Effect of Number of Carbons in Added Disulfide on Breadmaking Quality

Masaharu Seguchi[1,2] and Makoto Abe[3]

ABSTRACT

Cereal Chem. 81(6):710–713

Breadmaking properties such as bread height and specific volume were improved in bread from wheat flour with added disulfides such as dimethy disulfide (M_2S_2), diethyl disulfide (E_2S_2), dipropyl disulfide (Pro_2S_2), and dibutyl disulfide (B_2S_2). However, the amount of disulfides for maximum breadmaking properties was various and strongly related to the number of carbons (C) in the disulfide. Brabender farinographs of disulfide-wheat flour showed an interesting profile, and the properties of the modification of the width of the tail. Size-exclusion high performance liquid chromatography (SE-HPLC) of the wheat proteins in the control and the disulfide-added flours showed profiles of low, medium, and high molecular weight peaks. The area of the high molecular weight peak was larger in the disulfide-added flours than in the control, indicating that this protein was important for the improvement of breadmaking properties.

Seguchi and Abe (2003) reported that Welsh onion (*Allium fistulosum* L.) blended with wheat flour could improve breadmaking properties and they also observed that other *Allium* such as scallions (*A. chinense* L.) and leeks (*A. ampeloprasum* L.) showed the same effects on the breadmaking properties. *Allium* is known to have many medicinal properties such as antibiotic action, lowering of blood glucose and plasma cholesterol levels, antihyperlipidemia, thrombolysis, antiplatelet aggregation, prevention of rheumatic arthritis, and diuretic effects (Whitaker 1976). They reported that *Allium* has beneficial effects on human health and the improvement of breadmaking properties by *Allium* powder suggests that it may have a possible use in breadmaking. *Allium* contains a high level of disulfide, and Seguchi and Abe (2003) suggested that the enhancing effects of *Allium* on breadmaking properties are strongly related to disulfides. Alkyl cysteine sulfoxides are substrates for the production of these volatiles and are derivatives of the amino acid cysteine. These derivatives give rise through several reactions to the sulfur-containing volatiles of disulfides. Disulfides such as dimethyl disulfide (Me_2S_2), di-*n*-propyl disulfide (Pro_2S_2), and diallyl disulfide (Al_2S_2) are enzymatically produced in *A. chinense, A. tuberosum, A. cepa, A. fistulosum,* and *A. sativum* (Saghir et al 1963). Sulfur-containing amino acids and peptides such as cysteine, cystine, and glutathione act as redox agents in dough and improve breadmaking properties (Eliasson and Larsson 1993). Schofield and Chen (1995) established procedure measuring glutathione (G) and oxidized glutathione (GSSG) in wheat flour, and Li et al (2004a) suggested that glutathione and related thiol compounds do not lead to significant differences in the rheological properties of dough and the baking performance of flour. Li et al (2004b), however, indicated that the level of protein-bound glutathione (PSSG) in gel protein from flours and gluten protein with poor breadmaking performances was constantly higher and significantly different from that of flours with good breadmaking performance. In this experiment, disulfides such as dimethy disulfide (M_2S_2), diethyl disulfide (E_2S_2), dipropyl disulfide (Pro_2S_2), and dibutyl disulfide (B_2S_2) were used for a breadmaking test, and the relationship between the C number of these disulfides and the maximum breadmaking properties was investigated.

MATERIALS AND METHODS

Wheat Flour and Reagents

Red Knight wheat flour (13.6% MC) (Nitto Flour Milling Co., Ltd., Tokyo, Japan) was used in this experiment. Protein conversion was N × 5.7. Ash was determined by Approved Method 08-01 (AACC 2000). The analytical data of the wheat flour included protein 14.8% and ash 0.37%; however, the free SH group profile in this flour was not determined. Dimethyl disulfide (Me_2S_2) [$(CH_3)_2S_2$ MW 94.20], diethyl disulfide (E_2S_2) [$(C_2H_5)_2S_2$ MW 122.25], di-*n*-propyl disulfide (Pro_2S_2) [$(C_3H_7)_2S_2$ MW 150.31], and di-*n*-butyl disulfide (B_2S_2) [$(C_4H_9)_2S_2$ MW 178.36] were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan).

Breadmaking Test

When Me_2S_2, E_2S_2, Pro_2S_2, and B_2S_2 were added to wheat flour, the addition levels of each disulfide were 1.6–100 mg%, 0.05–0.15%, 0.10–0.40%, and 0.05–0.40%, respectively (Table I). The baking absorption of each disulfide-added flour was determined by farinograph with 300 g of flour (Approved Method 54-21, AACC 2000). Flour (290 g), compressed yeast (8.7 g), sugar (14.5 g), salt (2.9 g), and water (estimated by farinograph at 500 BU) were mixed in a computer-controlled National Automatic Bread Maker (SD-BT6, Matsushita Electric Ind. Co., Ltd., Japan) with the first proof for 2 hr and 20 min at 30°C. The time profile was 15 min for the first mixing, 50 min of rest, 5 min for the second mixing, and 70 min of fermentation. The mixing action (time and temperature) was all carefully controlled by computer in the bread maker. Next, the bread dough was removed from the bread maker and divided into 120-g pieces that were rounded, molded, and placed in baking pans (Approved Method 10-10A, AACC 2000). The bread dough was further proofed for 22 min at 38°C, and baked at 210°C for 30 min in a model DN-63 deck-style oven (Yamato Scientific Co., Ltd., Japan). After baking, the bread was removed from the pan and cooled for 1 hr at a room temperature of 26°C and relative humidity of 43%. Bread height (mm), weight (g), and volume (cm^3) were measured, and the crumb grains were evaluated visually.

Farinograph and SE-HPLC Tests

Me_2S_2 (6.30 mg%), E_2S_2 (0.10%), Pro_2S_2 (0.16%), and B_2S_2 (0.20%) were added to Red Knight wheat flour. Flour (300 g) mixed with 202 mL of water was subjected to a farinograph test for 30 min at 30°C and freeze-dried. The farinograph tests were performed twice. Freeze-dried powder (5.0 g) was suspended in 50 mL of 1% SDS solution and shaken overnight. Extracted proteins were dialyzed against water and freeze-dried.

For SE-HPLC analysis at 280 nm, a Shimadzu liquid chromatograph LC-3A connected to a Shodex Protein KW 803 column (5009010) was used.

---

[1] Faculty of Home Economics, Laboratory of Food Technology, Kobe Women's University, Suma-Ku, Kobe City, 654-8585, Japan.
[2] Corresponding author. E-mail: seguchi@suma.kobe-wu.wu.ac.jp
[3] Faculty of Intercultural Studies, Dept. of Japanese Studies, Gakushuin Women's College, Tokyo, 162-8650 Japan.

Publication no. C-2004-1013-04R.

Statistical Analysis

A statistical software package (SPSS Inc., Chicago, IL) was used for statistical analyses. Four loaves of bread were baked for each treatment and bread height (mm) and specific volume (cm^3/g) were measured four times for each sample and averaged. These were replicated in the analyses from one set of bread. SE-HPLC of each samples was performed twice. Significant *F* values were produced by analysis of variance, followed by Duncan's multiple range test for comparison of means.

RESULTS AND DISCUSSION

Effects of Disulfides on Breadmaking Properties

Disulfides such as Me_2S_2, E_2S_2, Pro_2S_2, and B_2S_2 were added to wheat flour, and breadmaking was performed. The effects of disulfides on breadmaking properties (bread height [mm] and specific volume [cm^3/g]) are indicated in Table I and Fig. 1. The addition levels of disulfides were 1.60–100 mg% Me_2S_2, 0.05–0.15% E_2S_2, 0.10–0.40% Pro_2S_2, and 0.05–0.40% B_2S_2. From these baking results, the maximum baking properties were obtained when added to wheat flour at 6.30 mg% Me_2S_2, 0.10% E_2S_2, 0.10–0.20% Pro_2S_2, and 0.20% B_2S_2. When higher levels of disulfides were added, the breadmaking properties of bread height and specific volume decreased. Loaf shape and interior grain are indicated in Fig. 1, which shows the changes in bread height and interior grain. From these results, it was concluded that every disulfide is remarkably related to the improvements of breadmaking properties. The baking data for each disulfide-added wheat flour were treated statistically and we obtained the maximum amount (%) of disulfide for the maximum baking properties. As indicated in Table I, baking results of Pro_2S_2 (0.10 and 0.20%)/wheat flour were rather similar, so we obtained the 0.16% for maximum baking properties from statistical analysis. Figure 2 shows that an increase in the number of carbons (C) in the disulfides required an increase in the amount (%) of the disulfide reagent for the maximum baking properties, which suggests that disulfides of smaller molecular weight may be more hydrophilic and could enter easily into the gluten matrix and react with them. From these results, the presence of disulfides would allow many SS/SH exchanges to occur in breadmaking.

Brabender Farinograph and SE-HPLC Profiles

The most suitable amount of disulfides for maximum baking properties (6.25 mg% Me_2S_2, 0.10% E_2S_2, 0.16% Pro_2S_2, and 0.20% B_2S_2) were added to wheat flour, and each farinograph profile was observed for 30 min (Fig. 3). Every disulfide-added flour showed the same unique profile which was rather different from wheat flour (Fig. 3C). Wheat flour shows a BU value that is almost constant for 30 min, and also the width of the tail is the same. However, when each disulfide was added to the wheat flour, the width of the tail after 30 min was rather narrower, and some interesting mixing behaviors modified the peak mixing time (arrow mark) and BU value, which were later and higher than those of the control. The farinogram profile of is mainly dominated by wheat proteins, and when each disulfide was added, the dynamic change would first occur in a short time (arrow mark) and the BU value increased due to interaction between proteins by sulfide. Although the width of tail gradually decreased, the BU value did not so decrease, which would indicate good stability of the interacted proteins and was related to the actual breadmaking

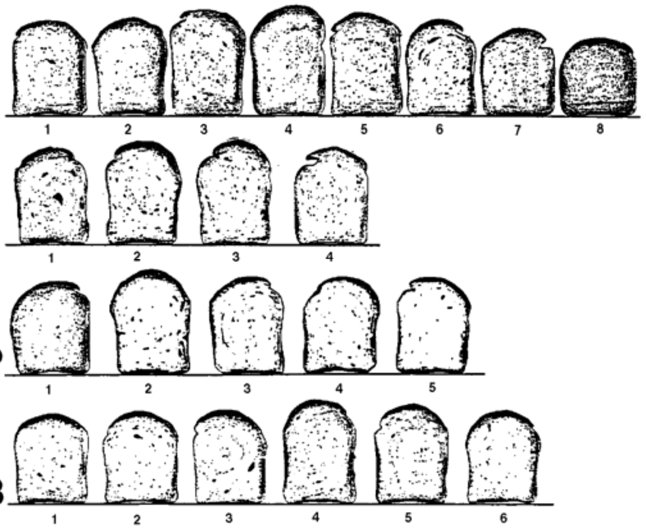

Fig. 1. Appearance of sectioned bread baked from disulfide-blended Red Knight wheat flour. Amount added to Red Knight wheat flour: **M** (mg% of Me_2S_2) (1) 0.00; (2) 1.60; (3) 3.10; (4) 6.30; (5) 12.5; (6) 25.0; (7) 50.0; (8) 100. **E** (% of E_2S_2) (1) 0.00; (2) 0.05; (3) 0.10; (4) 0.15. **P** (% of Pro_2S_2) (1) 0.00; (2) 0.10; (3) 0.20; (4) 0.30; (5) 0.40. **B** (% of B_2S_2) (1) 0.00; (2) 0.05; (3) 0.10; (4) 0.20; (5) 0.30; (6) 0.40.

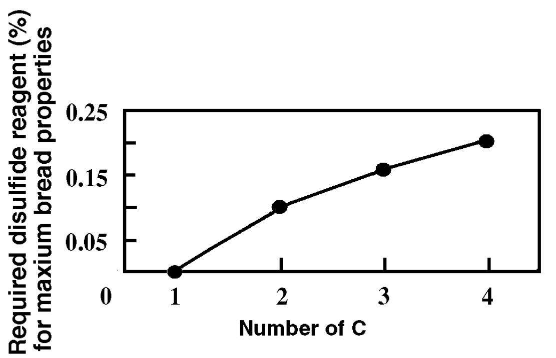

Fig. 2. Relationship between number of carbons (C) in disulfide and required disulfide reagent (%) for maximum bread properties such as bread height (mm) and specific volume (cm^3/g).

TABLE I
Effect of Disulfide on Breadmaking Properties[a]

Me_2S_2 (mg%)	Height (mm)	Sp. Vol. (cm^3/g)	E_2S_2 (%)	Height (mm)	Sp. Vol. (cm^3/g)	Pro_2S_2 (%)	Height (mm)	Sp. Vol. (cm^3/g)	B_2S_2 (%)	Height (mm)	Sp. Vol. (cm^3/g)
0.00	72.9a (1.43)[b]	3.08a (0.07)	0.00	71.1a (0.48)	3.54a (0.09)	0.00	67.8a (3.52)	3.32a (0.20)	0.00	70.1a (3.11)	3.38a (0.07)
1.60	79.2b (2.05)	3.42b (0.11)	0.05	74.3a (2.69)	3.65b (0.10)	0.10	72.9b (1.18)	3.61b (0.11)	0.05	71.1a (1.73)	3.46a (0.11)
3.10	83.9c (1.14)	3.80c (0.13)	0.10	77.0b (3.69)	3.86c (0.10)	0.20	73.0c (4.48)	3.69c (0.09)	0.10	70.3 (1.65)	3.43 (0.11)
6.30	81.5bc (2.70)	3.65d (0.12)	0.15	70.2a (3.56)	3.45a (0.01)	0.30	69.1a (3.15)	3.33a (0.12)	0.20	73.8a (3.32)	3.75b (0.12)
12.5	79.7b (2.07)	3.43b (0.07)	…	…	…	0.40	67.6a (2.13)	3.15a (0.13)	0.30	72.1a (3.84)	3.53c (0.07)
25.0	82.4bc (3.07)	3.55bd (0.06)	…	…	…	…	…	…	0.40	67.9a (4.49)	3.31a (0.04)
50.0	76.5b (1.31)	3.37b (0.02)	…	…	…	…	…	…	…	…	…
100.0	78.7b (2.54)	3.37b (0.09)	…	…	…	…	…	…	…	…	…

[a] Dimethy disulfide (M_2S_2), diethyl disulfide (E_2S_2), dipropyl disulfide (Pro_2S_2), and dibutyl disulfide (B_2S_2).

[b] Values represent means of four replicates with standard deviation in parentheses. Values followed by the same letter in the same column are not significantly different ($P < 0.01$).

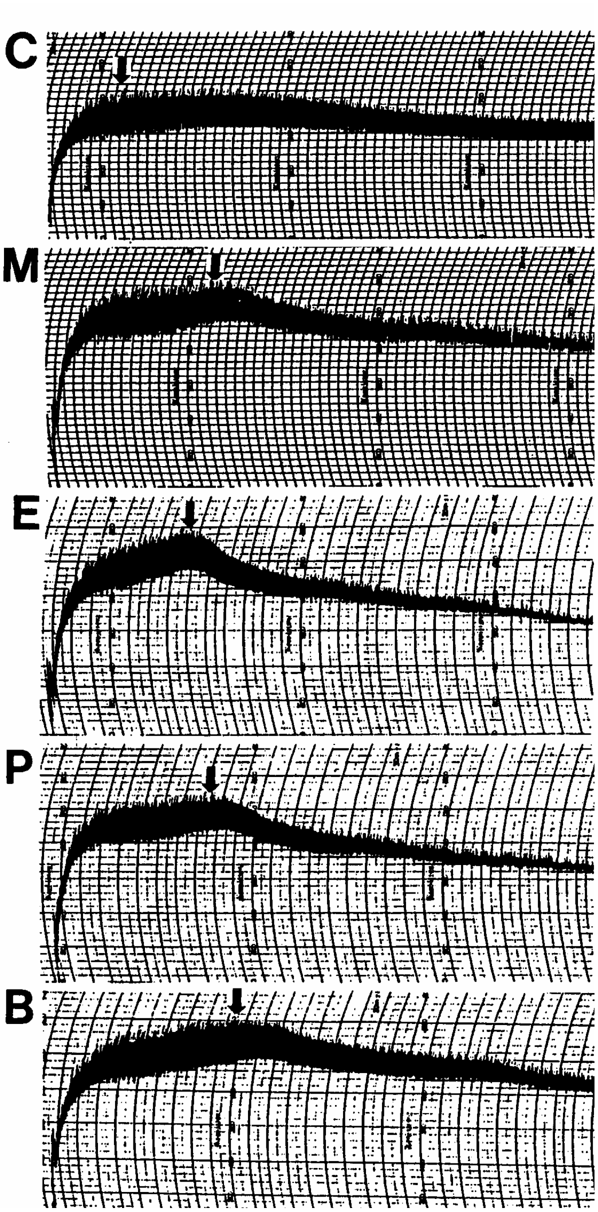

Fig. 3. Effect of disulfides on Brabender farinograph profiles of Red Knight wheat flour. **C,** Red Knight wheat flour plus **M,** 6.25 mg% Me_2S_2; **E,** 0.10% E_2S_2; **P,** 0.16% Pro_2S_2; **B,** 0.20% B_2S_2. Arrows show peak times.

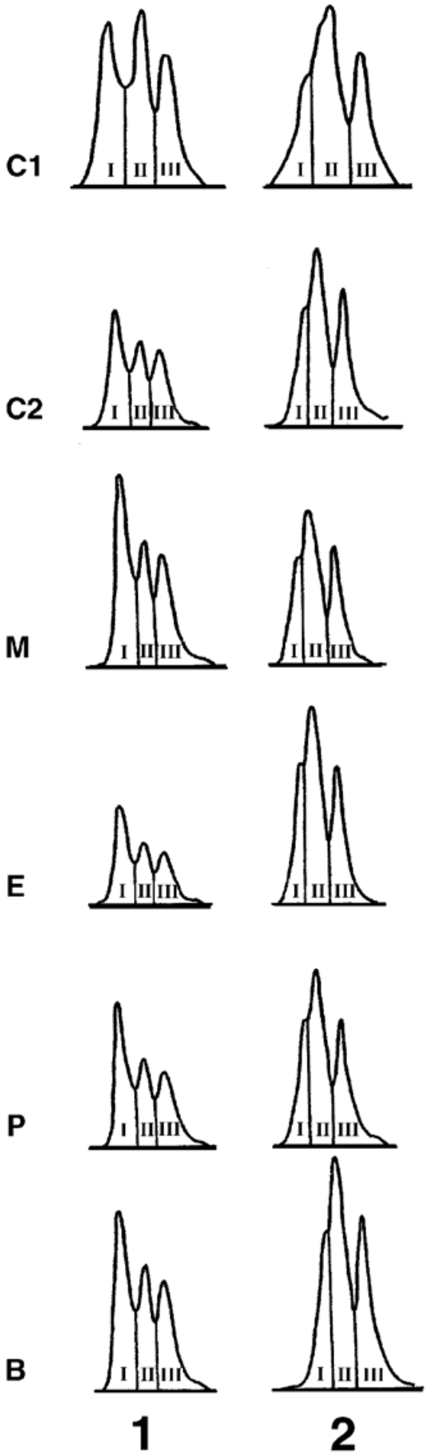

Fig. 4. Size-exclusion high-performance liquid chromatography (SE-HPLC) profiles of proteins extracted from Red Knight wheat flour control (**C1** without homogenizing and **C2** with homogenizing) with added disulfide 6.25 mg% Me_2S_2 (**M**), 0.10% E_2S_2 (**E**), 0.16% Pro_2S_2 (**P**), 0.20% B_2S_2 (**B**). Proteins (1.0 mg) were dissolved in 1.0 mL of 1.0% SDS and eluted from a column with the same solution (column1 C1, C2, M, E, P, and B). Proteins were reduced by boiling 5 min in 1% SDS containing 1% 2-mercaptoethanol and eluted with the same solution (column 2 C1, C2, M, E, P, and B).

properties that were observed. The profiles of the disulfide-added wheat flour were the same as the farinograph profiles of the *Allium*-blended wheat flour (Seguchi and Abe 2003). From those results, it was suggested that disulfides combine with the dough proteins through formation of disulfide bridges.

For further investigation, proteins from flour dough mixed for 30 min in a farinograph bowl at 40°C were extracted with 1% SDS and subjected to SE-HPLC. The most suitable amount of disulfides for maximum baking properties were also added to wheat flour. Figure 4 (column 1) indicates the SE-HPLC profiles of the 1% SDS-extracted wheat proteins. The column was equilibrated with 1% SDS, and the proteins were eluted from the column with 1% SDS. The mixing (30 min) effects on the HPLC profiles of dough in a farinograph bowl were observed. Figure 4 (1-C1) shows peak I (36.9%), II (32.4%), and III (33.9%) flour proteins without mixing, however, peak I in Fig. 4 (1-C2) after 30 min of dough mixing increased to 39.3%, and peaks II and III decreased to 27.6 and 33.3%, respectively, which indicated that the procedure of mixing dough recombined the flour proteins due to air oxidation. Under normal dough mixing conditions, the interpolypeptide disulfide bonds can be mobilized through the disulfide interchange reaction (Hamer and Hoseney 1998). The HPLC profiles in Fig. 4 (1-M, E, P, and B), in which the proteins were extracted from disulfide-added wheat flour, show that the areas of the peaks changed. The HMW I became larger and the

TABLE II
Effect of Disulfides on Ratio of HMW I, MMW II, and LMW III Protein (%) in HPLC Profile (1% SDS)

	HMW I (%)	HMW II (%)	HMW III (%)
Wheat flour	39.3a (0.6)	27.6a (0.5)	33.3a (0.2)
+6.30 mg% Me$_2$S$_2$	44.3b (1.3)	26.2b (0.4)	29.6b (0.8)
+0.10% E$_2$S$_2$	46.4b (2.5)	24.4b (0.0)	29.3b (2.5)
+0.16% Pro$_2$S$_2$	45.7b (1.3)	25.6b (0.6)	28.7b (0.7)
+0.20% B$_2$S$_2$	43.7b (0.1)	26.5 (0.2)	29.9b (0.4)

[a] Dimethy disulfide (M$_2$S$_2$), diethyl disulfide (E$_2$S$_2$), dipropyl disulfide (Pro$_2$S$_2$), and dibutyl disulfide (B$_2$S$_2$).

[b] Values represent means of four replicates with standard deviation in parentheses. Values followed by the same letter in the same column are not significantly different ($P < 0.01$).

TABLE III
Effect of Disulfides on Ratio of HMW I, MMW II, and LMW III Protein (%) in HPLC Profile (1% SDS)

	HMW I (%)	HMW II (%)	HMW III (%)
Wheat flour	20.9 (0.9)	48.2 (0.6)	31.0 (0.6)
+6.30 mg% Me$_2$S$_2$	21.7 (0.7)	47.9 (0.2)	30.5 (0.9)
+0.10% E$_2$S$_2$	21.2 (0.3)	48.8 (0.4)	29.9a(0.4)
+0.16% Pro$_2$S$_2$	21.6 (2.2)	48.4 (1.7)	30.1b(0.5)
+0.20% B$_2$S$_2$	19.5 (0.1)	48.9 (0.0)	31.7c(0.0)

[a] Dimethy disulfide (M$_2$S$_2$), diethyl disulfide (E$_2$S$_2$), dipropyl disulfide (Pro$_2$S$_2$), and dibutyl disulfide (B$_2$S$_2$).

[b] Values represent means of four replicates with standard deviation in parentheses. Values followed by the same letter in the same column are not significantly different ($P < 0.01$).

others became smaller than the proteins extracted from wheat flour (1-C2), which suggests that MMW II and LMW III proteins are rearranged by disulfides and changed to HMW I proteins. Table II shows data of the areas of HMW I, MMW II, and LMW III, in which the area of HMW I in flour increased from 39.3% to 44.3, 46.4, 45.7, and 43.7% when Me$_2$S$_2$, E$_2$S$_2$, Pro$_2$S$_2$, and B$_2$S$_2$ were added, respectively. The areas of HMW I (44.3, 46.4, 45.7, and 43.7%) in each disulfide addition are almost same regardless of different addition levels, which indicates that the most suitable amount of disulfides for maximum baking properties could give the same areas of HMW I in wheat flour. The SDS-extracted wheat flour proteins and the SDS-extracted proteins from disulfide-added wheat flour were reduced in 1% 2-mercaptoethanol and eluted with the same 1% SDS solution containing 1% 2-mercaptoethanol from the SE-HPLC column. Both elution profiles of the proteins from flours with or without disulfides (Fig. 4 [2-C1–B]) were almost the same. This shows (Tables II and III) that the increase in the area of the HMW I proteins by the addition of disulfides in the 1% SDS-eluted column profile may possibly be caused by the rearrangement of dough proteins by disulfides. From these results, it seems that disulfides act as a key material in the arrangement of dough proteins and in making HMW proteins through disulfide bridges. Increased number of carbons in disulfides (Me$_2$S$_2$ < E$_2$S$_2$ < Pro$_2$S$_2$ < B$_2$S$_2$) decrease hydrophilic and increase hydrophobic natures. Smaller molecular weight and more hydrophilic disulfides can easily enter into the gluten matrix and make interpolypeptide disulfide bonds, however, with increased number of carbons, the disulfide would need higher addition levels to obtain the same level of HMW proteins and resulting good baking properties.

CONCLUSIONS

The improvement of breadmaking properties by the addition of disulfides such as dimethy disulfide (Me$_2$S$_2$), diethyl disulfide (E$_2$S$_2$), dipropyl disulfide (Pro$_2$S$_2$), and dibutyl disulfide (B$_2$S$_2$) to flour may be caused by forming the interpolypeptide disulfide bonds. It was suggested that the smaller number of carbons in the disulfide molecule is related to the ease of making the high molecular weight proteins and improvement of the breadmaking properties.

LITERATURE CITED

American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th Ed. Methods 08-01, 10-10A, and 54-21. The Association: St. Paul, MN.

Eliasson, A. C., and Larsson, K. 1993. Cereals in Breadmaking. Pages 273-281. Marcel Dekker: New York.

Hamer, R. J., and Hoseney, R. C. 1998. Interactions: The Keys to Cereal Quality. Am. Assoc. Cereal Chem: St. Paul, MN.

Li, W., Bollecker, S. S., and Schofield, J. D. 2004a. Glutathione and related thiol compounds. I. Glutathione and related thiol compounds in flour. J. Cereal Sci. 39:205-212.

Li, W., Tsiami, A. A., Bollecker, S. S., and Schofield, J. D. 2004b. Glutathione and related thiol compounds. II. The importance of protein bound glutathione and related protein-bound compounds in gluten proteins. J. Cereal Sci. 39:213-224.

Saghir, A. R., Mann, L. K., Bernhard, R. A., and Jagobsen, J. V. 1963. Am. Soc. Horticult, Sci. 84:386-398.

Schofield, J. D., and Chen, X. 1995. Analysis of free reduced and free oxidized glutathione in wheat flour. J. Cereal Sci. 21:127-136.

Seguchi, M., and Abe, M. 2003. Effect of Welsh onion (*Allium fistulosum* L.) on breadmaking properties. J. Food Sci. 68:1810-1813.

Seguchi, M., Hayashi, M., and Matsumoto, H. 1997. Effect of gaseous acetic acid on dough rheological and breadmaking properties. Cereal Chem. 74:129-134.

Seguchi, M., Morimoto, N., Abe, M., and Yoshino, Y. 2001. Effect of maitake (*Grifola frondosa*) mushroom powder on bread properties. J. Food Sci. 66: 261-264.

Whitaker, J. R., 1976. Advances in Food Research, Vol. 22. Academic Press: New York.

[Received April 22, 2004. Accepted August 10, 2004.]