

**Rheological properties  
of wheat flour dough and gluten.  
Effect of water content and native lipids**

**Theofanis Georgopoulos**



**LUND INSTITUTE  
OF TECHNOLOGY**  
Lund University

**Licentiate Thesis**

**Department of Food Technology, Engineering and Nutrition  
Division of Food Technology  
Lund University, Lund, Sweden**

**2003**



# Publications

The thesis is based on the following publications, referred to by Roman numerals:

- I.** A comparison of the rheological properties of wheat flour dough and its gluten prepared by ultracentrifugation (2004).  
Theofanis Georgopoulos, Helena Larsson and Ann-Charlotte Eliasson.  
Food Hydrocolloids 18 (1), 143-151.  
Available on line since 1<sup>st</sup> May 2003. [www.sciencedirect.com/science/journal/0268005X](http://www.sciencedirect.com/science/journal/0268005X).
  
- II.** The influence of native lipids on the rheological properties of wheat flour dough and gluten.  
Theofanis Georgopoulos, Helena Larsson and Ann-Charlotte Eliasson.  
Submitted to Journal of Texture Studies (2003).

## CONTENTS

<b>Summary</b>	<b>5</b>
<b>Sammanfattning</b>	<b>6</b>
<b>Περίληψη</b>	<b>7</b>
<b>1. Aim of the study</b>	<b>8</b>
<b>2. Introduction</b>	<b>9</b>
<b>2.1 Rheological properties of viscoelastic materials</b>	<b>9</b>
<b>2.2 Wheat flour</b>	<b>10</b>
<b>2.2.1 Wheat flour lipids</b>	<b>10</b>
<b>2.3 Dough</b>	<b>12</b>
<b>2.3.1 Rheological properties of dough</b>	<b>13</b>
<b>2.4 Gluten</b>	<b>14</b>
<b>2.4.1 Rheological properties of gluten</b>	<b>15</b>
<b>3. Sample preparation</b>	<b>17</b>
<b>3.1 Preparation of flour with different lipid content</b>	<b>17</b>
<b>3.2 Gluten preparation and ultracentrifugation</b>	<b>17</b>
<b>4. Rheological properties of dough and gluten</b>	<b>19</b>
<b>4.1 The effect of water content</b>	<b>19</b>
<b>4.2 The effect of native wheat lipids</b>	<b>20</b>
<b>4.3 Frequency dependence</b>	<b>22</b>
<b>5. Conclusion</b>	<b>25</b>
<b>Acknowledgments</b>	<b>26</b>
<b>References</b>	<b>27</b>

## Summary

The aim of this thesis was to study the influence of native lipids on the rheological properties of wheat flour dough and gluten and to investigate the effect of water and native lipids on the rheological properties of wheat flour dough and on the gluten prepared by ultracentrifugation. The study considered the rheological properties of doughs with water contents varying from 42.6 to 47.1% and doughs with different native lipid composition. Lipids were removed from the flour by using three different solvents differing in polarity (ethanol, chloroform and diethylether). The extracted lipids were identified by thin layer chromatography. Three flours were produced differing in lipid composition. The rheological properties of dough are sensitively dependent on the dough water and native lipid content. Increased water content led to a decrease of storage modulus for the dough. Increased water content for gluten led to a smaller decrease of storage modulus. The results indicate that the native wheat lipids are important for the rheological functionality of dough, but less important for gluten. The removal of the lipids led a increase in  $G'$  of dough, much greater than the effect of a decrease in the water content of the dough. The frequency dependence of dough and gluten was affected by water content and the flour lipid content.

## Sammanfattning

Syftet med denna studie var att använda ultracentrifugering för att separera gluten från deg. I studien har vattenhalten och de nativa lipidernas inverkan på de reologiska egenskaperna i deg från vetemjöl samt korresponderande gluten. De reologiska egenskaperna bestämdes för degar med vattenhalt mellan 42.6 och 47.1% samt degar med olika sammansättning av nativa lipider. Lipiderna extraherades från mjölet med tre olika lösningsmedel med varierande polaritet (etanol, dietyleter och kloroform). De extraherade lipiderna identifierades med tunnskikt-kromatografi. Tre mjöl med olika lipidsammansättning tillverkades. De reologiska egenskaperna i deg är starkt beroende av vattenhalt och sammansättningen av nativa lipider. Ökad vattenhalt gav en minskning i lagringsmodul för deg. För gluten gav ökad vattenhalt en mindre minskning av  $G'$ . Resultaten visar att de nativa vetelipiderna är viktiga för degens reologiska funktionalitet, men mindre viktiga för gluten. Borttagande av lipiderna åstadkommer stora förändringar i degens viskoelastiska egenskaper, mycket större än en sänkning av degens vattenhalt åstadkommer. Deg och glutens frekvensberoende påverkades av vattenhalt och mjölets lipidinnehåll.

## Περίληψη

Ο σκοπός αυτής της μελέτης ήταν να χρησιμοποιηθεί η μέθοδος της υπερφυγοκέντρωσης για να παραγάγει γλουτένη από το ζυμαρί. Το αντικείμενο της μελέτης ήταν η επίδραση του νερού και των του αλευριού στις ρεολογικές ιδιότητες του ζυμαριού αλευριού σίτου και στη γλουτένη που προετοιμάστηκε με τη μέθοδο της υπερφυγοκέντρωσης. Η μελέτη θεώρησε τη ρεολογικές ιδιότητες του ζυμαριού με το περιεχόμενο του νερού που ποικίλλει από 42,6 σε 47,1% και του ζυμαριού με τη διαφορετική σύνθεση λιπιδίων αλευριού. Τα λιπίδια αφαιρέθηκαν από το αλεύρι με τη χρησιμοποίηση τριών διαφορετικών διαλυτών που διαφέρουν στην πολικότητα (αιθυλική αλκοόλη, χλωροφόρμιο και διαιθυλαιθέρας). Τα εκχυλισμένα λιπίδια προσδιορίστηκαν από τη χρωματογραφία λεπτής στοιβάδας. Τρία αλεύρια παρήχθησαν που διαφέρουν στη σύνθεση λιπιδίων. Οι ρεολογικές ιδιότητες του ζυμαριού εξαρτώνται από το νερό του ζυμαριού και το περιεχόμενο των λιπιδίων αλεύρου. Το αυξανόμενο περιεχόμενο νερού οδήγησε σε μια μείωση του συντελεστή ελαστικότητας για τη ζύμη. Το αυξανόμενο περιεχόμενο ύδατος για τη γλουτένη οδήγησε σε μια μικρότερη μείωση του συντελεστή ελαστικότητας. Τα αποτελέσματα δείχνουν ότι τα λιπίδια αλευριού είναι σημαντικά για τις ρεολογικές ιδιότητες του ζυμαριού, αλλά λιγότερο σημαντικά για τη γλουτένη. Η αφαίρεση των λιπιδίων προκάλεσε τη μεγάλη αλλαγή στις ιξωδοελαστικές ιδιότητες του ζυμαριού, πολύ μεγαλύτερη από την επίδραση μιας μείωσης στο περιεχόμενο νερού του ζυμαριού. Η εξάρτηση συχνότητας του ζυμαριού και της γλουτένης επηρεάστηκε από την περιεκτικότητα σε ύδωρ και την περιεκτικότητα σε λιπίδια αλευριού.

## **1. Aim of the study**

Dough is the intermediate product in the transformation of flour to bread or to other baked products. Rheological properties of dough and gluten are important since they affect the quality of the baked product. They are also important because they provide information on dough structure. As with any other material, the rheological properties of dough are determined by its composition (protein, starch, lipid and water content) and structure.

The aim of this thesis was to study the influence of native lipids on the rheological properties of wheat flour dough and gluten. Gluten was prepared by ultracentrifugation and as this is a new method for gluten preparation another aim of the thesis was to evaluate the rheological properties of such gluten. Ultracentrifugation of dough provides a tool for preparation of fresh gluten, which has experienced the same mixing procedure as the corresponding dough. Centrifugation of dough at 100,000 x g results in a fractionation of dough into liquid phase, gel phase, gluten phase, starch phase, and an unseparated fraction. Doughs with water contents varying from 42.6 to 47.1%, and doughs with different native lipid composition, were studied in terms of their viscoelastic properties (storage modulus, loss modulus). The lipids were removed using three different solvents differing in polarity (ethanol, chloroform and diethylether) resulting in flours with different lipid composition. The extracted lipids were identified by thin layer chromatography (TLC).

## 2. Introduction

### 2.1 Rheological properties of viscoelastic materials

Studies on synthetic polymers show that fundamental rheological properties reflect polymer properties such as degree and type of cross-linking (Ferry, 1980). Measurements at low strains within the linear viscoelastic region offer a possibility of studying material properties without destroying the inherent structure (Barnes, 1989). The linear viscoelastic region shows the range of strain or stress where the storage modulus and loss modulus are independent of the strain or stress.

When a specimen responds linearly to a sinusoidal shear strain (or stress), the resulting stress (or strain) is sinusoidal. The characteristic response at circular frequency ( $\omega$ ) can be represented by the complex modulus,  $G^*$ :

$$G^* = G' + iG''$$

The storage modulus  $G'$  is in phase with the strain (or stress) while the loss modulus  $G''$  is out of phase with strain (or stress). The storage modulus represents storage of elastic energy, while the loss modulus represents the dissipation of energy. Another commonly used dynamic viscoelastic property, the loss tangent [ $\tan\delta = G''/G'$ ], denotes relative proportion of viscous and elastic components in a viscoelastic material. The loss tangent is high ( $>1$ ) for materials that are liquid-like, and low ( $<1$ ) for materials that are solid-like. For linear response,  $G^*(\omega)$  is independent of strain amplitude, being dependent solely on the frequency and temperature.

Frequency sweeps reveal information about the structure of the samples (Ross-Murphy, 1995). The frequency dependence of  $G'$  and  $G''$  can be illustrated by the power law exponents  $n'$  and  $n''$  in the following equations:

$$G' = G_0' \omega^{n'}$$
$$G'' = G_0'' \omega^{n''}$$

where  $\omega$  is frequency and  $G_0'$ ,  $G_0''$  are the intercepts of the power law model for frequency sweeps.

The slope of  $\log G'$  against  $\log \omega$  reflects the time dependence of the moduli. In the case of a three-dimensional network we expect the slope to be near zero. In practice, the three-dimensional network such as dough consists of a mixture of highly cross-linked material with uncross-linked material (Kokini et al., 1994). This leads to slopes that are non-zero but relatively small. Increasing values demonstrate that the sample contains increasing fractions of uncross-linked material (Ferry, 1980). Gels can be characterized as "true gels" when  $\log G'$  versus  $\log \omega$  or  $\log G''$  versus  $\log \omega$  plots give nearly zero slopes (Ross-Murphy, 1984), while for "weak gels" and highly concentrated solutions the plots result in slopes with values higher than zero. According to Ferry (1980), when the slope of  $\log G'$  vs.  $\log \omega$  has a value approaching 0, the material behaves like a rubbery material, while material flowing like a liquid has a slope approaching 2.

## **2.2 Wheat flour**

Wheat is one of the oldest crops cultivated by man and it is the most abundant food crop in the world. The most important use of wheat flour is for making bread. The wheat kernels are milled into flour, bran and germ. White flour, mainly consisting of the starchy endosperm of the kernel, has three major components: carbohydrates (70-80% on dry matter, dm), proteins (8-18%, dm) and lipids (1.5-2.5%, dm) (MacRitchie, 1984). Wheat is a unique cereal in several aspects. Firstly, when mixed with water it forms a cohesive dough with viscoelastic properties. A second unique aspect, related to the first, is that wheat flour dough is more capable of retaining gas during the breadmaking process than any other dough (He. and Hoseney, 1988). Thirdly, wheat flour dough sets in the oven during baking due to the gelatinisation of the starch and changes that occur in part of the proteins (Blokma, 1990).

### **2.2.1 Wheat flour lipids**

Although the lipids make up only about 1.5-3% of wheat flour, they can have a major effect on baking (MacRitchie, 1984). Based on their functional properties, i.e. their ability to

interact with water, wheat flour lipids can be classified into a polar and a non-polar fraction (Larsson, 1986). Native wheat flour lipids consist of 51% non-polar lipids, 26% glycolipids and 23% phospholipids (MacRitchie, 1983). The detailed composition is given in Table 1. The wheat flour lipids are likely to play an important role in the stabilisation of the air-water interface in dough (Carlson, 1981). In aqueous media, the polar lipids interact with water to form liquid-crystalline phases (Larsson, 1986). The addition of lipid fractions to wheat flour dough after lipid removal by extraction can give totally different effects depending on their physical state (Larsson, 1986). The same lipid can exist in different forms (oil, emulsion, liquid-crystalline phases) and the breadmaking is directly related to this state (Eliasson and Larsson, 1993). The lamellar liquid-crystalline phase is expected to stabilise the thin films that develop during oven rise when the gas cells are expanding rapidly.

One way of studying the role of lipids in baking is to remove the lipids and then reconstitute the flour with various lipid fractions. Lipid fractions can also be added to untreated flours (MacRitchie, 1985). Certain solvents (e.g. water-saturated butanol) cause functional changes in the gluten that are reflected in extended mixing time of the dough (Chung et al, 1977, Morrison, 1988). Among the solvents that extract non-starch lipid efficiently, chloroform has been frequently used (MacRitchie, 1985). Diethylether extracts only a part of the non-starch lipid (approximately 70%), whereas chloroform and ethanol have been shown to also extract the polar lipids (Paper II, MacRitchie and Gras, 1973).

**Table 1.** Composition of wheat flour lipids (Morrison, 1988)

Lipid	Weight (%)
Steryl ester	7.5
Triglyceride	20.8
1,2-Diglyceride	6.2
1,3-Diglyceride	6.0
Free sterol	2.1
Free fatty acid	7.0
Monoglyceride	1.3
6-O-Acyl-monogalactosyldiglyceride	3.6
6-O-Acyl-sterylglucoside	1.6
Monogalactosyldiglyceride	4.9
Sterolglucoside + ceramidemonoglycoside	1.8
Monogalactosylmonoglyceride	0.4
Digalactosyldiglyceride	13.5
Ceramidediglyceride	0.03
Digalactosylmonoglyceride	0.6
N-Acyl-phosphatidylethanolamine	4.9
N-Acyl-lysophosphatidylethanolamine	2.9
Phosphatidylethanolamine	0.8
Phosphatidylcholine	5.8
Lysophosphatidylethanolamine	0.9
Lysophosphatidylcholine	7.1
Phosphatidylinositol	0.1
Phosphatidylserine	0.2

## 2.3 Dough

The wheat flour dough can be illustrated as an aqueous continuous medium of gluten and starch containing the dispersed gas phase. There are also interfaces such as the air-water interface, and the starch-gluten interface. The continuum of the dough is essentially the gluten gel but the starch also forms a continuous starch-water phase (Eliasson and Larsson, 1993).

On contact with water, gluten proteins form fibrils spontaneously (Bloksma and Bushuk, 1988). These fibrils then become aligned during the mixing process to form the gluten matrix. If there is insufficient water to meet the hydration needs of the dough components,

gluten does not become fully hydrated and the elastic nature of the dough does not fully develop (MacRitchie, 1980). If there is excessive water, the viscous component of a dough will become dominant, with a decreased resistance to extension, increased extensibility, and a sticky mass will develop (Kokelaar, 1994).

### **2.3.1 Rheological properties of dough**

. Dough is a viscoelastic material since it exhibits both storage and loss moduli in dynamic oscillatory measurement, stress overshoot at the start of shear flow, stress relaxation at the cessation of flow, viscosity that depends on shear, and normal stress differences (Bloksma, 1986, Bloksma, 1990, Faubion and Hosenev, 1990). Dynamic measurements have been used in fundamental, non-destructive rheological characterisation of wheat flour dough (Hibberd, 1970; Hibberd and Parker, 1975; Tsiami et al., 1997a). The rheological properties of dough depend on the volume fraction of the starch. Starch, the most abundant component in dough, is in high enough concentration to form a continuous network of particles that give rise to viscoelastic behaviour. Dynamic measurements on dough prepared from different wheat qualities have shown these measurements are affected by starch-starch, starch-protein and protein-protein interactions (Petrofsky and Hosenev, 1995). The water content affects both the rheological properties of the dough and the quality of the finished baked product (Abdelrahman and Spies, 1986). As the water content of a dough increases, both  $G'$  and  $G''$  decrease (Paper I, Navickis et al., 1982).

Few studies have been made of the effect of the lipids on the rheological properties of dough. The stress-relaxation modulus in rheological experiments increased when lecithin was added to wheat flour dough (Eliasson and Tjerneld, 1990; Larsson and Eliasson, 1996b). Fu and co-workers (1997) reported that both moduli,  $G'$  and  $G''$ , decreased over the entire frequency range (0.01 Hz to 20 Hz), as the amount of added shortening was increased from 0% to 7.5% fat (on flour weight). The addition of solid fat also decreased the  $G'$  and increased viscous behaviour of the dough (Watanabe et al., 2003). In contrast, the addition of oil gave rise to more elastic behaviour (Watanabe et al., 2003).

## 2.4 Gluten

Gluten is insoluble in water and it may be isolated by washing dough to remove starch or by ultracentrifugation (Paper I and Paper II). The gluten proteins, gliadins and glutenins, represent around 80% of the total proteins in flour (Wrigley and Bietz, 1988). When wheat flour is mixed with water, glutenins and gliadins combine to form the viscoelastic mass of gluten (LeGrys et al. 1981). The glutenin fraction is higher in molecular weight and contributes elasticity, while the lower molecular weight gliadin provides extensibility (MacRitchie, 1980).

Wheat proteins have been classified into four types according to their solubility (Osborne 1907):

- albumins are soluble in water or dilute salt solutions and are coagulated by heat,
- globulins are insoluble in pure water, but soluble in dilute salt solutions and insoluble at high salt concentrations,
- gliadins are soluble in aqueous alcohol,
- the glutenins are soluble in dilute acid or bases, detergents, or dissociated in urea, or reduced in beta-mercaptoethanol agents.

Glutenins are an extremely heterogeneous set of proteins. They are cross-linked via intermolecular disulphide bonds to form linear, concatenated multi-polymers with molecular weights ranging from 100,000 to millions (Wahlund et al., 1996, Schofield and Booth, 1983). Glutamic acid is the dominant amino acid of glutenins (Schofield and Booth, 1983). The high molecular weight, the low charge and the high level of hydrogen bonding of amino acids in glutenin contribute to the low solubility of gluten in neutral water solutions (Krull and Wall, 1969).

The gliadin fraction contains mainly single polypeptide chains that associate by hydrogen bonding and hydrophobic interactions (Shewry, et al. 1986). The fraction is characterised by an extremely high content of glutamic acid (around 37%), (Schofield and Booth, 1983). Gliadins exhibit little or no disulphide bonding between chains (Shewry et al., 1986). They

are classified as  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\omega$ -gliadin, according to their mobility in one-dimensional gel electrophoresis at low pH (Woychick et al., 1961).

When subsequently dried, gluten contains residual starch (5-15%) and lipid (5-10%) in addition to the protein (Bloksma and Bushuk, 1988, Janssen, 1992). Carlson et al (1979) reported the existence of MGDG (monogalactodiglyceride), DGDG (digalactodiglyceride), PC (phosphatidylcholine) and the lack of LPC (lyso-phosphatidylcholine) in the lipids extracted by ethanol from gluten. According to their work, the extractable lipid content of dry gluten was 6.8% (w/w). The lipid distribution in gluten fractions depends on the fractionation method (ethanol fractionation or acid fractionation). In 1967, Ponte and co-workers found that gliadin proteins contained significantly more lipids than glutenin proteins. Gliadins contained most of the phospholipids of gluten. Hosney and co-workers (1970) found that gliadins contained 8.2% lipids, corresponding to 68% of the total gluten lipids. Glutenins contained only 4.4% lipids, corresponding to 32% of the total gluten lipids. The DGDG were the major galactolipids, followed by MGDG (Hosney et al., 1970).

### **2.4.1 Rheological properties of gluten**

Gluten forms a continuous macromolecular network, provided that enough water for hydration is present, and sufficient mechanical energy is supplied during mixing. The rheological properties of gluten are more related to protein structure and composition than the rheological properties of dough (Lefebvre, 2003). Gluten shows a larger viscoelastic linear region than dough. The linear region extends up to at least 5-10% strain amplitude in dynamic mode (Lefebvre et al., 2000), whereas the linearity limit of dough is low (around 0.2%) (Phan-Thien et al., 1997). A critical step in performing rheological measurements on gluten is, of course, to prepare the gluten from dough. Gluten can be prepared from a wheat flour dough, either by hand washing or by automatic gluten washing devices. Dreese et al. (1988) studied the differences between hand-washed and commercially prepared glutes. These differences were related in part to the drying procedure, but also to the flour:water ratio during washing. The hand-washed gluten gave a higher storage modulus than the commercially produced gluten. The importance of standardising the gluten preparation method for rheological measurements can be noted. Introducing starch into gluten results in non-linear behaviour. The storage modulus and loss modulus increase (Smith et al., 1970;

Hibberd, 1970). The values for and the complex modulus increased with increasing fraction of starch granules from about 2 kPa for gluten to about 10 kPa for a gluten-starch mixture in which the volume fraction of starch granules was 0.5 (Funt Bar-David and Lerchenthal, 1975). Studies of the effect of lipids on rheological properties show the following. Up to a level of 3% added shortening (based on gluten weight) a decrease of  $G'$  and  $G''$  was noted for the gluten (Cumming and Tung, 1977). Hargreaves et al. (1995) concluded that the storage modulus and the loss modulus of gluten (coming from flour where the lipids had been removed by chloroform) did not change significantly.

### **3. Sample preparation**

#### **3.1 Preparation of flour with different lipid content**

To study the effect of native wheat lipids, three solvents differing in polarity (ethanol, chloroform and diethylether) were used to remove the lipids from the wheat flour (Kosack, a Swedish winter wheat). Diethylether extracted a smaller amount of lipids than the more polar solvents (chloroform and ethanol), and ethanol more than chloroform (Paper II). More DGDG, PC and LPC were extracted by ethanol than by diethylether and chloroform, whereas MGDG seemed to be extracted to the same extent by all three solvents. Consequently, three flours differing in lipid content and in lipid composition were obtained from the native wheat flour, by removing the lipids with solvents differing in polarity (Paper II). These flours were mixed with water in the farinograph to produce doughs with same amount of water.

#### **3.2 Gluten preparation and ultracentrifugation**

Ultracentrifugation was used to prepare gluten samples. Ultracentrifugation of dough results in a separation into a starch, a gluten, a gel, and a liquid phase, and an unseparated fraction (Mauritzen and Stewart, 1965, Larsson and Eliasson, 1996a). Gluten prepared by ultracentrifugation has an advantage over other separation methods, in that we can separate and study the fresh gluten that has been mixed under exactly the same conditions as the corresponding dough. Thus, gluten is produced by ultracentrifugation without rehydration and mixing of a dried powder (Paper I). In this study, we performed rheological measurements on freshly isolated gluten rather than on freeze-dried gluten rehydrated to a constant water content as described by Cornec et al (1994). Freeze-drying induces structural changes, as shown by electron microscopy (Hermansson and Larsson 1986). It was observed that fresh gluten had a more homogeneous structure than hydrated commercial gluten.

During the dough mixing process, the water added is distributed between the flour components (pentosans, gluten, lipids, and starch), and the remainder forms the liquid phase (Paper I). The size of the gluten phase increased as the dough water content increased from

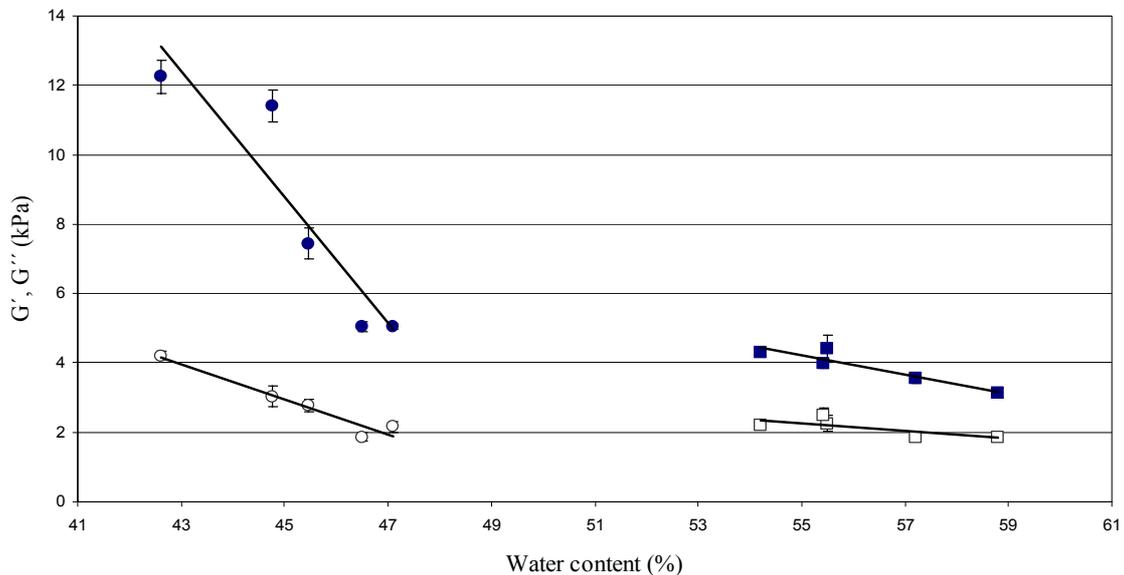
42.6 to 47.1%. This result was similar to previous results on cultivar Kosack (Larsson and Eliasson, 1996a).

The gluten phase was decreased by removing the lipids. The water content of gluten (53.8-55.4%) was higher than for dough (46.5%) (Paper II). A small reduction in the gluten water content was observed when chloroform, instead of diethylether or ethanol, was used to remove the lipids (Paper II). Our findings suggest that removing lipids from the flour reduced the volume of the gluten phase, but no general effect on the water content of gluten could be established.

## 4. Rheological properties of dough and gluten

### 4.1 The effect of water content

The effect of water content on the rheological properties of dough and the fresh gluten was studied.  $G'$  and  $G''$  are shown as functions of water content for both dough and gluten in Figure 1 (Paper I). The values of  $G'$  and  $G''$  for gluten decreased slightly with an increase in water content, although  $G''$  was not affected to the same extent as  $G'$ . Gluten with the highest water content (58.8%) resulted in the lowest value for  $G'$  and the gluten with the lowest water content gave the highest value for  $G'$ . Other studies found that  $G'$  decreased with increasing water content of gluten (Attenburrow et al. 1990; Dreese et al. 1988; Janssen, 1992). Dreese et al. (1988) reported that  $G'$  of gluten decreased when the water content increased from 46 to 61%, i.e. a much broader range than that studied in the present work. Attenburrow et al. (1990) reported values of the storage modulus of gluten in the range of 2 kPa–8 kPa.

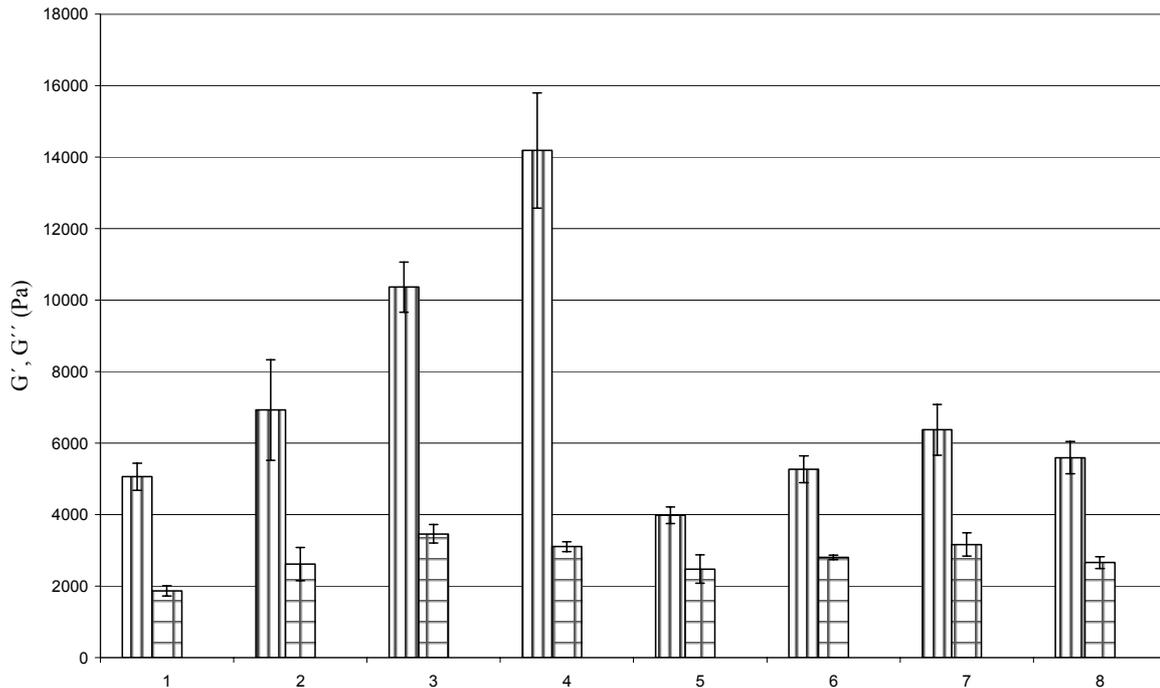


**Figure 1.** Storage modulus ( $G'$ ) and loss modulus ( $G''$ ) for dough and gluten respectively, at different water contents. Data was obtained at a frequency of 1 Hz.  $G'$  for gluten (■),  $G''$  for gluten (□),  $G'$  for dough (●),  $G''$  for dough (○). Error bars show standard deviation from mean value of at least four measurements.

Figure 1 shows that the values of  $G'$  and  $G''$  for dough decreased as the water content increased from 42.6 to 47.1% (w/w). The rheological properties of dough were much more affected by water content than the rheological properties of gluten. It can be concluded that the rheological properties of dough cannot be used to predict the rheological properties of gluten. The amount of water present in a wheat flour dough is known to significantly affect the quality of the finished baked product (Abdelrahman and Spies, 1986). The rheological properties of wheat flour dough are extremely sensitive to water content (Menjivar, 1990, Eliasson and Larsson, 1993, Paper I). The dependence of  $G'$  and  $G''$  on water content is related to the protein content of the flour. When the protein content increases,  $G'$  and  $G''$  both become less sensitive to the water content (Navickis et al., 1982). The trend for both  $G'$  and  $G''$  to decrease as water content of dough increased was in agreement with earlier studies (Hibberd, 1970; Hibberd and Parker, 1975; Kokelaar, 1994; Mani et al. 1992, Masi et al. 1998). The values of  $G'$  and  $G''$  from the present work were in the same range as the values from the previously mentioned works.

## **4.2 The effect of native wheat lipids**

In the present work, we studied the rheological properties of dough and gluten prepared by ultracentrifugation, after removal of the flour lipids using solvents differing in polarity (Paper II). Removal of lipids from the flour resulted in doughs with increasing  $G'$  values in the following order: diethylether < chloroform < ethanol (Figure 2, Paper II). The different lipid composition of the flours thus affected the rheological behaviour of the corresponding dough. The removal of lipids from the flour by a solvent may increase the friction between the starch granules and lead to higher  $G'$ .



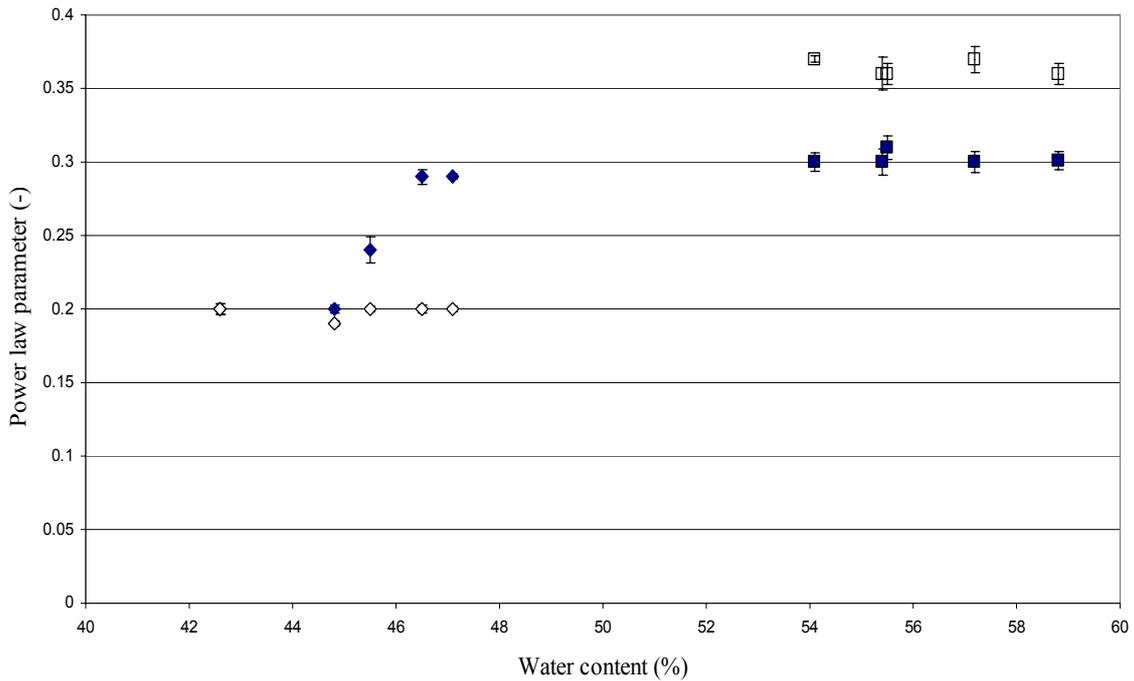
**Figure 2.** Storage modulus ( $G'$ , striped) and loss modulus ( $G''$ , squares) for dough and gluten at a frequency of 1 Hz,  $T = 25^\circ\text{C}$ . The water content of the dough was 46.5%. Doughs made with native flour (1), and flours where the lipids had been removed by diethylether (2), chloroform (3) and ethanol (4). Gluten from doughs made with native flour (5), and flours where the lipids had been removed by diethylether (6), chloroform (7) and ethanol (8).

Gluten originating from dough where the lipids were removed by chloroform gave significantly higher values for  $G'$  compared with those for native gluten. The water content of the gluten originating from dough where the lipids were removed was only moderately affected. It seems that the removal of the lipids directly affected the rheological properties of gluten and that the increase in  $G'$  was not caused by a reduction in the water content. However, the increase in  $G'$  of gluten was still small in comparison with the increase observed for dough. Hargreaves et al (1995) reported that the viscoelastic properties of gluten were not significantly altered when chloroform was used to remove lipids. The authors concluded that lipids do not play a significant role in the rheological properties of hydrated gluten networks. The value of  $G'$  of gluten coming from the flour where the lipids were removed by chloroform was around 6 kPa (gluten water content of 62%) (Hargreaves et al, 1995). In the present study (Paper II), the  $G'$  was 6.4 kPa (gluten water content of 53.8%) for the gluten coming from flour where the lipids were removed by chloroform. According to Hargreaves et al (1995), the change of the rheological properties of the gluten would be small due to the low lipid content (6.4%) and the deformability of the lipid vesicles. The present work (Paper II) showed that removing the lipids from the flour affected the rheological

properties of dough more than the rheological properties of gluten. The solvent polarity affected the rheological properties of dough, but this could not be established for gluten.

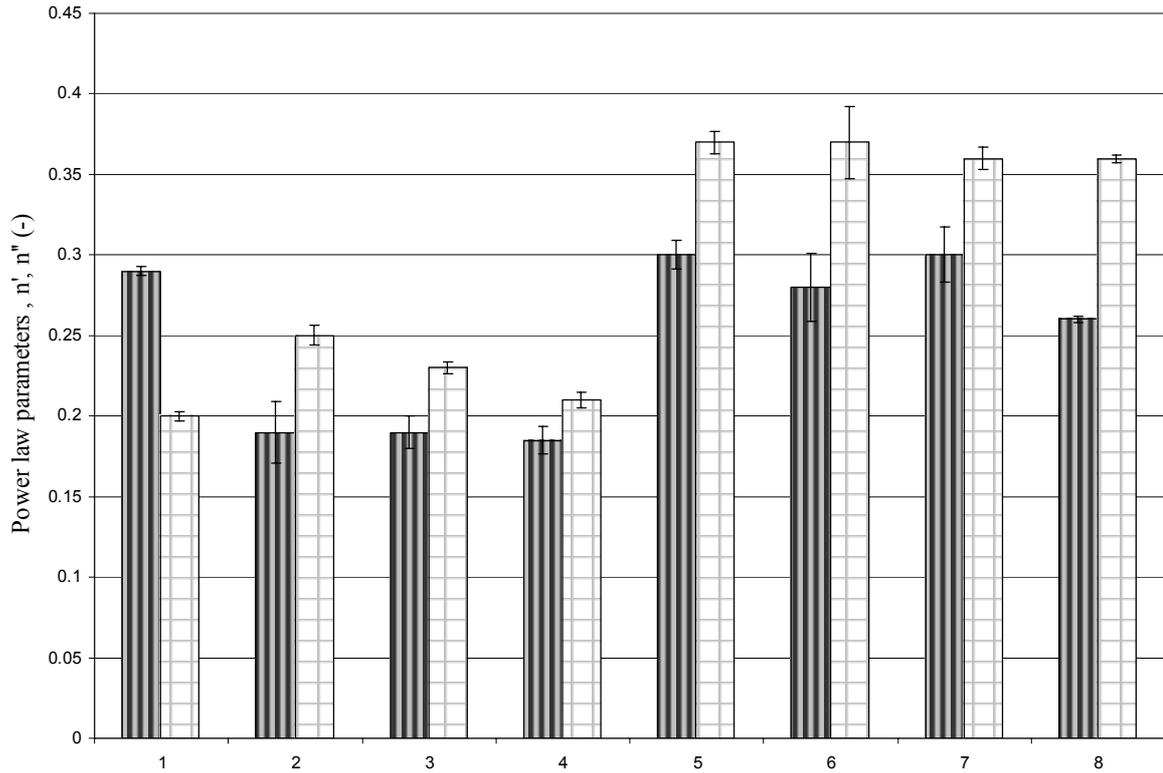
### 4.3 Frequency dependence

The frequency dependence of  $G'$  and  $G''$  provides information about the three dimensional network of dough and gluten. For a three dimensional network we expect the frequency dependency of  $G'$  to be near zero, which is characteristic for a highly cross-linked material (Kokini et al. 1994). The values of  $n'$  and  $n''$  for both dough and gluten were below 0.5 for all the studied water contents, indicating the existence of a three-dimensional network (Paper I). Similar results were shown for the doughs and glutens coming from the flours where the lipids were removed. The values of  $n'$  for gluten were independent of water content and at the same level as  $n'$  of dough determined at the highest dough water content (Fig. 3, Paper I). For gluten,  $n''$  was always higher than  $n'$  for all the studied water contents. A greater frequency dependence for  $G''$  compared with  $G'$  ( $n'' > n'$ ) has also been reported for commercial vital gluten (Redl et al., 1999), but the slope values were slightly higher ( $n''= 0.4$  and  $n'= 0.33$ ) than those reported here for fresh gluten recovered by ultracentrifugation (Paper I).



**Figure 3.** Power law parameters ( $n'$  and  $n''$ ) of the frequency sweep for dough and gluten in the range of water contents 42.6-58.8%. Error bars show standard deviation from mean value of at least four measurements. Power law parameters for  $n'$  of gluten (■) and for  $n''$  of gluten (□), for  $n'$  of dough (●), for  $n''$  of dough (○).

The values of  $n'$  and  $n''$  for dough in the same range that we found in the present study have been reported previously. Frequency dependence has been reported for a soft white winter wheat and a hard red winter wheat at the dough water content of 43%, where  $n' = 0.28$  and  $n'' = 0.27$ , and  $n' = 0.23$  and  $n'' = 0.22$  respectively (Schluentz et al., 2000). Earlier studies (Bohlin and Carlson, 1980, Le Grys et al. 1981) reported similar frequency dependence for  $G'$  ( $n' = 0.27$  and  $n' = 0.29$ ) for dough. The present study (Paper I) also confirms the increasing frequency dependence for  $G'$  at higher dough water content as observed for some of the flours studied by Navickis et al. (1982). The observation by Navickis and co-workers contradicted the earlier study by Hibberd where the frequency dependence of wheat flour dough was found to be unaffected by dough water content (Hibberd, 1970). Moreover, Smith et al (1970) reported an increase of  $n'$  with increasing protein content of dough.



**Figure 4.** Power law parameters of the frequency sweep ( $n'$  striped,  $n''$  squares), doughs made with native flour (1), flours where the lipids had been removed by diethylether (2), by chloroform (3) by ethanol (4) and for gluten separated from doughs made with native flour (5), flours where the lipids had been removed by diethylether (6), chloroform (7) and ethanol (8). Error bars show standard deviation from mean value of at least four measurements.

The value of  $n'$  was higher for the native flour dough than for dough made from flour where lipids had been removed (Figure 4). The frequency dependence of  $G'$  was decreased for dough made with flour where the lipids had been removed. No such obvious effect was observed on gluten, although removing the lipids by ethanol reduced  $n'$ . Fu et al 1997 reported that adding fat up to the level of 5% (on flour weight) did not affect the values of  $n'$  and  $n''$  for dough, but  $n'$  decreased when 7.5% fat was added. According to our work, removal of only 0.6-0.9% of the native lipids reduced the frequency dependence of  $G'$  considerably.

## 5. Conclusions

- The rheological properties of dough are sensitively dependent on dough water as was expected.
- Use of ultracentrifugation provides a method of producing gluten with the same rheological properties as in other studies.
- An increase in water content caused a decrease in  $G'$  for dough. An increase in water content for gluten gave a smaller decrease in  $G'$ .
- The removal of flour lipids by solvents differing in polarity resulted in flours that differed in lipid composition.
- The different lipid content and composition of flours affected the rheological properties of doughs.
- The removal of lipids caused a large increase in the  $G'$  of dough that was much greater than that caused by a reduction in the water content of the dough.
- The frequency dependence of dough made with flours where lipids had been removed was higher than for the native dough. However, the increase in water content decreased the frequency dependence of dough.
- The frequency dependence of gluten from native flour was not affected by water content. In addition, the frequency dependence of gluten from dough made with flour where the lipids had been removed was not influenced.
- The results indicate that the native wheat lipids are important for the rheological functionality of dough, and less important for gluten.

## **Acknowledgments**

First of all I would like to express my gratitude to my supervisors Ann-Charlotte Eliasson and Helena Larsson for their constant and friendly supervision and guidance during the course of this work.

I gratefully acknowledge the help and encouragement as well as the suggestions in all matters relating to this work and about life given to me by Bjorn Bergenståhl.

I gratefully acknowledge the help relating to the manuscript given to me by Anita Sandberg.

I would like to thank all my colleagues at the Division of Food Technology for discussions, being friends and creating a nice atmosphere to work.

I thank my parents for their ongoing giving. My deep gratitude to Jenny, for understanding my feelings, motivating me to carry on during my difficult times.

Special thanks to Rianne, Fernando, Lars, Malin, Anastasia, Babis, Angie for their acceptance of me and their warm support.

## References

Abdelrahman, A. A., and Spies, R.D. (1986). Dynamic rheological studies of dough systems. In H. Faridi and J. M. Faubion (eds), *Fundamentals of Dough Rheology*, pp. 87-103. St. Paul, MN: AACC.

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

Barnes, A.H. (1989). *Introduction to Rheology*. Elsevier, UK.

Bloksma, A.H., (1986). Rheological aspects of structural changes during baking. In J. M. V. Blanshard, P. J. Frazier and T. Galliard (eds), *Chemistry and Physics of Baking*. The Royal Society of Chemistry, London, 170-178.

Bloksma, A. and Bushuk, W. (1988). Rheology and chemistry of dough. In Y. Pomeranz, *Wheat: Chemistry and Technology*, Pomeranz, (Vol. 2) (pp. 131-218). Am. Assoc. Cereal Chem., St Paul, Minnesota.

Bloksma, A.H., (1990). Rheology of the breadmaking process. *Cereal Foods World* 35(2), 228-236.

Bohlin, L., and Carlson, T.L.G. (1980). Dynamic viscoelastic properties of wheat flour dough: dependence on mixing time. *Cereal Chemistry* 57, 174-177.

Carlson, T. L.-G. (1981). *Law and order in wheat flour dough: colloidal aspects of the wheat flour dough and its lipid protein constituents in aqueous media*. Thesis. Lund University, Sweden.

Carlson, T.L.-G., Larsson, K., Mieziš, Y., and Poovarodom. (1979). Phase equilibria in the aqueous system of wheat gluten lipids and in the aqueous salt system of wheat lipids. *Cereal Chemistry* 56(5), 417-419.

Cornec, M., Popineau, Y., and Lefebvre, J. (1994). Characterization of gluten subfractions by SE-HPLC and dynamic rheological analysis in shear. *J. Cereal Science* 19, 131-139.

Chung, O.K., Pomeranz, Y. Finney, K. F., and Shogren, M, D., (1977). Defatted and reconstituted wheat flours III. Effects of flour moisture content and aqueous binary azeotropes on functional (breadmaking) properties. *Cereal Chemistry* 55(1), 31-43.

Cumming, D.B. and Tung, M.A. (1977). Modification of the ultrastructure of rehydrated commercial wheat gluten. *Can. Inst. Food Sci. Technol. J.* 10(2), 109-119.

Dreese, P. C, Faubion, J. M., and Hosenev, R. C. (1988). The effect of different heating and washing procedures on the dynamic rheological properties of wheat gluten. *Cereal Foods World* 33(2), 225-228.

Eliasson, A-C. and E. Tjerneld (1990). On the influence of added lipid phases on the rheological properties of wheat flour doughs. *Z. Lebensm. Unters. Forsch* 191, 35-39.

Eliasson, A.-C, and K.Larsson, (1993). *Cereals in Breadmaking: a Molecular Colloidal Approach*. New York, Basel, Hong Kong, Marcel Dekker.

Faubion, J. M., and Hosenev, R. C. (1990). The viscoelastic properties of wheat flour doughs. In H. Faridi and Faubion J. M., *Dough rheology and baked product texture* (pp.29-66). New York: Van Nostrand Reinhold.

Ferry, J. D. (1980). *Viscoelastic properties of polymers*, John Wiley: New York.

Fu, J, Mulvaney, S.J. and Cohen, C. (1997). Effect of added fat on the rheological properties of wheat flour doughs. *Cereal Chemistry* 74(3), 304-311.

Funt Bar-David, C.B., and Lerchenthal, CH.H (1975). Rheological and thermodynamic properties of gluten gel. *Cereal Chemistry* 52, 154r-169r.

Hargreaves, J., Popineau, Y., Marion, D., Lefebvre, J. and Meste Le M. (1995). Gluten viscoelasticity is not lipid-mediated. A theological and molecular study on lipid and non-prolamin protein depleted gluteins. *J. Agric. Food Chem.* 43, 1170-1176.

He, H. and Hosenev, R.C., (1988). Study of bread baking using the electric resistance oven system. *Cereal Foods World* 33, 694

Hermansson, A.-M. and K. Larsson (1986). The structure of gluten gels. *Food Microstructure* 5, 233-239.

Hibberd, G.E. and W.J. Wallace (1966). Dynamic viscoelastic behaviour of wheat flour doughs. Part I: Linear aspects. *Rheol. Acta* 5(3), 193-198.

- Hibberd, G. E. (1970). Dynamic viscoelastic behaviour of wheat flour doughs. *Rheol. Acta* 9, 487-500.
- Hibberd, G. E. and Parker, N. S. (1975). Measurements of the fundamental rheological properties of wheat flour doughs. *Cereal Chemistry* 52, 1r-23r.
- Hoseney, R.C., Pomeranz, Y., and Finney, K. F. (1970). Functional (breadmaking) and biochemical properties of wheat flour components. VI. Gliadin-lipid-glutenin interaction in wheat gluten. *Cereal Chemistry* 47, 135-140.
- Janssen, A. (1992). *Obelisk and Katepwa wheat gluten. A study of factors determining bread-making performance*. Ph.D. thesis. Wageningen Agricultural University.
- Kokelaar, J.J. (1994). *Physics of breadmaking*. Ph.D. thesis. Wageningen Agricultural University.
- Kokini, J.L. Cocero, A.M., Madeka, H., and de Graaf, E. (1994). The development of state diagrams for cereal proteins. *Trends in Food Science Technology* 5, 281-288.
- Krull, L.H. and J. S. Wall (1969). Relationship of amino acid composition and wheat protein properties. *Bakers Digest* 43, 30-39.
- Larsson, K. (1986). Functionality of wheat lipids in relation to gluten gel formation. In J.M.V. Blanshard, P.J. Frazier and T. Galliard (eds), *Chemistry and Physics of Baking*. Royal Society of Chemistry, London, 62.
- Larsson, H., and Eliasson A-C. (1996a). Phase separation of wheat flour dough studied by ultracentrifugation and stress relaxation. I. Influence of water content. *Cereal Chemistry* 73(1), 18-24.
- Larsson, H., and Eliasson A-C. (1996b). Phase separation of wheat flour dough studied by ultracentrifugation and stress relaxation. II. Influence of mixing time, Ascorbic Acid, and Lipids. *Cereal Chemistry* 73(1), 25-31.
- LeGrys, G.A., Booth, M.R., and Al-Baghdadi, S.M. (1981). The physical properties of wheat proteins. In Y. Pomeranz and L. Munck, *Cereals: A Renewable Resource. Theory and Practice* (243-264). AACC, St. Paul.

Lefebvre, J., Popineau, Y., Deshayes, G. and Lavenant, L., (2000). Temperature-induced changes in the dynamic rheological behaviour and size distribution of polymeric proteins for gluteins from wheat near-isogenic lines differing in HMW glutenin subunit composition. *Cereal Chemistry* 77, 193-201.

Lefebvre, J., Pruska-Kedzior, A., Kedzior, Z. and Lavenant, L. (2003). A phenomenological analysis of wheat gluten viscoelastic response in retardation and in dynamic experiments over a large time scale. *Journal of Cereal Science* 38, 257-267.

MacRitchie, F. and Gras, P.W. (1973). The role of flour lipids in baking. *Cereal Chemistry* 50, 292.

MacRitchie, F. (1980). Physicochemical aspects of some problems in wheat research. In Y. Pomeranz (ed), *Advances in Cereal Science and Technology*. Page 271, Vol. 3. Am. Assoc. Cereal Chem: St. Paul, MN. Minnesota.

MacRitchie, F. (1983). Role of lipids in baking. Pages 165-188 in P.J. Barnes (ed), *Lipids in Cereal Technology*. Academic Press, London.

MacRitchie, F. (1984). Baking quality of wheat flours. *Advances in Food Research* 29, 201-277.

MacRitchie, F. (1985). Studies of the methodology for fractionation and reconstitution of wheat flours. *J. Cereal Science* 3, 221-230.

Mani, K., Trägårdh, C., Eliasson, A.C., and Lindahl, L. (1992). Water content, water soluble fraction, and mixing affect fundamental rheological properties of wheat flour doughs. *Journal of Food Science* 57(5), 1198-1200, 1209.

Masi, P. Cavella, S. and Sepe, M. (1998). Characterization of dynamic viscoelastic behavior of wheat flour doughs at different moisture contents. *Cereal Chemistry* 75(4), 428-432.

Mauritzen, C.M., and Stewart, P.R. (1965). The ultracentrifugation of doughs from wheat flour. *Aust. J. Biol. Sci.* 18,173.

Menjivar, J.A. (1990). Fundamental aspects of dough rheology. In Faridi and J.M. Faubion, *Dough Rheology and Baked Product Texture* (pp.1-28), AVI, Van Nostrand Reinhold, NewYork.

Morrison, W.R. (1988). Lipids in "Wheat Chemistry and Technology", edited by Y. Pomeranz, AACC, St. Paul, Minnesota, USA, p. 373-439.

Navickis, L. L., Anderson, R. A., Bagley, E.B. and Jasberg, B. K. (1982). Viscoelastic properties of wheat flour doughs. Variation of dynamic moduli with water and protein content. *Journal of Texture Studies* 13, 249-264.

Osbourne, T. B. (1907). *The proteins of the wheat kernel*. Press of Judd & Detweiler, Inc, pp 1-119.

Petrofsky, K.E., and Hosney, R.C. (1995). Rheological properties of dough made with starch and gluten from several cereal sources. *Cereal Chemistry* 72(1), 53-58.

Phan-Thien, Nh., Safari-Ardi. M., Morales-Patino, A., 1997. Oscillatory and simple shear flows of a flour-water dough: a constitutive model. *Rheol. Acta* 36, 38-48.

Ponte, J. G., Jr., Destefanis, V. A., Titcomb, S. T., and Cotton, R. H. (1967). Study of gluten proteins as influenced by certain organic solvents. *Cereal Chemistry* 44, 211-216.

Redl, A., Morel, M. H., Bonicel, J., Guilbert, S., and Vergnes, B. (1999). Rheological properties of gluten plasticized with glycerol: dependence on temperature, glycerol content and mixing conditions. *Rheologica Acta* 38(4), 311-320.

Ross-Murphy, S.B. (1984). Rheological methods. In H.W.S. Chan (Ed.). *Biophysical methods in food research* (pp.138-199). London: Blackwell Scientific Publications.

Ross-Murphy, S.B. (1995). Structure-properties relationships in food biopolymer gels and solutions. *Journal of Rheology* 39, 1451-1463.

Schofield, J.D. and Booth, M.R. (1983). Wheat proteins and their technological significance. *Dev. Food Proteins* 2, 1-65. Ross-Murphy, S.B. (1984). *Rheological methods*. In *Biophysical Methods in Food Research*, ed. H.W.S. Chan, 138-199. London: Blackwell Scientific Publications.

Shewry, P.R., Tatham, A.S., Forde, J., Kreis, M. and Miflin, B.J., (1986). The classification and nomenclature of wheat gluten proteins: a reassessment. *J. Cereal Science* 4, 97-106.

Schluntz, E.J., Steffe, J.F. and Ng K.W. P. (2000). Rheology and microstructure of wheat dough developed with controlled deformation. *Journal of Texture Studies* 31, 41-54.

Smith, J.R., Smith T.L. and Tschoegl N. W. (1970). Rheological properties of wheat flour doughs. III. Dynamic shear modulus and its dependence on amplitude, frequency, and dough composition. *Rheol. Acta* 9(2), 239-252.

Tsiami, A.A., Agterof, W.G.M., and Groot, R.D. (1997a). Rheological properties of glutenin subfractions in relation to their molecular weight. *Journal of Cereal Science* 26, 15-27.

Wahlund, K.-G., Gustavsson, M., MacRitchie, F., Nylander, T. and Wannerberger, L. (1996). Size characterisation of wheat proteins, particularly glutenin, by asymmetrical flow field-flow fractionation. *Journal of Cereal Science* 23(2), 113-119.

Watanabe, Akihiro, Kazuhisa Yokomizo, and Ann-Charlotte Eliasson (2003). Effect of physical states of non-polar lipids on rheology, ultracentrifugation and microstructure of wheat flour dough. *Cereal Chemistry* 80(3), 281-284.

Wrigley, C.W. and Bietz, J.A., (1988). Proteins and amino acids. In Y. Pomeranz (ed), *Wheat, Chemistry and Technology*, Vol. I. Am. Assoc. Cereal Chem., Inc., St. Paul, Minnesota, USA, 159-275.

Woychick, J.H., Boundy, J.A., and Dimler, R.J. (1961). Starch gel electrophoresis of wheat gluten proteins with concentrated urea. *Arch. Biochem. Biophys* 94, 477-482.