The influence of native lipids on the rheological properties of wheat flour dough and gluten

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Abstract

In this study work, we report on the viscoelastic properties of dough and gluten (prepared by ultracentrifugation) after removal of the flour lipids using solvents differing in polarity (chloroform, ethanol and diethylether). The extracted lipids were fractionated by thin layer chromatography (TLC). It was concluded that the flours differed in lipid composition after the extraction. Ethanol removed more polar lipids than the other solvents. Removal of lipids (0.6 to 0.9 % on flour weight) altered the viscoelastic properties of dough significantly, whereas gluten was only marginally affected. The storage modulus (G´) of dough increased with solvent polarity. The highest value of G´ was observed for the dough made with the flour where the lipids were removed by ethanol. This was consistent with a marked decrease in the frequency dependence of G´ of dough when the lipids were removed.

1. Introduction

Wheat flour contains only a small amount of lipids,-1.5 % to 3 % by weight (MacRitchie 1981). Wheat lipids can be divided into two types with regard to their interaction with water. About 50 % of the non-starch lipids are non-polar (mainly triglycerides), forming no aqueous phases (Larsson 1983). The rest of the lipids are of the polar type and form liquid-crystalline phases

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(Larsson 1983). It has been observed that lipids can form vesicles that are embedded in the gluten and dough, and thus could thereby act as fillers (Hargreaves et al. 1995). The polar lipids have a beneficial effect in baking, whereas the nonpolar fraction causes a deterioration of loaf volume and texture when added to defatted flour (Daftary et al. 1968; MacRitchie 1976; MacRitchie and Gras 1973).

The composition of the wheat lipids as follows (Morrison 1988; MacRitchie 1983). The non-polar or neutral lipids (NL) consist of triglycerides, with small amounts of steryl esters, diglycerides, monoglycerides, and minor amounts of acylated glycolipids. Free fatty acids (FFA) are included in the NL. The polar lipids are comprised of several glycolipids (GL) and phospholipids (PL). The principal GL are the di- and mono-galactosyldiglycerides (DGDG, MGDG) with smaller amounts of the corresponding monoacyl lipids. The principal PL includes phosphatidyl-choline, ethanolamine and glycerol (PE) and two unusual N-acyl derivatives of PE.
One way of studying the role of lipids in baking is to remove the lipids and then reconstitute the flour with various lipid fractions (MacRitchie 1980). 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 also the polar lipids (MacRitchie and Gras 1973; Morrison et al. 1975).

To our knowledge previous studies do not provide much information about the rheological properties of dough made with defatted flour. However, studies have considered the influence of the addition of lipids on the rheological properties. The stress-relaxation modulus increased when lecithin was added to wheat flour dough in its lamellar liquid-crystalline phase (Eliasson and Tjerneld 1990; Larsson and Eliasson 1998). Fu et al. (1997) reported that both moduli, G′ (storage modulus) and G′′ (loss modulus), in small-amplitude decreased over the entire frequency range studied (0.01 Hz to 20 Hz), as the amount of added shortening was increased (from 0 % to 7.5 % fat on flour weight). In the present work, we studied the rheological properties (i.e. G′, G′′) of dough and its corresponding gluten (prepared by ultracentrifugation)

when the flour lipids had been removed by solvents differing in polarity. The extracted lipids were identified by thin layer chromatography (TLC).

2. Materials and Methods

2.1.1 Materials

One wheat flour of medium baking quality (Kosack, Swedish winter wheat flour) was selected for this study. The flour was milled in a Quadrumat Senior Mill to an extraction rate of 65 % (Svalöf Weibull AB, Svalöv, Sweden). Chemical analysis of the flour gave the following contents, calculated on a dry basis (w/w): protein 8 % (Nx5.7), ash 0.42 %, water content 14.4 %, starch 85.9 %, damaged starch 4.4 % (American Association of Cereal Chemists, 1994). The Falling number was 333 (American Association of Cereal Chemists, 1994). Ethanol (99.5 %) was purchased from Kemetyl AB, Haninge, Sweden. Chloroform (99 % p.a.) and diethylether (99 % p.a.) were purchased from Merck, Darmstadt, Germany.

2.2 Removal of the lipids

Lipids were extracted from wheat flour with three different solvents (ethanol, diethyethyl ether, chloroform). In the case of ethanol, the following procedure was followed. Flour (100g) and ethanol (500 ml) were stirred for 2 hours at 20ºC (Sahasrabudhe 1979). The extract was then filtered through a filter paper in a Buchner funnel. When the chloroform and diethyether solvents were used the flour (100g) was extracted with each solvent (200 ml) for 30 min (MacRitchie 1985), and the slurry was filtered. The procedure was repeated twice. Afterwards, the solvent was removed by rotary vacuum evaporation performed at a temperature below 40ºC. Higher temperatures would damage the phospholipids, which are sensitive to heat. Not only lipids are extracted by ethanol so a purification step was needed. When the ethanol was removed, the remaining crude lipids were dissolved in a chloroform: methanol: water mixture (2.5:1.25:1) (Saharabudhe 1979). The mixture was shaken in a separator funnel and the mixture left to equilibrate for 9 hours until a sharp distinction between the layers was obtained. After the lower

chloroform layer containing lipids had been drained and evaporated, the lipid residue was dried over P_2O_5 until constant weight was attained (about 24 hours). The lipids were dissolved in chloroform and stored at -18ºC in a tube saturated with N_2 until characterization by TLC. Extraction and weighing of lipids were repeated twice.

2.3 Thin-Layer Chromatography (TLC)

TLC was performed on the lipids extracted from flour. A thin layer plate (silica GF 254, from Merck) with the dimension of 20 x 20 cm and a layer thickness of 0.25 mm was activated in an oven at 110-120ºC for 30 minutes. References used were DGDG, MGDG, PC (Phosphatidyl choline), LPC (Lysophosphatidyl choline) and linoleic acid (FFA), (Larodan, Malmö, Sweden). The lipid samples were dissolved in a chloroform: methanol mixture (ratio 2:1), 20 mg lipid/ml sample. 10µl of references were used while 30µl were used for the lipid samples. The samples were applied as spots 1.5 cm from the edge and then the plate was placed in a chamber containing the mobile phase for development of the chromatogram. The mobile phase was chloroform: methanol:H_2O (ratio 65:25.4). The development time of the chromatogram was about 70 minutes. A cupric acetate (CuAc) reagent solution was sprayed on the plate to give colored products when heated at 180ºC for 15 minutes.

2.4 Preparation of dough and gluten

The defatted flours and the native flour (10 g) were mixed with distilled water to produce doughs with a water content of 46.5% (w/w). They were mixed in a 10-g Farinograph bowl (Brabender Do. Corder, OHG, Duisburg, Germany) at 30ºC, 60 rpm, for 10 minutes.

2.5 Ultracentrifugation of dough

Details concerning the ultracentrifugation of dough have been reported previously (Georgopoulos, Larsson and Eliasson, 2003). Doughs were centrifuged for 1 hr at 100,000 x g in

an LE 80K OPTIMA ultracentrifuge (Beckman Instruments, Stockholm, Sweden). The gluten recovered after ultracentrifugation was used for rheological characterization. The water content of gluten was determined by drying for 1 hr at 130 ºC. Four replicas of each sample were prepared for drying. The water content of the gluten was obtained with a coefficient of variation of less than 4%.

2.6 Dynamic rheological measurements

Dynamic oscillatory measurements were performed in a controlled stress rheometer (RTI Stress-Tech International Ltd., Lund, Sweden). All measurements were conducted at 25ºC using the 15 mm diameter parallel plate geometry with a gap between the plates of 2 mm. Dough was removed from the farinograph bowl, and placed between the plates of the rheometer. In the case of gluten, the freshly prepared gluten phase was removed from the tube immediately after the ultracentrifugation, and placed in the rheometer. To prevent drying, a thin layer of silicon oil was spread over the dough or gluten surfaces exposed to air. Before starting any measurements, the sample (dough or gluten) was held at rest for 30 min to allow relaxation of stresses generated during sample loading. Dynamic frequency sweep tests of dough and gluten were carried out in the linear viscoelastic region. The frequency range 0.1 to 10 Hz was investigated. The viscoelastic properties are described by the values of G′ and G′′ at 1 Hz and the frequency dependence of G′ and G′′. The frequency dependence of G′ and G′′ is described by the power law parameters (n′ and n′′ respectively) in the following equations:

- $G' = G'_o \omega^{n'}$, (equation 1)
- $G'' = G''_o \omega^{n''}$ (equation 2)

where ω is frequency, and G'_o, G''_o are the intercepts of the power law model for frequency sweeps. The experiments were performed in tetraplicate and the values reported are the means and standard deviation of these measurements.

2.7. Statistical analysis

The results of the rheological measurements were analyzed statistically using the univariate analysis of variance (ANOVA) at a level of significance of $P<0.001$ with one single factor (Microsoft Excel 2000). Mean values were compared by the least significant difference test with a level of significance of $P<0.01$.

3. Results and discussion

3.1 Preparation of flours differing in lipid content and composition

Wheat flours differing in lipid content were obtained by extracting the flour lipids using ethanol, chloroform and diethylether. The amount of lipids extracted with each solvent is given in Table 1. Diethylether extracted a smaller amount of lipids than the more polar solvents (chloroform and ethanol), and ethanol more than chloroform. The total yield was therefore dependent on the polarity of the solvents, which was in agreement with the results reported by Finney et al. 1976. The results of the TLC analysis are shown in Figure 1.

Table 1. The quantity of lipids extracted by three solvents differing in polarity

Solvent used for extraction	Polarity*	Quantity of extracted lipids (mg/100 g flour, dry basis)
Diethyether	0.117	580 ± 60
Chloroform	0.259	770 ± 72
Ethanol	0.654	900 ± 70

* According to Grulke (1989).

MGDG	DGDG	FFA	PC	LPC	Lipids extracted by ethanol	Lipids extracted by chloroform	Lipids extracted by diethylether

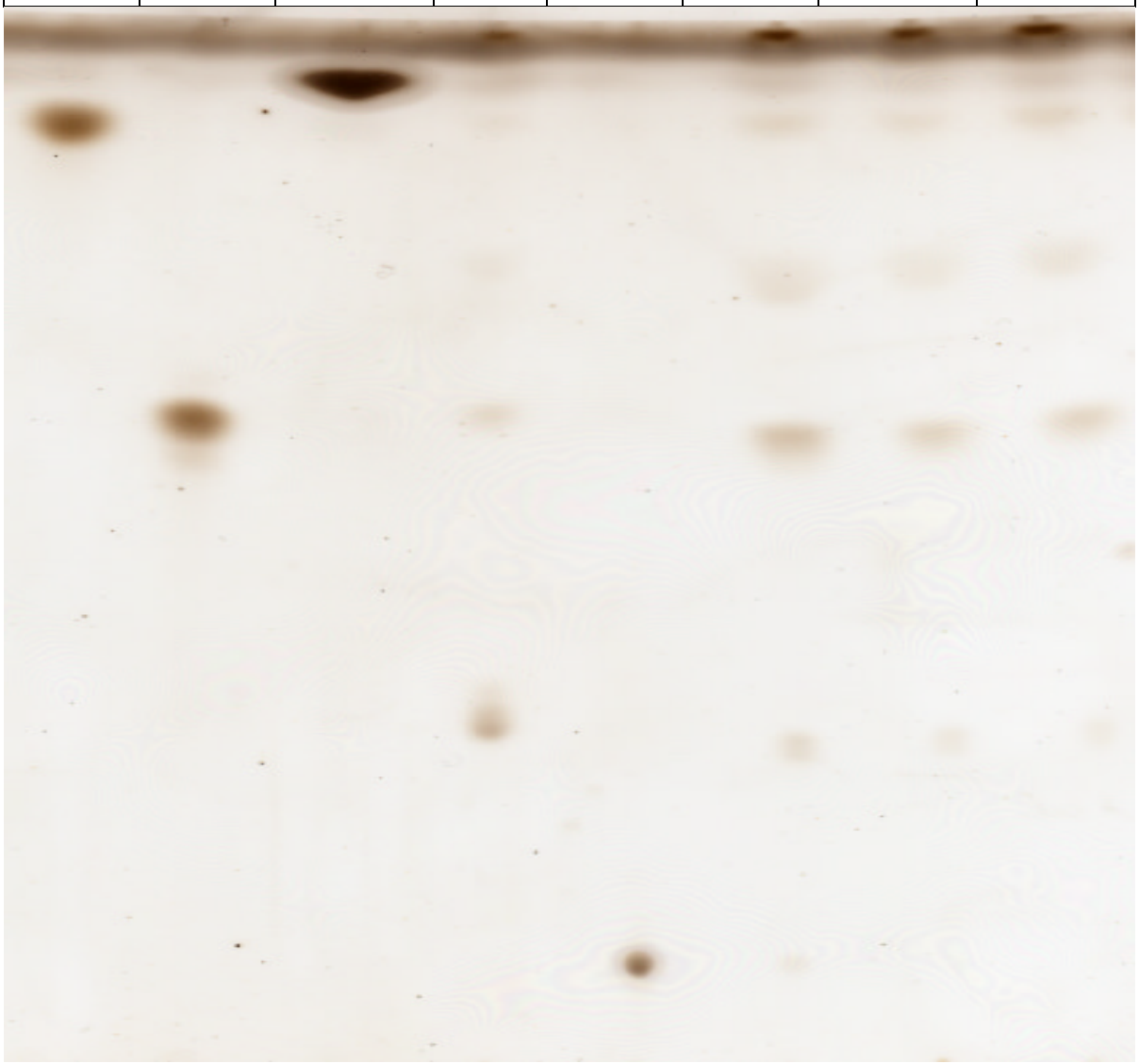

Figure 1. Thin-layer chromatography plate of lipids extracted by ethanol, chloroform and diethylether.Standards:Monogalactosyldiglyceride (MGDG), Digalactosyldiacylglyceride (DGDG), L-α-phospahtidylcholine (PC), L-α-lysophospahtidylcholine (LPC).

The TLC patterns clearly show that all solvents extracted FFA, MGDG, DGDG and PC although the respective amounts of the individual lipids differed considerably 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. LPC was extracted only by ethanol, while

chloroform and diethylether did not seem to extract any LPC. We can assume that more polar lipids remained in the flour extracted by diethylether than in the flours extracted by ethanol or chloroform. 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.

3.2 Ultracentrifugation of dough

Ultracentrifugation was used to prepare gluten for the rheological measurements and to observe effects on the separation properties of the doughs. Five phases were obtained when doughs with 46.5 % water were subjected to ultracentrifugation: liquid, gel, gluten, starch and unseparated (Figure 2). It was interesting to see that the boundaries between the phases were clearer when the lipids had been removed from the flour. Moreover, the liquid phases had a slightly larger volume and were more viscous than those separated from dough made of native flour. Similarly, the gluten phases were smaller compared with those originating from the native flour. The size of the gel phase was not affected by removing the lipids from the flours.

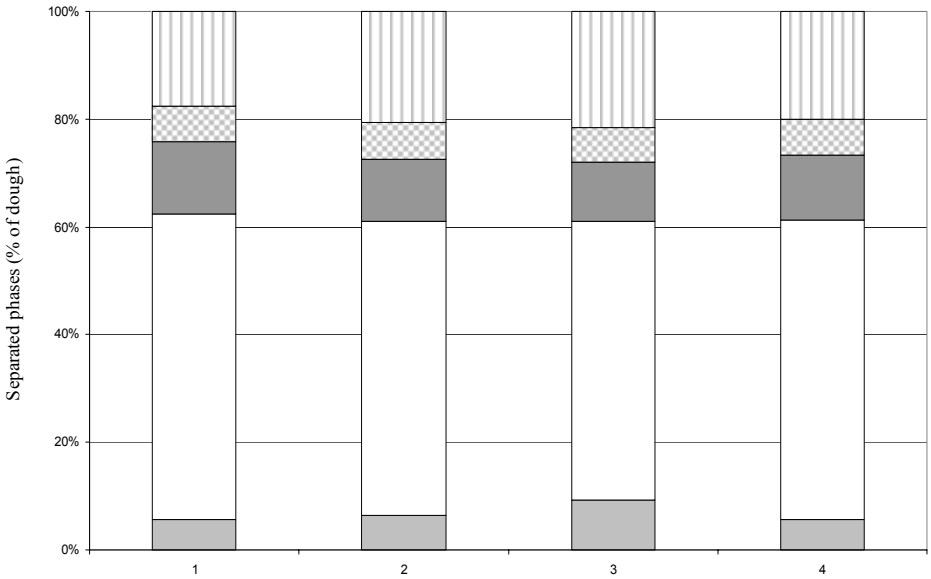

Figure 2. Separation of wheat flour dough (water content 46.5 % (w/w)) by ultracentrifugation. Dough made with Kosack flour (1), and Kosack flour where the lipids had been removed by diethylether (2), chloroform (3) and ethanol (4). The separated phases are (from the top): liquid, gel, gluten, starch and unseparated dough.

The starch phase resulting from the flours where the lipids had been removed was smaller and the unseparated phases were larger. Table 2 shows the water content of the gluten phases separated by ultracentrifugation. The water content of gluten (53.8-55.4 %) was higher than for dough (46.5%). A small reduction in the gluten water content was observed when chloroform instead of diethylether or ethanol was used to remove the lipids (significant difference, $P<0.01$). The water content of the other gluten phases did not differ significantly. Our findings suggest that removing of lipids from the flour reduced the size of the gluten phase, but no general effect on the water content of gluten could be established.

Table 2. Water content of the gluten phase recovered after ultracentrifugation of dough. Native flour and flours where the lipids had been removed by ethanol, chloroform and diethylether were used for dough mixing. The water content of dough was 46.5 %.

Solvent	Water content of gluten phase, weight (%)
Native flour	55.4 ± 0.5^{a}
Diethylether	54.9 ± 0.2^{a}
Chloroform	53.8 ± 0.7^{b}
Ethanol	55.0 ± 0.7^{a}

Data followed by the same superscript did not differ significantly ($P<0.01$).

3.3 Rheological behavior of gluten and dough made with flour where the lipids have been removed

The values of G´ and G´´ for dough and gluten made with the flours from which lipids had been removed are shown in Figure 3 and the values of tanδ are shown in Figure 4. The values of G´ were higher than G´´ in all cases for both dough and gluten. Removal of lipids from the flour resulted in doughs with increasing G´ values in the following order: diethylether<chloroform<ethanol. Thus, removing the lipids with the most polar solvent, i.e. ethanol, resulted in the highest values of G´ and the lowest values of tan δ of dough (both

differed significantly, P<0.01). The values of G´´ were less affected. The highest value of tan δ resulted for the dough made with the flour where the lipids had been removed by diethylether.The different lipid composition of the flours clearly affected the rheological behavior 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´ Removing the lipids by ethanol resulted in the highest value of G´. This may be due to the smaller amount of lipids remaining in this flour compared to the other flours (Table 1). Presumably, this flour also contained less polar lipids than the other flours (Figure 1).

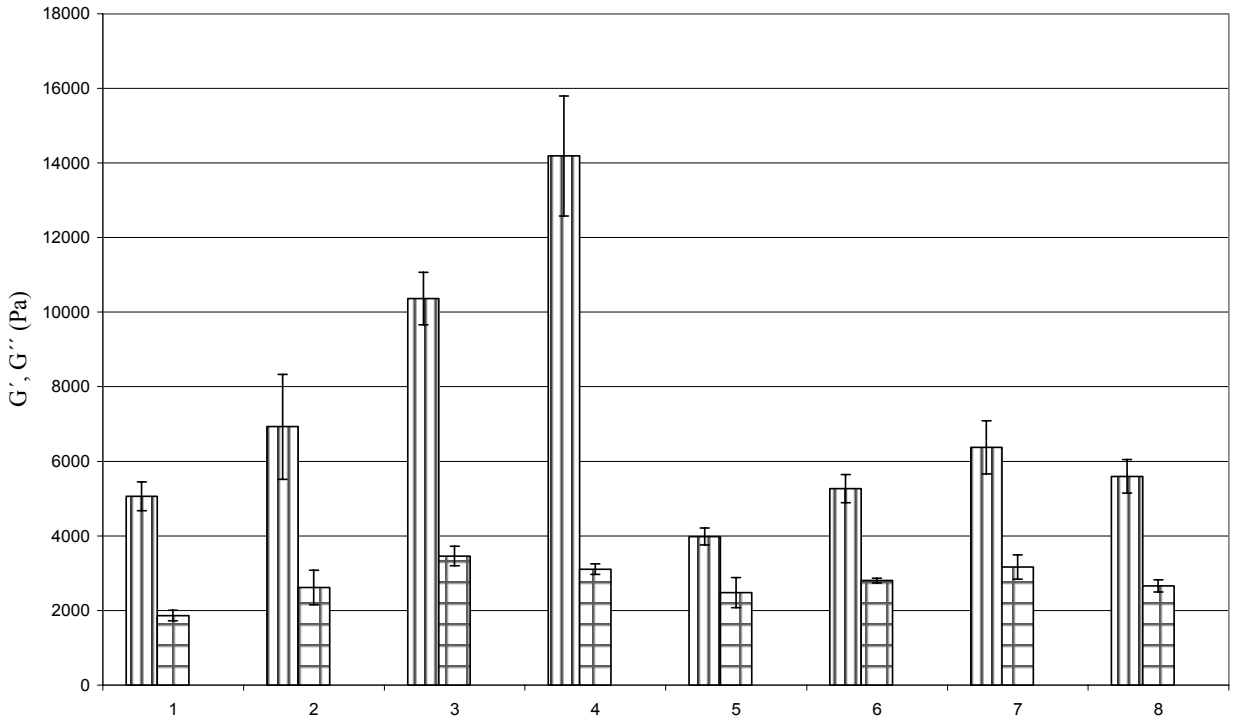

Figure 3. Storage modulus (G´, stripped) and loss modulus (G´´, squares) for dough and gluten at a frequency of 1 Hz, T=25°C. The water content of the dough was 46.5%. Doughs made with native flour (1), and flours where the lipids had been removed by diethylether (2), chloroform (3) and ethanol (4). Gluten from doughs made with native flour (5), and flours where the lipids had been removed by diethylether (6), chloroform (7) and ethanol (8).

Removing the lipids from the flour produced gluten with significantly higher values of G´ compared with those for native gluten. However, the increase in G´ of gluten was still small in comparison with the increase observed for dough. The values of G´´ were not affected significantly (P<0.01). Removing the lipids by chloroform reduced the gluten water content slightly (approx 1 %) compared with the other ones (Table 2), but the higher G´ value observed

for this gluten (Figure 3) was still higher than expected due to a reduction in the water content (Georgopoulos et al., 2003). According to the previous study, where the same wheat flour was used, native gluten with a slightly lower water content (approx 54 %) resulted in a G´ value of 4.5 kPa, instead of the 6.4 kPa as for the gluten recovered after the flour lipids had been removed by chloroform. Therefore, the removal of the lipids seemed to affect the rheological properties of gluten directly and that the increase in G´ was not caused by a reduction in the water content. The native gluten had the highest value of tan δ, and it differed significantly ($P<0.01$) compared to the other glutens. In conclusion, removing lipids from the flour affected the rheological properties of dough more than the rheological properties of gluten. It seems that the solvent polarity was important for the effect of the dough, but this could not be established for gluten.

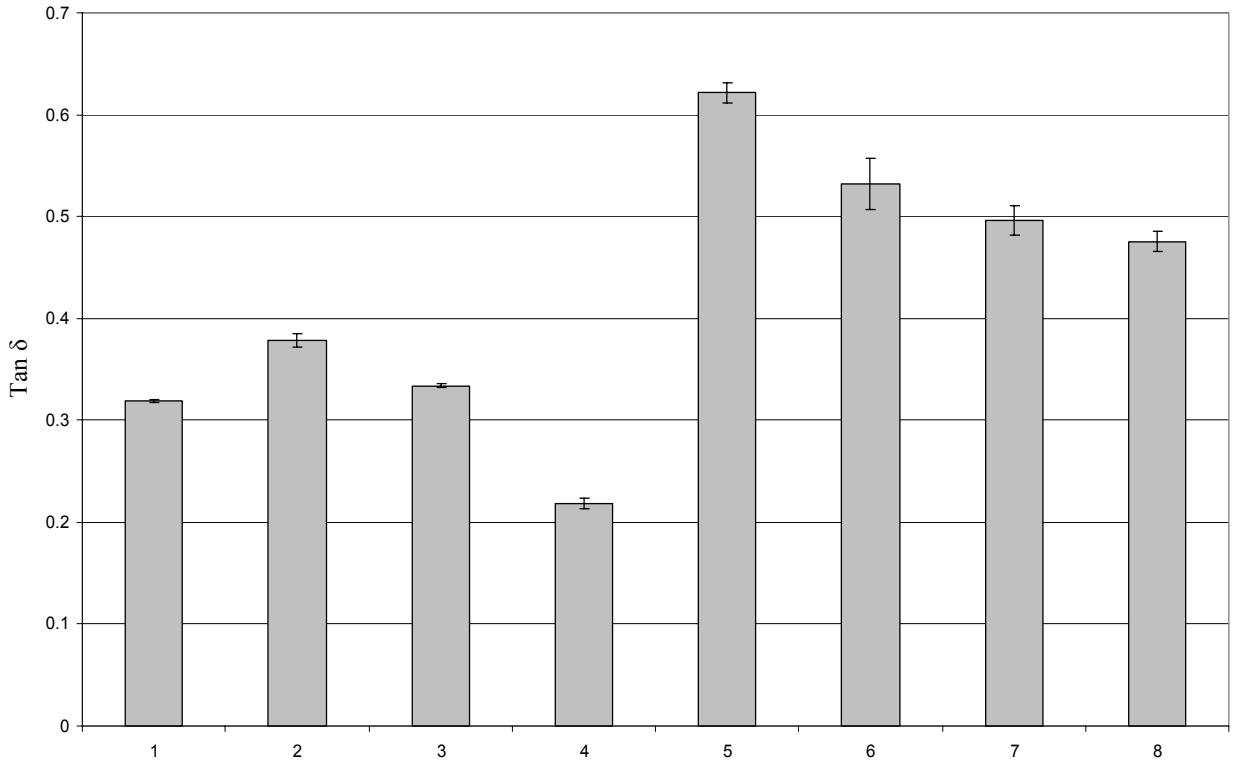

Figure 4. Tan δ at a frequency of 1 Hz for doughs made of native flour (1) and of flours where the lipids had been removed by diethylether (2), chloroform (3) and ethanol (4) and of gluten prepared from doughs of native flour (1) and of flours where the lipids had been removed by diethylether (2), chloroform (3) and ethanol (4).

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). This means that increasing the concentration of uncross-linked material will cause the frequency dependency of G′ to increase, and vice-versa. Figure 5 gives the effect on the frequency dependence of G′ and G′′ (n′ and n′′, equations 1 and 2) for removing the lipids. The value of n′ was higher for the native flour dough than for dough made of flour were lipids had been removed. On the other hand, the removal of the lipids increased the frequency dependence of G′′ (n′′). When the lipids had been removed the values of n′′ were higher than the values of n′, whereas n′>n′′ for the native flour. The removal of lipids reduced the value of n′ while there was an increase in n′′ for the doughs made with flours where lipids had been removed. No effect of solvent polarity was established on the frequency dependence.

For all gluten samples the frequency dependence was higher for G′′ compared with G′ (n′′>n′). The only significant effect ($P<0.01$) on gluten of removing the lipids that could be established was for the solvent ethanol, where the frequency dependence of G′ (n′) was reduced. There was no significant difference between the values of n′′ for the native gluten and gluten recovered from the flours where the lipids had been removed**.** The removal of lipids decreased the frequency dependence of G′ (n′) for dough recovered from flour where the lipids had been removed**.** No such obvious effect was observed on gluten, although removing the lipids by ethanol reduced n′. This behaviour suggests that the amount of uncrossed-linked material was smaller for doughs made with flours where the lipids had been removed compared with the native dough. Similarly, this conclusion can also be drawn for gluten resulting from the flour where the lipids had been removed by ethanol. Furthermore, it seems that the frequency dependence of G′ for gluten and dough was not influenced by the polarity of the solvent used. Fu et al. (1997) reported that the addition of fat up to the level of 5 % (on flour weight) did not affect the values of n′ and n′′ of dough, but adding 7.5 % fat reduced the frequency dependence of G′. According to our work, removal of only 0.6-0.9 % of the native lipids reduced the frequency dependence of G′ considerably.

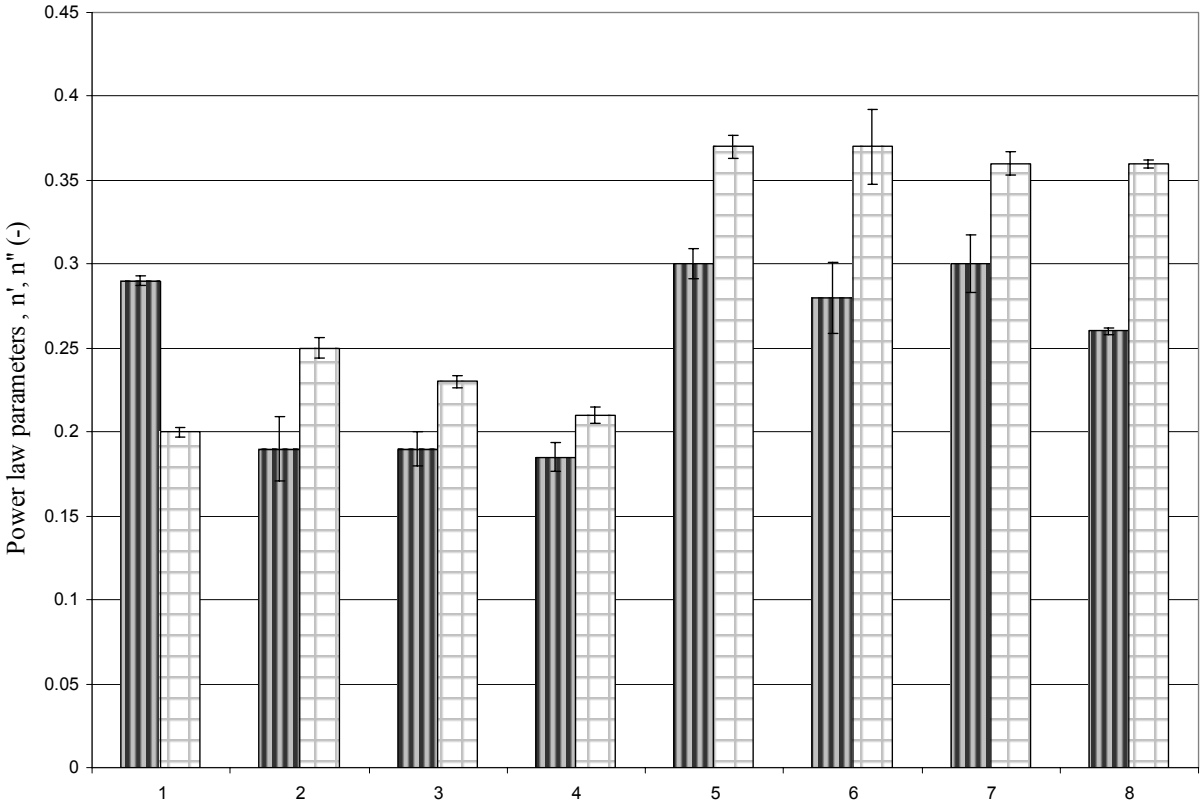

Figure 5. Power law parameters of the frequency sweep (n´ stripped, n´´ squares), doughs made with native flour (1), flours where the lipids had been removed by diethylether (2), by chloroform (3) by ethanol (4) and for gluten separated from doughs made with native flour (5), flours where the lipids had been removed by diethylether (6), chloroform (7) and ethanol (8).

4. Conclusions

Three wheat flours differing in lipid content and composition were prepared by extracting the wheat lipids using three solvents differing in polarity. The effect of lipids was more pronounced on the rheological properties of dough than of gluten. Thus, the results indicate that the native wheat lipids are very important for the rheological functionality of dough, and less important for gluten. Removing the lipids by ethanol increased G´ of dough three times while G´of the corresponding gluten only increased by less than 50 %. Similarly a considerable decrease in the frequency dependence of G´ was established for dough, while the corresponding effect on gluten was only marginal.

Acknowledgements

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