Effects of Enzymes and Oxidizing Agents on Shear Stress Relaxation of Wheat Flour Dough: Additions of Protease, Glucose Oxidase, Ascorbic Acid, and Potassium Bromate

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ABSTRACT

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Measuring shear stress relaxation with an established research rheometer after slowly applying large strain proved useful for characterizing the effects of different chemical and enzymatic additives. Baking tests done with and without added ascorbic acid indicated that the method can be used for predicting effects of such additives on breadbaking quality. The relaxation process for dough is discussed in terms of two flow processes, one occurring at short time periods and the other at longer time periods. The slowly applied large strain shear stress relaxation method provides information about stress relaxation behavior during the second flow process. The effects of two enzymes (protease and glucose oxidase) and two chemical additives (ascorbic acid and potassium bromate) were studied. The results are presented as the stress relaxation curve with the corresponding rate plot and the initial value of the stress. Addition of the oxidizing agents increased the second flow process times and decreased the relaxation rate during the second process. Protease shortened the second flow process times and increased the relaxation rate during the second process. Glucose oxidase separated the two flow processes by increasing the relaxation time of the second process and decreasing the relaxation time of the first process.

For a long time, the use of exogenous enzymes in the baking industry was limited to the addition of amylase for adjustment of flour quality as reflected by the falling number. Today, several enzyme manufacturers offer a wide range of enzymes produced especially for breadmaking. Exogenous enzymes can be used to reduce the effects of quality variations in wheat flours, as well as to favorably alter the baking properties. For example, a high-protein flour that is "too strong" can, with the addition of protease, increase in extensibility and give an expanded bread volume (Drapron and Gordon 1987). The bakers (users) knowledge of the enzyme products offered by the enzyme manufacturers is often very limited. Side activities of enzymes other than the main enzymes are not always declared or even known. Because the function of the enzymes in the dough is not very well known, it is difficult to control the final result. An enzyme that has a positive effect on one flour might be negative for another. Therefore, it is desirable to find a method that can characterize the influence of exogenous enzymes on the baking quality of wheat flours. The rheological properties of a dough have often been correlated with the bread baking quality (Johnson et al 1943, Janssen 1992, Mani et al 1992, Wikström and Bohlin 1996). Moreover, fundamental rheological measurements can give more information about the character of a dough and, hence, the quality of the flour than baking can alone.

Traditionally, a stress relaxation measurement is obtained by applying a sudden step in strain, normally applied in less time than the shortest relaxation time of the sample. Preferably, the measurement is made at a strain in the linear region to avoid disturbing the structure of the sample. Such an experiment gives useful information about the rheological properties of viscoelastic materials. Shear stress relaxation measurements on dough and gluten were performed by Bohlin and Carlson (1981). Their results were analyzed according to a theory of cooperative flow (Bohlin 1980). They found that the shear stress relaxation process in both wheat flour dough and gluten can be separated into two different flow processes, one occurring at short time periods (1–10 sec) and one at longer time periods (10–10^4 sec).

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The character of the second flow process should be most important for the ability of a dough to expand in volume during fermentation. Dough fermentation time is normally ≈40–80 min, which is within the time period of the second flow process. It appears that a good baking quality wheat flour requires that a second relaxation process occur within a certain time period with a certain relaxation rate during that time (Mita and Bohlin 1983). Poor baking quality will result if the second relaxation process is too slow or occurs too late. Baking quality will be equally bad if the second process occurs too early or perhaps not at all.

In the present work, we show that a rheological method we call large strain stress relaxation (the relaxation that occurs after a large strain is applied) is a most useful method for characterizing the influence of different enzymes and other additives on dough structure and flow behavior. This method is not as sensitive to variations in the initial relaxation during the strain rise. Moreover, it brings out the second process, which contains important information on dough quality properties during fermentation.

Three wheat cultivars were used in the experiments. The effects of two enzymes (protease and glucose oxidase) and two chemical additives (ascorbic acid and potassium bromate) were studied. The results are presented as stress relaxation plots together with the corresponding relaxation rate curve and the initial stress value for each dough.

MATERIALS AND METHODS

Flours

Three flours were obtained from Nord Mills (Malmö, Sweden) and Svalöf-Weibull AB (Svalöv, Sweden): a spring wheat (Thasos) and two winter wheats (Kosack and Ritmo). The flours were carefully selected for baking quality. The spring wheat Thasos was a "too strong". Ritmo was a winter wheat with poor baking quality, and Kosack was a normal winter wheat. The flours were analyzed for protein (near-infrared reflectance), wet gluten (ICC 1960), simple sugars (AACC 1995), falling number (AACC 1995), and glutogram value (Sietz 1987). They were test baked according to Olered (1979) at the Swedish Cereal laboratory (Svalöv, Sweden) with and without ascorbic acid (75 ppm).

Additives

Four different additives were studied, each at three different concentrations. The additives were ascorbic acid (5, 50, and 500 ppm) from Merck, potassium bromide (3, 30, and 300 ppm) from Merck, a protease (*Aspergillus oryzae)* from Röhm (1, 10, and 50 ppm), and a glucose oxidase (*Aspergillus niger*) from Novo Nordisk (Bagsvaerd, Danmark) (0.36, 1.8, and 3.6 ppm). Water solutions of the additives were freshly made each day and stored in a water bath at 30°C.

Mixing of Doughs

Wheat flours were mixed with distilled water in a Brabender Farinograph using the 10-g bowl. Water absorption for each flour was estimated according to farinograph standards (AACC 1995). The same flour-to-water ratio was used in the preparation of sam-

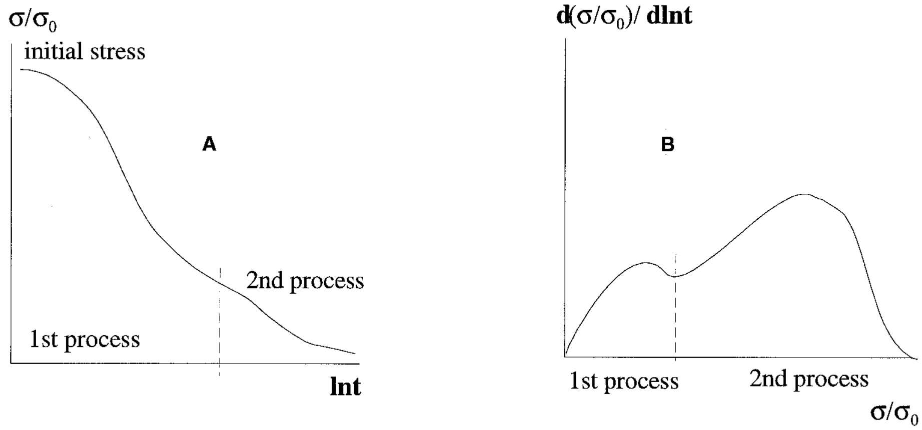

Fig. 1. Example of a typical stress relaxation curve (**A**) with a corresponding rate plot for a wheat flour dough (**B**).

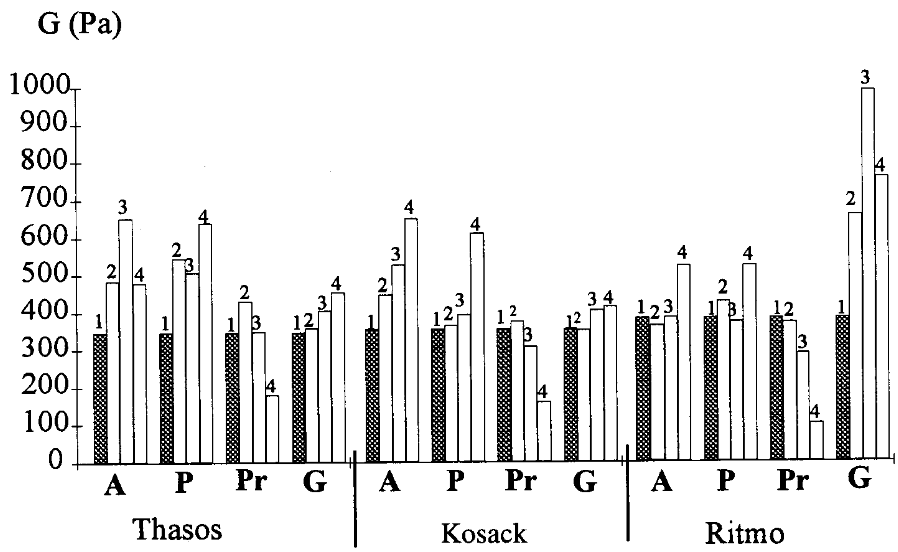

Fig. 2. Histogram bars represent the initial stress for untreated and treated doughs from three wheat cultivars. A = Ascorbic acid (1–4 at 0, 5.00, 50.0, and 500 ppm, respectively). P = Potassium bromate (1–4 at 0, 3.00, 30.0, and 300 ppm, respectively). Pr = Protease (1–4 at 0, 1, 10, and 50 ppm, respectively). G = Glucose oxidase (1–4 at 0, 0.36, 1.8, and 3.6 ppm, respectively). Each bar represents the average of three measurements.

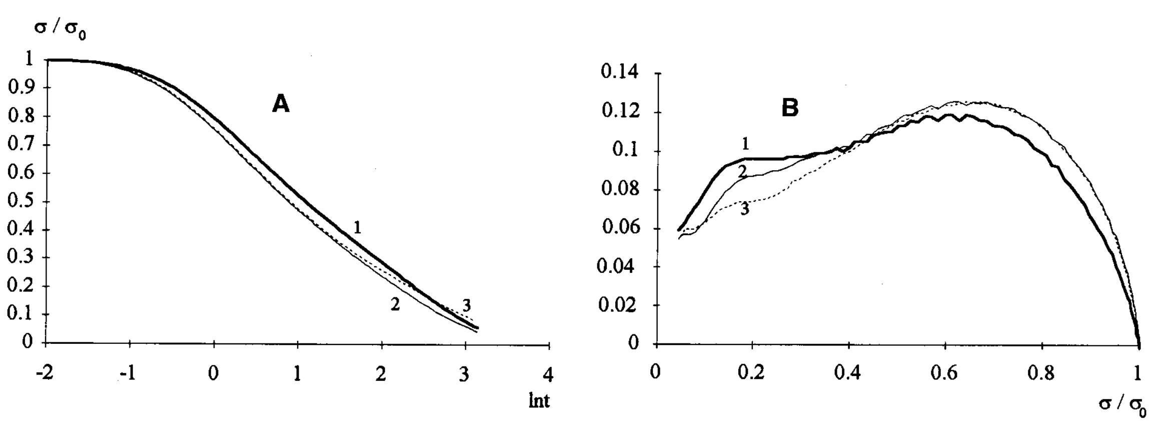

Fig. 3. Normalized stress versus linear time (**A**) [$\sigma(t)/\sigma_0$ vs. lnt] and the corresponding relaxation spectrum versus normalized stress (**B**) [$d(\sigma/\sigma_0)$/dlnt vs. $\sigma(t)/\sigma_0$] for Thasos (1), Kosack (2), and Ritmo (3) wheat dough samples. Each curve represents the average of three measurements.

ples containing additives. The samples were mixed for 5 min at 30°C. After mixing, the dough samples were allowed to rest for 20 min in a 30°C water bath before being transferred to the rheometer.

Rheological Measurements

A Bohlin VOR rheometer (Metric Analys, Stockholm, Sweden) was used to study the stress relaxation behavior of the samples subjected to large strain. The strain was applied in the flow relaxation

TABLE I
Analytical and Baking Test Results for Three Wheat Cultivars

Wheat	Protein (%)	Falling No.(sec)	Water Absorption %	Wet Gluten (%)	Glutogram (sec)	Glucose (%)	Bread Volume (cm^2)	
							1	2
Thasos[a]	13.8	396	60.0	29.0	124.2	0.05	844	779
Kosack[b]	11.5	419	59.0	24.8	19.8	0.03	620	936
Ritmo[b]	8.2	355	57.5	18.5	30.4	0.03	632	785

[a] Without ascorbic acid.
[b] With ascorbic acid (75 ppm).

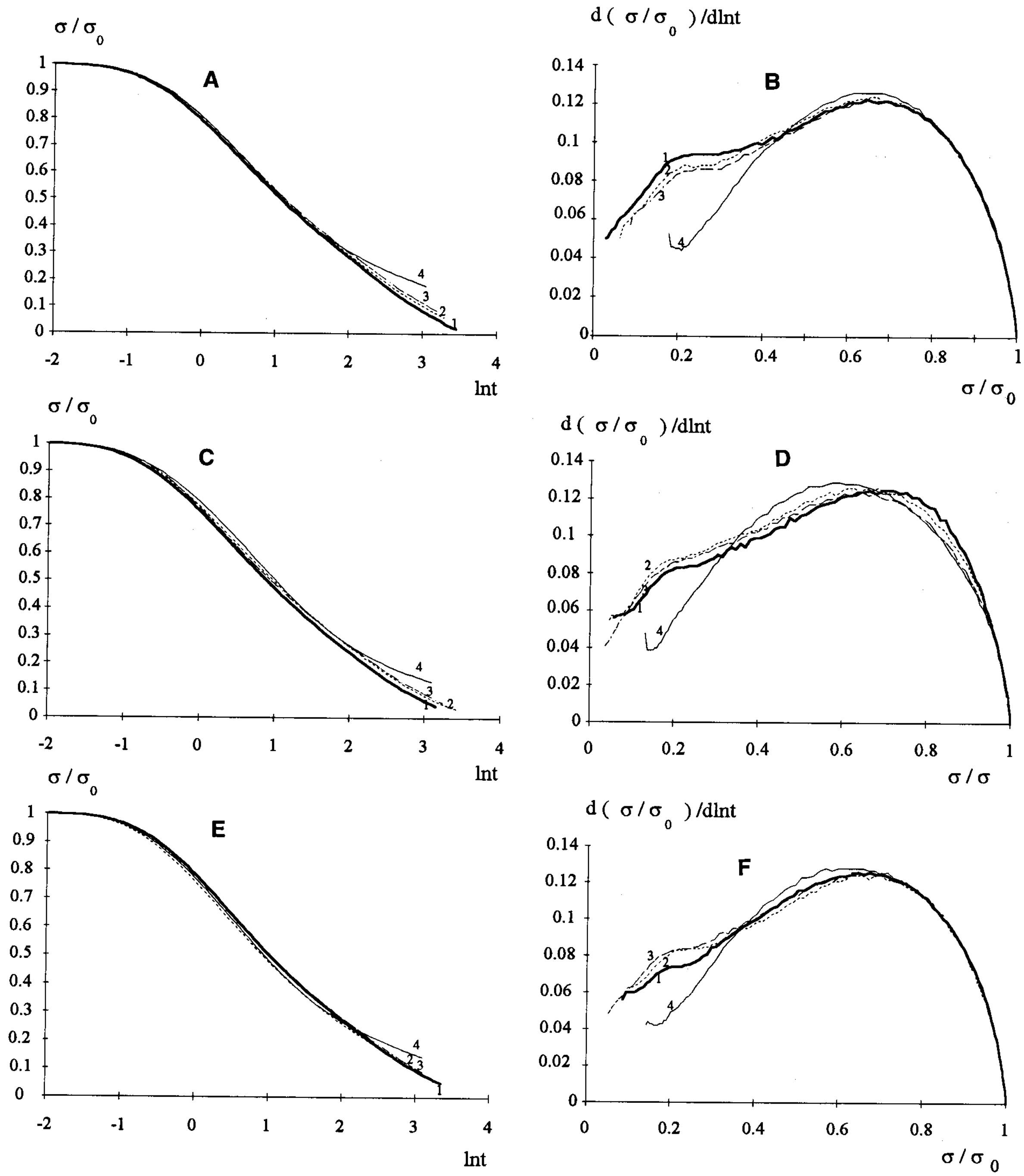

Fig. 4. Normalized stress versus linear time [$\sigma(t)/\sigma_0$ vs. lnt] and the corresponding relaxation spectrum versus the normalized stress [$d(\sigma/\sigma_0)$/dlnt vs. $\sigma(t)/\sigma_0$] for Thasos (**A,B**), Kosack (**C,D**), and Ritmo (**E,F**) wheat dough samples. Potassium bromate added at 0, 3.00, 30.0, and 300 ppm (1–4 respectively). Each curve represents the average of three measurements.

mode which is normally used to measure shear stress relaxation behavior after the cessation of steady flow. Because the magnitude of the strain in our work was only 0.367, it cannot be characterized as steady flow in the dough but rather as the application of a large strain before a stress relaxation measurement. The parallel plate system (30 mm diameter) was used with the gap between the plates set at 1.00 mm. The exposed dough surfaces at the rim were covered with silicon oil to prevent water loss.

All experiments were performed at 30°C. Large strain relaxation measurements were started after an initial equilibration time of 1,200 sec. A strain rise time of 10 sec and a strain rate of 0.0367/sec resulted in a total strain of 0.367. After the strain was applied, the stress was allowed to relax for 1,200 sec. Each experiment was replicated at least three times (each replicate was from a separate batch) and then averaged. Variation between replicates was <8%.

The results are presented as: 1) normalized stress [$\sigma(t)/\sigma_0$)] versus logarithmic time (lnt), where $\sigma(t)$ is the stress at time t and σ_0 is the initial stress; and 2) rate plot $-[d(\sigma/\sigma_0)/\text{dln}t]$ (relaxation spectrum) by Alfrey's rule (Leaderman 1958) versus the normalized stress; and 3) the initial value of the stress, σ_0. The normalization of the stress eliminates effects on the relaxation behavior that do not relate to the fundamental structure (e.g., changes in the water content).

Figure 1 shows a typical relaxation curve and a typical rate plot for a dough with the two flow processes indicated. The relaxation curve shows how the stress changes with time. The initially rapid relaxation of stress is relevant to how the dough is perceived and also how it responds to kneading and mixing. The relaxation of stress over long time periods is relevant to how the dough behaves during fermentation. The existence of two relaxation processes is not always obvious in the relaxation curve itself. In the rate plot, however, the existence of two processes can be seen clearly, and their relative importance can be characterized. It should be noted that because the relaxation rate is plotted against normalized stress, the first process appears to the right and the second process

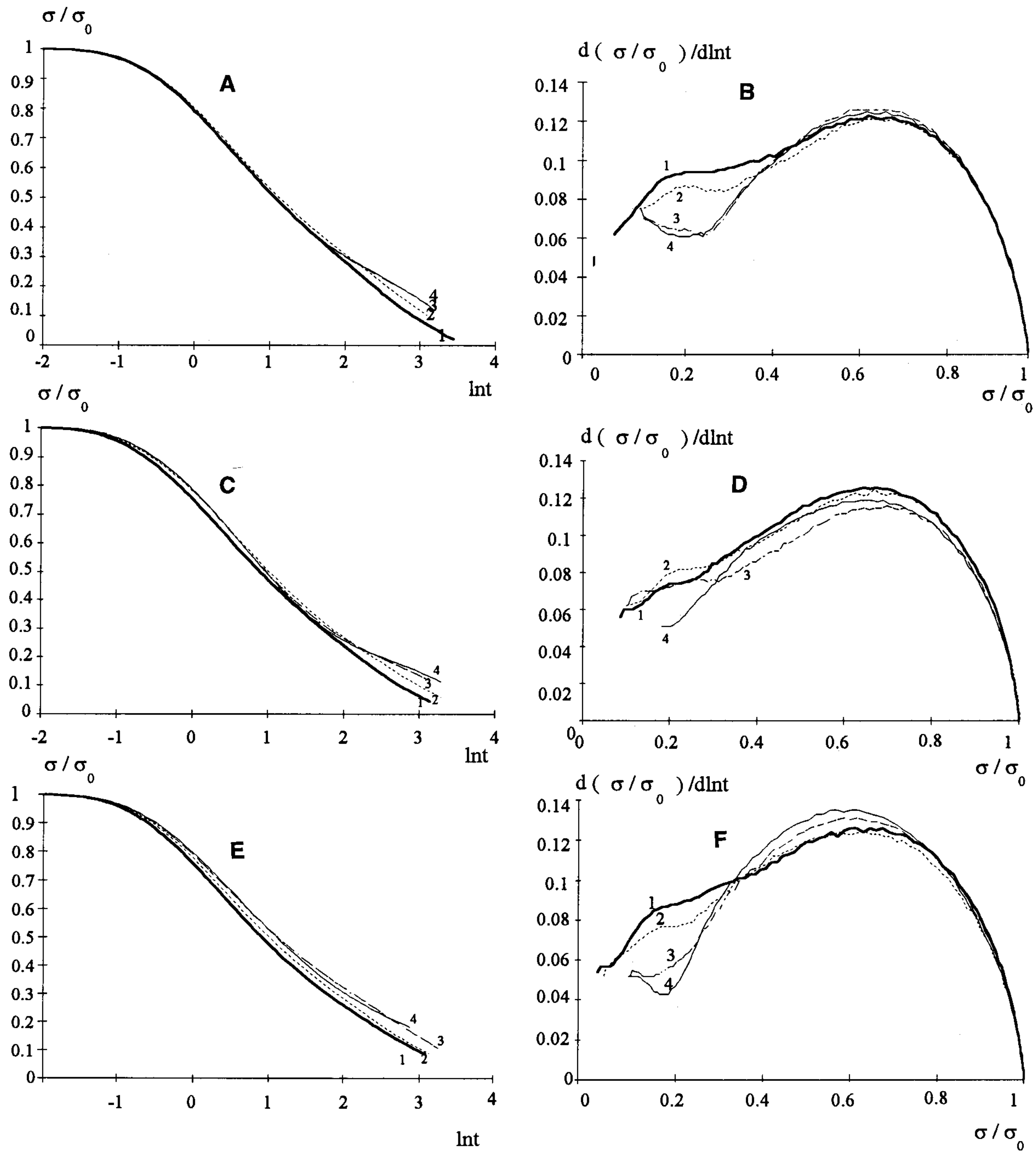

Fig. 5. Normalized stress versus linear time [$\sigma(t)/\sigma_0$ vs. lnt] and the corresponding relaxation spectrum versus the normalized stress [$d(\sigma/\sigma_0)/\text{dln}t$ vs. $\sigma(t)/\sigma_0$] for Thasos (**A,B**), Kosack (**C,D**), and Ritmo (**E,F**) wheat dough samples. Ascorbic acid added at 0, 5.00, 50.0, and 500 ppm (1–4 respectively). Each curve represents the average of three measurements.

appears to the left, which closer to the origin on the *x*-axis. To obtain the best understanding of the relaxation behavior in our measurements, the normalized stress relaxation curve, the corresponding rate plot, and the initial stress value will be considered together.

RESULTS AND DISCUSSION

The histogram in Fig. 2 shows the effect of the different additives on the initial stress value (σ_0). The gray bars represent the untreated samples. The concentration of additives in each group increases from left to right. The results in this figure are discussed in parallel with the corresponding relaxation curves and rate plots.

Figure 3 shows the normalized relaxation curve and the rate plot curve for Thasos, Kosack, and Ritmo flours, respectively. The flow relaxation method clearly distinguished between the three untreated wheat cultivars. Primarily, they differed in the relaxation rate of the second process. The spring wheat Thasos had the highest rate, while the winter wheat Ritmo had the lowest rate. The initial stress value (Fig. 2) was similar for all three flours.

Figures 4–7 show the normalized relaxation curve and the rate plot for doughs treated with potassium bromate, ascorbic acid, protease, and glucose oxidase, respectively. Each additive showed pronounced effects on the rheological behavior of each of the doughs. The effect on the relaxation pattern depended on the type of additive as well as on the wheat cultivar.

Potassium bromate is an oxidizing agent that tends to improve the dough by increasing the resistance and decreasing the extensibility of the dough (Dempster et al 1952, 1956). This would correspond to an increased value of the initial stress, an increased stress relaxation time of the second flow process, and a decrease in the

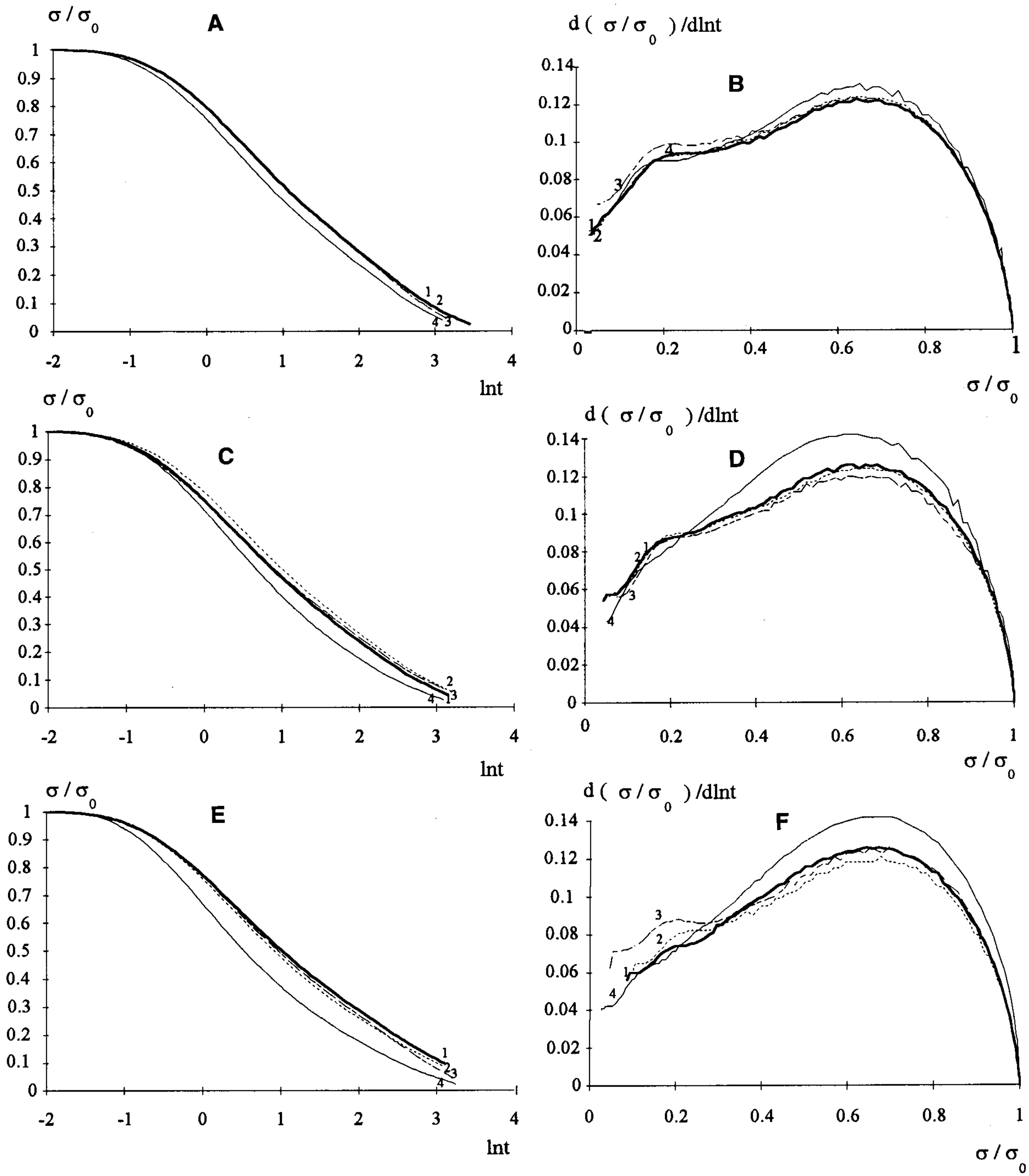

Fig. 6. Normalized stress versus linear time [$\sigma(t)/\sigma_0$ vs. lnt] and the corresponding relaxation spectrum versus the normalized stress [$d(\sigma/\sigma_0)/d\ln t$ vs. $\sigma(t)/\sigma_0$] for Thasos (**A,B**), Kosack (**C,D**), and Ritmo (**E,F**) wheat dough samples. Protease added at 0, 1, 10, and 50 ppm (1–4 respectively). Each curve represents the average of three measurements.

relaxation rate at long time periods. Typical usage is 20–50 ppm. The initial stress value (Fig. 1) for Thasos increased at all addition levels, while the initial stress value for Kosack and Ritmo increased only at the highest concentration (300 ppm). Figure 4 reveals that at concentrations of 3 and 30 ppm of potassium bromate, Thasos showed a decrease in the relaxation rate for the second process, Kosack showed a small increase, and Ritmo a larger increase. At 300 ppm, the relaxation time for the second process is largely increased for all three wheat cultivars.

Ascorbic acid is in itself a reducing agent with effects on dough properties similar to those of the oxidizing agents added to improve bread dough (Bloksma and Bushuk 1988). Ascorbic acid, however, at low and moderate concentrations had quite a different effect than that of potassium bromate (Fig. 5). Adding ascorbic acid increased the initial stress as well as the relaxation time for the second flow process. This is in agreement with earlier studies with ascorbic acid (Mita and Bohlin 1983; Larsson and Eliasson 1996a,b). Ritmo differed from the other two flours by showing a much weaker effect on the initial stress and on the relaxation time with a distinct increase in the latter occurring only at the highest concentration.

Protease from *A. oryzae* has a weakening action on gluten and is added to increase extensibility of an overly strong dough (Hamer

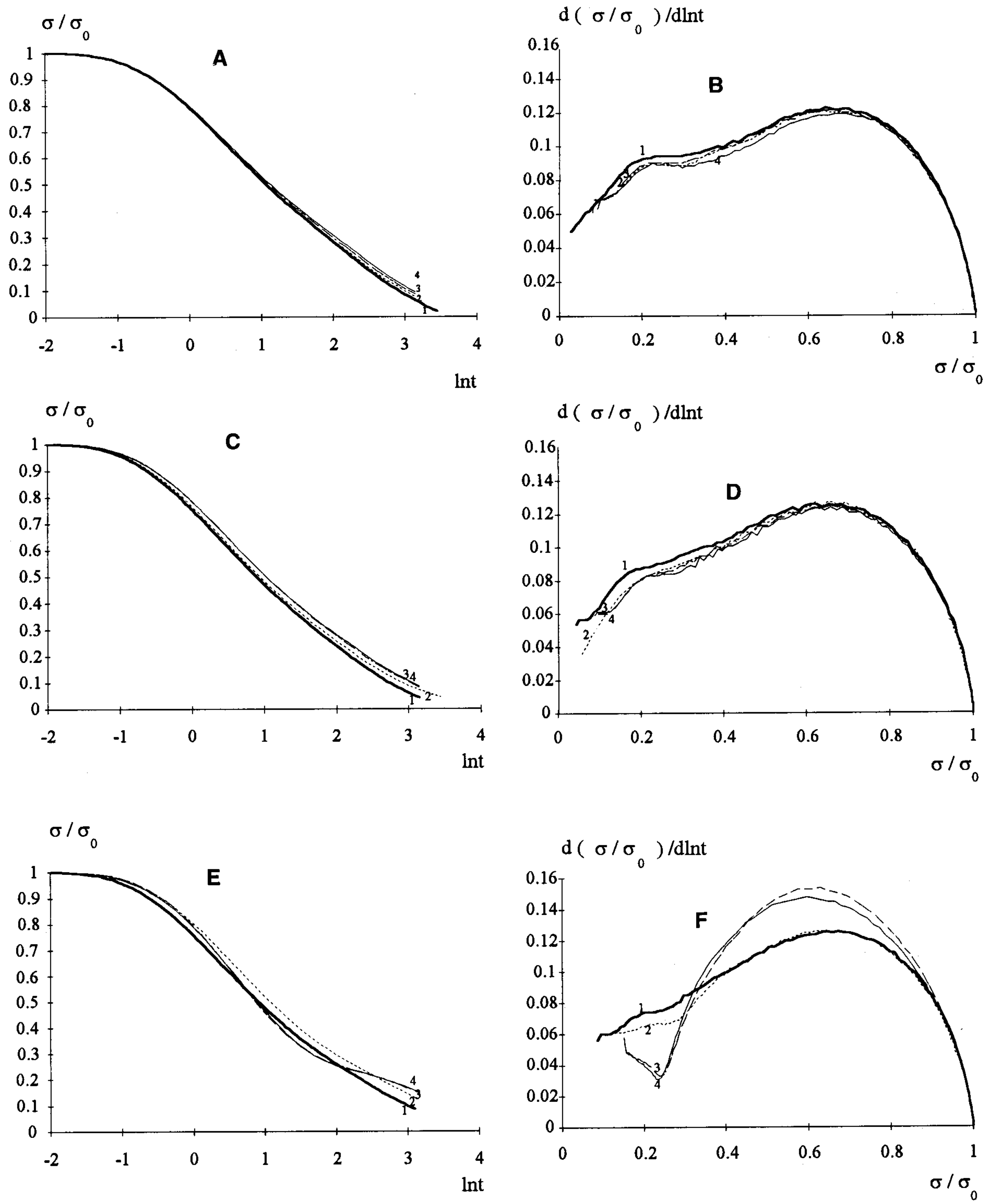

Fig. 7. Normalized stress versus linear time [$\sigma(t)/\sigma_0$ vs. lnt] and the corresponding relaxation spectrum versus the normalized stress [$d(\sigma/\sigma_0)$/dlnt vs. $\sigma(t)/\sigma_0$] for Thasos (**A,B**), Kosack (**C,D**), and Ritmo (**E,F**) wheat dough samples. Glucose oxidase added at 0, 0.36, 1.8, and 3.6 ppm (1–4 respectively). Each curve represents the average of three measurements.

1991). This would result in one or more rheological effects including: 1) an increase in the rate of the second process, 2) a decrease in relaxation time of the second process, and 3) a decrease in the initial stress. Typical usage is 5–20 ppm. Figure 6 shows that effect 1 occurred in Thasos and Ritmo at 10 ppm. Effect 3 occurred in Ritmo and Kosack at higher concentrations. Thasos showed effect 3 only at the highest concentration. At this concentration, the rate of the second process for Thasos is the same as for untreated dough, while the second process disappears for Ritmo.

Glucose oxidase is an oxidizing enzyme that has an effect similar to that of chemical oxidants such as potassium bromate (J. H. G. M. Mutsaers, *unpublished data*), especially in the presence of hemicellulases (J. P. Van Der Lugt, *unpublished data*). This would, again, correspond to an increased relaxation time and a decreased rate for the second process. Because the enzyme uses glucose as a substrate, the amount of glucose available in the dough is important. Also, because degradation of starch can produce more substrate, the falling number should be considered. The relaxation patterns are shown in Fig. 7. Glucose oxidase had a major effect on Ritmo. The initial stress increased almost threefold at 1.8 ppm. The higher concentrations (1.8 and 3.6 ppm) gave a remarkable change in the relaxation pattern, separating the two processes by increasing the relaxation time of the second process and decreasing it for the first process. Glucose oxidase had a minor effect on Kosack and Thasos with a small increase in the initial stress and a small decrease in the relaxation rate of the second process.

Finally, the analytical results, which are presented in Table I, are considered. Thasos had a glutogram value fivefold higher than the other two, indicating a very high elasticity. Without any ascorbic acid added, Thasos gave the highest bread volume, while both Kosack and Ritmo gave ≈30% lower bread volume than Thasos. Addition of ascorbic acid had a negative effect on Thasos, which had an 8% decrease in bread volume. On the other hand, Ritmo and Kosack exhibited volume increases of 19 and 51%, respectively. The simple sugar levels (represented by the glucose value in Table I) were similar for all three wheats, while the falling number was somewhat lower for Ritmo than for the other two.

CONCLUSIONS

The results clearly showed that shear stress relaxation measurements after large strain can be useful for distinguishing between different wheat cultivars and for characterizing the effects of different chemical and enzymatic additives. The stress relaxation curves show two flow processes, one at shorter time periods and the other at longer time periods.

We have shown that addition of oxidizing agents such as ascorbic acid and potassium bromate increased the time of the second flow process and also decreased the relaxation rate during the second process. This effect was less pronounced for Ritmo, the poor baking wheat, with a correspondingly low increase in bread volume on addition of ascorbic acid. On the other hand, the effect was greater on the highly elastic Thasos, with a corresponding decrease in bread volume when ascorbic acid was added.

Protease decreased the time of the second flow process and also increased the relaxation rate during the second process, unless this process disappeared, as it did for the poor baking wheat Ritmo.

Glucose oxidase had a major effect on the poor baking wheat Ritmo that could not be explained by differences in the available substrate in the three wheats. It separated the two flow processes by increasing the relaxation time of the second process and by decreasing the relaxation time for the first process.

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