening were not correlated ($r < 0.2$).

For puff pastry strong correlations were found between properties of the final product and several stress-related dough properties at large deformations. Puff pastry volume was negatively correlated with the strain rate-dependency of the stress ($r = 0.95$; Fig. 6) and positively with strain hardening of doughs in uniaxial extension ($r = 0.94$; Fig. 7). No significant correlation was found between puff pastry volume and fracture strain ($r < 0.40$). The primary aim of pastry dough processing is the formation of thin alternating layers of dough and fat [36]. In the production of puff pastry by the French method dough thickness is decreased with a factor three in each rolling step [32]. This equals an uniaxial strain of 1.1. In total 6 of these steps are performed which should result in a decrease of dough layer thickness with a factor 729 (3^6) to a value of about 15 μm and a strain in uniaxial extension of 3.3. It has to be noted that in the French method after two rolling steps the doughs are allowed to relax for 30 minutes. It is therefore the question if these calculated strains are really reached. Intuitively one would expect a positive relationship between the fracture strain of sheeted doughs and product volume. The more spots of fracture the more gas can escape, the lower the gas pressure and thus the lower product volume. At high deformation rates such as applied during sheeting the variation in fracture strains between flour doughs was however relatively small. It is therefore to be expected that differences in puff pastry volumes are caused by variation in strain hardening and fracture stress of the dough films between the bubbles.

In an earlier paper we reported that dough mixing time is associated with several factors related with dough strength: positively with strain hardening and the stress at fracture and negatively with the strain rate-dependency of the stress [11]. As mentioned above, the correlation between bread volume and dough mixing time was rather weak. Highest loaf volumes were found for those cultivars which exhibited an intermediate stress-level at relatively large strains (see Tables 6 and 7). In bread making long mixing flours and/ or strong doughs apparently do not give the highest loaf volumes. This is likely to be caused by a low deformability of dough films between gas cells of dough with a high tensile stress, which results in a limitation of the expansion of gas cells during fermentation and baking and with that of the loaf volume that can be obtained. Lowest loaf volumes were found for those cultivars which exhibited a low stress-level, low strain hardening and small to intermediate fracture strains.

***Table 7A**. Water addition, mixing time, stress, strain-dependency of the stress and strain rate-dependency of the stress determined in uniaxial extension for optimally mixed flour doughs of six European and two Canadian wheat cultivars. T = 25°C. Mixing was done in a 10 g Mixograph.*

Cultivar	**Water addition**	**Mixing time**	**σ (kN.m^{-2})**	**dlnσ/dε (-)**	**dlnσ/dln$\dot{\varepsilon}$ (-)**
	(% (w/w) flour)	(min)	(ε=1.75, $\dot{\varepsilon}$=0.01 s^{-1})	($\dot{\varepsilon}$=0.01 s^{-1})	(ε=0.75)
Glenlea	72	14	12.4	1.6	0.15
Minaret	64	14	20.7	1.6	0.16
Soissons	64	16	17.7	1.7	0.13
Hereward	64	14	17.0	1.9	0.15
Katepwa	70	9	12.7	1.5	0.18
A6/13	67	11	12.1	1.6	0.24
Vivant	63	7	7.7	1.3	0.23
Apollo	60	7	4.9	1.1	0.27

***Table 7B**. Stress and strain at fracture in uniaxial extension at two hook displacement speeds for optimally mixed flour doughs of six European and two Canadian wheat cultivars.*
T = 25°C.

Cultivar	**σ_f (kN.m^{-2})**		**ε_f (-)**	
	(v=12 mm. min^{-1})	(v=120 mm. min^{-1})	(v=12 mm. min^{-1})	(v=120 mm. min^{-1})
Glenlea	18	36	2.1	2.3
Minaret	18	26	1.7	1.8
Soissons	16	26	1.9	2.0
Hereward	13	27	1.7	2.0
Katepwa	14	29	2.0	2.2
A6/13	11	24	1.9	2.1
Vivant	5	11	1.6	2.0
Apollo	4	9	1.8	2.0

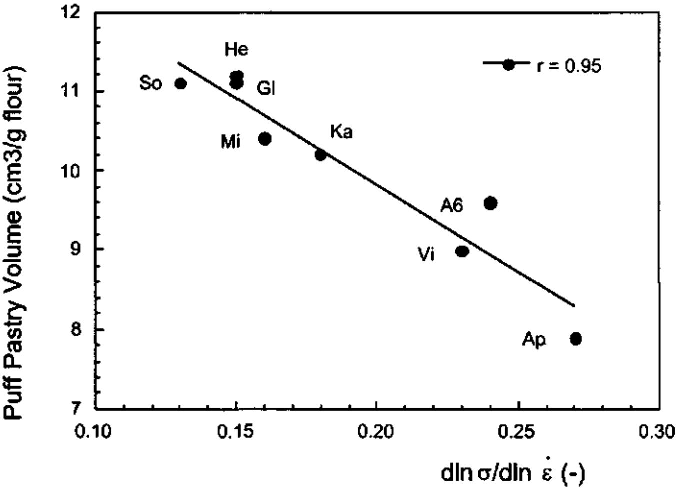


Figure 6. *Correlation between puff pastry volume and the strain rate-dependency of the stress of flour dough for six European and two Canadian wheat flours.*

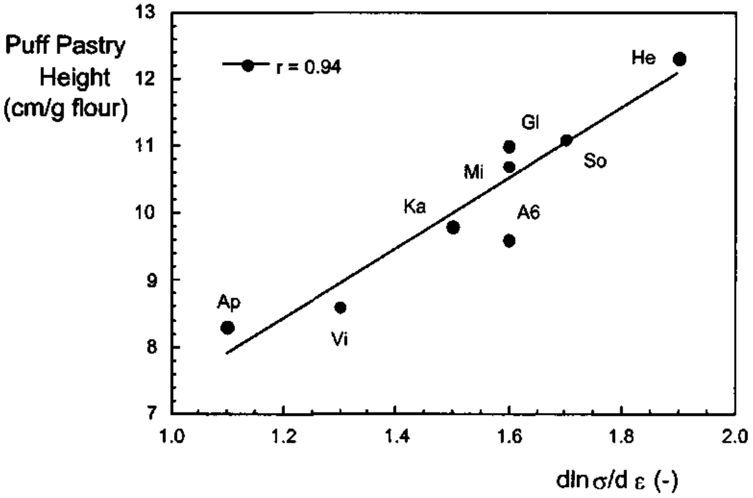


Figure 7. *Correlation between puff pastry height and strain hardening of flour dough for six European and two Canadian wheat flours.*

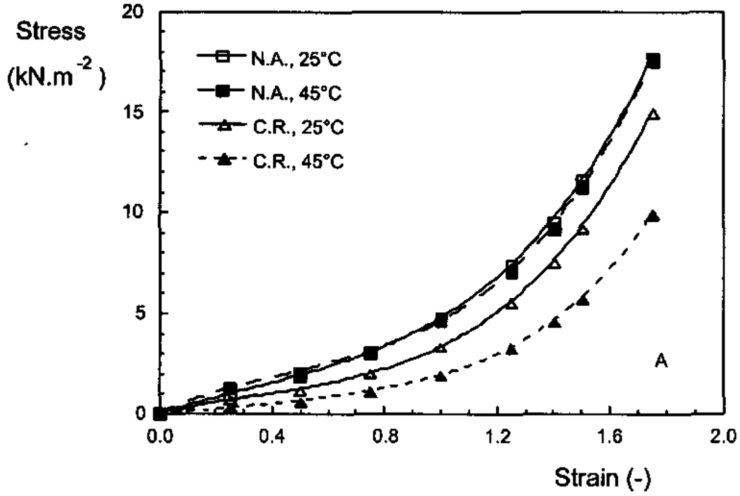
This largely explains the positive correlation between loaf volume and fracture stress at the higher displacement speed of the hook (r = 0.87). It can however seriously be questioned if there is a causal relationship between loaf volume and the fracture stress at high deformation rates. At the lower displacement speed the correlation between loaf volume and fracture stress was less strong (r = 0.75). Strain rates occurring during fermentation are considered to be about a factor 10 lower than those applied by us in uniaxial extension tests [4,6]. Thus, with decreasing displacement speed of the hook the correlation between loaf volume and fracture stress becomes worse. The correlation between bread volume and fracture strain in uniaxial extension was 0.57 and 0.75 for displacement speeds of the hook of 120 $mm.min^{-1}$ and 12 $mm.min^{-1}$, respectively. Thus, the lower the displacement speed the stronger the correlation between loaf volume and fracture strain. It might therefore well be that the correlation between loaf volume and fracture strain improves further with decreasing deformation rate. Both bread volume and fracture strain are positively correlated with gluten protein content; the lower the deformation rate the stronger the correlation. These correlations suggest that for optimally prepared doughs the bread volume that can be obtained is determined to a large extent by the extensibility of dough films. Based on results from baking tests and rheological tests on sheeted doughs Morgenstern and others arrived at the same conclusion [38]. They observed a close similarity between the dependence of the fracture strain and bread volume on the number of sheeting passes.

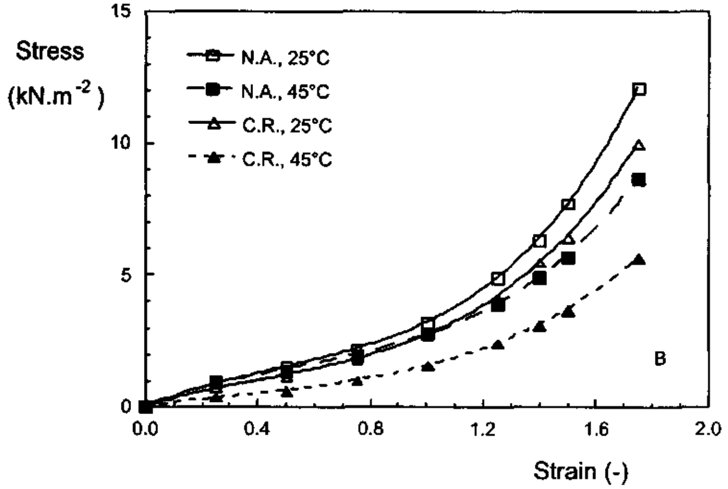
Part 2: three wheat cultivars

2.1 Dough rheology

The effect of temperature and addition of a mix of additives on the rheological and fracture properties of flour doughs was studied for three wheat cultivars that are representative for the whole set of flours, i.e. Vivant, A6/13 and Hereward. It is known that some additives may affect surface-rheological properties [7]. But they may also affect bulk rheological properties. Because the rheological behaviour of dough in baking is not only of importance at room temperature, but as well at elevated temperatures, the doughs were tested also at 45°C.

In figure 8 the stress-strain curves calculated at a constant strain rate ($\dot{\varepsilon}$ = 0.01 s^{-1}) are shown for optimally mixed flour doughs of Hereward, A6/13 and Vivant with and without the mix of additives at 25°C and 45°C. In tables 8A and 8B the most important characteristics that were deduced from these graphs and the fracture properties are shown. In figure 9 stress-strain curves are shown for two displacement speeds of the hook up to fracture. In all cases the use of the mix of additives resulted in a decrease of the stress at relatively small strains. From a strain of 0.5 the relative difference in stress between the curves for flour-water doughs and for doughs containing the mix decreased. For flour doughs of Hereward containing the mix of additives the stress was considerably lower at 45°C than at 25°C, whereas hardly any difference was found between the stress-strain curve at 25°C and 45°C for base flour-water doughs. For this cultivar addition of the mix of additives resulted in a significant increase of the strain hardening coefficient to 2.2, both at 25°C and 45°C. For A6/13 the increase of the strain hardening coefficient as a result of addition of the mix of additives was largest at 45°C, namely from 1.4 to 1.8.





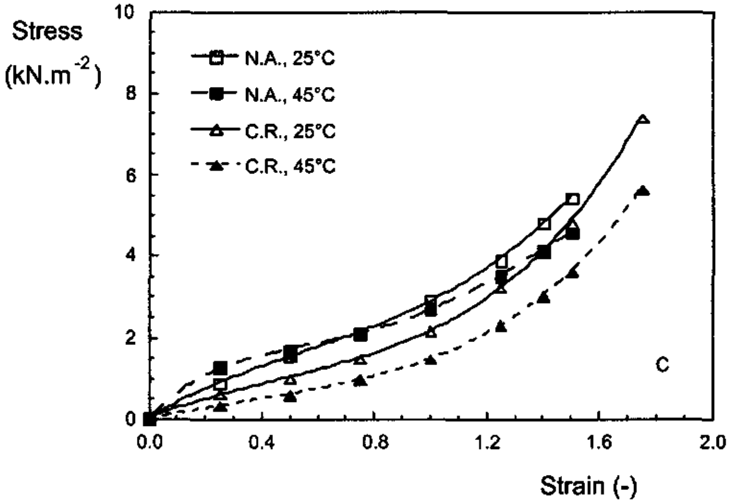
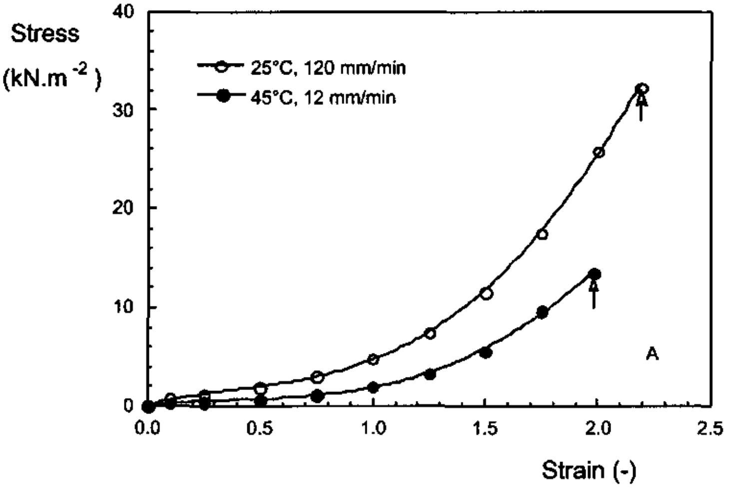


Figure 8. *Uniaxial stress as a function of strain at a constant strain rate of 0.01* s^{-1} *for Hereward (8A), A6/13 (8B) and Vivant (8C) flour dough. N.A. (No Addition) stands for lean flour-water doughs and C.R. (Complete Recipe) stands for flour doughs with mix of additives.*



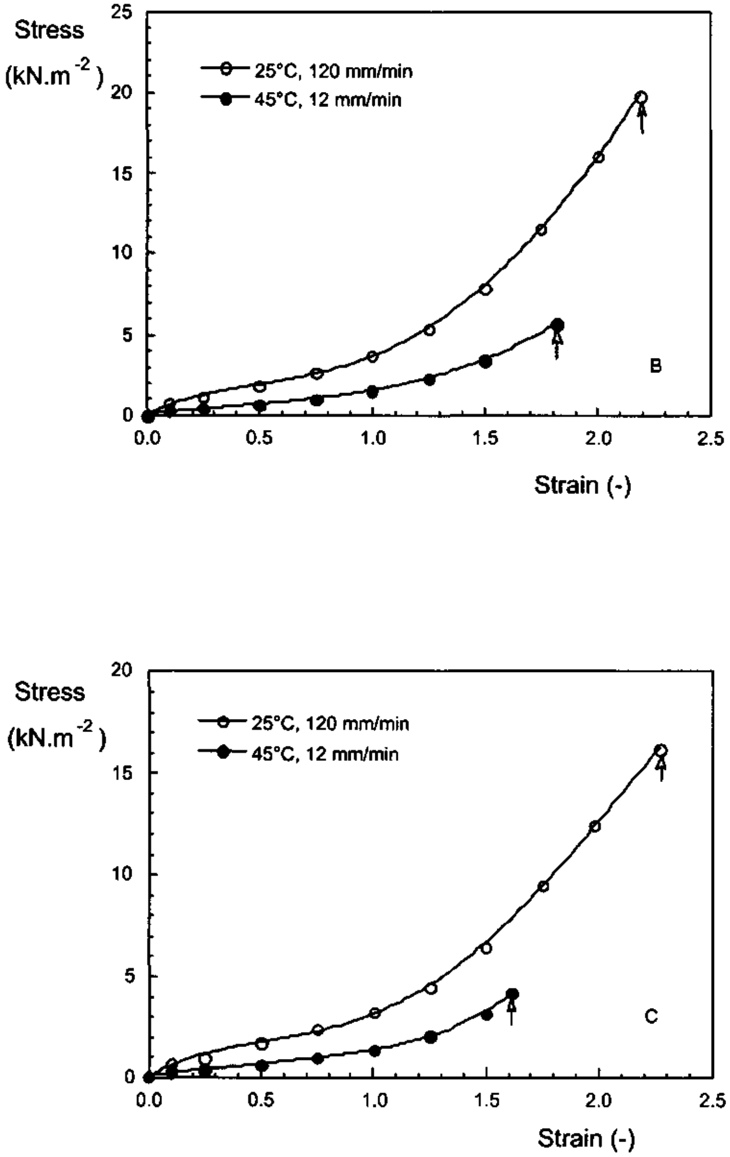


Figure 9. *Uniaxial stress as a function of strain at 25°C and a displacement speed of the hook of 120 mm.min*$^{-1}$ *and at 45°C and a displacement speed of 12 mm.min*$^{-1}$ *for flour doughs with the mix of additives of the wheat cultivars Hereward (A), A6/13 (B) and Vivant (C). The point of fracture is indicated by an arrow.*

***Table 8A**. Stress, strain-dependency of the stress and strain rate-dependency of the stress determined in uniaxial extension for Vivant, A6/13 and Hereward flour doughs mixed for 7, 11, 14 minutes, respectively. N.A. (No Addition) stands for lean flour-water doughs and C.R. (Complete Recipe) stands for flour doughs with mix of additives.*

Cultivar	**Condition**	**σ (kN.m^{-2})**	**σ (kN.m^{-2})**	**$d\ln\sigma/d\varepsilon$ (-)**	**$d\ln\sigma/d\ln\dot{\varepsilon}$ (-)**
		(ε=0.50, $\dot{\varepsilon}$=0.01 s^{-1})	(ε=1.50, $\dot{\varepsilon}$=0.01 s^{-1})	($\dot{\varepsilon}$=0.01 s^{-1})	(ε=0.75)
Hereward	N.A./25°C	1.9	11.6	1.8	0.15
	N.A./45°C	2.0	11.3	1.7	0.17
	C.R./25°C	1.0	8.7	2.2	0.17
	C.R./45°C	0.6	5.7	2.2	0.11
A6/13	N.A./25°C	1.5	7.7	1.6	0.24
	N.A./45°C	1.4	5.7	1.4	0.13
	C.R./25°C	1.2	6.4	1.7	0.18
	C.R./45°C	0.6	3.7	1.8	0.15
Vivant	N.A./25°C	1.6	5.4	1.3	0.23
	N.A./45°C	1.7	4.6	1.0	0.15
	C.R./25°C	1.0	4.8	1.6	0.20
	C.R./45°C	0.6	3.6	1.8	0.20

Also for Vivant a large difference between the stress-strain curves of flour-water doughs with and without the set of additives was found. Starting from a strain of 0.25 the stress increased less strongly for flour-water doughs than for doughs to which the mix was added. This resulted in large differences in strain hardening coefficients (Table 8A), indicating a striking improvement of the dough properties at large deformations.

Addition of the mix resulted in a remarkable increase of the fracture strain for Vivant dough and a concomitant increase of the fracture stress. Addition of the mix of additives to Hereward flour also resulted in a remarkable increase of the fracture strain. However, no increase in fracture stress was seen. For A6/13 addition of the mix of additives only slightly affected the fracture strain and resulted in lower fracture stresses for mix-containing than for flour-water doughs. So, regarding the fracture properties the three wheat cultivars reacted differently on the change of recipe. As a result of these changes the difference in fracture properties between A6/13 and Vivant decreased strongly. A similar conclusion could be drawn after analysis of the stress-strain curves at a constant strain rate of 0.01 s^{-1}. However, at the lower displacement speed of the hook the fracture strain was significantly smaller for Vivant than for A6/13 dough. And because during proofing and baking relatively low strain rates are applied ($\dot{\varepsilon} = 10^{-4} - 10^{-3}$ s^{-1}) the difference in bread making performance between A6/13 and Vivant is probably related to this difference in strain rate and temperature-dependency of the fracture strain.

To summarise, addition of the mix resulted in a decrease of the stress and an increase of the strain hardening coefficient for all cultivars. The effect of addition of the mix on fracture strain differed between the cultivars. The effect of addition of the mix was particularly large at 45°C. An increase of strain hardening and fracture strain should have a positive effect on baking performance. The same probably holds for the lower stress level at low strain rates. For a better understanding of the relation between the behaviour of flour dough and baking quality it seems necessary to use similar recipes for both experiments.

Table 8B*. Stress and strain at fracture in uniaxial extension at two hook displacement speeds for Vivant, A6/13 and Hereward flour doughs mixed for 7, 11, 14 minutes, respectively. N.A. (No Addition) stands for lean flour-water doughs and C.R. (Complete Recipe) stands for flour doughs with mix of additives.*

Cultivar	**Condition**	σ_f (kN.m^{-2})		ε_f (-)	
		(v=12 mm. min^{-1})	(v=120 mm. min^{-1})	(v=12 mm. min^{-1})	(v=120 mm. min^{-1})
Hereward	N.A./25°C	13	27	1.7	2.0
	N.A./45°C	17	24	1.8	1.9
	C.R./25°C	17	32	2.0	2.2
	C.R./45°C	13	25	2.0	2.2
A6/13	N.A./25°C	11	24	1.9	2.1
	N.A./45°C	7	14	1.8	2.0
	C.R./25°C	11	20	2.0	2.2
	C.R./45°C	6	8	1.8	1.9
Vivant	N.A./25°C	5	11	1.6	2.0
	N.A./45°C	3	6	1.3	1.6
	C.R./25°C	8	16	2.0	2.3
	C.R./45°C	4	8	1.6	1.9

2.2 Dough rheology and baking quality

The effect of addition of a mix of additives on the large-deformation properties of flour dough was studied for three wheat cultivars. Of these three cultivars the lowest bread volume was obtained with Vivant and the highest with A6/13. As a result of addition of the mix the differences between the rheological and fracture properties of the flour doughs of these two cultivars strongly diminished, because the dough properties of these two cultivars improved to a different extent. The rheological and fracture properties of mix-containing doughs depended to a higher extent on strain rate and temperature for Vivant than for A6/13. At 25°C and the highest displacement speed of the hook fracture stress and strain were similar for both cultivars, while at 45°C and the lowest displacement speed fracture stress and strain were clearly lower for Vivant. So, fracture strains for Vivant dough will be smaller at lower strain rates and higher temperatures. The possibility of fracture of dough films and with that a decrease of loaf volume and a deterioration of baking quality is the largest during the baking stage [4,6]. In the oven dough films are deformed at relatively low strain rates at higher temperatures. Under these conditions the fracture properties of Vivant flour dough are inferior to those of A6/13. It is this difference in rheological behaviour that presumably causes the difference in baking performance between the two flours. And it confirms the previously reported finding for flour-water doughs that the strain rate- and temperature-dependency of the fracture stress and strain is an important factor in dough rheology [11].

Of the three more intensively studied cultivars Vivant gave the lowest puff pastry volume and Hereward the highest. The puff pastry volume of A6/13 flour was in between but closer to that of Vivant. For the production of high pastry volumes thin dough laminates are required [37]. It is therefore easily understood that strain hardening is such an important property in puff pastry production. For mix-containing flour doughs stress-level was much higher and strain hardening was much stronger for Hereward than for Vivant and A6/13. Additionally, the strain rate- and temperature-dependency of the stress and fracture strain were lowest for Hereward. For lean flour-water doughs it was concluded that differences in puff pastry volume are mainly due to variation in strain hardening and fracture stress between flours. Addition of the mix did not give rise to change this conviction.

CONCLUSIONS

The bread making performance of white flours is strongly influenced by the amount of water added to a flour and the time a dough is mixed. For most cultivars best baking results are obtained at the highest water content yielding doughs that are still manageable at the stage of panning. It appears that in puff pastry production where lower water additions are being used, the amount of damaged starch is relatively more important in determining the required water addition than for the production of bread.

A flour type can be well-suited for one product and less suited for another. These differences may reflect differences in dough composition and processing. A positive correlation was found between the mixing time for bread dough and puff pastry volume. In other words flours that require longer mixing in bread making give higher volumes in puff pastry production. Apparently, both phenomena depend on the same basic properties. As well in mixing as in rolling dough is deformed in a combination of uniaxial extension and shear at relatively high deformation rates. It is therefore to be expected that puff pastry volume strongly depends on the rheological properties of the dough before baking.

Addition of a mix of additives as used in bakery practice resulted in most cases in a decrease of the stress at a certain strain, an increase of the strain hardening coefficient and an increase of the fracture strain. The effect of the mix of additives was largest at 45°C. From the increase in strain hardening and fracture strain a positive effect is to be expected on baking performance. For a better understanding of the relation between the behaviour of flour dough and baking quality the use of similar recipes for both experiments is to be preferred.

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Abbreviations

CV=coefficient of variation
DTT=dithiothreithol
EDTA=ethylenediamidetriammonium
G'=storage modulus
HMWgs=high molecular weight glutenin subunits
LMWgs=low molecular weight glutenin subunits
MMW=medium molecular weight proteins
P1=polymeric protein of large size
P2=polymeric protein of relatively small size
SDS=sodiumdodecylsulphate
SDS-PAGE=sodiumdodecylsulphate-polyacrylamide gelelectrophoresis
tan δ=tangent of the phase angle
UEP=SDS-unextractable polymeric protein

Summary

Flour composition, dough rheology and baking quality

Grain samples of Six European and two Canadian wheat cultivars that perform differently in cereal products were selected and milled to a milling degree of approximately 70% and an ash content of about 0.47%. Protein contents varied from 10.3 to 13.4%. Using a modified Osborne sequential extraction method and a combination of SDS-PAGE and laser scanning densitometry the gluten protein composition of the flours was studied. The size distribution of the glutenin fraction of glutens isolated from the flours was studied by gel filtration after extraction in a SDS-buffer. We concluded that the flours involved in this study differ considerably regarding gluten protein composition. The total amount of HMWgs of the flours was strongly associated with protein characteristics that are commonly used to describe differences in gluten protein composition as the gliadin to glutenin ratio, glutenin content, the LMWgs to HMWgs ratio and glutenin size distribution. This is no surprise, since an increase in HMWgs content should lead to an increase of the amount of glutenin and to a decrease of the LMWgs to HMWgs ratio and the gliadin to glutenin ratio. Therefore the HMWgs content of wheat is an important parameter for flour quality and as we shall see later for the large-deformation properties of wheat flour and gluten dough. In our data set the content of y-type HMWgs was fairly constant for all flours. On the contrary large differences were found in the content of x-type HMWgs. Therefore the variation in x-type HMWgs is responsible for most of the variation in HMWgs content between the flours.

Extensive baking tests were performed as a function of water addition and mixing time for bread and as a function of water addition for puff pastry. The results of the bread making tests are presented as working areas for every flour individually. Combinations of water addition and mixing time within the working area of a flour resulted in an acceptable to good product quality, while combinations outside the working area led to inferior product quality. Large differences were found between the cultivars in the size and place of the working areas within which good baking quality could be expected. Correlations between loaf volume and bread dough mixing time were positive, but weak ($r = 0.61$), while correlations between loaf volume and gluten protein content were positive and strong ($r = 0.93$). This suggests that, since long mixing flours contain relatively more glutenin protein, for optimally developed bread doughs the loaf volume that can be obtained depends to a larger extent on gluten protein content and to a lesser extent on gluten protein composition.

For all cultivars higher volumes were obtained per gram of flour with the puff pastry baking test than with the bread making test. Flours that require short mixing times in bread making, gave low volumes for both bakery products. Flours requiring intermediate mixing times, gave a good bread, but a low puff pastry volume, while flours requiring long mixing times gave intermediate loaf volumes and high puff pastry volumes. Glenlea is exceptional, because for this flour the longest mixing times, the highest loaf volumes and the best puff pastry products were found. The amount of damaged starch was relatively more important for determining the required water addition in puff pastry production where lower water additions are used than in the production of bread. For all cultivars the percentage of shrinkage parallel to the direction of the last sheeting action was higher than the shrinkage percentage perpendicular to that direction. On average shrinkage percentage was lower for low volume flours than for flours with which high puff pastry volumes could be obtained.

The rheological and fracture properties of the flour doughs of all cultivars were determined in uniaxial extension using the Kieffer Extensibility Rig. All cultivars displayed a more than proportional increase of the stress with increasing strain, a phenomenon called strain hardening. Between the flours considerable differences were observed in stress-level, strain hardening, strain rate-dependency of the stress, fracture stress and fracture strain. The stress at a chosen strain was positively correlated with the percentage of polymeric protein of large size, while a negative correlation was found between the strain rate-dependency of the stress and the percentage of HMWgs on total protein. These findings are consistent with the known strong dependence of rheological properties on molecular weight and molecular weight distribution for polymers in general. No correlation, positive or negative, was found between fracture strain and gluten protein composition. Fracture strain was positively correlated with gluten protein content, the higher the displacement speed of the hook the stronger the correlation. For all doughs fracture stress and strain increased with increasing displacement speed of the hook. The observed time-dependency of fracture properties can best be explained by inefficient transport of energy to the crack tip. Presumably, this is caused by energy dissipation due to inhomogeneous deformation resulting in energy dissipation by friction processes between structural elements, e.g. between dispersed particles and the network. For flour dough the rate-dependency of the fracture properties is likely to be caused by the abundance of starch granules.

Several stress-related dough properties of the set of flours under study were strongly correlated with mixing time of bread dough. That mixing time and dough strength are strongly correlated is no surprise, but consistent with what should be expected for this kind of viscoelastic materials. In this context the observation that stress relaxation in wheat flour dough occurs over a spectrum of relaxation times is essential. The latter is easy to understand if one realises that flour dough has a very complex composition (starch, proteins, non-starch carbohydrates), most for network formation essential gluten proteins are present in aggregates with a wide size distribution and the structure elements in dough are connected by several types of bonds. Taking this into account a picture starts to develop for the mechanical properties of flour and gluten dough in which the tendency for aggregation plays a central role. Doughs in which relatively more strong bonds with a long relaxation time are formed in and between the protein aggregates will exhibit on average a relatively long relaxation time. If such a material is deformed at a chosen strain rate only a relatively low amount of bonds will relax during deformation and the stress will strongly increase deformation. In a dough in which relatively weak bonds are formed many will relax during deformation causing that at the same strain rate the stress will increase less. Apparently, longer mixing times are required to reach a certain level of dough development for doughs in which stronger bonds are formed.

Puff pastry volume was positively correlated with strain hardening and negatively with the strain rate-dependency of the stress. To produce high puff pastry volumes apparently strong flours are required that contain relatively high contents of glutenin protein (of large size). The primary aim of pastry dough processing is the formation of thin alternating layers of dough and fat. Intuitively one would expect a positive relationship between the fracture strain of sheeted doughs and product volume. The more spots of fracture the more gas can escape, the lower the gas pressure and thus the lower product volume. At high deformation rates such as applied during sheeting the variation in fracture strains between flour doughs was however relatively small. It is therefore to be expected that differences in pastry volumes are caused by variation in strain hardening and fracture stress.

Mixing time of bread dough was negatively correlated with the strain rate-dependency of the stress of flour-water dough and positively with strain hardening and fracture stress. The correlation between bread volume and dough mixing time was rather weak. Highest loaf volumes were found for those cultivars which exhibited an intermediate stress-level at relatively large strains. In bread making long mixing flours and/or strong doughs apparently do not give the highest loaf volumes. The most likely explanation is that a high internal stress limits the deformability of dough films between gas cells and with that the loaf volume that can be obtained.

Large-deformation properties of wheat flour and gluten dough in uni- and biaxial extension

Rheological and fracture properties of flour and gluten doughs from three wheat cultivars that were representative for the whole set of flours were studied in uniaxial and biaxial extension. Besides doughs were tested in small angle sinusoidal oscillation. In accordance with previously published results the linear region of flour dough was found to be very small. The rheological properties at small deformations hardly depended on the cultivar. A higher water content of the dough resulted in a lower value for the storage modulus and a slightly higher value for tan δ. At 25°C the linear region of gluten dough was in the same range as that of flour dough, while at a higher temperature (45°C) the linear region had increased by more than an order of magnitude. At 45°C the storage modulus and tan δ were lower than at 25°C. Variations in moduli between cultivars were much more pronounced for gluten than for flour doughs.

For flour dough a more than proportional increase in stress was found with increasing strain, a phenomenon called strain hardening, in both uniaxial and biaxial extension. Differences in strain hardening between flours were larger in uniaxial than in biaxial extension. Stresses at a certain strain were much higher and the stress was less dependent on the strain rate in uniaxial than in biaxial extension, indicating that in elongational flow orientational effects are of large importance for the mechanical properties of flour dough.

Similarly to flour dough also gluten dough displayed strain hardening in both uniaxial and biaxial extension. The stress at a chosen strain and strain hardening depended much stronger on the type of deformation for gluten than for flour dough: the stress was higher in biaxial extension for gluten than for flour dough, but was much higher in uniaxial extension. This indicates that orientational effects in elongational flow are of even larger importance for the mechanical properties of gluten than of flour dough. A strong orientational effect is consistent with published data on birefringence of stretched gluten. Presumably it is the glutenin fraction that because of its large size confers these direction dependent properties to gluten and flour doughs.

Differences in fracture strain between the flour doughs were relatively small at 25°C and at the higher displacement speed of the hook. At the lower displacement speed of the hook fracture strain increased in the order Vivant < Hereward < A6/13. At both displacement speeds of the hook values for fracture stress were highest for Hereward, somewhat lower for A6/13 and much lower for Vivant.

The large-deformation properties of flour and gluten doughs were also studied in uniaxial extension at 45°C. For Vivant and A6/13 flour dough the stress, the strain hardening coefficient and the strain rate-dependency of the stress decreased with increasing temperature. For Hereward only a decrease in the strain hardening coefficient was observed with increasing temperature, while the other values hardly changed. For Hereward gluten the stress-level increased slightly with increasing temperature, while the stress-level for Vivant and A6/13 gluten decreased. Strain hardening coefficients of gluten doughs of the three cultivars were similar no matter gluten type and temperature.

With increasing temperature fracture strain remained unchanged for Hereward flour dough and decreased slightly for A6/13. The most drastic effects were noted for Vivant: The fracture strain decreased from 1.6 to 1.3 at the lower displacement speed of the hook and from 2.0 to 1.6 at the higher speed. So, at lower deformation rates and higher temperatures differences in fracture strain between cultivars were very large, while at 25°C and the higher extension rate the difference was only small. For a weaker flour dough (i.e. which has a low protein content and/or a low glutenin content) the fracture strain apparently depends to a larger extent on strain rate and temperature than for a stronger flour.

The observed changes in the stress with increasing temperature were similar for flour and gluten dough for each cultivar. It appears therefore that for flour dough the effect of temperature on the stress is determined to a large extent by the gluten fraction. This trend is also reflected in the fracture stress. However, contrary to what was observed for flour dough the fracture strain for gluten dough did not decrease with increasing temperature, but showed a slight increase. Moreover, the fracture strain depended to a lesser extent on the strain rate for gluten than for flour dough. Another difference is that for flour dough the smallest fracture strain was found for the cultivar with the lowest glutenin content (Vivant), while for gluten dough the smallest fracture strain was found for the cultivar with the highest glutenin content (Hereward). So, regarding the effect of protein composition, strain rate and temperature on the fracture strain the behaviour of flour and gluten dough differs. The difference in the effect of protein composition on the fracture strain between flour and gluten dough might well be caused by the fact that in flour dough the amount of glutenins of large size is limiting, while in gluten dough it is the amount of gliadin that can be too low. It shows that to reach maximum fracture strains the content of glutenin polymers of large size in flour and gluten dough should be not too low, but also not too high.

The effect of addition of a mix of additives on the rheological and fracture properties of flour doughs was studied at 25°C and at 45°C. In all cases the use of the mix of additives resulted in a decrease of the stress at relatively small strains. For Hereward addition of the mix resulted in a significant increase of the strain hardening coefficient to 2.2 and a remarkable increase of the fracture strain. For A6/13 the increase of the strain hardening coefficient was largest at 45°C, namely from 1.4 to 1.8, while fracture strains were only slightly affected. For Vivant a striking improvement of the dough properties was observed: strain hardening coefficients increased from 1.3 to 1.6 at 25°C and from 1.0 to 1.8 at 45°C, while fracture strains increased from 2.0 to 2.3 at 25°C and from 1.6 to 1.9 at 45°C. A concomitant increase of the fracture stress was found. As a result of these changes the difference in fracture properties between A6/13 and Vivant decreased strongly. However, at the lower displacement speed of the hook and the higher temperature the fracture strain remained significantly smaller for Vivant than for A6/13 dough. And because during proofing and baking relatively low strain rates are applied ($\dot{\varepsilon} = 10^{-4} - 10^{-3}\ s^{-1}$) the difference in bread making performance between A6/13 and Vivant in the presence of the mix of additives is probably related to this difference in strain rate and temperature-dependency of the fracture strain.

Premature fracture of a dough film at a thin spot is prevented by the occurrence of strain hardening, which is defined as a more than proportional increase of the stress with increasing strain. If strain hardening occurs depends if the proportionality limit is exceeded or not. For uniaxial extension the relative increase of the stress with a relative increase of the strain should be higher than 1.0, while for biaxially extended dough films strain hardening coefficients should be higher than 2.0. When strain hardening coefficients come close to the criterion value, as was found for Vivant flour dough in uniaxial extension, fracture occurred at much smaller strains. Since the values for strain hardening in biaxial extension we have found were lower than 2.0, the specific power limit for flour dough in uniaxial extension is never exceeded. Fracture of dough films should therefore always occur at relatively small strains. However in reality the criterion in biaxial extension is less strict, among others because variations in strain rate with strain should be taken into account. More information on the effect of the type of deformation on the fracture strain and on the relationship between strain hardening and fracture strain is strongly desired.

During fermentation and oven rise dough films are extended biaxially. In the baking industry an alternative method for the production of cookies, pizza, bread and pastry doughs is sheeting and laminating. In this method the development of a two-dimensional structure in the dough rather than an unidirectional one is promoted by combining the sheeting action of the rollers with rotation of the axis over 90° of the dough between successive passages. Dough development using sheeting rolls is about 6 times as efficient with regard to energy consumption than the use of the conventional pin mixer. Additionally with dough rollers bread with an exceptionally fine texture can be produced. It seems therefore that the development of a cross-hatched, two-dimensional structure in dough is superior to the use of conventional mixers. Also this aspect deserves further study.

SAMENVATTING

Bloemsamenstelling, deegreologie en bakkwaliteit

Voor het onderzoek werden graanmonsters van zes Europese en twee Canadese tarwerassen van verschillende bakkwaliteit geselecteerd en gemalen tot een uitmalingsgraad van ongeveer 70%. De eiwitgehaltes in de bloem varieerden van 10.3 tot 13.4%. De gluten eiwitsamenstelling van de bloemsoorten is bestudeerd met behulp van een aangepaste Osborne's extractiemethode en een combinatie van SDS-PAGE en laser scanning densitometrie. De grootteverdeling van de gluteninefractie van de uit de bloemsoorten geïsoleerde gluten werd bestudeerd met gelfiltratie na extractie in een SDS-buffer. Wij concludeerden dat de bloemsoorten aanzienlijk behoorlijk verschillen in gluten eiwitsamenstelling. De totale hoeveelheid HMWgs van de bloemsoorten was sterk geassocieerd met eiwitkarakteristieken die gewoonlijk gebruikt worden om de variatie in gluten eiwitsamenstelling te beschrijven zoals de gliadine-glutenine verhouding, het glutenine gehalte, de LMWgs-HMWgs verhouding de deeltjesgrootteverdeling van glutenine. Dit is niet verrassend, omdat een toename van de hoeveelheid HMWgs zou moeten leiden tot een toename in de hoeveelheid glutenine en een afname van de LMWgs-HMWgs verhouding en de gliadine-glutenine verhouding. Het is daarom dat het gehalte HMWgs een belangrijke parameter is voor bloem kwaliteit en zoals we verderop zullen zien ook voor de grote vervormingseigenschappen van deeg van tarwebloem en gluten. In onze data set was het gehalte y-type HMWgs redelijk constant voor alle bloemsoorten. In het gehalte x-type HMWgs werden echter grote verschillen gevonden. Daarom is de variatie in x-type HMWgs verantwoordelijk voor het grootste deel van de variatie tussen bloemsoorten in de totale hoeveelheid HMWgs.

Een groot aantal bakproeven werden uitgevoerd voor brood als functie van de vochtgift en de kneedtijd voor brood en voor bladerdeeg als functie van de vochtgift. De resultaten van de broodbakproeven zijn voor iedere individuele bloem gepresenteerd als werkgebieden in een vochtgift/kneedtijd diagram. Combinaties van vochtgift en kneedtijd binnen het werkgebied van een bloem resulteerden in een acceptabele tot goede productkwaliteit, terwijl combinaties buiten het werkgebied leidden tot een inferieure productkwaliteit. Tussen de rassen werden grote verschillen gevonden in de grootte en de ligging van de werkgebieden waarbinnen de bakkwaliteit goed was. Correlaties tussen broodvolume en kneedtijd van brooddeeg waren positief, maar zwak ($r = 0.61$), terwijl correlaties tussen broodvolume en gluteneiwitgehalte positief en sterk waren ($r = 0.93$). Aangezien bloemsoorten die een langere kneedtijd vereisen relatief meer glutenine bevatten, suggereert dit dat voor optimaal ontwikkelde degen het broodvolume dat bereikt kan worden sterker afhangt van de hoeveelheid gluteneiwit in de bloem, dan van de samenstelling ervan.

Voor alle rassen werden per gram bloem hogere volumes verkregen met de bakproef voor bladerdeeg dan met die voor brood. De bloemsoorten die in de broodbereiding korte kneedtijden vereisen, gaven lage volumes voor beide bakkerijproducten. Bloemsoorten die een gemiddelde kneedtijd nodig hadden, gaven goed brood, maar lage bladerdeegvolumes, terwijl bloemsoorten die een lange kneedtijd nodig hebben, gemiddelde volumes gaven voor brood en hoge voor bladerdeeg. Glenlea is een uitzondering, omdat met deze cultivar de langste kneedtijden, de hoogste broodvolumes en de beste bladerdeegproducten werden verkregen. De benodigde hoeveelheid vocht was sterker afhankelijk van de hoeveelheid beschadigd zetmeel bij de bereiding van bladerdeeg waar lagere vochtgiften worden gebruikt dan bij de broodbereiding. Voor alle rassen was het percentage krimp tijdens het bakken parallel aan de richting van de laatste deegtoer hoger dan het percentage krimp in de richting daar loodrecht op. Gemiddeld genomen was het percentage krimp lager voor bloemsoorten die lage volumes gaven dan voor bloemsoorten waarmee hoge bladerdeegvolumes verkregen konden worden.

De reologische en breukeigenschappen van bloemdegen van alle cultivars werden onderzocht in uniaxiale rek met de ‘Kieffer Extensibility Rig’. Alle rassen lieten een meer dan evenredige toename van de spanning zien bij een toenemende vervorming, een fenomeen dat rekversteviging heet. Tussen de bloemsoorten werden aanmerkelijke verschillen gevonden in de hoogte van de spanning, de mate van rekversteviging, de mate waarin de spanning afhangt van de vervormingssnelheid, de breukspanning en de breukvervorming. De spanning bij een bepaalde vervorming was positief gecorreleerd met het percentage hoogpolymeer gluteneiwit. Een negatieve correlatie werd gevonden tussen de snelheidsafhankelijkheid van de spanning en het percentage HMWgs op totaal eiwit. Deze bevindingen zijn consistent met de bekende sterke afhankelijkheid van reologische eigenschappen van molecuulgewicht en molecuulgewichtsverdeling van polymeren in het algemeen. Geen correlatie, positief of negatief, werd gevonden tussen de breukvervorming en de gluteneiwitsamenstelling. De breukvervorming was positief gecorreleerd met het gluteneiwitgehalte, des te hoger de verplaatsingssnelheid van de haak des te sterker de correlatie. Voor alle degen was de breukspanning en de breukvervorming hoger bij een hogere verplaatsingssnelheid van de haak. De waargenomen tijdsafhankelijk van de breukeigenschappen kan het best verklaard worden uit inefficiënt energietransport naar de breukpunt. Het is aannemelijk dat dit veroorzaakt wordt door energiedissipatie tengevolge van inhomogene vervorming, resulterend in energiedissipatie door frictieprocessen tussen de structuurelementen, bijvoorbeeld tussen gedispergeerde deeltjes en het netwerk. Voor bloemdeeg wordt de snelheidheidsafhankelijkheid van de breukeigenschappen waarschijnlijk veroorzaakt door de overvloed aan zetmeelkorrels.

Een aantal spanningsgerelateerde deegeigenschappen van de set van bloemsoorten was sterk gecorreleerd met de kneedtijd van brooddeeg. Dat kneedtijd en deegsterkte sterk gecorreleerd zijn is geen verrassing, maar consistent met wat verwacht mag worden voor dit soort viscoelastische materialen. In dit verband is essentieel dat spanningsrelaxatie in deeg van tarwebloem plaatsvindt over een breed spectrum van relaxatietijden. Dit laatste is eenvoudig te begrijpen als men zich realiseert dat bloemdeeg een complexe samenstelling heeft (zetmeel, eiwit, niet-zetmeel koolhydraten), de meeste voor netwerkvorming belangrijke gluteneiwitten aanwezig zijn in de vorm van aggregaten met een brede grootteverdeling en de structuurelementen in deeg onderling verbonden zijn door verschillende typen bindingen. Dit resulteert in een beeld waarin de mechanische eigenschappen van deeg van bloem en gluten sterk bepaald worden door de neiging tot aggregatie van de glutendeeltjes. Degen waarin relatief meer sterke bindingen met een langere relaxatietijd gevormd worden tussen en in de eiwitaggregaten zullen gemiddeld genomen als geheel een lange relaxatietijd laten zien. Als een dergelijk materiaal wordt vervormd bij een bepaalde vervormingssnelheid zal gedurende de vervorming slechts een relatief klein deel van de bindingen relaxeren en de spanning sterk toenemen met de vervorming. In een deeg waarin relatief zwakke bindingen zijn gevormd zullen veel meer bindingen relaxeren gedurende de vervorming wat er toe leidt dat bij een gelijke vervormingssnelheid de spanning minder zal toenemen. Blijkbaar zijn langere kneedtijden nodig om een bepaald niveau van deegontwikkeling te bereiken voor degen waarin sterkere bindingen zijn gevormd.

Het volume voor bladerdeeg was positief gecorreleerd met de rekversteviging en negatief met de snelheidsafhankelijkheid van de spanning in bloemdeeg bij een bepaalde vervorming. Om hoge bladerdeegvolumes te bereiden zijn blijkbaar sterke bloemsoorten nodig die relatief veel glutenine bevatten. Het belangrijkste doel bij de bereiding van bladerdeeg is de vorming van dunne afwisselende lagen van deeg en vet. Intuïtief zou men een positieve relatie verwachten tussen de breukvervorming van getoerde degen en het productvolume. Des te meer breukplaatsen, des te meer gas kan ontsnappen, des te lager de gasdruk en dus des te lager het productvolume. Bij de hoge vervormingssnelheden zoals die worden toegepast bij de bereiding van bladerdeeg was de variatie tussen de bloemsoorten in breukvervorming echter relatief klein. Het is daarom te verwachten dat verschillen in bladerdeegvolumes veroorzaakt worden door variatie in rekversteviging en breukspanning.

De kneedtijd voor brooddeeg was negatief gecorreleerd met de snelheidsafhankelijkheid van de spanning in bloemdeeg bij een bepaalde vervorming en positief met de rekversteviging en de breukspanning. De correlatie tussen broodvolume en kneedtijd was zwak. De hoogste broodvolumes werden gevonden voor degen van de rassen die een gemiddeld spanningsniveau lieten zien bij relatief grote vervormingen. In de broodbereiding geven bloemsoorten die een lange kneedtijd nodig hebben en/of sterke degen blijkbaar niet de hoogste volumes. De meest waarschijnlijke verklaring hiervoor is dat een hoge spanning benodigd voor het vervormen van deeg beperkingen oplegt aan de uitrekbaarheid van deegfilms tussen gasbellen als gevolg van een bepaalde gasproductie en daarmee aan het broodvolume dat kan worden verkregen.

Grote vervormingseigenschappen van deeg van tarwebloem en gluten in uni- en biaxiale rek

De reologische en breukeigenschappen van degen van bloem en gluten van drie tarwerassen die representatief zijn voor de hele set werden bestudeerd in uniaxiale en biaxiale rek. Daarnaast werden dynamische metingen bij kleine vervormingen uitgevoerd. In overeenstemming met eerder gepubliceerde resultaten werd voor bloemdeeg een erg klein lineair gebied gevonden. De reologische eigenschappen bij kleine vervormingen hingen nauwelijks af van het ras. Een hoger watergehalte van het deeg resulteerde in een lagere waarde voor de opslagmodulus en een enigszins hogere waarde voor tan δ. Bij 25°C was het lineaire gebied voor glutendeeg vergelijkbaar met dat van bloemdeeg, terwijl het bij een hogere temperatuur (45°C) met meer dan een orde van grootte was toegenomen. Bij 45°C waren de opslagmodulus en tan δ voor glutendeeg lager dan bij 25°C. Verschillen in moduli tussen cultivars waren veel uitgesprokener voor gluten dan voor bloemdeeg.

Voor bloemdeeg werd een meer dan evenredige toename van de spanning met toenemende vervorming gevonden, een fenomeen dat rekversteviging heet, in zowel uniaxiale en biaxiale rek. Verschillen in rekversteviging tussen bloemsoorten waren groter in uniaxiale dan in biaxiale rek. De rekspanning bij een bepaalde vervorming was veel hoger en minder afhankelijk van de vervormingssnelheid in uniaxiale dan in biaxiale rek. Dit duidt erop dat in rekstroming orientatie-effecten van groot belang zijn voor de mechanische eigenschappen van bloemdeeg.

Net als bloemdeeg vertoonde glutendeeg rekversteviging in zowel uniaxiale als in biaxiale rek. De rekspanning bij een bepaalde vervorming en de mate van rekversteviging waren voor gluten veel sterker afhankelijk van het type vervorming gluten dan voor bloemdeeg: de spanning was ook in biaxiale rek hoger voor gluten dan voor bloemdeeg, maar in uniaxiale rek was het verschil veel groter. Dit duidt erop dat orientatie-effecten in rekstroming van nog groter belang zijn in gluten dan in bloemdeeg. Een sterk effect van orientatie in gluten is consistent met gepubliceerde gegevens over dubbelbreking van gerekte gluten. Het is waarschijnlijk de gluteninefractie die vanwege de grootte van de glutenine aggregaten verantwoordelijk is voor deze richtingsafhankelijkheid van de mechanische eigen-schappen van gluten en bloemdeeg.

Bij 25°C en de hogere verplaatsingssnelheid van de haak waren de verschillen in breukspanning tussen de bloemdegen klein. Bij de lagere verplaatsingssnelheid van de haak was de breukvervorming het kleinst voor Vivant, iets groter voor Hereward en het grootst voor A6/13. Bij beide verplaatsingssnelheden van de haak was de breukspanning het hoogst voor Hereward, iets lager voor A6/13 en veel lager voor Vivant.

De eigenschappen van gluten en bloemdegen bij grote vervormingen in uniaxial rek werden ook bestudeerd bij 45°C. Voor bloemdeeg van Vivant en A6/13 namen de spanning, de rekverstevigingscoëfficiënt en de snelheidsafhankelijkheid van de spanning af met een toename van de temperatuur. Voor Hereward werd met toenemende temperatuur alleen een afname van de rekverstevigingscoëfficiënt waargenomen, terwijl de andere waarden vrijwel gelijk bleven. Voor Hereward gluten nam het spanningsniveau iets toe met toenemende temperatuur, terwijl het voor Vivant en A6/13 gluten afnam. De mate van rekversteviging van de glutendegen was onafhankelijk van type gluten en temperatuur.

Bij toenemende temperatuur bleef de breukvervorming van Hereward bloemdeeg onveranderd, terwijl die van A6/13 enigszins afnam. De meest drastische effecten werden gevonden voor Vivant: Bij de lagere verplaatsingssnelheid van de haak nam de breukvervorming af van 1.6 tot 1.3 en bij de hogere van 2.0 tot 1.6. Bij lagere vervormingssnelheden en hogere temperaturen waren de verschillen in breukvervorming tussen de rassen dus erg groot, terwijl bij 25°C en de hogere snelheid het verschil maar klein was. Voor deeg van een zwakke bloem (wat een lager eiwit- c.q. gluteninegehalte inhoudt) hangt de breukvervorming blijkbaar in sterkere mate af van de vervormingssnelheid en de temperatuur dan voor deeg van een sterkere bloem.

De waargenomen veranderingen in de rekspanning bij toenemende temperatuur waren gelijk voor de gluten en bloemdegen van de bestudeerde rassen. Dit duidt erop dat voor bloemdeeg het effect van temperatuur op de rekspanning voor een groot deel wordt bepaald door de glutenfractie. Deze trend is ook zichtbaar bij de breukspanning. In tegenstelling tot wat was gevonden bij bloemdeeg nam de breukvervorming voor glutendeeg niet af bij een toenemende temperatuur, maar vertoonde een lichte toename. Daarnaast hing de breukvervorming voor glutendeeg in mindere mate af van de vervormingssnelheid dan voor bloemdeeg. Een ander verschil is, dat voor bloemdeeg de kleinste breukvervorming werd gevonden voor het ras met het laagste eiwit-/gluteninegehalte (Vivant), terwijl voor glutendeeg de kleinste breukvervorming werd gevonden voor het ras met het hoogste gluteninegehalte (Hereward). Dus, gluten en bloemdeeg verschillen wat betreft het effect van eiwitsamenstelling, vervormingssnelheid en temperatuur op de breukvervorming. Het verschil in het effect van eiwitsamenstelling op de breukvervorming tussen bloemdeeg en glutendeeg zou heel goed veroorzaakt kunnen worden door het feit dat in bloemdeeg de hoeveelheid hoogpolymeer gluteneiwit limiterend is, terwijl in glutendeeg het de hoeveelheid van monomere gliadines is die te laag zou kunnen zijn. Dit laat zien dat om een maximum in de breukvervorming voor bloemdeeg en gluten deeg te bereiken het gehalte hoogpolymeer gluten eiwit niet te laag mag zijn, maar ook niet te hoog.

Het effect van de toevoeging van een mix van additieven, zoals die gewoonlijk in de bakkerij gebruikt worden, op het reologisch en breukgedrag van bloemdeeg is bestudeerd bij 25°C en bij 45°C. In alle gevallen leidde toevoeging van de mix van additieven tot een afname van de spanning bij relatief kleine vervormingen. Voor Hereward leidde toevoeging van de mix tot een significante toename van de rekverstevigingscoëfficient tot 2.2 en een opmerkelijke toename van de breukvervorming. Voor A6/13 was de toename van de rekverstevigingscoëfficient het grootst bij 45°C, namelijk van 1.4 tot 1.8, terwijl de breukvervorming nauwelijks veranderde. Voor Vivant werd een opvallende verbetering van de deegeigenschappen waargenomen: de rekverstevigings-coëfficient nam toe van 1.3 tot 1.6 bij 25°C en van 1.0 tot 1.8 bij 45°C, terwijl een toename werd gevonden van de breukvervorming van 2.0 tot 2.3 bij 25°C en van 1.6 tot 1.9 bij 45°C en een hiermee samengaande toename van de breukspanning. Tengevolge van deze effecten namen de verschillen in breukeigenschappen tussen Vivant en A6/13 sterk af. Bij de lagere verplaatsingssnelheid van de haak en de hogere temperatuur was de breukvervorming nog steeds significant kleiner voor deeg van Vivant dan van A6/13. En omdat tijdens het rijzen en het bakken relatief lage vervormingssnelheden worden toegepast ($\dot{\varepsilon} = 10^{-4} - 10^{-3}\ \mathrm{s}^{-1}$) kan het verschil in bakkwaliteit tussen Vivant en A6/13 waarschijnlijk gerelateerd worden aan het verschil in snelheids- en temperatuursafhankelijkheid van de breukvervorming.

Voortijdige breuk van een deegfilm op een dunne plek wordt voorkomen door het mechanisme van rekversteviging, wat is gedefinieerd als een meer dan evenredige toename van de spanning met een toename van de vervorming. Of rekversteviging optreedt, hangt ervan af of de evenredigheidsgrens wordt overschreden of niet. Bij uniaxiale rek moet de relatieve toename van de spanning met een relatieve toename van de vervorming hoger zijn dan 1.0, terwijl bij biaxiaal uitgerekte deegfilms rekverstevigingscoëfficienten hoger moeten zijn dan 2.0. In het geval dat rekverstevigingscoefficienten de criteriumwaarde naderen, zoals was gevonden voor Vivant in uniaxiale rek, vond breuk plaats bij veel kleinere vervormingen. Aangezien de waarden voor rekversteviging in biaxiale rek die wij hebben gevonden lager zijn dan 2.0, wordt voor bloemdeeg de specifieke grens van 2.0 nooit overschreden. Breuk van deegfilms in biaxiale rek zou daarom dus altijd al bij relatief kleine vervormingen moeten plaatsvinden. Het criterium voor rekversteviging is in de praktijk echter minder strikt, o.a. omdat variaties in vervormingssnelheid in beschouwing moeten worden genomen. Meer informatie over het effect van het type vervorming op de breukvervorming en over de relatie tussen rekversteviging en breukvervorming is dringend gewenst.

Tijdens het rijzen en in de oven worden deegfilms biaxiaal uitgerekt. In de bakkerijwereld bestaat een alternatieve methode voor de bereiding van deeg voor koekjes, brood, en bladerdeeg waarbij deeg ontwikkeld wordt door het te vervormen tussen twee rollen. Bij deze aanpak wordt de ontwikkeling van een tweedimensionale structuur in het deeg bevorderd boven een eendimensionale door de vorming van lagen te combineren met draaiing van het deeg over een as van 90° tussen opeenvolgende passages. Deegbereiding m.b.v. rollen is ongeveer zes keer zo efficiënt met betrekking tot energieverbruik dan de traditionele haakkneders. Daarbij kan met de rollenmethode een brood worden gemaakt met een buitengewoon fijne kruimstructuur. Het lijkt er dus op dat de ontwikkeling van een kruislingse, tweedimensionale structuur in deeg door gebruik te maken van "sheeting" tussen rollen superieur is aan het gebruik van traditionele kneders. Ook dit aspect verdient nader onderzoek.

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Curriculum Vitae

Edward Lucian Sliwinski werd op 1 mei 1959 geboren in Arnhem. In 1977 behaalde hij het VWO diploma aan het Thomas à Kempis college in Arnhem. Twee jaar later begon hij te studeren aan de Socale Akademie Den Bosch. Daar behaalde hij in 1983 het hbo-diploma Kultureel Werk met de aantekening Opbouwwerk. Na vier jaar gewerkt te hebben op zijn eigen bedrijf als biologisch tuinder begon hij in 1987 met de studie planteziektenkunde aan de Landbouw Universiteit Wageningen. Tijdens zijn studie deed hij afstudeervakken bij de vakgroep Virologie (LUW), de vakgroep Biochemie (LUW) en de vakgroep voor Fysische en kolloidchemie (LUW). De stage werd uitgevoerd bij el Centro Biologia Molecular (Universidad Autonoma, Madrid, España). In september 1993 studeerde hij af in de vrije orientatie met de nadruk op de virologie.

Van januari 1994 tot april 1998 was hij als assistent in opleiding in dienst van de Landbouwuniversiteit. In deze periode heeft hij het in dit proefschrift beschreven onderzoek uitgevoerd bij de sectie Zuivel en Levensmiddelennatuurkunde van de Landbouwuniversiteit en bij de afdeling Industriële eiwitten van het Agrotechnologisch Onderzoeksinstituut (ATO-DLO). Ook was hij in deze periode drie maanden aangesteld als toegevoegd docent bij de leerstoelgroep Levensmiddelennatuurkunde. Van april 1998 tot april 2002 was hij in dienst van de Landbouwuniversiteit als toegevoegd docent Zuiveltechnologie bij de leerstoel Product ontwerpen en kwaliteitskunde, waarna hij zich heeft gewijd aan het schrijven van dit proefschrift, diverse andere publicaties en de begeleiding van een promovendus.

Met ingang van oktober 2003 is hij werkzaam voor Numico Research in Wageningen als onderzoeker op de afdeling Technology Development & Quality.

Edward is getrouwd met Doret van Dreumel. Zij hebben een zoon en een dochter, Arthur en Anna, die beide geboren zijn op 3 mei 2001 in Oss.

The investigations described in this thesis were carried out at the Agrotechnological Research Institute (ATO-DLO) in Wageningen, The Netherlands and at the Department of Agrotechnology and Food Sciences of Wageningen University in Wageningen, The Netherlands and were part of the Graduate School VLAG (Food Technology, Agrobiotechnology, Nutrition and Health Sciences). This research was financed by the Ministry of Economical Affairs through the IOP-Industrial Protein program (project-number IIE92013).

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