Phase Separation of Wheat Flour Dough Studied by Ultracentrifugation and Stress Relaxation. II. Influence of Mixing Time, Ascorbic Acid, and Lipids

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ABSTRACT

Separation by ultracentrifugation provides a simple tool for studies of the aqueous phases of wheat flour dough. A separation into liquid, gel, gluten and starch, phases was maintained. More or less unseparated dough was also found at the bottom of the test tube. In the present study, the separation behavior was studied for three wheat cultivars when influenced of factors known to affect the baking behavior. The separation properties differ among cultivars. Increased mixing time caused an approximately linear increase for incorporation of water into the gluten phase. For winter wheat, the amount of unseparated dough decreased with increased mixing time, while the separation of spring wheats was not influenced. The general effect of adding ascorbic acid to dough or using defatted flour was improved separation. Lecithin impaired separation. An advantage of this technique is that the phases could be studied without disturbing the composite system. This resulted in valuable information for the interpretation of rheological measurements on dough. It was possible to relate effects on the modulus to either gluten strength, the liquid phase, or interactions between starch and gluten.

One of the unique features of wheat flour is the phase separation of two continuous aqueous phases when the flour is mixed into a dough. The bicontinuous phases are the gluten phase (water-swollen protein) and the liquid phase (containing the dispersed starch granules and soluble components) (Eliasson and Larsson 1993). In a previous study, in which ultracentrifugation was used to illustrate the phase separation of dough, it was observed that the separation of dough occurred when the water content was high enough to enable a gluten phase to separate (Larsson and Eliasson 1996). The rheological behavior at small deformations was correlated with the phase separation behavior over a wide range of water contents. However, it was not possible to establish a simple relationship between the rheological behavior and the amount or properties of the separated phases when cultivars were compared. In the present study, the influence of factors such as mixing time, ascorbic acid, defatting, and lecithin, which are known to affect both the baking behavior, and the rheological behavior at small deformations has been investigated. These factors, which may modify the properties of either or both of the bicontinuous phases, are compared for three wheat cultivars.

Results from rheological measurements on dough at small deformations may appear conflicting. For example, for an increasing flour protein content, both an increase (Abdelrahman and Spies 1986) and a decrease (Hibbered 1970) in the modulus have been observed. Also, for increasing mixing time, different studies have shown both an increase (Bohlin and Carlsson 1980) and a decrease (Mani et al 1992) in the modulus measured at small deformations. The present technique demonstrates that an effect on the rheological behavior at small deformations, such as stress relaxation, may be attributed to different properties of the two aqueous phases present in dough.

MATERIAL AND METHODS

Material

The protein (Kjeldahl, N × 5.7), starch (Åman et al 1994), and damaged starch contents (method 76-30A, AACC 1983) of the studied flours are given in Table I. Kosack is a Swedish winter wheat (medium baking performance); Dragon and Sport are Swedish spring wheats (good baking performance). The flours were milled in a Quadrumat Senior Mill (Svalöf Weibull AB, Svalöv, Sweden) to the following extraction rates: Kosack (69%), Dragon (66%), Sport (61%). The lecithin used was Epicuron 200 (Lucas Meyer, Hamburg, Germany); and ascorbic acid was obtained from Merck.

Dough Mixing

The wheat flour doughs were prepared and the farinograph water absorption was determined according to Larsson and Eliasson (1996). Flour (10 g) was mixed with the amount of distilled water corresponding to the farinograph water absorption (Table I). The same amount of water, on a dry weight basis, was used when ascorbic acid and lecithin were added and when defatted flour was used. The mixing was continued for 8 min, except when the effects of mixing time were to be studied. An increased mixing time, 8 min compared with 5 min (Larsson and Eliasson 1996), was desired in the present study as ascorbic acid and lecithin was added. At least three doughs were mixed under each set of conditions and used for ultracentrifugation and rheological tests. The farinograms of the flours are given in Figure 1.

Ultracentrifugation of Dough

The phase separation of dough was studied by ultracentrifugation as described in Larsson and Eliasson (1996). The volume fraction of the separated dough phase was determined with an accuracy of ±6%, except for some of the unseparated fractions and the smallest liquid phases, which deviated by up to 15%. The water content of the separated phases was obtained with an accuracy of ±1.5%.

Defatting

To remove the lipids, flour (100 g) was extracted with chloroform (200 ml) for 30 min (MacRitchie 1985), and the slurry was filtered. The procedure was repeated twice. The water content of the flour was determined according to the method 44-19 (AACC 1983).

Ascorbic Acid

Solutions of ascorbic acid were prepared to give 230 ppm on a dry flour weight basis.

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Lecithin

Lecithin was shaken with distilled water and ultrasonicated to form a homogeneous lamellar liquid-crystalline dispersion (liposomes). The dispersions were prepared to give 2.3% lecithin on a dry flour weight basis. In earlier studies, an addition of 2% lecithin improved dough strength and bread volume (Rajapaksa et al 1983, Eliasson and Tjerneld 1990). According to the phase diagram for lecithin and water, the lamellar phase is formed at the concentration chosen (Bergenståhl and Fontell 1983). The liposomal birefringence was also confirmed under the polarized microscope.

Dough Rheology

A Bohlin VOR rheometer (Metric Analys, Stockholm, Sweden) was used in the stress-relaxation mode to determine the rheological behavior of the doughs. The details are described in Larsson and Eliasson (1996). The initial value of the stress-relaxation modulus (G_0) and two relaxation times ($t_{0.5}$ and $t_{0.1}$) were chosen to characterize the rheological behavior. The relaxation times were taken as the times at which G was reduced to 50% ($t_{0.5}$) and 10% ($t_{0.1}$) of G_0, respectively. The reproducibility of G_0 and the relaxation times were within 7 and 10%, respectively, except for $t_{0.1}$ of ascorbic-acid-treated Dragon wheat dough, where the repro ducibility was ±15%.

RESULTS

The phase separation of wheat flour dough and its significance in bicontinuous dough is best illustrated by the separation of doughs prepared with a range of water contents (Larsson and Eliasson 1996). In Figure 2, the separation of three Kosack doughs is shown for a mixing time of 5 min (results from Larsson and Eliasson 1996). The dough water contents chosen were, on total weight basis, 39.8% (low), 45.3% (farinograph water absorption), and 48.6% (high). At the lowest water content, no separation was obtained at all. The separation started somewhere before the water content corresponding to the farinograph water absorption. When the amount of water was further increased, separation into four or five phases was observed. From the top, the separated phases were: liquid, gel, gluten, and starch. Depending on the efficiency of the separation, more or less unseparated dough was found at the bottom of the test tube. In the 45.3% sample, some unseparated dough was seen (Fig. 2), but at the highest water level, this unseparated fraction more or less disappeared. Not surprisingly, the separation properties were favored by the higher water content. It was concluded that a certain amount of water was needed to separate the water-swollen protein phase.

TABLE I
Chemical Composition and Farinograph Water Absorption of Flours

	Protein[a]	Starch[a]	Damaged Starch[a]	Water Absorption[b]
Kosack (winter wheat)	11.1	81.8	6.1	45.3
Dragon (spring wheat)	13.4	80.8	5.8	44.8
Sport (spring wheat)	16.7	76.6	4.5	47.1

[a] % on dry, total basis.
[b] % based on dough weight.

Phase-Separation Properties

Cultivar. The separation properties differed between the cultivars when they were compared after 8 min of mixing (Fig. 3a–c). The volume fractions of the separated phases are given in Table II as a complement to Figure 3a–c. The efficiency of separation, which was observed as less sharp boundary lines between the phases, was lower for Sport. The separation of gluten and starch was also lower for Sport than for Kosack and Dragon. This was judged simply from looking at the centrifuged phases in the test tubes. The amount of liquid phase was higher for Kosack than for Dragon and Sport. The largest volume of gluten was obtained for Sport, the flour with the highest protein content. The water contents of the separated dough phases are listed in Table III. At 8 min of mixing, the water content of the gluten phases varied between 54.4 and 56.7%. This should be compared with the water content obtained for swollen wheat storage protein fractions, where the lower molecular weight fraction contained 56% water and the higher molecular weight fraction contained 54% water (Eliasson and Larsson 1993). The lowest amount of water associated with the gluten phase was obtained for Dragon, which also resulted in the lowest farinograph water absorption (Table I). The

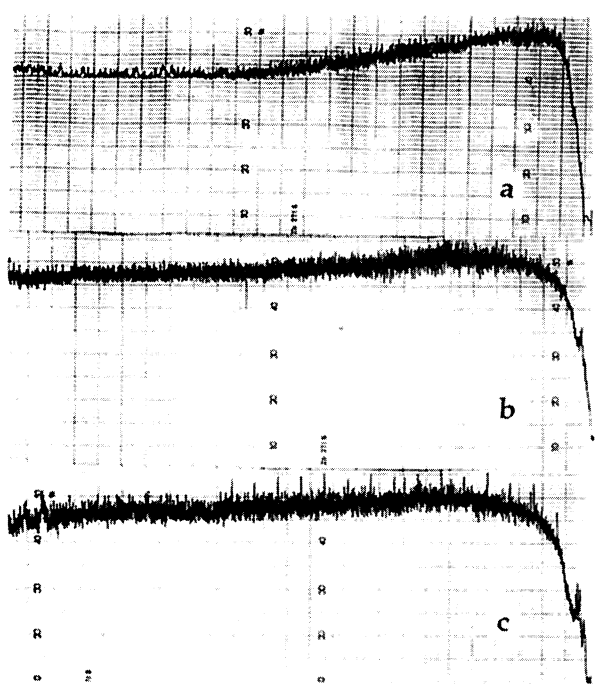

Fig. 1. Farinograms of Kosack (a), Dragon (b), and Sport (c).

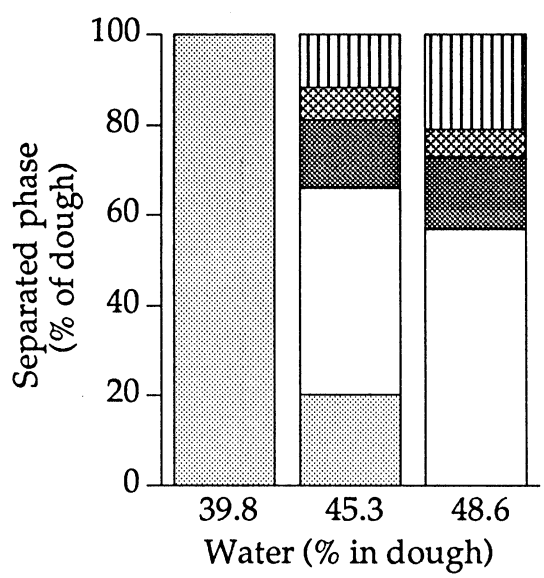

Fig. 2. Separated dough made from Kosack flour with water contents of 39.8% (low), 45.3% (corresponding to the farinograph water absorption), and 48.6% (high). Symbols for separated phases are: liquid (striped), gel (crossed), gluten (dark gray), starch (white), and unseparated dough (light gray).

amount of water associated with all the separated phases was highest for Sport doughs.

Mixing. The influence of mixing time was studied by mixing the doughs for 3, 8, and 25 min. A decrease in the amount of separated liquid phase was obtained after prolonged mixing for Dragon and Sport, whereas for Kosack the amount of separated liquid phase was not influenced (Table II, Fig. 3a–c). The amount of gel phase increased somewhat with prolonged mixing for Dragon, but was hardly influenced at all for Kosack. For Sport, the amount of gel phase was somewhat lower at 8 min of mixing than at 3 and 25 min. The amount of mixed phase was essentially unaffected by more than 8 min of mixing for Dragon and Sport. Kosack, on the other hand, produced less unseparated dough with prolonged mixing. As a consequence of this improved separation, the amount of gluten increased moderately for Kosack but was not affected for the other two cultivars. The amount of separated starch phase increased with prolonged mixing for Kosack and Sport, whereas no increase was observed for Dragon. When the mixing time was increased from 3 to 25 min, the water content of the gluten phase increased significantly for all the cultivars studied (Fig. 4). The increase was less for the winter wheat Kosack. The highest gluten water content was obtained for Sport mixed for 25 min. The water content of the gel and starch phases was not appreciably affected by mixing time (Table III).

Ascorbic acid. The effect of ascorbic acid differed between the cultivars. Doughs including ascorbic acid were compared with the corresponding (8 min of mixing) nonmodified dough. Improved separations were obtained for all the cultivars when ascorbic acid was included but was most pronounced for Sport (Table II, Fig. 3a–c). This improved separation was observed as less unseparated dough and more distinct boundary lines between the separated phases. The amount of separated gluten was not influenced by ascorbic acid for any of the cultivars. A larger amount of liquid phase was found for Kosack, while a reduced amount was observed for Dragon. No separated liquid phase was observed for Sport when ascorbic acid was included. Compared with the nonmodified reference dough, ascorbic acid did not influence the water content of any of the phases (Table III). A slight decrease in the water content of the gel phase was, however, observed for Kosack.

Defatted flour. When defatted flour dough was compared with the corresponding nonmodified dough, the first effects noticed were improved separation (observed as sharper boundary lines), a gray gluten phase, and a clear liquid phase. The liquid phase also became sticky and was more viscous, i.e., less pourable. A slight decrease in the amount of liquid phase was observed for all the cultivars when they were defatted (Fig. 3a–c). The amount of gel phase increased for defatted Kosack, decreased slightly for defatted Dragon, and was not influenced for defatted Sport. The amount of gluten was reduced in defatted Sport flour; however, this was not observed for the two other cultivars. The reduction in

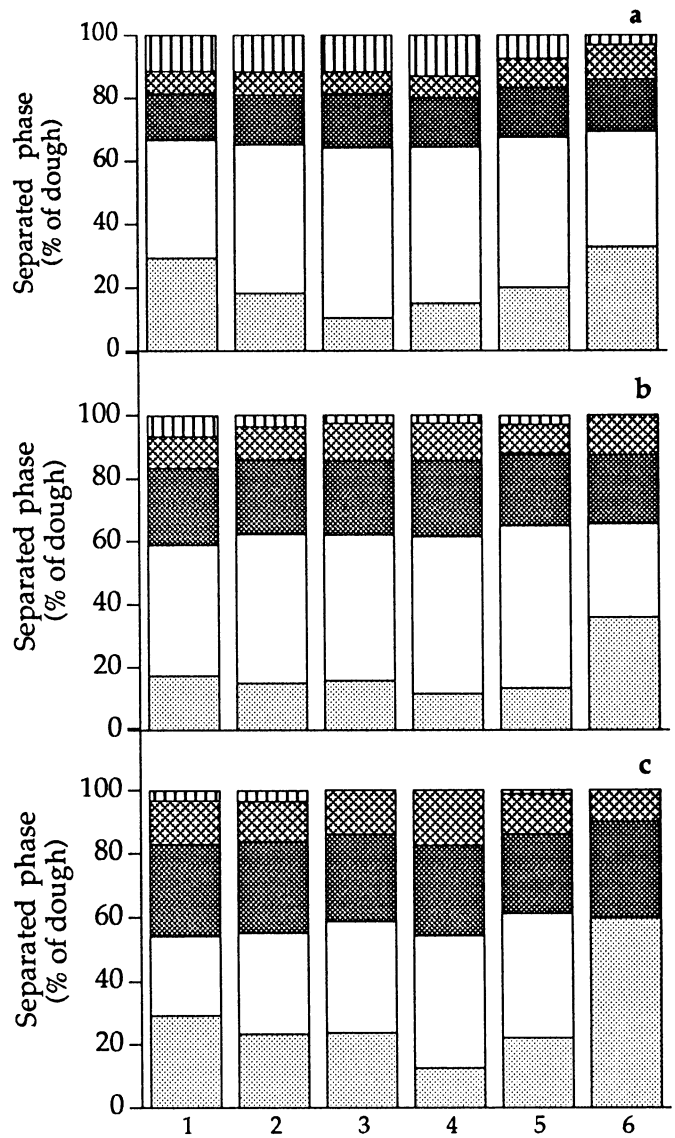

Fig. 3. Separated dough of Kosack (a), Dragon (b), and Sport (c). Columns 1–6 indicate the influence of mixing time: 3, 8, 25 min (1, 2, 3), ascorbic acid (4), defatted flour (5), and lecithin (6). Symbols as in Fig. 2.

TABLE II
Volume Fractions of Separated Phases (%)

Flour	Separated Phase	Mixing Time			Mixing Time (8 min)		
		3 min	8 min	25 min	Ascorbic Acid	Defatted Flour	Lecithin
Kosack	Liquid	11.5 ± 0.0	11.7 ± 0.1	11.7 ± 0.1	13.1 ± 0.2	7.8 ± 0.5	3.2 ± 0.5
	Gel	7.1 ± 0.0	7.2 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	9.1 ± 0.2	10.8 ± 0.4
	Gluten	14.9 ± 0.1	15.9 ± 0.1	17.2 ± 0.2	15.6 ± 0.0	15.6 ± 0.5	16.6 ± 0.2
	Starch	37.4 ± 0.2	47.1 ± 0.1	54.0 ± 0.5	49.5 ± 0.2	47.7 ± 0.2	36.7 ± 3.4
	Unseparated dough	29.2 ± 0.1	18.1 ± 0.2	10.3 ± 0.5	15.0 ± 0.2	19.9 ± 0.2	32.7 ± 4.3
Dragon	Liquid	6.6 ± 0.1	3.3 ± 0.0	2.3 ± 0.2	2.3 ± 0.2	2.7 ± 0.1	···
	Gel	10.1 ± 0.2	10.7 ± 0.3	12.0 ± 0.0	12.0 ± 0.5	9.3 ± 0.0	12.5 ± 0.1
	Gluten	24.5 ± 0.1	23.6 ± 1.0	23.5 ± 0.5	24.1 ± 0.2	23.1 ± 0.0	22.0 ± 0.1
	Starch	41.5 ± 0.2	47.4 ± 0.5	46.4 ± 0.4	50.0 ± 2.0	51.6 ± 0.3	29.5 ± 0.1
	Unseparated dough	17.3 ± 0.1	15.0 ± 0.5	15.8 ± 0.2	11.6 ± 1.7	13.3 ± 0.5	36.0 ± 0.2
Sport	Liquid	3.4 ± 0.1	3.6 ± 0.1	···	···	1.3 ± 0.2	···
	Gel	13.7 ± 0.2	12.8 ± 0.0	13.9 ± 0.1	17.6 ± 0.0	12.9 ± 0.2	10.1 ± 0.1
	Gluten	28.7 ± 0.3	28.3 ± 0.1	27.2 ± 0.0	28.2 ± 0.1	24.7 ± 0.1	30.0 ± 0.0
	Starch	24.9 ± 0.3	32.0 ± 0.2	35.2 ± 0.2	41.8 ± 1.7	39.3 ± 0.2	···
	Unseparated dough	29.3 ± 0.4	23.3 ± 0.1	23.7 ± 0.2	12.5 ± 1.9	22.0 ± 0.5	59.9 ± 0.1

TABLE III
Water Contents of Separated Phases (%)

Flour	Separated Phase	Mixing Time			Mixing Time (8 min)		
		3 min	8 min	25 min	Ascorbic acid	Defatted Flour	Lecithin
Kosack	Gel	81.4	80.7	81.3	79.3	80.2	82.6
	Gluten	54.8	56.0	59.1	55.4	56.4	59.6
	Starch	29.8	29.7	29.7	29.5	30.1	30.9
Dragon	Gel	80.9	81.6	81.2	81.0	78.1	79.3
	Gluten	53.0	54.4	58.5	54.4	54.9	57.4
	Starch	29.3	30.7	29.9	29.7	30.1	31.5
Sport	Gel	83.3	84.0	83.3	84.2	82.8	80.0
	Gluten	54.6	56.7	62.5	56.3	58.1	56.3
	Starch	30.2	30.2	30.2	30.3	31.8	35.5[a]

[a] Unseparated fraction.

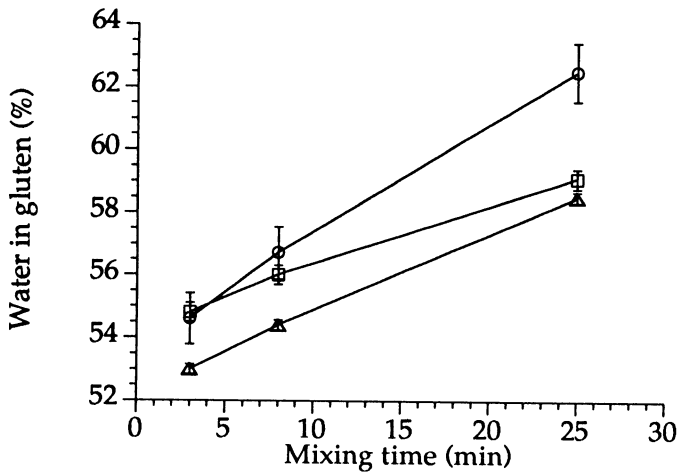

Fig. 4. Water content of gluten as a function of mixing time. Kosack (□), Dragon (Δ), Sport (○).

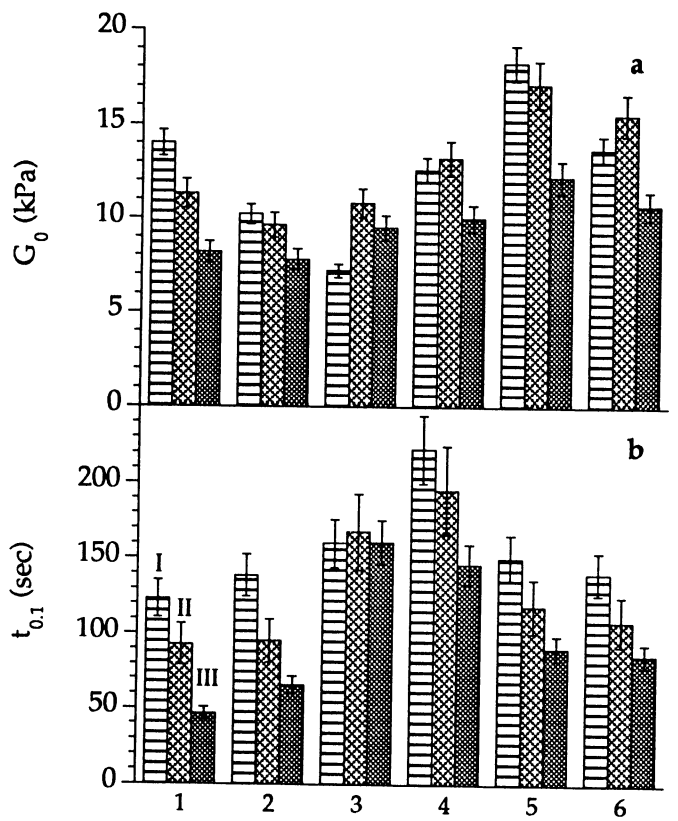

Fig. 5. Influence on G_0 (a) and $t_{0.1}$ (b) of mixing time 3,8, 25 min (columns 1, 2, 3), ascorbic acid (4), defatted flour (5), lecithin (6) for Kosack (I, striped), Dragon (II, crossed), and Sport (III, gray).

the amount of gluten observed for defatted Sport corresponded to an increase in the amount of separated starch. A slight increase in the amount of starch was also observed for Dragon, but not for Kosack. No change was observed in the amount of unseparated dough for any of the cultivars. The water content of the separated phases of Kosack was not influenced by defatting (Table III). With defatted Dragon and Sport flours, a decrease in the water content of the gel phase was noted. The water content of the gluten and the starch phases increased for defatted Sport but were not influenced for Dragon. Considering the poorer separation properties of Sport, the reduced amount of gluten phase observed after defatting is consistent with the higher water content and, thus, less contaminating starch in the gluten phase.

Lecithin. The most striking effects on the separation properties of the dough were observed when lecithin was added. Compared with the nonmodified dough mixed for 8 min, less efficient separation and a lower amount of separated liquid phase were observed (Fig. 3a–c). A larger amount of water was absorbed as no liquid phase separated for Dragon and Sport, and the liquid phase of Kosack was reduced from 11.7 to 3.2%. When lecithin was added, poorer separation was observed for all the cultivars. Sport showed the most extreme behavior with no proper separation of starch and gluten. The boundary lines were diffuse and no starch phase was distinguishable, instead a large amount was classified as unseparated. The gluten phase increased somewhat for Sport when lecithin was added, which may be explained by a higher starch content. The gluten phases of Kosack and Dragon were not significantly affected by lecithin. The thin white layer with a high concentration of small starch granules observed between the gluten and the gel phases in all other samples (Larsson and Eliasson 1996) was not present in the lecithin doughs. Although the separation properties were poorer when lecithin was added, the technique still exhibited high accuracy. The addition of lecithin resulted in an increase in the amount of water associated with the gel phase for Kosack and a decrease for Dragon and Sport (Table III). The water content of the gluten phase increased for Kosack and Dragon but was unaffected for Sport. The unaffected water content of the gluten phase of Sport dough does not necessarily involve a lower amount of water associated with the gluten phase but is more likely a result of the larger amount of starch in the gluten phase as the separation properties were strongly reduced when lecithin was added. The starch phases also contained elevated amounts of water when lecithin was added. As no pure starch phase was obtained for Sport, the unseparated fraction was considered. The water content of the unseparated fraction of Sport was higher (36.2%) than the separated starch phase when no lecithin was added (30.2%). This indicates the presence of nonseparable gluten in the starch phase.

Influence on Rheological Behavior

The half relaxation times ($t_{0.5}$) are not reported, as they paralleled the G_0 values.

Cultivar. The influence of cultivar on the stress relaxation was compared at the fixed mixing time of 8 min. The modulus of Sport was lower than that of Dragon and Kosack (Fig. 5a). The relaxation time decreased in the order: Kosack, Dragon, Sport (Fig. 5b).

Mixing. The optimum mixing times derived from the farinograms were 3 min for Kosack and 7–8 min for Dragon and Sport. The stress-relaxation modulus of Dragon and Sport were not significantly influenced by the mixing time. Kosack, on the other hand, with a lower mixing tolerance, exhibited a decreased modulus at longer mixing times (Fig. 5a). Both higher and lower dynamic moduli have been observed for doughs mixed passed their optimum in the farinograph (Bohlin and Carlsson 1980, Mani et al 1992).

The relaxation time ($t_{0.1}$) increased with longer mixing times for all the cultivars. The value of $t_{0.1}$ for Kosack increased gradually with mixing time already after 3 min of mixing (Fig. 5b). For the spring wheats, it increased mainly after 8 min of mixing, which corresponded to their optimal mixing times according to the farinograms. The largest increases were noted for Dragon and Sport at 25 min of mixing. Thus, $t_{0.1}$ increased when the doughs were overmixed, according to the farinogram. The same value of the relaxation time was obtained for all the cultivars when the doughs were mixed for 25 min.

Ascorbic acid. The stress-relaxation parameters, both the modulus and the relaxation time, increased for all wheat cultivars, although to different extents. The greatest increase in modulus was observed for Dragon, followed by Sport and then Kosack (Fig. 5a). This should be compared with the increase in $t_{0.1}$ (greater for Sport than for Dragon and Kosack) (Fig. 5b).

Defatted flour. A large increase in stress-relaxation modulus was observed with defatted flours (Fig. 5a). The increase was greatest for Dragon and Kosack. The value of $t_{0.1}$ was only slightly affected by defatting (Fig. 5c).

Lecithin. Mixing the doughs with the lamellar dispersion of lecithin influenced the stress-relaxation parameters in a manner similar to that of defatting but the effect of lecithin on the modulus was less than that of using defatted flour. The greatest effect on the modulus was observed for Dragon.

DISCUSSION

The gluten strength, the interaction of starch granules with gluten, and the interaction between starch granules are the main elements that determine the overall dough strength (Smith et al 1970). The phase separation properties of a mixed dough provide some additional aspects to consider for the evaluation of the rheological behavior at small deformations. The rheological properties of the separated phases will all contribute to the rheological properties of the composite dough. In the present study, the results are discussed as: 1) strength of the gluten phase; 2) amount and viscosity of the liquid phase; 3) amount of unseparated dough. The amount of liquid phase, the main medium in which the starch granules are dispersed, determines the volume fraction of the granules in the particle-filled system, i.e., the starch-liquid phase. The modulus of a composite polymer system is dependent on the volume fraction of the filling particles and on the modulus of the matrix in which they are suspended (Nielsen 1975). The amount of unseparated dough is the fraction regarded as contributing to an increased modulus because of the high degree of interaction between the starch granules and the gluten phase.

The relaxation times ($t_{0.5}$ and $t_{0.1}$) are related to the two flow processes occurring when dough relaxes (Bohlin and Carlsson 1981, Larsson and Eliasson 1996). Values of $t_{0.5}$ are, however, not reported here, as they parallel those of G_0. The second relaxation process has been related to a lamellar gluten phase in dough (Carlsson 1981). In the present study, $t_{0.1}$ was chosen to represent an effect on the gluten phase.

Cultivar

A large amount of separated gluten phase, and hence less of starch phase, seems to result in both a lower stress-relaxation modulus and shorter relaxation time (Fig. 5). The possibility that the properties of all the separated phases may differ between the cultivars cannot be ruled out. Therefore, no simple relationship was found between the studied rheological parameters and cultivars. The influence of cultivar on the correlation between the rheological measurements and the separation behavior is discussed elsewhere (Larsson and Eliasson 1996).

Mixing

Dreese and co-workers (1988) compared overmixed dough with dough mixed with excess amounts of water. A loss of water binding capacity similar to that observed when dough is mixed with excess amounts of water was suggested for doughs mixed past their optimum. In the present study, the incorporation of water in the gluten phase increased almost linearly with mixing time (Fig. 4). This may be compared with no, or a very limited, increase in the water content of the gluten phase when excess water was added to the dough (Larsson and Eliasson 1996). Excess water added to dough was recovered in the liquid phase after separation instead of being incorporated into the gluten phase. A comparison of these results clearly shows the different location of water in an overmixed dough and a dough made with excess water. The continuous incorporation of water seemed to depend on the work input, which provided a finer distribution of water in the gluten phase. The increase in water content of the environment and the higher work input when overmixing may cause increased protein solubility as observed by Tanaka and Bushuk (1973), and higher lipid solubility as observed by Pomeranz et al (1968).

Another explanation for the increased water content of gluten when dough is overmixed (Fig. 4) may be related to the separation properties of gluten and starch. The resulting water content of the gluten phase may be higher if less starch is recovered in the gluten phase after separation. Mixing for a longer period of time may lead to more efficient extraction of starch and will favor separation.

Doughs mixed for 25 min had a wet and sticky appearance, independent of cultivar and farinogram mixing stability. The stickiness of overmixed dough seemed to be related to the higher amount of water incorporated in the gluten phase (Table III). The Sport dough was judged by manual examination to be the most sticky dough after both 8 and 25 min of mixing.

Kosack had a dough development time of ≈3 min according to the farinogram, and prolonged mixing resulted in reduced mixing resistance (Fig. 1). A pronounced decrease in the amount of unseparated dough (Table II, Fig. 3a) and a decrease in the stress-relaxation modulus (Fig. 5a) seemed to be correlated to the lower tolerance to mixing observed for this winter wheat. As the amount of liquid phase was constant, the reduction in the amount of unseparated dough is suggested to explain the decrease in G_0 when mixing was extended beyond the optimum. The spring wheats, on the other hand, showed a high mixing tolerance. The optimum mixing time of the spring wheats was 7–8 min (Fig. 1). The amount of unseparated dough did not change from 8 to 25 min of mixing (Table II, Fig. 3a). A slight increase in the modulus at 25 min of mixing was, however, obtained for Dragon and Sport (Fig. 5a). This may be explained by the reduced amount of liquid phase or its elimination, which is equivalent to a higher volume fraction of starch granules in the liquid phase. Over all, the modulus seemed to be strongly influenced by the amount of unseparated dough.

It seems reasonable to suggest that the large amount of liquid phase obtained for Kosack caused starch to become washed out of the gluten phase during prolonged mixing as it resulted in a lower amount of unseparated dough. A decrease in the amount of

unseparated dough was also observed when excess water was added (Fig. 2). Separation of the spring wheat doughs resulted in a low amount of liquid phase, which may explain the constant amount of unseparated dough obtained for doughs mixed 25 min. The mixing stability seemed to be correlated to the amount of unseparated dough and possibly also to the amount of liquid phase.

Gluten is expected to become weaker when mixing is extended beyond the optimum as a reduction in molecular weight because of overmixing is generally assumed to take place (Tanaka and Bushuk 1973, Danno and Hoseney 1982). Also, the larger amount of water incorporated in the gluten phase may result in a softer gluten. A general decrease in rheological parameters was, however, not observed. G_0 decreased for Kosack but was only slightly affected for Dragon and Sport, even after 25 min of mixing (Fig. 5a). On the other hand, when optimum mixing time was exceeded according to the farinogram, $t_{0.1}$ increased for all the cultivars (Fig. 5b). This behavior may indicate that a change in the gluten phase took place after optimum mixing. Evaluation of the rate-stress plots according to Bohlin and Carlsson (1981) showed a decrease in the peak corresponding to the second relaxation process for doughs mixed for 25 min. Mita and Bohlin (1983) concluded that reduction in the influence of either relaxation process was because of arrested flow in this process, such as that caused by crosslinking. It also seems reasonable that the smaller influence of the second relaxation process can result from a decrease in the amount of elements causing it. In this case, a smaller influence of the second relaxation process may be caused by disruption of the structure after intense overmixing. Micrographs at different stages of dough mixing have shown that parallel gluten sheets, with starch granules in between, were formed at optimum mixing (Moss 1972). The sheets became thinner during prolonged mixing. In overmixed doughs, very thin gluten films covered all the starch granules. The amount of gluten sheets was strongly reduced by overmixing.

Similar relaxation of the second flow process was observed for the winter wheat and the spring wheats when they were mixed far beyond their optimum. The same $t_{0.1}$ values obtained after 25 min of mixing may be a coincidence but may also be related to a change in the protein phase, which was similar for all the cultivars mixed far beyond their optimum.

Ascorbic Acid

A pronounced increase in stress-relaxation parameters was observed in $t_{0.1}$ when ascorbic acid was added; however, G_0 also increased (Fig. 5a–b). The effect of ascorbic acid on $t_{0.1}$ can be considered mainly as influencing the gluten phase, especially as no effect on $t_{0.1}$ was observed when dough was mixed with defatted flour or when lecithin was added. The larger increase in $t_{0.1}$ observed for Dragon and Sport may indicate that their gluten was strengthened more by ascorbic acid than the gluten of Kosack. The effect on G_0 also seemed to be caused by a strengthening of the gluten phase as the amount of unseparated dough decreased and the amount of liquid phase was only moderately influenced and, consequently, could not explain the effect on the modulus. According to Mita and Bohlin (1983), ascorbic acid increased both the modulus and the half relaxation time ($t_{0.5}$) of gluten. The largest increase in G_0 was obtained for Dragon, while $t_{0.1}$ increased most for Sport. This may indicate different dominating effects of ascorbic acid on the two flours.

The stress-rate plots for ascorbic acid doughs were similar to those of doughs mixed 25 min, but differed from those of dough containing defatted flour and those containing lecithin. Although the apparent similar influence on the second relaxation processes for overmixed doughs and ascorbic acid doughs, the behavior may be attributed to different phenomena. The overmixed dough is discussed above. For the ascorbic acid treated doughs, the decrease in the peak corresponding to the second relaxation process is in accordance with an arrested flow in the second relaxation process. This was attributed to the formation of high molecular weight proteins by Mita and Bohlin (1983). It is widely accepted that ascorbic acid favors the formation of high molecular weight proteins (Mair and Grosch 1979).

Although the liquid phase was eliminated for Sport, i.e., there was no extracting liquid to help wash out starch from the gluten phase, the amount of unseparated dough decreased when ascorbic acid was added (Fig. 3c). It is suggested that the unseparated dough reflects the degree of interaction between starch and the gluten phase. It appears that larger molecular weight gluten proteins improves the separation of starch in the gluten gel. This is in contrast to the results from the study by Eliasson (1990), where the higher molecular weight protein fraction adsorbed more strongly to the starch granule surface than the lower molecular weight fraction. The starch granule-gluten interface may also be influenced by ascorbic acid. For example, Seguchi (1993) showed that a higher degree of hydrophobicity was obtained for the starch granule surface after chlorine treatment, heating, or aging. Another quality of oxidized dough that appears to be important for its separation properties is the homogeneity of the dough. Light microscopic studies have shown that oxidized dough is less homogeneous than its nonoxidized equivalent (Moss 1974).

Defatting

The high modulus and improved separation properties were the most striking observations made for defatted flour. It should be pointed out that the amount of unseparated dough was not affected; however, separation of the gluten and starch phases was improved for defatted flour. The liquid phase remaining after defatting was considerably more viscous than that separated from nondefatted flour. The large increase in modulus (Fig. 5a) seems to result from the more viscous (qualitative remark) liquid phases separating from the defatted flours. Defatting with chloroform might permit soluble material, for example pentosans, to be released in the liquid phase to a greater extent rather than being associated with the gel phase. Soluble pentosans are known to form highly viscous solutions with intrinsic viscosities 15–20 times higher than those of soluble proteins (Udy 1956). The improved separation properties found in the present study, may be because of the absence of foam destabilizing lipids in the defatted flours. Extremely good foam stability of the liquid phase has been observed for flours defatted with chloroform (MacRitchie 1976). The increase in G_0 may also be a result of the reduced amount of lubricating lipids present in the gluten phase after defatting.

Lecithin

The tendency of a dough to phase separate was impaired by lecithin. The increase in modulus is explained by the increased amount of unseparated dough (Fig. 3a–c) and the higher volume fraction of starch in the liquid phase. By adding lecithin to the dough, the water content of the gluten phase increased and the amount of liquid phase was reduced or eliminated. The modulus may be expected to decrease if the starch granules were emulsified by lecithin as an adsorbed layer around a filler determines the actual rheological properties (van Vliet 1988). The centrifuged doughs, however, showed strongly reduced separation properties, which suggests a finer distribution of all the components to explain the effect of lecithin. The lower tendency to separate seemed to simply prove the strong emulsifying effect of lecithin added in its lamellar phase.

CONCLUSIONS

Separation by ultracentrifugation provides a simple tool for studies of the two aqueous phases present in wheat flour dough. An advantage of this technique is that the phases could be studied without disturbing the composite system. This resulted in valuable information for the interpretation of rheological measurements on dough. When a specific flour was influenced by mixing

time, ascorbic acid, lecithin, or defatting, the technique provided the effect on the modulus to be related to either gluten strength, the liquid phase, or interactions between starch and gluten. On the other hand, when different cultivars were compared, it was harder to attribute a lower or higher modulus to the amount or quality of a separated phase.

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