

# Optimized Methods for Incorporating Glutenin Subunits into Wheat Dough for Extension and Baking Studies

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## ABSTRACT

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In order to study the functional properties of glutenin subunits added to a dough, they must be incorporated into the glutenin polymer. This requires partial reduction to open up the polymer, followed by oxidation to incorporate the added monomer into the polymer. Existing methods for incorporating glutenin subunits were suitable only for studies on mixing properties and needed to be modified for use in studies on extension and baking. A range of concentrations and of reaction times was therefore tested for both the reductant and the oxidant. In addition, mixing time as well as relaxation time before extension were varied. Extension curves and loaf heights were used to evaluate the treatments. Optimum conditions were developed that provided extension curves of normal dimensions

but with altered shape. The conditions were reduction with 0.2 mg/mL of dithiothreitol (DTT) solution for 1 min followed by oxidation with 5 mg/mL of KIO<sub>3</sub> solution, then mixing the dough to 70% of the peak dough development time. For microbaking, the conditions of 2 mg/mL of DTT for 1 min, 2.5 mg/mL of KIO<sub>3</sub> for 5 min, and mixing the dough to peak development time allowed loaf height to be retained. The size distribution of the glutenin polymer was analyzed using size-exclusion HPLC and field-flow fractionation methods. This showed that the monomers were incorporated into the polymer and that polymer size was restored to control levels following reduction and oxidation.

Glutenin is the major group of proteins contributing to the strength and stability of dough. Glutenin polymers are composed of high molecular weight glutenin subunit (HMW-GS) and low molecular weight glutenin subunit (LMW-GS) proteins linked by disulfide bonds. Both qualitative and quantitative differences within these groups of proteins contribute to intercultivar variation in bread-making quality (Payne 1987). Nevertheless, knowledge about the functional properties of the various glutenin subunits has been based mainly on correlative studies in which the proportion of the protein fractions in flours of diverse quality is determined and statistically correlated with dough properties and breadmaking quality. The importance of the ratio of HMW-GS to LMW-GS has also been demonstrated by correlative studies and for a fixed glutenin content, the higher this ratio, the better the breadmaking quality (Gupta et al 1992).

More information can be obtained in structural or structured studies, where only one aspect of the composition is varied and all others are held constant (Uthayakumar et al 1999). In applying this principle to the study of the functionality of glutenin subunits, chemical incorporation of these subunits into the native glutenin polymer is necessary. A method for incorporation of glutenin subunits was developed by Bekes et al (1994a) and has been used in several subsequent studies (Bekes et al 1994b, Bekes et al 1995, Sapirstein and Fu 1996, Sissons et al 1998, Lee et al 1999). To date, reports of the direct assessment of the effects of specific subunits on dough properties have been restricted to the effects on mixing properties, the ostensible reason being that the reduction-oxidation conditions optimized for mixing studies were not suitable for extension and baking studies. While direct assessments of mixing properties address the need to understand the mechanisms underlying dough mixing, they do not address the effects of specific proteins on the extensibility or baking properties of the dough. Therefore, new techniques for estimation of the effects of incorporated glutenin subunits on extension and baking properties had to be developed. The aim of this study was, therefore, to optimize the reduction-oxidation conditions for extension and baking.

## MATERIALS AND METHODS

Wheat flours used were derived from Australian cultivars Banks (13% protein content, HMW-GS 2\*, 7+8, 2+12) and Hartog (12.4% protein, 1, 17+18, 5+10) obtained from BRI Australia Limited (North Ryde, NSW, Australia). A commercial bakers' flour blend (Queensland Bakers', 11.2% protein content, 1, 17+18, 2+12) made up of cvs. Janz and Cunningham obtained from Weston Milling Enfield, NSW, Australia was also used. These bread wheats were selected because they covered a wide range of quality (Uthayakumar et al 1999). Doughs for the test were mixed on a 2-g mixograph (TMCO, Lincoln, NE).

### Extension Testing

The total quantity of water to be used was calculated, using the protein and moisture contents of ingredients (AACC 2000). The water absorptions were Banks 64.7%, Hartog 63.0%, and Queensland Bakers' 65.1%. The water volume was divided into three portions, two fixed for the reductant and the oxidant, and the third variable to give the appropriate water absorption. Dough was prepared by mixing the flour, 450  $\mu$ L of dithiothreitol (DTT) solution and the variable water volume for 30 sec. It was then allowed to rest for the determined reduction time. In the last few seconds of the resting period, 250  $\mu$ L of oxidant solution (KIO<sub>3</sub>) was added and mixing resumed for 30 sec before the dough was allowed to rest further for the determined oxidation time. The dough was mixed to a predetermined proportion of the peak dough development time (including the initial two 30-sec mixing). Dough samples (subsample 1.7 g/test) were molded into cylinders  $\approx$ 6 mm in diameter with a prototype molder consisting of a 153 mm diameter drum rotating at 20 rpm within a fixed 167 mm diameter partial outer drum. Dough pieces (1.7 g) were introduced and rolled around the annular space for  $\approx$ 300°. The molded pieces,  $\approx$ 45 mm long, were mounted on a sample carrier and rested at 30°C and >90% rh for 45 min before extension testing (Gras and Bekes 1996). Extension was performed on a microextension tester with a 19 mm gap and 6 mm hook operating at 1 cm/sec. The dough resistance and the sample carrier position were determined 100 times/sec and recorded by a personal computer using LabTech Notebook software. Maximum resistance to extension ( $R_{max}$ , N) and extension before rupture (Ext, cm) were calculated (Rath et al 1994). In addition, distance to  $R_{max}$  ( $D_{max}$ , cm) was determined. Duplicate dough samples were prepared for each set of conditions and each sample yielded two subsamples for the extension tests, giving a total of four replicates.

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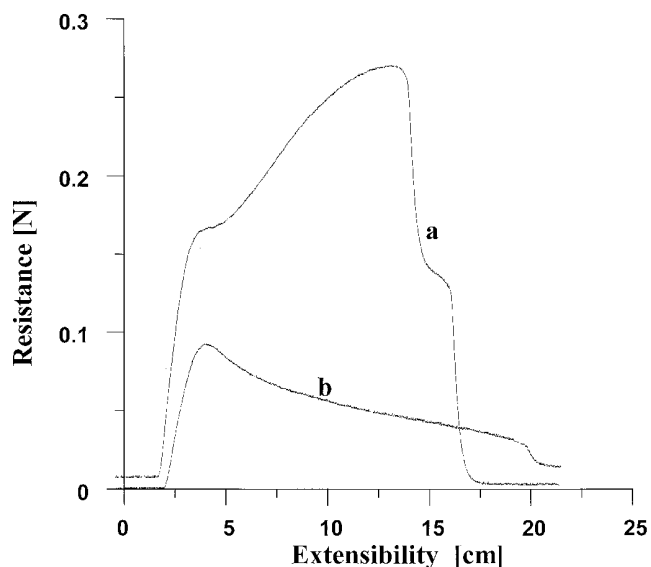
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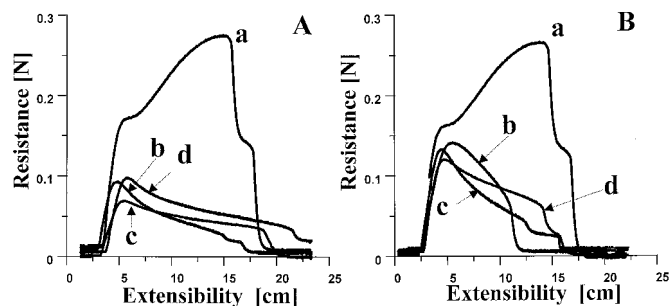
<sup>4</sup> CSIRO Plant Industry, Grain Quality Research Laboratory, North Ryde, NSW, 1670, Australia.

The reduction-oxidation conditions as optimized for mixing studies (Bèkès et al 1994a) did not produce extension curves similar those of the controls (Fig. 1). Therefore, conditions needed to be optimized specifically for extension testing. The conditions playing a role in the determination of optimum conditions are 1) the relaxation time of dough between mixing and extension testing; 2) concentration of reducing agent; 3) time allowed for the reduction reaction to take place; 4) concentration of oxidizing agent; 5) time allowed for the oxidation to take place; and 6) the mixing time as a proportion of optimum.

Each reduction-oxidation step was tested with certain variations. The overall experiment was not factorial since that would have required 7,200 treatments. Instead, each treatment was varied individually and all other treatment conditions were maintained at standard levels. The relaxation time was tested at 0 (dough was pulled immediately after mixing), 15, 30, 45, and 60 min at the standard level of 2 mg/mL of reductant, 4 min of reduction time, 5 mg/mL of oxidant, 5 min of oxidation time. The concentration of the reductant (DTT) and time of its application were varied, with all concentrations (0.2, 1, and 2 mg/mL) being tested at one time (4 min) and all times (1, 2, 3, and 4 min) tested at two reductant concentrations (0.2 mg/mL and 2 mg/mL). All concentrations (2, 2.5, 3, 5, 7.5, and 10 mg/mL) of oxidant (KIO<sub>3</sub>) were tested at one



**Fig. 1.** Extension curve for water control (a) and reduction-oxidation conditions optimized for mixing studies (b). Standard errors for  $R_{\max}$ , Ext, and  $D_{\max}$  are 0.017, 1.1, and 0.5, respectively.



**Fig. 2.** Extension curves for different reductant concentrations and time allowed for reduction reaction. Reduction time of 4 min (A) and 1 min (B). Water control (a) and 0.2, 1, 2 mg/mL of DTT, respectively (b-d). Relaxation time 45 min; KIO<sub>3</sub> concentration 5 mg/mL; oxidation time 5 min; mixing time 100%. Standard errors for  $R_{\max}$ , Ext, and  $D_{\max}$  are 0.014, 1.0 and 0.5, respectively.

time (5 min), and all oxidation times (1, 2, 3, 4, and 5 min) tested at one oxidant concentration (5 mg/mL). The mixing time was also altered (70, 80, 90, and 100% of maximum dough development time) using 0.2 mg/mL of reducing agent, 1 min of reduction time, 5 mg/mL of oxidant and 5 min of oxidation time. Hartog flour was not used in tests of reductant concentration, reduction time, and oxidation time.

Reduction-oxidation had profound effects on  $R_{\max}$ , Ext, and shape (as indicated by  $D_{\max}$ ) of the extension curve. To characterize these changes, an extension parameter function (EPF) was calculated, consisting of the square root of the sum of the squares of the deviations of the treatment values from the control values of  $R_{\max}$ , Ext, and  $D_{\max}$ , normalized by dividing by the corresponding control values:

$$EPF = \sqrt{[(R_{\max C} - R_{\max T}/R_{\max C})^2 + (Ext_C - Ext_T/Ext_C)^2 + D_{\max C} - D_{\max T}/D_{\max C}]^2}$$

where the subscript C indicates the control value and T indicates for the treated value. As this function approaches 0, the curve of

**TABLE I**  
Polymer Sizes and Extension Curve Dimensions of Doughs Prepared with Optimized Mixing Conditions<sup>a</sup>

Treatment	Flour	% UPP	Extension Properties			EPF
			$R_{\max}$ (N)	Ext (cm)	$D_{\max}$ (cm)	
Control	Banks	67.3	0.300	15.2	13.1	...
	Hartog	68.0	0.400	9.2	8.0	...
	QB	62.0	0.303	15.0	12.5	...
Optimum	Banks	38.8	0.080	21.3	4.0	1.068
	Hartog	49.0	0.180	15.3	2.9	1.007
	QB	40.3	0.095	21.0	4.0	1.045
SE		0.8	0.017	1.1	0.5	

<sup>a</sup> UPP, unextractable polymeric protein;  $R_{\max}$ , maximum resistance to extension; Ext, extension before rupture;  $D_{\max}$ , distance to  $R_{\max}$ ; EPF, extension parameter function; QB, Queensland Bakers'; SE, standard error.

**TABLE II**  
Extension Curve Dimensions and Polymer Sizes of Doughs Prepared with Varying Relaxation Times after Mixing<sup>a</sup>

Relaxation Time (min)	Flour	% UPP	Extension Properties			EPF
			$R_{\max}$ (N)	Ext (cm)	$D_{\max}$ (cm)	
Control	Banks	67.3	0.248	15.2	13.0	...
	Hartog	68.0	0.372	9.2	8.1	...
	QB	62.0	0.303	15.0	12.7	...
0	Banks	62.0	0.280	10.8	6.7	0.577
	Hartog	71.4	0.435	4.5	2.8	0.854
	QB	66.0	0.383	9.5	5.9	0.694
15	Banks	54.7	0.160	15.3	6.4	0.674
	Hartog	57.0	0.260	7.0	2.8	0.781
	QB	50.0	0.200	15.9	6.5	0.592
30	Banks	34.0	0.098	16.8	5.1	0.905
	Hartog	55.0	0.205	10.8	3.2	0.767
	QB	40.0	0.120	18.0	5.4	0.851
45	Banks	38.0	0.080	21.3	4.9	1.025
	Hartog	50.0	0.185	15.5	3.6	0.963
	QB	42.0	0.095	21.0	4.8	1.003
60	Banks	41.0	0.060	22.5	4.7	1.112
	Hartog	55.0	0.153	17.5	3.8	1.130
	QB	43.0	0.080	22.9	4.8	1.092
SE		0.8	0.015	0.9	0.4	

<sup>a</sup> UPP, unextractable polymeric protein;  $R_{\max}$ , maximum resistance to extension; Ext, extension before rupture;  $D_{\max}$ , distance to  $R_{\max}$ ; EPF, extension parameter function; QB, Queensland Bakers'; SE, standard error.

the treatment approaches the control curve, and the aim of the study was, therefore, to establish a set of conditions that minimized EPF. Means across replicates were used.

In addition, the chemical changes caused by the treatments were monitored. After extension testing, the dough samples were frozen in liquid nitrogen and freeze-dried for protein size-distribution analysis. The unextractable polymeric protein (%UPP) was determined using SE-HPLC (Singh et al 1990, Batey et al 1991).

**TABLE III**  
Extension Curve Dimensions and Polymer Sizes of Doughs Prepared with Varying Reductant Concentrations and Reduction Time of 1 min<sup>a</sup>

Reductant (mg/mL)	Flour	% UPP	Extension Properties			EPF
			R <sub>max</sub> (N)	Ext (cm)	D <sub>max</sub> (cm)	
Control						
	Banks	67.3	0.288	15.5	13.0	...
	Hartog	68.0	0.380	10.0	7.7	...
	QB	62.0	0.303	15.0	12.6	...
0.2						
	Banks	44.0	0.155	11.0	5.4	0.803
	Hartog	51.0	0.179	10.3	4.6	0.685
	QB	48.0	0.142	13.0	7.5	0.674
1						
	Banks	40.0	0.125	15.5	4.6	0.860
	Hartog	42.0	0.155	14.0	4.2	0.881
	QB	41.0	0.120	16.0	5.2	0.841
2						
	Banks	41.0	0.102	15.0	4.3	0.930
	Hartog	42.0	0.120	13.0	4.0	0.915
	QB	40.5	0.090	19.0	4.7	0.976
SE						
		0.8	0.014	1.0	0.5	

<sup>a</sup> UPP, unextractable polymeric protein; R<sub>max</sub>, maximum resistance to extension; Ext, extension before rupture; D<sub>max</sub>, distance to R<sub>max</sub>; EPF, extension parameter function; QB, Queensland Bakers'; SE, standard error.

**TABLE IV**  
Extension Curve Dimensions and Polymer Sizes of Doughs Prepared with Varying Oxidant Concentrations and Oxidation Time of 5 min<sup>a</sup>

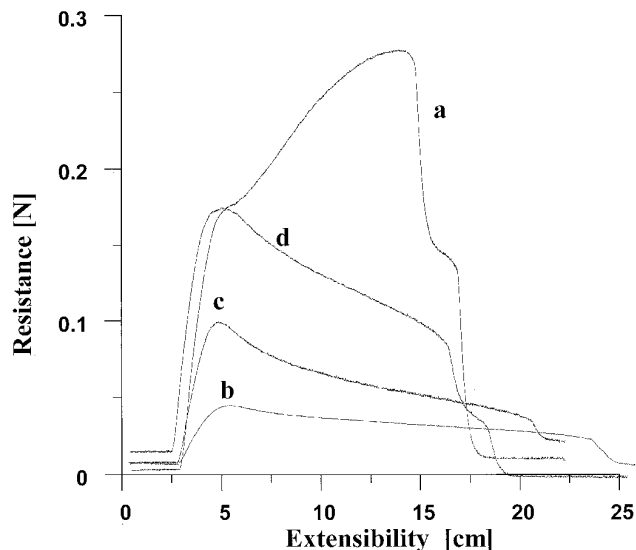
Oxidant (mg/mL)	Flour	% UPP	Extension Properties			EPF
			R <sub>max</sub> (N)	Ext (cm)	D <sub>max</sub> (cm)	
Control						
	Banks	67.3	0.288	15.5	13.0	...
	Hartog	68.0	0.388	9.8	7.9	...
	QB	62.0	0.303	15.0	12.6	...
2						
	Banks	31.0	0.063	24.8	5.0	0.963
	Hartog	42.0	0.163	19.5	4.0	1.260
	QB	36.0	0.090	25.0	4.5	1.259
2.5						
	Banks	40.0	0.075	23.8	5.2	1.092
	Hartog	45.0	0.175	20.0	4.4	1.268
	QB	39.0	0.075	24.0	4.9	1.137
3						
	Banks	41.0	0.080	21.3	5.3	1.008
	Hartog	46.0	0.180	18.5	4.1	1.153
	QB	39.0	0.070	22.0	4.8	1.089
5						
	Banks	47.0	0.080	20.3	5.3	0.970
	Hartog	50.0	0.183	16.0	4.0	0.984
	QB	47.0	0.100	16.5	5.0	0.903
7.5						
	Banks	49.0	0.083	17.3	5.5	0.925
	Hartog	54.0	0.188	14.5	4.6	0.827
	QB	48.0	0.130	15.0	5.0	0.827
10						
	Banks	52.0	0.145	15.0	8.7	0.599
	Hartog	55.0	0.245	8.0	4.3	0.617
	QB	53.0	0.200	12.0	6.7	0.607
SE						
		0.8	0.013	0.9	0.4	

<sup>a</sup> UPP, unextractable polymeric protein; R<sub>max</sub>, maximum resistance to extension; Ext, extension before rupture; D<sub>max</sub>, distance to R<sub>max</sub>; EPF, extension parameter function; QB, Queensland Bakers'; SE, standard error.

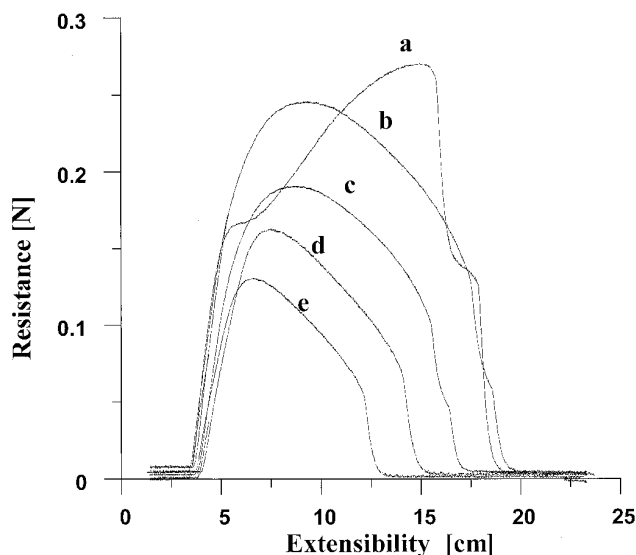
Where final treatments were optimum (resulting in smallest EPF value), the size distribution of the samples was further characterized using field-flow fractionation techniques (FFF) on proteins extracted by varying lengths of sonication time. Native polymer size was estimated by extrapolation to zero sonication time (Larroque et al 1999). Elution times were converted to molecular size in kDa with a calibration obtained using DNA fragments of known mass (Daqiq et al 2000).

### Microbaking

The reduction-oxidation conditions optimized for mixing studies and extension studies did not produce loaf heights comparable to those of untreated controls in microbaking (the conditions were



**Fig. 3.** Extension curves of different oxidation reaction times (5 mg/mL of KIO<sub>3</sub>). Water control (a) and 2, 3, and 5 min, respectively (b-d). Results for 4 min were similar to 5 min (data not shown). Relaxation time 45 min; KIO<sub>3</sub> concentration 5 mg/ mL; DTT concentration 2 mg/ mL; reduction time 4 min; mixing time 100%. Standard errors for R<sub>max</sub>, Ext, and D<sub>max</sub> are 0.018, 1.0 and 0.5, respectively.



**Fig. 4.** Extension curves after different mixing times. Water control (a) and 70, 80, 90, and 100% peak dough development time, respectively (b-e). Relaxation time 45 min; DTT concentration 0.2 mg/ mL; reduction time 4 min, KIO<sub>3</sub> concentration 5 mg/ mL; oxidation time 5 min. Standard errors for R<sub>max</sub>, Ext, and D<sub>max</sub> are 0.016, 0.8 and 0.4, respectively.

apparently toxic to yeast and did not produce desirable loaf height), so the appropriate conditions for microbaking were determined. The concentration of the reductant and time of its application were varied with two concentrations (0.2 and 2 mg/mL) being tested at one time (4 min), and all times (1, 2, 3, and 4 min) tested at one concentration (2 mg/mL). Similarly, all concentrations of oxidant (2.5, 5, 7.5, and 10 mg/mL) were tested at one time (5 min), and all times (1, 2, 3, 4, and 5 min) were tested at one concentration (2.5 mg/mL). Hartog flour was not used in these experiments.

The total quantity of water to be used to prepare doughs for microbaking was calculated using the protein and moisture contents of ingredients (AACC 2000). Doughs were mixed using a no-time formulation (flour 100%, yeast 2.5%, sodium chloride salt 2%, and improver 0.5%). The improver used was a rapid dough formulation improver which provides 100 ppm of ascorbic acid and 0.5 SKB units of cereal  $\alpha$ -amylase per 100 g of flour. Flour, 450  $\mu$ L of DTT solution, and additional water (175  $\mu$ L for Banks, 235  $\mu$ L for Queensland Bakers') were placed in the mixing bowl. The mix-

ture was mixed for 30 sec and allowed to rest for the determined reduction time. In the last few seconds of the resting period, 250  $\mu$ L of oxidant solution ( $KIO_3$ ) was added along with the yeast solution (10 g of compressed yeast + 8 g of sodium chloride salt + 2 g of improver in 100 mL of water; 515  $\mu$ L for Banks, 505  $\mu$ L for Queensland Bakers'). Mixing was resumed for 30 sec and the dough was allowed to rest further for the determined oxidation time. The dough was then mixed to the peak dough development time (including the initial two 30-sec mixings). Loaves were prepared from 2.4 g of the resulting dough which was molded, rested for 20 min at 40°C in a small airtight container, then remolded, proofed for 45 min (40°C and 90% rh) and baked at 200°C for 17 min (Gras and Bekes 1996). Loaf height was measured with vernier calipers. Baking tests were done in triplicate.

Statistical analysis of variance and analysis of covariance used MSSTAT v 4.1 (Richard E. Lund, Montana State University, Bozeman, MT) and Super Anova v 1.11 (Abacus Concepts, Berkeley, CA).

## RESULTS AND DISCUSSION

### Extension Testing

In all of the experiments, the treatments had a significant effect on the extension measurements. In most, the exception being where oxidation time was varied, the effect of cultivar was significant as well, but in no case was the cultivar  $\times$  treatment interaction significant. Thus, the three flours behaved in a parallel fashion to the experimental treatments. Representative curves are shown for each treatment for Banks and results are summarized in tables for all three flours.

TABLE V

Extension Curve Dimensions and Polymer Sizes of Doughs Prepared with Varying Oxidation Times and Oxidant Concentration of 5 mg/mL<sup>a</sup>

Oxidation Time (min)	Flour	% UPP	Extension Properties			
			R <sub>max</sub> (N)	Ext (cm)	D <sub>max</sub> (cm)	EPF
Control	Banks	67.3	0.288	15.5	13.1	...
	QB	62.0	0.303	15.0	12.5	...
2	Banks	34.0	0.043	23.0	5.6	1.134
	QB	47.0	0.050	20.0	5.0	1.080
3	Banks	49.0	0.098	21.0	5.3	0.957
	QB	48.0	0.080	17.0	4.8	0.968
5	Banks	64.0	0.168	17.3	5.2	0.741
	QB	58.0	0.270	16.0	5.0	0.612
SE		0.8	0.018	1.0	0.5	

<sup>a</sup> UPP, unextractable polymeric protein; R<sub>max</sub>, maximum resistance to extension; Ext, extension before rupture; D<sub>max</sub>, distance to R<sub>max</sub>; EPF, extension parameter function; QB, Queensland Bakers'; SE, standard error.

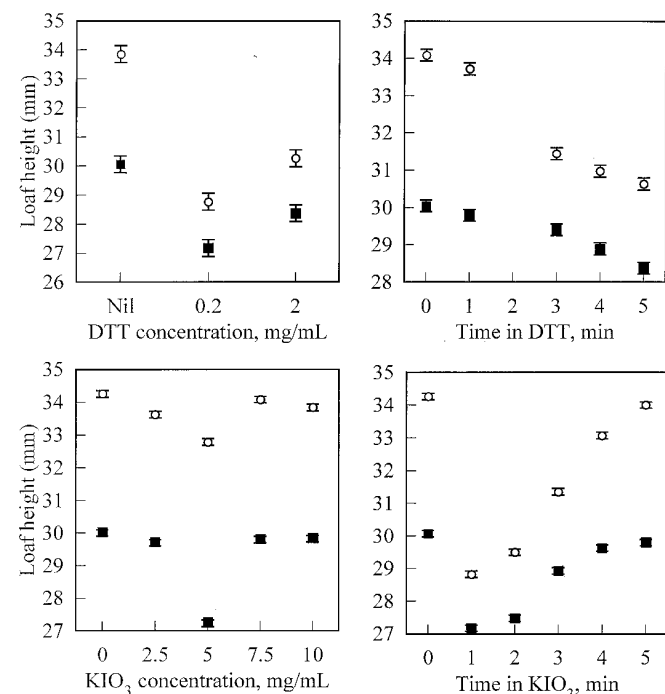


Fig. 5. Effect of reduction-oxidation on loaf height of Banks (■) and Queensland Bakers' (○). Error bars  $\pm$ 1 standard error.

TABLE VI

Extension Curve Dimensions and Polymer Sizes of Doughs Prepared with Varying Mixing Times<sup>a</sup>

Mixing Time (% optimum)	Flour	% UPP	Extension Properties			
			R <sub>max</sub> (N)	Ext (cm)	D <sub>max</sub> (cm)	EPF
Control	Banks	67.3	0.288	18.5	12.9	...
	Hartog	68.0	0.363	9.8	7.8	...
	QB	62.0	0.303	17.0	12.4	...
70	Banks	67.9	0.263	18.5	11.0	0.264
	Hartog	68.7	0.360	10.0	6.7	0.179
	QB	63.0	0.310	17.5	10.8	0.214
80	Banks	51.4	0.173	14.8	10.0	0.466
	Hartog	55.0	0.273	7.5	5.1	0.522
	QB	50.0	0.210	14.3	9.6	0.385
90	Banks	48.7	0.155	13.8	8.7	0.580
	Hartog	50.0	0.255	7.3	4.5	0.611
	QB	46.0	0.220	12.3	7.3	0.527
100	Banks	45.3	0.120	12.0	5.1	0.873
	Hartog	47.0	0.220	5.8	2.6	0.900
	QB	47.0	0.170	9.3	4.2	0.882
SE		0.8	0.016	0.8	0.4	

<sup>a</sup> UPP, unextractable polymeric protein; R<sub>max</sub>, maximum resistance to extension; Ext, extension before rupture; D<sub>max</sub>, distance to R<sub>max</sub>; EPF, extension parameter function; QB, Queensland Bakers'; SE, standard error.

TABLE VII

Optimum Conditions for Mixing, Extension, and Microbaking

Treatment	Mixing	Extension	Microbaking
Reductant conc. (mg/mL)	2	0.2	2
Reduction time (min)	4	1	1
Oxidant conc. (mg/mL)	5	5	2.5
Oxidation time (min)	5	5	5
Mixing time (% of peak dough development time)	100	70	100

The reduction-oxidation treatments had profound effects on the shape of the extension curve (Fig. 1, Table I). Relaxation times of 0, 15, and 30 min gave shorter extensibility than the controls. The 45 and 60 min of relaxation time gave the longest extensibilities (Table II). Because 45 min of relaxation time is conventional, this time was used for further experiments.

For 0.2, 1.0, and 2.0 mg/mL of reductant, 1 min of reduction time provided greater  $R_{\max}$  than 4 min (Fig. 2). At 1 min of reduction time, 0.2 mg/mL of reductant provided greater  $R_{\max}$  than higher concentrations, although extensibility was reduced (Fig. 2B, Table III). Higher reductant concentrations were also associated with dough stickiness. Therefore a reduction concentration of 0.2 mg/mL and 1 min of reduction time was used.

For the oxidation step, though 10 mg/mL of  $KIO_3$  gave extensibility closer to that of the control (Table IV), the 5 mg/mL solution was selected because with all other combinations (reductant, reductant time, relaxation time, and proportion of mixing time) it gave the lowest EPF value. Oxidation times of 4 or 5 min were required to obtain %UPP values similar to those of the control (Fig. 3, Table V) and 1 min of oxidation time produced sticky dough that could not be used. Reducing the mixing time to 70% of peak dough development time gave an extension curve closer to the control than did longer mixing times (Fig. 4, Table VI). The optimized conditions (Table VII) providing extensibility and maximum resistance to extension values comparable to that of the controls, with somewhat different overall curve shape, were used for further investigations.

The average molecular weights (kDa) as determined by FFF for insoluble polymeric proteins of untreated control dough and dough obtained by the optimized reduction-oxidation conditions for extension testing were similar (Banks 714 kDa and 721 kDa respectively; Hartog 719 kDa and 734 kDa, respectively; and Queensland Bakers' 657 kDa and 684 kDa, respectively;  $LSD_{0.05} = 29$ ), indicating that the reduction-oxidation treatment was valid.

### Microbaking

In all of these experiments, the effects of treatment, flour, and the flour  $\times$  treatment interaction were all statistically significant, but the interaction term was small, as shown by the largely parallel performance of the two flours shown in Fig. 5. Using a reductant concentration of 2 mg/mL provided a loaf height closest to that of the control. Longer reduction times decreased loaf height and, hence, 1 min was selected as a suitable reduction time. For the oxidation, 2.5, 7.5, and 10 mg/mL of  $KIO_3$  solution provided equally good results and, hence, the lowest concentration (2.5 mg/mL) among them was selected as suitable. The 5.0 mg/mL concentration provided anomalously low results. The longest oxidation time, 5 min gave a loaf height closest to that of the control. Therefore, an oxidant concentration of 2.5 mg/mL and 5 min of oxidation time were used for further investigations. The observations for the various treatments were similar for the two flours studied (Fig. 5).

The optimized conditions for mixing, extension, and microbaking studies are given in Table VII. In extension studies, the concentration of reductant (DTT) had to be reduced to 10% of the original value (optimum for mixing studies) (Bèkès et al 1994a) because the extensibility curve obtained with the original DTT concentration differed considerably from that of the control (especially for maximum resistance). The original concentration of DTT was, however, suitable for baking studies. In both extension and baking studies, the time allowed for the reductant to act was reduced to 1 min from 4 min. The concentration of oxidant was kept the same for extension testing and the same could be used for baking studies but for these it was halved, because the lower concentration gave the same results as those of the concentration used for either mixing or extension testing and was considered less likely to have adverse effects. In both extension and baking studies, the time allowed for the oxidant to react was the same as was used for the mixing

experiments. The results showed that it was not possible to have a single reduction-oxidation technique for all three tests with the reductant (DTT) used in these experiments. However, a single-oxidation procedure was suitable. Other reductants may in the future allow the development of a single technique common to all three tests.

## CONCLUSIONS

A carefully selected reduction-oxidation procedure provides a method for studying the contribution of glutenin composition to functional properties of dough. Results also indicate that small-scale techniques are very suitable to study the direct effect of dough components on functionality.

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