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The effect of protein disulphide isomerase on dough rheology assessed by fundamental and empirical testing

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Fundamental rheological tests were carried out on wheat flour dough containing protein disulphide isomerase (PDI, E.C. 5.4.3.1.) and compared with their respective controls. The Controlled Stress Rheometer (CSR) and the Dynamic Mechanical Thermal Analyzer (DMTA) were used. Results from these instrumental tests which are essentially non-destructive were compared with results obtained when using a traditional Extensometer. The possibility of PDI duplicating the effect of potassium bromate was evaluated. Fundamental testing provided more information about the structure of the dough when used under a wide range of different testing conditions. The Extensometer, under the conditions used in this work, was better than the CSR and the DMTA for showing the effect of PDI (50 ppm, flour basis), which seemed to make the dough more elastic the longer the resting time. Although the effect was basically the same as that produced by potassium bromate, it was not of the same magnitude. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Protein disulphide isomerase (PDI, E.C. 5.4.3.1.) is an enzyme which has been shown *in vitro* to catalyse disulphide bond interchange in several 'scrambled' proteins ('scrambled' proteins were prepared by reducing a disulphide bonded protein and allowing it to reoxidise under denaturing conditions, thus causing it to form non-native disulphide bonds) (Freedman and Hillson, 1980). PDI has been found in many tissues, including wheat endosperm cells during their development (Roden, 1983). Whether or not it has any role in controlling the nature of disulphide-linked aggregates *in vivo* has not yet been established (Miflin *et al.*, 1983). However, if PDI is capable of such an action *in vitro*, it presents a new area of research, and may be a substitute for potassium bromate, a substance no longer allowed as an additive in bread doughs made for major world markets.

A variety of different instruments has been developed in the past 60 years for the study of dough rheology (Weipert, 1990). Based on the principle employed, techniques have been classified as empirical/imitative or basic/fundamental. In most of the dough rheology research done so far, empirical tests such as the Farinograph, the Mixograph and the Extensigraph, have been used. The relatively high deformation applied by these machines leads to a large-scale disorientation of molecules and eventually to the destruction of the structure of the dough. These machines have an added disadvantage of recording data only in arbitrary units and hence the results are unique to the test instrument rather than to the material. This research has provided a great deal of information, but is limited to certain empirical correlations (Dreese *et al.*, 1988). Fundamental (non-destructive) testing which employs a sinusoidal stress-strain technique is now more commonly used for following the rheological changes in dough systems. These methods yield a very small deformation of the sample and therefore do not significantly destroy the dough structure. They work over a much broader range of test conditions and the results can be obtained in absolute physical units (Weipert, 1990). In such cases, the results obtained using fundamental tests would be meaningful to another scientist, even if the type of apparatus used to make the measurement was unknown (Mitchell, 1984).

Dough is a viscoelastic material and, when assessed by dynamic sinusoidal stress-strain testing, the results may be expressed as G' and G'', the storage and loss moduli, respectively. The storage modulus (G') is defined as the stress in phase with the strain in a sinusoidal shear deformation divided by the strain; it is a measure of the energy stored and recovered per cycle.

The loss modulus (G″) is defined as the stress 90° out of phase with the strain divided by the strain; it is a measure of the energy dissipated or lost as heat per cycle of sinusoidal deformation. The ratio of the loss modulus to the storage modulus is called tan delta, which is very useful since it may reflect any phase change in the sample (Ferry, 1980).

The rheological behaviour of a wheat flour dough is non-linear. That is, over all of the measured range, the stress and the strain are not directly proportional to one another (Hibberd and Parker, 1978; Bloksma and Bushuk, 1988; Faubion and Hoseney, 1990). Dynamic sinusoidal stress-strain testing is particularly useful in measuring behaviour during small deformations and levels of strain (Faubion *et al.*, 1985), i.e. usually within the linear region of the sample. However, there is no agreement as to whether overall dough behaviour at low stresses can be approximated by linear theory (Hibberd and Parker, 1979) even at strains as low as 10^{-4} (Smith *et al.*, 1970).

MATERIAL AND METHODS

Wheat

Avalon wheat, a variety of hard wheat, grown at Churn State, Oxfordshire, UK, was used in this work. Milling was carried out using a Buhler laboratory mill, with a flour yield of 65%. Protein content (Kjeldahl, N*X*5.7) was assayed as 10.0% w/w.

Protein disulphide isomerase

PDI was kindly supplied by Dr Linda Blades of the Department of Food Science and Technology at the University of Reading. It was purified from bovine liver, according to the procedure of Lambert and Freedman, (1983). The PDI used in this work had a specific activity of 700 PDI units g^{-1}. When required, 50 ppm of PDI were dissolved in the water which was added to the flour at the mixing stage in the Farinograph.

Farinograph

The Farinograph was used according to the American Association of Cereal Chemists Approved Methods, method 54-21 (AACC, 1983), using the procedure for constant flour weight (300 g, 14% w/w moisture basis); water absorption was 63% w/w; mixing time was 2.0 min and stability was 2.5 min.

Simon 'research' extensometer

All work on the Simon Research Extensometer and on the Farinograph was carried out at 30°C ± 1°C. A dough containing 2% w/v of sodium chloride was mixed in the Farinograph to 500 Brabender units (BU) of consistency. Mixing was carried out for 1 min, stopped for 5 min, and continued until the full development time of the flour was reached (2 min). Pieces of dough, of 75 g were moulded into balls in the shaper unit of the Simon Research Dough Testing Equipment. The balls of dough were rested under plastic domed dishes, to prevent water loss, for 45 min. After the resting period, the pieces of dough were carefully impaled through the centre of the ball on the split pin of the Extensometer. The instrument was switched on and the samples were stretched. When the dough broke, the motor was stopped and the piece of dough was removed. It was then reshaped and, as before, rested for 45 more minutes, before being stretched again. A third test was made on the same piece of dough by reshaping it and allowing another resting period of 45 min before stretching. In this way, the samples of dough were tested at 45, 90 and 135 min total time. The results shown in Figs 4 (a,b) correspond to the mean of three readings (see below).

Controlled stress rheometer

The samples loaded on the CSR (RTI - Rheotech International, Forest Gate, London, UK) were obtained from the centre of a dough mixed in the Farinograph and moulded and stretched in the Extensometer after 90 min rest at 30°C ± 1°C, as explained in the Simon 'research' extensometer procedure.

After mixing, a sample of dough was loaded on the CSR between parallel plates (10 mm radius). The gap size (sample thickness) was 1.0 mm. The excess of dough was cut off and the edges of the sample were covered with a low viscosity sealing paste to prevent water loss. The sealing paste had a viscosity < 0.1% of the sample viscosity. The sample was allowed to rest for 30 more minutes at 30°C ± 0.1°C before oscillatory testing was carried out over the test frequency range (0.1 to 10 Hz, logarithmic scale) and at a torque amplitude of 0.1 mNm, which corresponds to a stress amplitude of 63.66 N m^{-2} at the rim of the plate. Frequencies sweeps were run from 0.1 to 10 Hz. The data shown on the graphs (Figs 1 (a,b)) correspond to the mean of at least three readings, for three different samples. According to Ferry, (1980), when any of the dynamic functions is plotted against frequency, the ordinate can assume an enormous range of magnitudes, changing over several decades. Therefore, both co-ordinates are usually plotted logarithmically. The reproducibility of the procedure was studied in a previous work of ours (Watanabe *et al.*, 1992*a*), where absolute values of G′ and G″ were variable between replicate experiments. However, it was shown that the overall shape of the curve remained essentially the same and trends within any given experiment were found to be consistent.

For the data shown in the 3-D plots (Figs 2 (a,b)), a sample of dough was loaded on the CSR immediately after being mixed for 2 min (peak or development time) in the Farinograph. For these samples, a sodium phosphate buffer 0.2 M, pH 7.5, was used instead of water. The sample was allowed to rest for 30 min. The temperature was set at 25°C ± 0.1°C and three torque amplitudes were used: 0.1, 0.5 and 1.0 mNm (which correspond to stress amplitudes of 63.66, 318.31 and 636.62 N m^{-2}, respectively).

Dynamic mechanical thermal analyzer

The DMTA Mark 2 (Rheometrics Scientific Ltd., Epsom, Surrey, UK) was used in this work. The mixing and the loading procedure was the same as on the CSR (3-D plots). The parallel plate geometry (24 mm length × 12 mm width) was also used with a gap size of 1.0 mm. In this case, one of the two plates which 'sandwich' the sample is oscillated sinusoidally at a set frequency and amplitude, while the other remains stationary. The samples of dough were tested from 25 to 90°C (1°C min^{-1}). The controlled variable, the strain, was fixed at $X1$ (approximately 1.6×10^{-2}) and the frequency was fixed at 1 Hz. The data reported in this work (Figs 3 (a,b)) correspond to the mean of three readings.

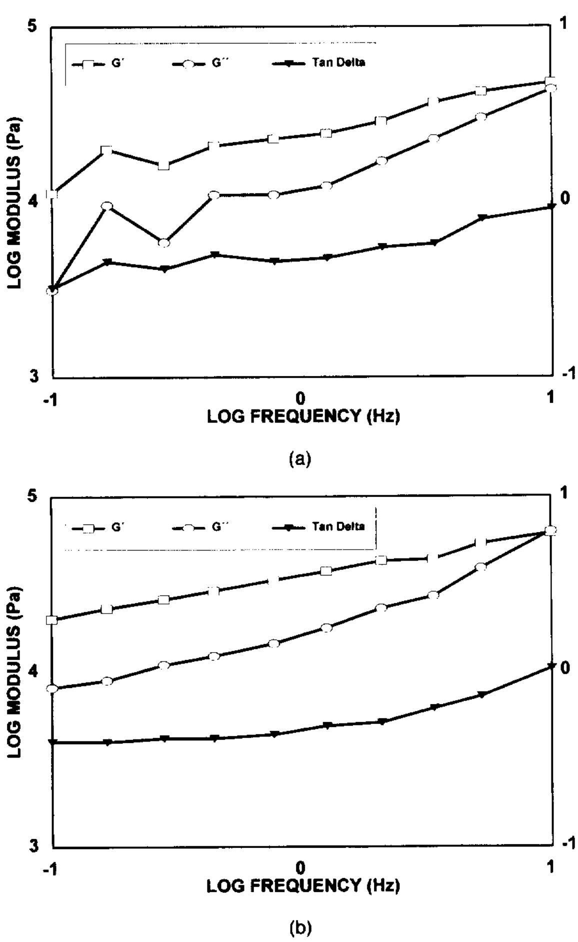

Fig. 1. (a) G′, G″ and tan delta temperature sweep on the CSR of plain dough samples (control) loaded between parallel plates. The samples were mixed in the farinograph and stretched in the extensometer. Torque amplitude: 0.1 mNm. (b) G′, G″ and tan delta temperature sweep on the CSR of dough samples treated with 50 ppm of PDI (flour basis) loaded between parallel plates. The samples were mixed in the farinograph and stretched in the extensometer. Torque amplitude: 0.1 mNm.

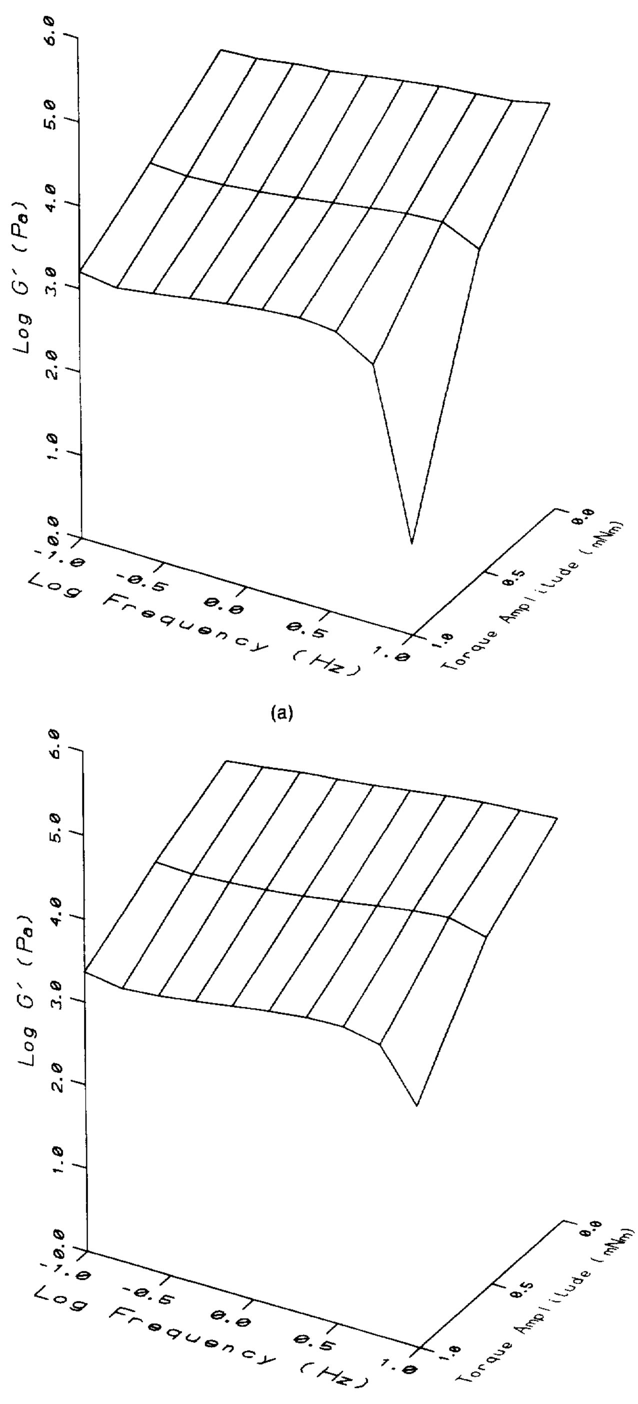

Fig. 2. (a) CSR 3-D plot. G′ against frequency at three different torque amplitudes of dough samples (control) mixed in the farinograph and loaded between parallel plates. Phosphate buffer 0.2 M was used instead of water. (b) CSR 3-D plot. G′ against frequency at three different torque amplitudes of dough samples treated with 50 ppm of PDI (flour basis) mixed in the farinograph and loaded between parallel plates. Phosphate buffer 0.2 M was used instead of water.

RESULTS AND DISCUSSION

The effect of potassium bromate on the rheology of wheat flour doughs has been reported elsewhere (Watanabe *et al.*, 1992*a*,*b*). In these studies, non-destructive dynamic oscillatory testing, as well as traditional testing, were carried out on samples of dough that had been mixed for different times. It was observed that, when these samples of dough were submitted to a range of different torque amplitudes, differences between treated and untreated samples could easily be detected. In this paper, the same studies were carried out on doughs containing PDI, to see if PDI could be a potential substitute for potassium bromate in bread-making.

Controlled stress rheometer

During the preliminary studies with PDI, doughs containing 50 ppm of PDI (flour basis) were tested on the CSR using a single torque amplitude (0.1 mNm) and compared with their respective controls (Figs 1 (a,b)). Apparently, the samples containing PDI were stronger (higher values of G and G′) than the controls. At the same time, the tan delta lines of both samples were very similar. Nevertheless, there were no readily detectable differences in the overall shape of the curves of the samples. It was observed that the samples of dough containing PDI seemed to be more homogeneous and smoother than the control. The plots resulting from these samples were also more 'linear', i.e. no sudden changes occurred during the tests. Thus, it was noticeable that PDI was playing a role in the structure of the dough, although not in a clear way.

In our previous work (Watanabe *et al.*, 1992*b*), samples of dough containing potassium bromate were tested under the same range of three different torque amplitudes (0.1, 0.5 and 1.0 mNm, which correspond to stress amplitudes of 63.66, 318.31 and 636.62 N m^{-2}, respectively). The effects of bromate were easier to verify, especially at the highest torque amplitude (1.0 mNm), when potassium bromate acted to 'reform' the structure of the dough, after it had been exposed to essentially 'destructive forces' Thus, in this work, samples contain-

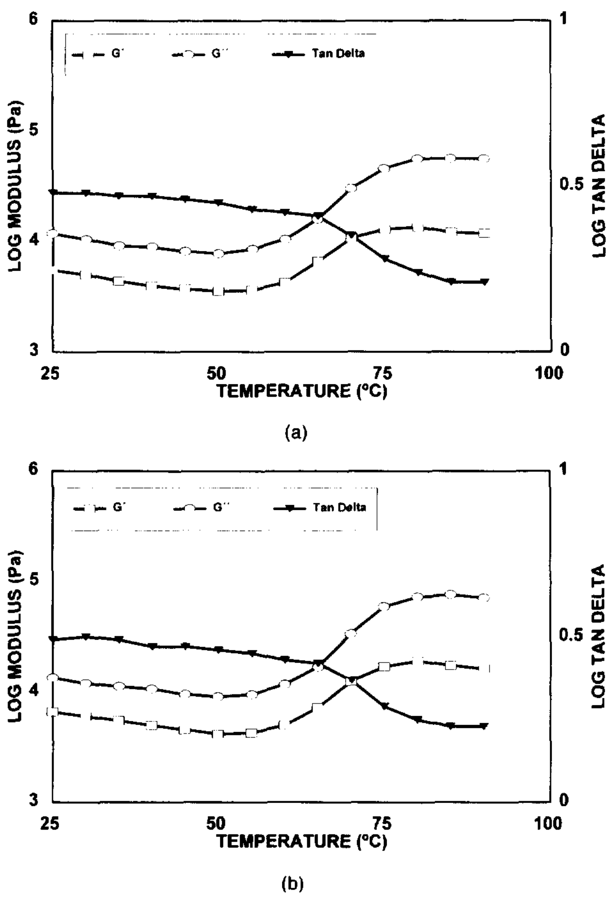

Fig. 3. (a) G′, G″ and tan delta temperature sweep on the DMTA of dough samples (control) mixed in the farinograph and loaded between parallel plates. Strain: XI (approximately 1.6×10^{-2}). Frequency: 1 Hz. (b) G′, G″ and tan delta temperature sweep on the DMTA of dough samples treated with 50 ppm of PDI mixed in the farinograph and loaded between parallel plates. Strain: XI (approximately 1.6×10^{-2}). Frequency: 1 Hz.

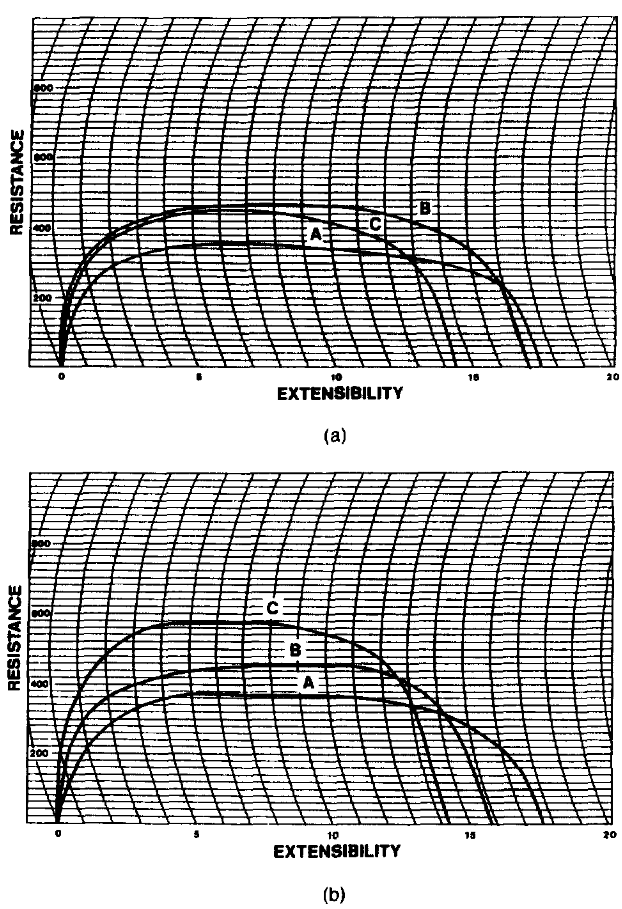

Fig. 4. (a) Extensigram of dough samples (control) mixed in the farinograph and rested for 45 min (A), 90 min (B) and 135 min (C). (b) Extensigram of dough samples treated with 50 ppm of PDI mixed in the farinograph and rested for 45 min (A), 90 min (B) and 135 min (C).

ing PDI were tested on the CSR using a similar range of torque amplitudes (0.1, 0.5 and 1.0 mNm).

Initially, the enzyme was dissolved in the water added to the dough during mixing. No beneficial rheological effects were observed (i.e. the values of G′ and G″ did not increase). PDI was then dissolved in sodium phosphate buffer, 0.2 M, pH 7.5, which was added to the dough during mixing instead of water. The same phosphate buffer, without PDI, was used for the controls. Because of the presence of salts, the consistency of the dough increased from 500 BU to approximately 560 BU when tested on the Farinograph (results not shown). This change in behaviour did not change the mixing time of the doughs and was exactly the same for both samples, with or without PDI (their farinograms were virtually identical).

Figures 2 (a,b) show the results obtained, where the storage modulus (G′) is plotted against the frequency and the torque amplitude, for the untreated and the treated samples, respectively. The fact that G′ at a constant frequency decreases with an increase of the stress amplitude shows that the stress amplitudes used were too high to be within the linear viscoelastic region of the sample, particularly the two highest ones. If both figures are superimposed, it is possible to observe that, for all the three torque amplitudes tested, G′ was higher for doughs containing PDI. This difference was very small at the lowest torque amplitude (0.1 mNm) and more marked at the other amplitudes. This indicates a system with overall improved elasticity and improved resistance to damage at higher frequencies/shear rates. This effect of the PDI was very similar to the one previously observed for potassium bromate, although not as marked.

Dynamic mechanical thermal analyzer

The DMTA is another instrument of the oscillatory type and was used to further study the rheological properties of wheat flour dough. On the DMTA, the strain is the controlled variable.

The DMTA was used to tentatively simulate the baking process. In the baking process, the temperature is raised from the fermentation temperature to about 95°C at the centre of the loaf (Eliasson, 1980). It is possible to test the rheology of dough while heating through a similar temperature range on the DMTA. In this way, the effects of PDI may be better evaluated, since the dough is exposed to the whole range of temperatures that would occur in the oven during baking.

It is known that, during the several stages in breadmaking (mixing, fermentation, baking, etc), the dough undergoes several different rates of deformation and that the reaction of dough to these actions will play a large role in governing its overall breadmaking properties (Bushuk, 1985; Faubion and Faridi, 1986; Bloksma and Bushuk, 1988; and Bloksma, 1990*a*). Therefore, all of these rates of deformation cannot be reproduced by the DMTA.Figures 3 (a,b) show the values obtained for G, G and tan delta for samples of plain flour dough and samples containing 50 ppm of PDI, respectively. There were no readily detectable differences between the treated and untreated samples. In both cases, G and G values start to increase at around 55°C. This is believed to be due to the onset of starch gelatinisation and to polymerisation of glutenin molecules as a result of thiol-disulphide interchange reactions (Schofield *et al.*, 1983). At around 80°C, the G and G lines seemed to achieve a value that remained constant until the end of the test, when the samples would contain gelatinised starch and denatured proteins. The rheological properties of both samples of dough remained the same at this strain, even at temperatures higher than 80°C. A higher strain might, however, have caused the breakdown of the structure of the samples and thus a change in G′, G′ and consequently tan delta. From 70°C, the G′ and G′ lines are slightly higher for the samples containing PDI. Even so, the difference observed was not enough to warrant any further consideration.

The tan delta lines were also very similar for both samples. They started to fall from the beginning of the test (there was a tendency throughout for the system to become more elastic). The fall in tan delta became more accentuated between 65 and 80°C, when the samples became more elastic. But this fall in tan delta was the same for both treated and untreated samples.

Simon 'research' extensometer

The results obtained for the untreated and treated samples are shown in Figs 4 (a,b), respectively. No differences were detected between the samples containing PDI and the controls at the mixing stage on the Farinograph (data not shown). After 45 min rest (Figs 4 (a,b), lines A), the curves for both samples were very similar. After 90 min rest (Figs 4 (a,b), lines B), the only apparent difference was that the sample containing PDI had its extensibility decreased. The elasticity (resistance values) for both samples, however, was the same. The opposite effect occurred after 135 min rest (Figs 4 (a,b), lines C). Both samples presented the same extensibility, but the samples containing PDI were more elastic.

CONCLUSIONS

The studies on the CSR and on the DMTA indicate that fundamental oscillatory testing may be used to assess the differences between samples of dough with respect to their rheological properties, which are not normally possible to observe using the Farinograph. Fundamental tests, although difficult to carry out, give useful information when carried out over a range of different torque amplitudes. The wider the range of torque amplitudes used, the better 'profile' of the samples obtained. On the DMTA, a broader range of strains would make this equipment considerably more suitable for dough research, since it would not be limited to the three fixed strains. Fundamental tests are much better for describing the rheological properties of dough, giving much more information.

The extensometer, under the test conditions used in this work, was better than the CSR and the DMTA for showing changes in the viscoelastic properties of dough. Although it destroys the structure of the samples, the extensometer was able to detect the effects of PDI. PDI made the dough more elastic with longer resting times and its effect was similar to that of potassium bromate, although it was observed that it was not of the same order of magnitude. Possibly, in order to better observe the effect of PDI using the CSR and the DMTA, a longer rest period should be allowed.

According to Bloksma (1990*a*,*b*), from the physical point of view, any rheological test on dough can reliably predict its behaviour in a bakery only if the rate and extent of the deformation in these tests are in the same range as those used during processing.

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