



# Variation in the Breadmaking Quality and Rheological Properties of Wheat in Relation to Sulphur Nutrition under Field Conditions

F. J. Zhao\*, S. E. Salmon†, P. J. A. Withers‡, J. M. Monaghan§, E. J. Evans§, P. R. Shewry¶ and S. P. McGrath\*

\* IACR-Rothamsted, Soil Science Department, Harpenden, Herts, AL5 2JQ, U.K.; † Campden & Chorleywood Food Research Association, Chipping Campden, Gloucestershire, GL55 6LD, U.K.;

‡ ADAS Bridgets, Martyr Worthy, Winchester, Hants, SO21 1AP, U.K.; § University of Newcastle-upon-Tyne, Department of Agriculture, NE1 7RU, U.K.; ¶ IACR-Long Ashton, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol, BS18 9AF, U.K.

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## ABSTRACT

Seven field experiments were conducted at four sites in England in the 1994/95 and 1995/96 cropping seasons to investigate the effects of S application on the breadmaking quality of the premium hard winter wheat variety Hereward. Two N levels (180 and 230 kg/ha) were combined with three S levels (0, 20 and 100 kg/ha) in all experiments. Loaf volume was increased significantly by S in four out of the seven experiments, whereas increasing the N rate significantly increased loaf volume in only one experiment. Responses of breadmaking quality to S were more common than responses in terms of grain yield. Sulphur application did not affect grain protein concentration directly, but tended to increase gel protein weight in flour and the proportion of polymeric proteins. The elastic modulus of gel protein and dough resistance were decreased consistently by S, whereas dough extensibility was increased by S. Correlation and regression analyses showed that grain protein concentration was a poor indicator of loaf volume, whereas grain S status (S concentration and N:S ratio) was more influential. These results indicate that there is a current need to apply S fertiliser to wheat in many areas of England in order to maintain breadmaking quality.

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## INTRODUCTION

It has been established that the sulphur (S) nutrition of wheat has an important influence on the breadmaking quality of flour<sup>1</sup>. This is due to the essential role of disulphide bonds in maintaining gluten functionality<sup>2</sup>. Disulphide bonds are formed from cysteine residues, either within the same

molecule (intra-chain) or linking two protein subunits (inter-chain). Viscosity (extensibility) of dough is mainly attributed to the monomeric gliadins, which form only intra-chain or no disulphide bonds, whereas dough elasticity is primarily associated with the polymeric glutenins, which form both intra- and inter-chain disulphide bonds<sup>2</sup>. It is the balance between viscosity and elasticity that determines the suitability and quality of wheat flour for different end uses.

Several studies have shown that S deficiency favours the synthesis of S-poor proteins, such as

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Corresponding author: Dr F. J. Zhao. Tel: 01582 763133 Ext 2667; Fax: 01582 760981; E-mail: [Fangjie.Zhao@bbsrc.ac.uk](mailto:Fangjie.Zhao@bbsrc.ac.uk)

$\omega$ -gliadins and high molecular weight (HMW) subunits of glutenin, at the expense of S-rich proteins, such as  $\alpha$ - and  $\gamma$ -gliadins and low molecular weight (LMW) subunits of glutenin<sup>3-6</sup>. These compositional changes were associated with decreased extensibility and increased elasticity of dough<sup>7,8</sup>. In the case of severe S deficiency, the resulting loaf volume was also significantly smaller<sup>6,8</sup>.

Wheat has a relatively low requirement for S, amounting to about 20 kg S/ha for an average crop with a grain yield of 8 t/ha<sup>9</sup>. Deficiency of S in wheat crops has occurred in England only recently, largely due to a massive decrease in the inputs of S from atmospheric deposition over the last three decades<sup>9</sup>. As a result of reduced inputs, the concentrations of S in British wheat grain decreased considerably from the early 1980s to the early 1990s, whereas the N:S ratio increased<sup>10</sup>. Recent studies have shown significant yield responses of wheat to S fertilisation, particularly in areas of low S deposition and with light textured or shallow calcareous soils<sup>9,11</sup>.

There have been no systematic studies to investigate whether the breadmaking quality of wheat would benefit from soil applied S fertilisers under field conditions in the U.K. The objectives of this study were, therefore, to evaluate responses of breadmaking quality parameters to the addition of S fertiliser using field trials on different locations, and to analyse relationships between these parameters and grain S status.

## EXPERIMENTAL

### Field experiments

Field experiments were carried out in the 1994/95 and 1995/96 seasons at four sites, which were located in the main wheat growing areas of England: Bridgets (Hampshire), Woburn (Bedfordshire), Raynham (Norfolk) and Wark Common (Northumberland). The crop at Raynham in 1995/96 was badly affected by drought; therefore the grain samples from this experiment were not included in milling and breadmaking tests.

Soil properties and previous cropping at each experimental site are shown in Table I. Soil samples were collected from the 0–30, 30–60 and 60–90 cm depth in early spring prior to fertiliser application, for the measurement of mineral N and extractable S. Mineral N (nitrate and ammonium) in the fresh soils was extracted with 2 M KCl and determined colorimetrically using a

continuous-flow analyser. Air-dried soils were used for the determination of extractable sulphate-S, which was extracted with 0.016 M  $\text{KH}_2\text{PO}_4$  and determined by ion chromatography. Both mineral N and extractable S concentrations are expressed on an oven-dried soil basis. Apart from the Bridgets and Wark Common sites in 1995/96, soils from all other sites contained  $\leq 3.0$  mg/kg of extractable sulphate-S (Table I). An extractable sulphate-S value below 3 mg/kg usually indicates a low S supply<sup>9</sup>.

The variety used in all experiments was Hereward, an autumn-sown hard wheat (*Triticum aestivum*) of high breadmaking potential. The experimental design was the same for all sites in each season. There were 12 treatments, consisting of factorial combinations of two N rates, 180 and 230 kg/ha, and six S rates, which were 0, 20, 40, 60, 80 and 100 kg/ha in the 1994/95 season, and 0, 10, 20, 40, 70 and 100 kg/ha in the 1995/96 season. All treatments were replicated in three plots in a randomised block design. Plot size varied between 36 and 50 m<sup>2</sup> at different sites. Crops were sown in autumn between mid September and early November. Nitrogen was applied as ammonium nitrate in two dressings in March and April, and S was applied as gypsum (18% S) in March. Herbicides, fungicides and insecticides were applied according to standard practices.

At maturity (mid August), grain yields were determined using a plot combine. Grain samples were collected for the determination of moisture content. Milling and breadmaking tests were carried out only on the grain samples collected from the S0, S20 and S100 treatments at both N rates. Grain yield responses to S addition were reported elsewhere<sup>11</sup>.

### Methods

#### Grain N and S

Grain samples were ground to pass a 0.5-mm sieve using a Retsch centrifugal mill. The concentration of N was determined using a Dumas combustion method (LECO CNS Analyzer). Grain protein concentration was calculated from the N concentration by multiplying by a factor of 5.7. For the determination of S, samples were digested with a mixture of  $\text{HNO}_3$  and  $\text{HClO}_4$ , followed by measurement of S in solution using inductively coupled plasma atomic emission spectroscopy<sup>12</sup>. The concentrations of N and S are expressed on

**Table I** Soil properties, concentrations of available N and S, and previous cropping at each site

	Bridgets		Raynham	Woburn		Wark Common	
	1994/95	1995/96	1994/95	1994/95	1995/96	1994/95	1995/96
Previous cropping	Winter oat	Oilseed rape	Pea	Winter oat	Lupin	Winter wheat	Oilseed rape
Soil texture	Clay loam	Clay loam	Sandy loam	Sandy loam	Sandy loam	Loam	Loam
Organic matter (%) <sup>*</sup>	3.82	3.49	1.38	1.03	1.35	2.87	2.48
Total N (%) <sup>*</sup>	0.29	0.31	0.09	0.06	0.08	0.16	0.16
pH <sup>*</sup>	8.3	7.9	8.3	6.9	7.4	6.2	6.2
Mineral N (mg/kg) <sup>†</sup>							
0–30 cm	21.7	12.0	4.1	1.5	3.5	14.7	9.4
30–60 cm	15.3	6.8	1.6	2.0	3.0	3.3	5.6
60–90 cm	7.0	5.4	1.4	1.6	1.9	3.2	2.8
Available S (mg/kg) <sup>†</sup>							
0–30 cm	2.1	6.5	1.2	1.3	2.1	0.9	4.4
30–60 cm	2.1	9.8	0.8	2.7	2.8	1.6	3.8
60–90 cm	1.9	6.8	0.9	3.2	1.8	3.0	6.2

<sup>\*</sup> Analyses were done on the topsoil (0–30 cm) samples collected in autumn before sowing. Organic matter and total N were determined using a LECO CNS Analyzer. pH was determined in a soil and water suspension with a glass electrode.

<sup>†</sup> Soils were collected in spring before fertiliser additions.

dry matter basis, whereas grain protein concentration was calculated on an 86% dry matter basis. Grain N:S ratio was calculated from the N and S concentrations.

### Milling and baking<sup>\*</sup>

Grain samples were milled in a Buhler MLU 202 mill to produce straight-run white flour. A Buhler MLU 203 Impact finisher was then used to remove adhering endosperm from the bran and offal fractions obtained during the initial milling. The additional flour produced was blended with the straight-run white flour for quality testing. Flour protein concentration and moisture content were measured by Near Infrared Reflectance<sup>13</sup>.

The water absorbing capacity of each flour sample was measured using the Brabender Farinograph working to the 600 BU line<sup>13</sup>. This test provides a measure of the water required to mix a dough to a fixed consistency which is used subsequently in test baking. A standard laboratory-scale Chorleywood Bread Process (CBP) baking test was used to produce 400 g white loaves<sup>13</sup>. The recipe used for the CBP bread was (all as a proportion of flour weight): 2.5% yeast; 2% salt; 1% hard fat; 0.01% ascorbic acid; water as determined by Farinograph 600 line; mixing work input 39.6 kJ/kg. The test was run in duplicate.

Loaf volume was measured by seed displacement. The quality of crumb structure was assessed visually by an expert. A high score (maximum 10) for crumb cell structure was awarded for a close and uniform structure of small, thin-walled cells.

### Gel protein and rheology

The gel protein fraction in white flour and its elastic modulus were determined according to Pritchard and Brock<sup>14</sup>. Flour (10 g) was defatted with 25 mL petroleum ether (b.p. 40–60 °C) for 1 h, filtered and dried. Defatted flour (5 g) was stirred with 90 mL of 1.5% (w/v) sodium dodecyl sulphate for 10 min at 10 °C before being centrifuged at 63 000 *g* for 40 min. The gel protein layer was removed and weighed. The elastic modulus (*G'*) of gel protein was measured using a small deformation, constant strain oscillatory rheometer (Bohlin VOR), after a 30 min relaxation period at 10 °C.

### Extensograph

Dough resistance and extensibility of the 1995/96 samples were determined using a Brabender Extensograph according to the manufacturer's instructions.

### SE-HPLC

White flour samples were used to extract and fractionate proteins on the basis of size according to the method of Batey *et al.*<sup>15</sup>. Flour samples were extracted with 0.5% (w/v) SDS in 50 mM Na-

<sup>\*</sup> Details of the methods of milling and baking tests are available upon request.

phosphate buffer (pH 6.9) with sonication, and then resolved into three fractions using size-exclusion HPLC (Beckman System with TSK Gel 3000SW column, mobile phase containing 50% acetonitrile and 0.06% TFA). The replicates of the SDS extract gave consistent readings of absorbance at 280 nm, with the coefficient of variation varying between 4 and 6% in 10 replicates. The proportion of the flour protein extracted was not determined in this study, but Batey *et al.*<sup>15</sup> reported a consistent high proportion of extraction (95%) for the method used. To identify the proteins present in these peaks, the three fractions were collected, freeze-dried, and then separated by SDS-PAGE under non-reducing conditions and also after reduction of disulphide bonds.

### Data analysis

Analysis of variance (ANOVA) was performed on all data sets in two steps: first to test the significance of effects of N and S treatments at each site; and then data from all sites were pooled to test the effects of sites, N and S treatments. There was no evidence of variance heterogeneity in the second step ANOVA, indicating that pooling the data from all sites was statistically valid. Data from all sites in each year were combined in correlation and regression analyses. Factors such as site, N and S treatments were not accounted for in these regression analyses. The statistical package Genstat 5 was used<sup>16</sup>.

## RESULTS

### Effects of S and N on grain composition, breadmaking quality, rheology and protein distribution

Tables II and III summarise the results of ANOVA for individual sites and for all sites combined in each season, respectively. It is not surprising that the differences between experimental sites were highly significant for most of the quality parameters determined (Table III), although the patterns of responses to N and S were generally similar across all sites in each year. Hence, interactions between site and N or S were not significant for most parameters, or if significant, their variance ratios were considerably smaller than those for the main factors N or S (Table III). For simplicity, mean values for each treatment across all sites in each season are presented in Figures 1–5.

### Grain protein concentration

In general, grain protein concentration was higher in 1994/95 than in 1995/96, ranging from 8.3 to 13.8% and from 8.5 to 12.2% in 1994/95 and 1995/96, respectively. Within each season, experimental site was by far the most important factor affecting the grain protein concentration, followed by N treatment (Table III). On average, increasing the N rate from 180 to 230 kg/ha increased grain protein concentration by 0.8 and 1% in 1994/95 and 1995/96, respectively (Fig. 1). In contrast, the S treatments had no significant effect on grain protein concentration (Fig. 1), except in one experiment at Bridgets in 1994/95, which showed a significant negative influence of S due to the dilution effect resulting from a large yield response to the S addition<sup>11</sup>.

Flour protein concentrations (data not shown) correlated closely with grain protein concentrations, but were 0.5–0.9% lower.

### Grain S concentration and N:S ratio

The ranges of grain S concentration were similar in both seasons, varying between 1.2 and 1.9 mg/g. Application of S had the most significant effect on grain S concentration (Fig. 2 and Table III). On average, applications of 20 and 100 kg S/ha increased grain S concentration by 5–10% and 18–19%, respectively, in the two seasons. Overall, increasing the N rate also increased grain S concentration (Fig. 2), the effect being significant in five out of the seven experiments (Table II).

Grain N:S ratios were higher in 1994/95 (range 12–21.7) than in 1995/96 (range 11.9–18.6), due to higher protein concentrations in the first season. For all experiments in 1994/95 the N:S ratios were above 16 when S was not applied, whereas in 1995/96 only for the experiment at Woburn were the ratios above 16 in the absence of S application. Application of S decreased grain N:S ratio significantly in all but the 1994/95 experiment at Wark Common, whereas increasing the N rate tended to increase the N:S ratio (Table II and Fig. 2).

### Loaf volume and crumb score

Flour yield varied slightly between sites and seasons, but was not significantly influenced by the N and S treatments. Mean flour yields were 75.3, 76.4, 80.8 and 80.4% for Woburn, Bridgets, Borders and Raynham in 1994/95, respectively, and 75.3, 75.4 and 72.5% for Woburn, Bridgets and

**Table II** Significance levels from ANOVA of the N and S treatment effects and N × S interactions on different quality parameters

	Grain protein			Grain S			Grain N:S ratio			Flour water absorption			Loaf volume			Crumb score			Gel protein weight			Gel protein G'			Dough extensibility			Dough resistance		
	N	S	N × S	N	S	N × S	N	S	N × S	N	S	N × S	N	S	N × S	N	S	N × S	N	S	N × S	N	S	N × S	N	S	N × S	N	S	N × S
1994/95																														
Bridgets	***	**	NS	**	***	NS	*	***	NS	***	***	*	NS	***	***	NS	NS	NS	***	*	*	NS	**	NS						
Raynham	***	NS	NS	**	**	NS	NS	***	NS	***	NS	NS	NS	*	NS	NS	NS	NS	***	NS	NS	*	*	NS						
Wark Common	NS	NS	NS	NS	*	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	**	NS						
Woburn	***	NS	NS	NS	***	*	NS	***	NS	**	**	NS	NS	NS	NS	NS	NS	NS	NS	***	NS	*	***	NS						
1995/96																														
Bridgets	***	NS	NS	***	***	NS	NS	***	NS	***	NS	NS	**	**	NS	NS	NS	NS	***	NS	NS	NS	*	NS	***	NS	NS	NS	**	*
Wark Common	***	NS	NS	***	***	NS	**	**	NS	***	NS	NS	NS	**	NS	NS	*	NS	*	**	NS	NS	**	NS	*	**	NS	NS	*	NS
Woburn	***	NS	NS	*	***	NS	*	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

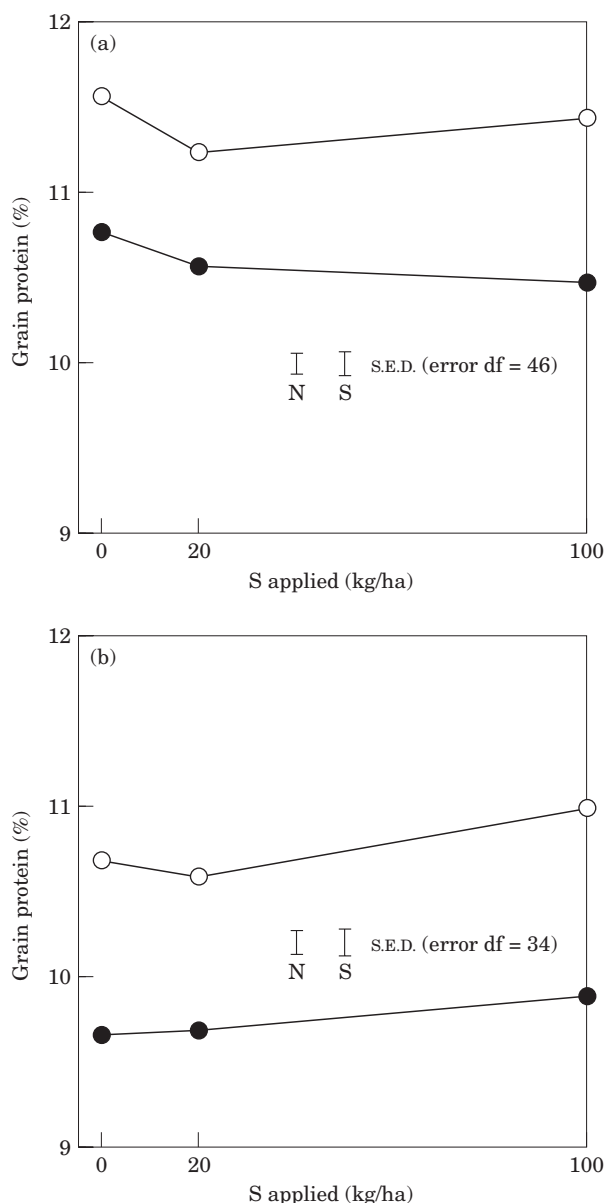
Significance levels: NS, not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

**Table III** Variance ratios and significance levels of site, N and S treatments in the two seasons

	Grain protein	Grain S	Grain N:S ratio	Flour water absorption	Loaf volume	Crumb score	Gel protein weight	Gel protein G'	Dough extensibility	Dough resistance
1994/95										
Site	171.7***	76.1***	19.9***	78.8***	99.1***	58.5***	54.7***	27.6***		
N	60.0***	20.8***	6.3**	49.3***	0.7 <sup>NS</sup>	0.6 <sup>NS</sup>	29.5***	16.2***		
S	2.2 <sup>NS</sup>	90.9***	66.7***	17.0***	13.0***	1.6 <sup>NS</sup>	21.9***	39.0***		
Site × N	1.5 <sup>NS</sup>	1.1 <sup>NS</sup>	0.3 <sup>NS</sup>	0.9 <sup>NS</sup>	1.7 <sup>NS</sup>	0.5 <sup>NS</sup>	4.2*	2.0 <sup>NS</sup>		
Site × S	1.9 <sup>NS</sup>	16.8***	9.6***	2.3*	0.7 <sup>NS</sup>	0.7 <sup>NS</sup>	8.7***	0.6 <sup>NS</sup>		
N × S	0.6 <sup>NS</sup>	1.5 <sup>NS</sup>	0.4 <sup>NS</sup>	0.8 <sup>NS</sup>	0.9 <sup>NS</sup>	0.5 <sup>NS</sup>	4.1*	0.1 <sup>NS</sup>		
1995/96										
Site	76.9***	18.7***	41.6***	14.6***	23.4***	2.1 <sup>NS</sup>	24.4***	57.0***	18.4***	5.0*
N	65.7***	16.1***	6.3*	37.4***	1.7 <sup>NS</sup>	2.1 <sup>NS</sup>	16.8***	0.1 <sup>NS</sup>	25.7***	3.6*
S	2.3 <sup>NS</sup>	44.4***	43.2***	3.4*	6.8**	1.0 <sup>NS</sup>	3.9*	5.2**	7.1**	10.7***
Site × N	3.5*	1.5 <sup>NS</sup>	0.2 <sup>NS</sup>	2.7 <sup>NS</sup>	0.3 <sup>NS</sup>	0.9 <sup>NS</sup>	0.1 <sup>NS</sup>	0.7 <sup>NS</sup>	1.0 <sup>NS</sup>	0.2 <sup>NS</sup>
Site × S	2.1 <sup>NS</sup>	1.8 <sup>NS</sup>	4.0**	1.6 <sup>NS</sup>	2.2 <sup>NS</sup>	2.7*	2.1 <sup>NS</sup>	1.0 <sup>NS</sup>	4.8**	0.4 <sup>NS</sup>
N × S	0.2 <sup>NS</sup>	0.5 <sup>NS</sup>	1.2 <sup>NS</sup>	1.2 <sup>NS</sup>	0.2 <sup>NS</sup>	1.8 <sup>NS</sup>	0.9 <sup>NS</sup>	0.3 <sup>NS</sup>	2.6 <sup>NS</sup>	2.2 <sup>NS</sup>

Significance levels: NS, not significant; \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$ .





**Figure 1** Effects of S and N on grain protein concentration in (a) 1994/95 and (b) 1995/96. The N fertilisation rates were 180 kg N/ha (●) and 230 kg N/ha (○) S.E.D., standard error of difference.

Borders in 1995/96, respectively. The flour water absorptions increased significantly with increasing N rate in six out of the seven experiments (Table II). In contrast, application of S decreased the flour water absorptions in two experiments, and had no significant effects in the other experiments.

Loaf volume ranged from 1269 to 1538 mL in 1994/95, and from 1481 to 1783 mL in 1995/96, respectively. Increasing the N rate from 180 to

230 kg/ha improved loaf volume significantly in only one experiment (Bridgets 1995/96), whereas application of S increased loaf volume in all experiments, with the effects being significant in four out of the seven experiments (Table II). When all sites in each season were combined in the ANOVA, it was clear that site and S treatment were the most significant factors affecting loaf volume, whereas applying an additional 50 kg N/ha had no significant effect overall (Table III). Averaging all experiments in each season, applications of 20 and 100 kg S/ha increased loaf volume by 25 and 41 mL in 1994/95, and 35 and 53 mL in 1995/96, respectively (Fig. 3). The largest response occurred in the Wark Common experiment in 1995/96, which showed an increase in loaf volume of more than 100 mL as a result of the application of 100 kg S/ha, representing a relative increase of 6.7%. Proportionally, application of the first 20 kg S/ha produced a larger response than the further dose of S (Fig. 3). Crumb structure was improved significantly by S in one experiment (Wark Common 1995/96), and was unaffected by the N treatment (Table II).

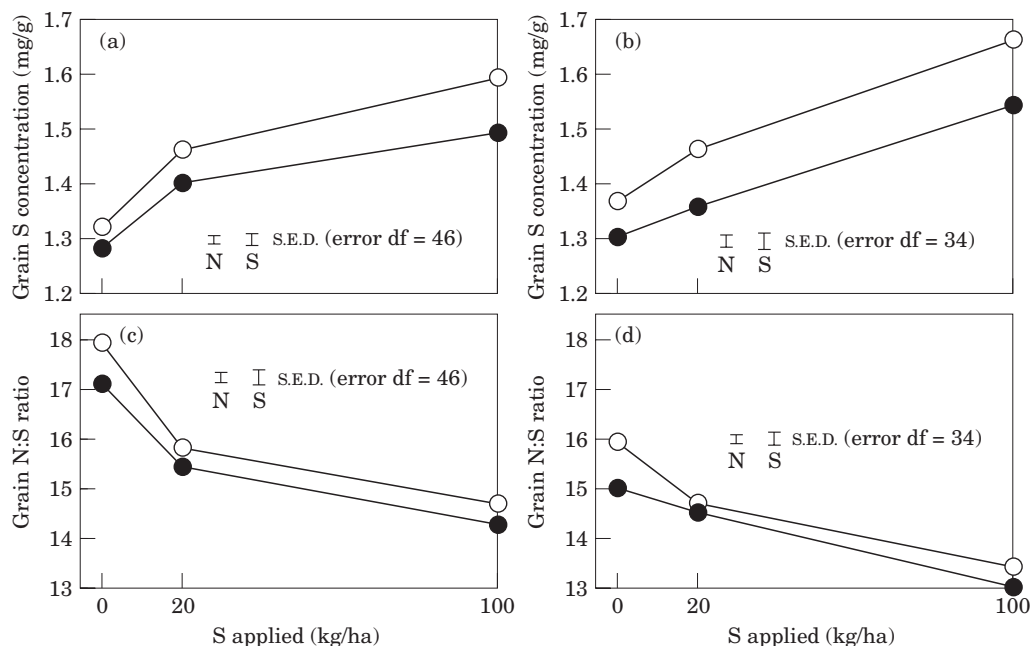
#### *Gel protein weight and elastic modulus*

The flours from 1994/95 had higher concentrations of gel protein (Fig. 4), which was consistent with their higher total protein concentrations. Increasing the N rate increased gel protein concentration significantly in five out of the seven experiments (Table II). Application of S also tended to increase gel protein concentration, and the effect was significant in three experiments (Table II). The S effect was most apparent for the first 20 kg/ha, beyond which little further increase was observed (Fig. 4).

The elastic modulus ( $G'$ ) of the gel protein was decreased significantly by the S treatment in all but one experiment (Table II), the effect being more pronounced in 1994/95 than in 1995/96 (Fig. 4). The influence of the N treatment was not consistent in the two seasons. Increasing the N rate increased  $G'$  in two experiments in 1994/95, but had no significant effect in the second season (Fig. 4).

#### *Dough extensibility and resistance*

Extensograph measurements were performed only on the 1995/96 samples. Application of N increased the dough extensibility significantly in two experiments, but had no significant effect on dough resistance (Table II and Fig. 5). Application of S



**Figure 2** Effects of S and N on grain S concentration in (a) 1994/95 and (b) 1995/96, and on grain N:S ratio in (c) 1994/95 and (d) 1995/96. The N fertilisation rates were 180 kg N/ha (●) and 230 kg N/ha (○). S.E.D., standard error of difference.

increased dough extensibility in one experiment, and decreased dough resistance significantly in two out of the three experiments (Fig. 5). It is clear from the combined ANOVA that N had the most significant effect on dough extensibility, whereas S had the most significant effect on dough resistance (Table III).

#### SE-HPLC

SE-HPLC was used to determine the size distribution of total protein fractions extracted from flour of the Woburn and Bridgets samples from 1994/95 and the Woburn and Wark Common samples from 1995/96. The proteins were resolved into three peaks, with peak 1 corresponding mainly to high  $M_r$  glutenin polymers, peak 2 to a mixture of medium  $M_r$  polymers and monomers and peak 3 to mainly monomers with some low  $M_r$  polymers. Nitrogen had no significant effects on the relative proportions of the three peaks in all four sets of samples. However, there were significant effects of the S treatment on the relative proportions of peaks 1 and 2 in the Woburn 1994/95 and Wark Common 1995/96 samples. These were the two experiments having the most significant effects of S on gel protein concentration (Table II). In both experiments, application of S increased the relative proportion of peak 1 and decreased that of peak

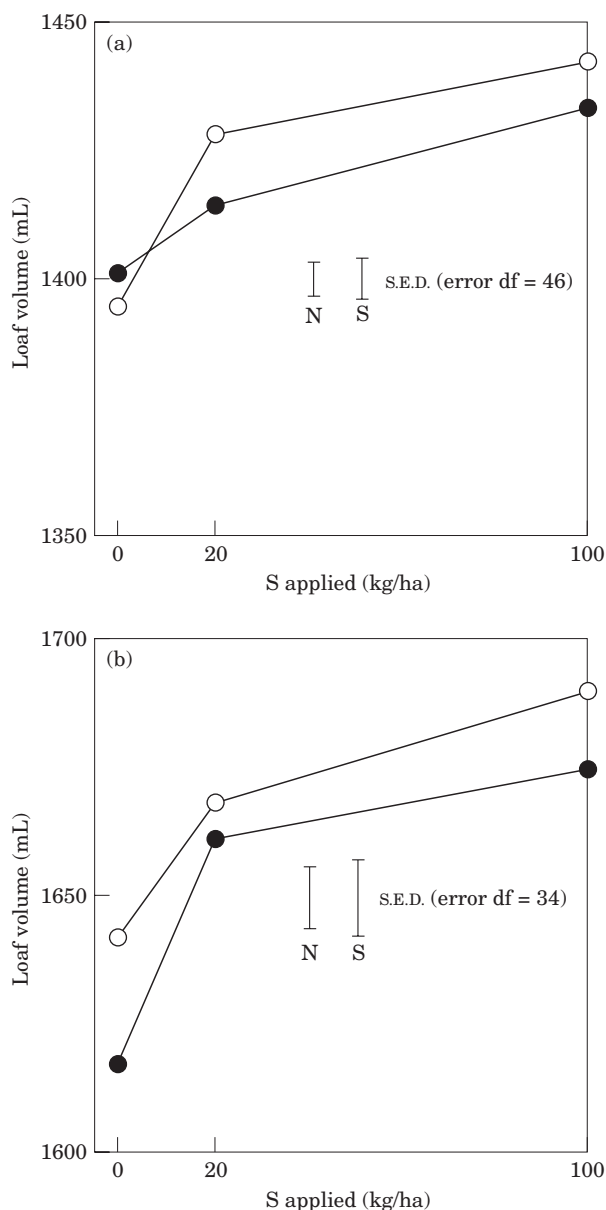
2, but had little effect on peak 3. Results for peaks 1 and 2 are shown in Figure 6.

#### Correlations between grain S status and breadmaking quality

Correlations were calculated between loaf volume and grain protein concentration, S concentration, N:S ratio, gel protein concentration and elastic modulus for the two seasons separately (Table IV). Correlations were generally poor in 1994/95, although loaf volume correlated negatively with grain N:S ratio ( $P < 0.001$ ) and with grain protein ( $P < 0.05$ ) [Table IV and Fig. 7(a)]. In 1995/96, loaf volume correlated positively with gel protein concentration, grain S concentration and grain protein concentration ( $P < 0.001$ ). Correlations between loaf volume and gel protein or grain S concentration were more significant than between loaf volume and grain protein concentration [Fig. 7(b)–(d)]. Grain protein, grain S concentration and flour gel protein alone explained 19, 39 and 46% of the variation in loaf volume, respectively.

Step-wise multiple regression was then used to identify which combinations of the above variables best explain the variations in loaf volume within each season. In both seasons, gel protein con-





**Figure 3** Effects of S and N on CBP loaf volume in (a) 1994/95 and (b) 1995/96. Note the different y-axis scales for (a) and (b). The N fertilisation rates were 180 kg N/ha (●) and 230 kg N/ha (○). S.E.D., standard error of difference.

centration and grain N:S ratio were the only two variables reaching a significance level of  $P < 0.05$  or better in the step-wise regression (Table V). In 1994/95, these two variables explained only 24% of the variance of loaf volume, with grain N:S ratio being more influential than gel protein concentration. In 1995/96, 54% of the variance of loaf volume could be explained by these two variables, with gel protein being more influential than grain

N:S ratio. In the regression equations, the coefficients for grain N:S ratio were negative and similar in both seasons, indicating that increasing N:S ratio was associated with decreasing loaf volume. The influence of gel protein concentration was opposite in the two seasons.

Step-wise multiple regression was also employed to obtain a best fit equation for dough resistance and extensibility from the Extensograph tests in 1995/96. For dough resistance ( $Y$ ), the two significant variables were grain S concentration ( $S$ ) and the elastic modulus of gel protein ( $G'$ ), as shown in the following equation:

$$Y = 465.4 - 141.6S + 2.2G'$$

( $R^2_{\text{adjusted}} = 0.36$ ; variance ratios for  $S$  and  $G'$  were 19.6 and 7.3, respectively.)

In contrast, for dough extensibility ( $Y$ ), gel protein concentration ( $GEL$ ) was the most significant variable, followed by grain protein concentration ( $GPC$ ) and the elastic modulus of gel protein ( $G'$ ):

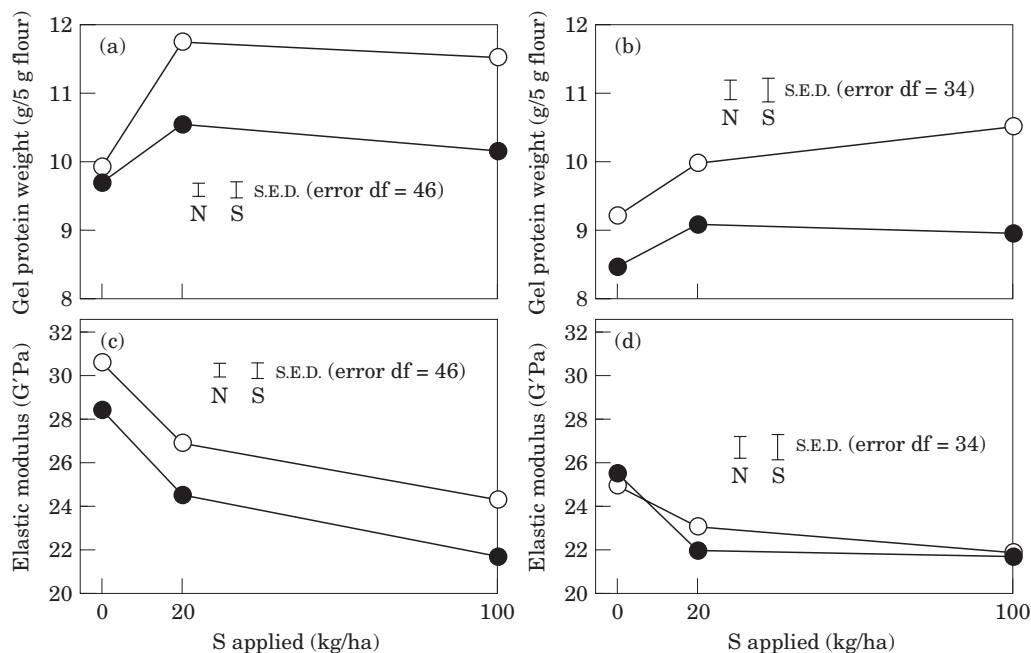
$$Y = 7.2 + 0.7GEL + 0.7GPC - 0.1G'$$

( $R^2_{\text{adjusted}} = 0.68$ ; variance ratios for  $GEL$ ,  $GPC$  and  $G'$  were 89.7, 6.2 and 4.6, respectively.)

## DISCUSSION

The results obtained in this study demonstrate that application of S fertiliser improved the breadmaking quality of winter wheat Hereward under field conditions in England, with the effects being significant in four out of the seven experiments conducted in two seasons. Loaf volume, obtained using the Chorleywood Bread Process, was increased between 20 and 105 mL by S application. In contrast, increasing the rate of N from 180 to 230 kg/ha, which is a common practice for producing breadmaking wheat in the U.K., had little effect on breadmaking quality. In the present series of experiments, responses of breadmaking quality parameters to both N and S additions bore little relationship with the concentrations of mineral N and available S in the soils in early spring (Table I). This suggests that the soil supply of both N and S is difficult to quantify in a single measurement, and the availability of fertilisers N and S to the crop's uptake also depends on factors such as weather conditions and rooting pattern.

Grain protein concentration is widely used as a criterion in determining premium prices for breadmaking wheat, with many studies showing a positive relationship between protein con-



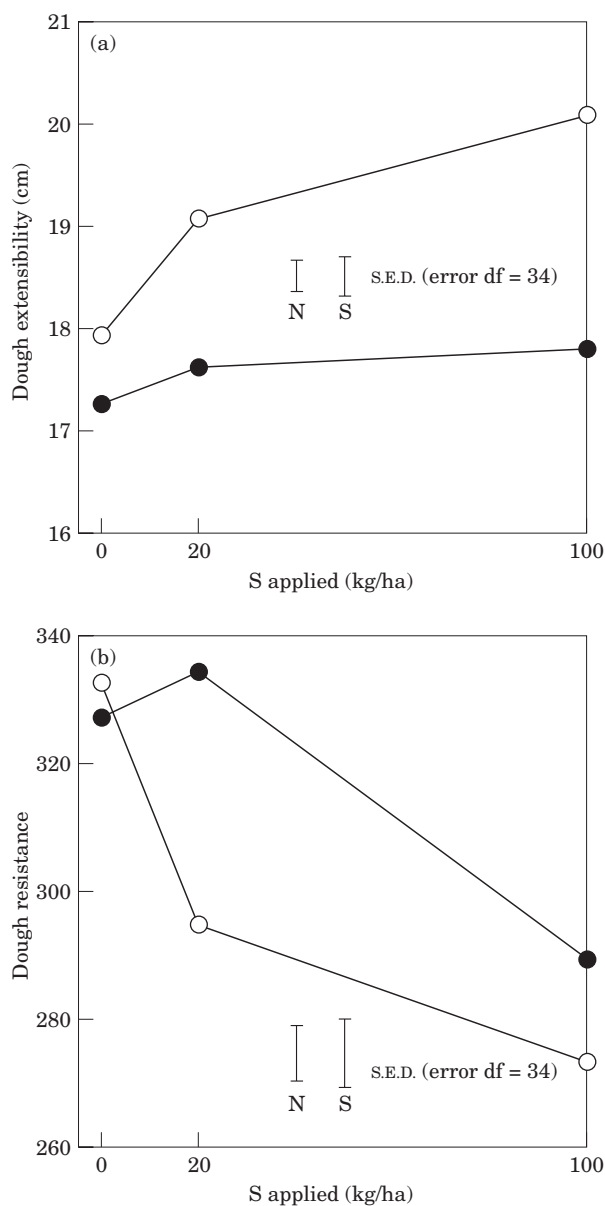
**Figure 4** Effects of S and N on gel protein concentration in (a) 1994/95 and (b) 1995/96, and on the elastic modulus ( $G'$ ) of gel protein in (c) 1994/95 and (d) 1995/96. The N fertilisation rates were 180 kg N/ha (●) and 230 kg N/ha (○). S.E.D., standard error of difference.

centration and loaf volume<sup>17–19</sup>. However, the present study showed a poor relationship between grain protein concentration and loaf volume for the variety Hereward. Loaf volumes obtained in 1995/96 were normal for this variety, but those obtained in 1994/95 were considerably lower than normal, despite the fact that protein concentrations were higher than in 1995/96. The reason for the unusually low loaf volumes in 1994/95 was not clear. In the 'normal' season of 1995/96, grain S concentration was found to correlate more closely with loaf volume than grain protein concentration. Furthermore, step-wise regression identified grain N:S ratio as an important parameter affecting loaf volume in both seasons, with higher N:S ratios, indicating a shortage of S, leading to lower loaf volumes. These results agree with other reports<sup>6,8,20</sup>, and indicate that balanced supplies of N and S are essential for breadmaking quality. Grain protein concentration alone appears to be a very unsatisfactory indicator of breadmaking quality, whereas grain S concentration and N:S ratio have been shown in this study to be more influential.

Responses of loaf volume to S application were observed more frequently than responses in grain yield in this series of experiments. Significant yield

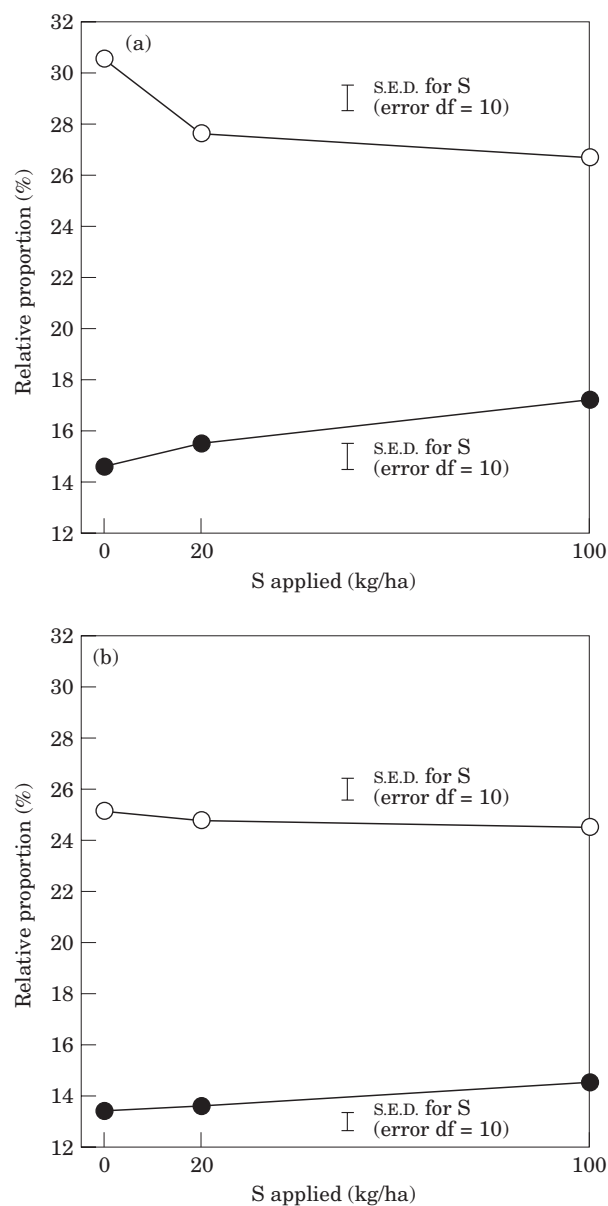
responses to S application were obtained only in two experiments (Bridgets and Woburn in 1994/95)<sup>11</sup>, whereas significant increases in loaf volume were obtained in four experiments. In addition, yield responses were largely limited to the application of the first 20 kg S/ha, whereas further increases in loaf volume occurred when the amount of S applied was increased from 20 to 100 kg/ha. The Woburn site was an exception, being S-deficient yet lacking significant responses in loaf volume. However, it was also drought prone and one explanation for the anomalous results could be lack of water during the grain filling period. The total rainfalls during the active growth period between 1 April and 31 July in 1995 and 1996 were 99 and 123 mm, respectively, both of which were considerably smaller than the 30 year average of 200 mm at the site.

Although there was no direct effect of S on the total concentration of crude protein in the grain, S application increased the concentration of gel protein, which consists mainly of glutenin<sup>14</sup>. This is consistent with the SE-HPLC results, which showed that S application increased the relative proportion of high  $M_r$  polymeric proteins (glutenins) eluted in peak 1. These results agree with MacRitchie and Gupta<sup>21</sup>, who showed that the



**Figure 5** Effects of S and N on (a) dough extensibility and (b) resistance in 1995/96. The N fertilisation rates were 180 kg N/ha (●) and 230 kg N/ha (○). S.E.D., standard error of difference.

proportion of polymeric protein in the total protein increased with S concentration. Glutenin consists of HMW and LMW subunits, which are linked through inter-chain disulphide bonds. LMW subunits are rich in S, and contain cysteine residues which form intra- and inter-chain disulphide bonds<sup>2</sup>. Several studies have shown that the proportion of the S-rich LMW subunits correlates positively with grain S, whereas that of the rel-



**Figure 6** Effects of S on the relative proportions of SE-HPLC peak 1 and peak 2 at (a) Woburn in 1994/95 and (b) Wark Common in 1995/96. ●, peak 1; ○, peak 2. S.E.D., standard error of difference.

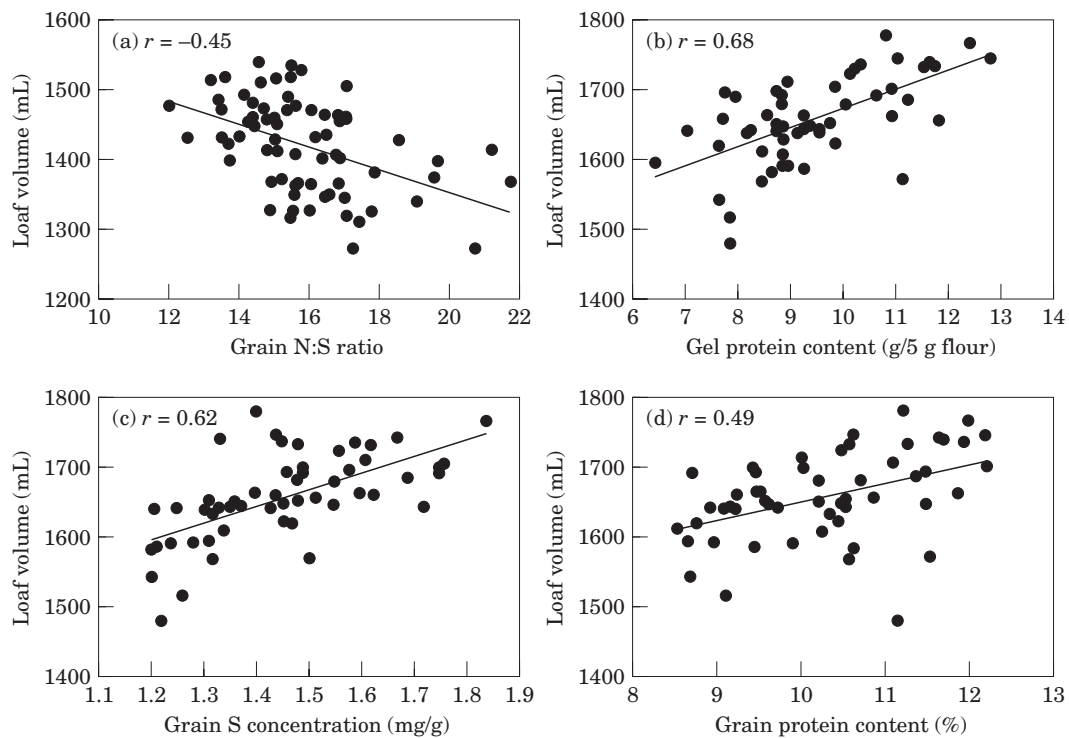
atively S-poor HMW subunits correlates negatively with grain S<sup>3-5</sup>. Because the LMW subunits are the major components of glutenin, the net effect of increasing grain S concentration would be to increase the proportion of polymeric protein in total, as observed previously by MacRitchie and Gupta<sup>21</sup>.

In agreement with Moss *et al.*<sup>7,8</sup>, application of S decreased dough resistance and increased dough

**Table IV** Correlation coefficients between loaf volume and grain protein concentration, S concentration, N:S ratio, gel protein concentration and elastic modulus

	Grain protein concentration	Grain S concentration	Grain N:S	Gel protein concentration	Elastic modulus of gel protein
1994/95	-0.27*	0.15	-0.45***	-0.22	-0.18
1995/96	0.49***	0.62***	-0.20	0.68***	0.28*

Significance levels: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

**Figure 7** Relationships between loaf volume and grain N:S ratio in (a) 1994/95, and with (b) gel protein concentration, (c) grain S concentration and (d) grain protein concentration in 1995/96.**Table V** Regression equations obtained from stepwise multiple regression

	1994/95	1995/96
Regression equation	$Y = 1774.1 - 9.5\text{GEL} - 16.1\text{GNS}$	$Y = 1533.0 + 32.7\text{GEL} - 12.9\text{GNS}$
$R^2_{\text{adjusted}}$	0.24***	0.54***
Variance ratio for GEL	5.1*	47.0***
Variance ratio for GNS	19.0***	8.8**

Y = loaf volume; GEL = gel protein concentration, GNS = grain N:S ratio.

Significance levels: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

extensibility. Also, step-wise multiple regression revealed that dough resistance was strongly correlated with grain S concentration, whereas dough extensibility was correlated mainly with gel protein

and crude protein concentrations. Sulphur application consistently decreased the elastic modulus of gel protein fractionated from white flour. As expected, dough resistance and extensibility

also correlated, in opposite ways, with the elastic modulus of gel protein. Decreases in dough resistance and in the elastic modulus of gel protein, as a result of increased S concentration, may be explained by a decrease in the ratio of HMW/LMW subunits of glutenins<sup>21</sup>.

In conclusion, grain S status (S concentration and N:S ratio) is an important factor influencing breadmaking performance of wheat. Application of S fertilisers is likely to benefit breadmaking quality of wheat in many areas of the U.K. at present, with wider benefits to be expected if atmospheric S inputs continue to decrease as planned<sup>9</sup>.

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