Changes in the Levels of Glutathione and Cysteine During the Mixing of Doughs with L-*threo*- and D-*erythro*-Ascorbic Acid[1]

RIDWAN SARWIN,[2] GUDRUN LASKAWY, and WERNER GROSCH[3]

ABSTRACT

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An isotope dilution assay was developed for free reduced glutathione (GSH), total glutathione (GSH, oxidized glutathione, and protein-glutathione mixed disulfides), free cysteine (Cys), and total Cys (Cys, cysteine, and protein-Cys mixed disulfides). The isotope dilution assay was checked by the determination of these compounds in some flours and in crude glutenins. It was applied to flours of two wheat cultivars (DNS and Kanzler) and to their doughs, which had been mixed for 3 min at 30°C. In the DNS doughs without additions, GSH (values in nmol/g) decreased from 100 to 44 and Cys increased from 13 to 42; in the K doughs, GSH decreased from 35 to 17 and Cys increased from 8 to 18. Addition of L-*threo*-ascoric acid accelerated the disappearance of GSH and partially inhibited the increase of Cys. In contrast, D-*erythro*-ascorbic acid was inactive. The results confirmed the hypothesis that the improver action of L-*threo*-ascorbic acid is caused by a rapid oxidation of GSH to its rheologically inactive disulfide.

The improver effect of ascorbic acid (L-*threo*-AA) in conventional doughmaking was recognized by Jørgensen (1935a,b), who found that small amounts (2–6 g of L-*threo*-AA per 100 kg of flour) caused a pronounced increase of both dough strength and bread volume. During dough mixing, L-*threo*-AA is oxidized rapidly to L-*threo*-dehydroascorbic acid (L-*threo*-DHAA) (Elkassabany et al 1980, Nicholas et al 1980), the active form of the improver (Melville and Shattock 1938, Maltha 1953, Lillard et al 1982).

The improver effects of the four ascorbic acid stereoisomers are different (Sandstedt and Hites 1945, Maltha 1953, Lillard et al 1982, Kieffer et al 1990): L-*threo*-AA has the greatest improver activity; both D- and L-*erythro*-AA are less active; and D-*threo*-AA is inactive. As this ranking corresponds to the substrate specificity of the enzyme glutathione dehydrogenase (GSH-DH; EC 1.8.5.1) occurring in wheat flour (Carter and Pace 1965, Boeck and Grosch 1976, Walther and Grosch 1987), it has been suggested (Mair and Grosch 1979, Kieffer et al 1990) that the improver action of L-*threo*-DHAA is due to the removal of GSH in wheat flour (Hird et al 1968, Tkachuk 1969, Archer 1972, Wierzbicka et al 1989, Sarwin et al 1992a) by the oxidation to the corresponding disulfide with L-*threo*-DHAA as the oxidant (Sandstedt and Hites 1945; Kuninori and Matsumoto 1963, 1964; Carter and Pace 1965). Otherwise, the GSH would diminish dough strength by sulfhydryl-disulfide (SH-SS) interchange with gluten proteins (Kuninori and Sullivan 1968, Jones and Carnegie 1969).

Recently, Sarwin et al (1992a) developed an isotope dilution assay (IDA) of wheat flours for GSH and total glutathione ([GSS], composed of GSH, oxidized glutathione [GSSG], and protein-glutathione mixed disulfide). The determination of GSH required extraction of flour samples with a buffer at pH 4.5 containing *N*-ethylmaleimide (NEMI) and ^{14}C-labeled *S*-(*N*-ethylsuccinimido)glutathione. GSS was quantified after reduction of the SS bonds with dithioerythritol (DTE).

The sensitivity of this method was improved by using *N*-phenylmaleimide (NPMI) instead of NEMI as reagent for SH compounds, because the molar extinction coefficient of GS-NPMI at 220 nm is 80% higher than that of GS-NEMI (*unpublished results*). Consequently, [^{14}C]-GS-NEMI in the extraction buffer was replaced by ^{14}C-labeled *S*-(*N*-phenylsuccinimido)glutathione ([^{14}C]-GS-NPMI).

The new method is also suitable for the determination of free cysteine (Cys), present in wheat flour in a concentration range of 59–86 nmol/g (Ewart 1988, 1990). ^{14}C-labeled *S*-(*N*-phenylsuccinimido)cysteine ([^{14}C]-Cys-NPMI) was used as an internal standard for Cys. Total cysteine ([CysSS] composed of Cys, cystine, and protein-Cys mixed disulfide) was assayed after reduction of the SS bond with DTE.

This new method determines GSH and Cys in some flours and in crude glutenins. The concentration changes of GSH and Cys were measured in doughs prepared from the flours of two wheat varieties. The effects of L-*threo*-AA and D-*threo*-AA were compared in this study.

MATERIALS AND METHODS

Flours

Wheat cultivars CWRS, DNS, Kanzler, and Maris Huntsman were milled into flour using a Quadrumat Junior mill (Brabender, Duisburg, Germany). The flour was passed through a 0.2-mm mesh sieve. The flours of the wheat cultivars Bussard, Columbus, Gambrinus, and Kraka, as well as the commercial flour A, were gifts from Unilever, Vlaardingen, The Netherlands. The commercial flour B was a gift of Seibel, Detmold, Germany.

Crude Glutenins

The residual proteinaceous masses (crude glutenins) obtained by the Osborne fractionation procedure (Graveland, *unpublished results*) from the wheat samples Bussard and flour A were gifts from Unilever. After lyophilization, their amino acid compositions were determined (Wieser et al 1978); the protein contents were calculated as 52.7% for flour A and 46.2% for Bussard.

Flour-Water Dough

Doughs were kneaded at 30°C in a Brabender microfarinograph from flour (10 g, db), NaCl (0.2 g), and 6.5 ml of water or an aqueous solution of L-*threo*-AA or D-*threo*-AA (300 μg each). The dough was mixed for 3 min, frozen with liquid nitrogen, and freeze-dried.

Chemicals

The chemical substances were purchased from commercial suppliers: NPMI and trifluoroacetic acid (protein-sequencing grade) (Sigma, Deisenhofen, Germany); D-*threo*-AA and DTE (Serva, Heidelberg, Germany); Titrisol 9884 buffer solution (pH 4.0), L-Cys, and L-*threo*-AA (Merck, Darmstadt, Germany); [2,3-^{14}C]-maleic acid anhydride, specific radioactivity 740 kBq/μmol (Amersham, Braunschweig, Germany); the [2,3-^{14}C]-maleic acid anhydride (0.15 mg) was diluted with 5.6 mg of the unlabeled anhydride scintillation reagent Permablend I (Packard, Frankfurt, Germany); Sephadex G-10 (Pharmacia, Freiburg, Germany); ODS-Hypersil, 5 μm, porosity 100 Å (Shandon, Frankfurt, Germany). The high-performance liquid chromatography (HPLC) column (20 × 0.46 cm) packed with LiChrospher RP-Select B was purchased from Merck. Scintillation fluids (Rauschenbach

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[2]Unilever Research Laboratory, Vlaardingen, The Netherlands.

[3]Author to whom correspondence should be addressed. Deutsche Forschungsanstalt für Lebensmittelchemie and Kurt-Hess-Institut für Mehl- und Eiweissforschung, Lichtenbergstrasse 4, D-85748 Garching, Germany.

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and Simon 1971) were 2-phenylethylamine (90 ml), methanol (125 ml), and Permablend I (2.75 g). They were mixed and then diluted with toluene to a total volume of 500 ml.

Syntheses

[2,3-^{14}C]-NPMI was prepared by following the indications of Orphanides (1977). Freshly distilled aniline (182 μl) and triethylamine (104 μl) were dissolved in absolute acetone (3.7 ml). An aliquot of this mixture (0.113 ml) was added to a solution of maleic acid anhydride (5.75 mg, specific radioactivity 19 kBq/μmol) in absolute acetone (0.3 ml). The reaction mixture was magnetically stirred in a vial of brown glass (5 ml volume) at room temperature for 3 hr and then left overnight. After adding a solution of acetic acid anhydride in absolute acetone (1:1, v/v, 20 μl) and some crystals of $MgCl_2 \cdot 6H_2O$, the reaction mixture was stirred again for 3 hr and left overnight. The mixture was diluted with water (2 ml) and then pipetted onto a C18 Sep-Pak cartridge (Waters, Eschborn, Germany) that was pretreated with methanol (5 ml) and water (5 ml). The vial was rinsed with 1 ml of water. The cartridge was washed with water (4 × 0.5 ml), and the [^{14}C]-NPMI (yield 50%) was eluted with aqueous acetone (1:1, v/v, 3 × 1 ml).

[^{14}C]-Cys-NPMI and [^{14}C]-GS-NPMI were prepared. SH compound (5 μmol) was dissolved in aqueous acetic acid (1.7 mmol/L, 0.5 ml) and added to a solution of [^{14}C]-NPMI (5 μmol, specific radioactivity 19 kBq/μmol) in aqueous acetone (1:1, v/v, 1 ml). After 2 hr at room temperature, the reaction mixture was pipetted onto a phenyl bond elut cartridge (ICT, Frankfurt/M, Germany) that was pretreated with methanol (5 ml) and water (5 ml). The NPMI derivative was eluted with aqueous acetic acid (0.1 mol/L) and with a mixture (1 ml) of aqueous acetic acid (0.1 mol/L) and acetone (9:1, v/v). After adjustment to pH 4.0 with drops of aqueous ammonia, the purity and the concentration of [^{14}C]-GS-NPMI were determined. The radioactivity was determined by liquid scintillation analysis. The solutions of [^{14}C]-Cys-NPMI and [^{14}C]-GS-NPMI (both with specific radioactivity 19 kBq/μmol) were divided into several portions and stored at −20°C.

Unlabeled Cys-NPMI was prepared according to Lee and Reynolds (1962). Unlabeled GS-NPMI was prepared according to Marrian (1949). The derivatives were purified by chromatography on Sephadex G-10.

Gel Chromatography

A 90- × 1.5-cm column was filled with Sephadex G-10 suspended in 0.1*M* aqueous acetic acid and methanol (9:1, v/v).

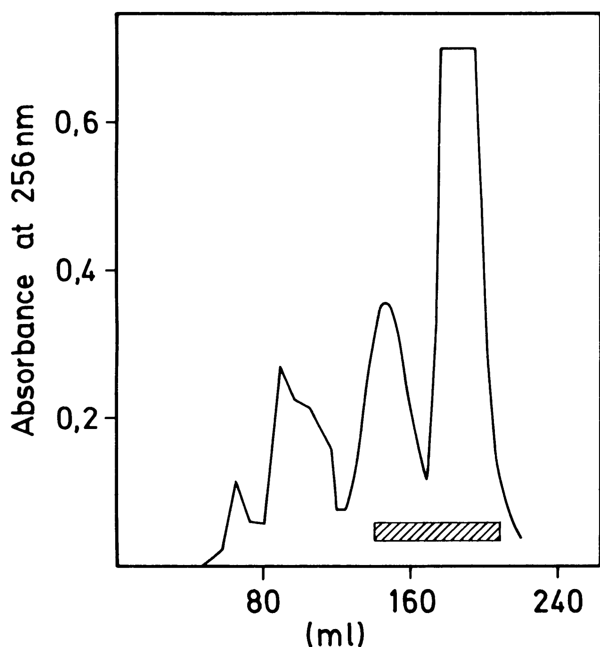

Fig. 1. Separation of a flour extract (wheat cultivar Kanzler) by gel chromatography on Sephadex G-10. Hatched area indicates the effluent range collected.

This mixture was also used as eluent (0.3 ml/min flow rate). The effluent was monitored at 256 nm. Between applications, eluent (~800 ml) was pumped through the column (20 ml/hr flow rate).

HPLC

The apparatus consisted of two pumps (type 112; Beckman Instruments, Munich, Germany) and a UV detector (Shimadzu, Duesseldorf, Germany). Two HPLC systems were used.

HPLC I. Column: ODS-Hypersil (250 × 4.6 mm i.d., 5-μm particle size, 100 Å pore size) with ODS-Hypersil precolumn (50 × 4.6 mm). Mobile phase A: a solution of KH_2PO_4 (2.72 g) in water (30 ml) was adjusted with concentrated phosphoric acid (85%, w/w) to pH 2.8 and diluted to 40 ml. One half of the solution was mixed with water (200 ml) and methanol (30 ml) and then filled up with water to 1 L. Mobile phase B: One half of the aqueous KH_2PO_4 solution (pH 2.8) was mixed with water (280 ml) and methanol (700 ml). Gradient (1.7 ml/min flow rate): 100% A for 10 min, linear to 20% B in 10 min, then 10-min hold, then to 100% B in 2 min, 18-min hold, and, finally, to 100% A in 2 min. Column temperature: 50°C. Detection: UV at 220 nm. Sample: 250 μl.

HPLC II. Column: Lichrospher RP-Select B (250 × 4.6 mm, i.d.). Mobile phase C: aqueous solution containing acetonitrile (6%, v/v) and trifluoracetic acid (0.1%, v/v). Mobile phase D: aqueous methanol (70%, v/v). Gradient (1.5 ml/min flow rate): 100% C for 15 min, linear to 100% D in 2 min, then 10-min hold, and, finally, to 100% C in 3 min. Column temperature: 40°C. Detection: UV at 220 nm. Sample: 250 μl.

IDA of GSH and Cys

Extraction. A buffer containing 28m*M* citric acid and 22m*M* HCl was prepared by diluting an ampoule of Titrisol 9884 with water (700 ml), adjusting to pH 4.5 with 0.5*M* NaOH (20 ml), and filling with water to 1 L. A solution of NPMI (140 mg) in acetone (10 ml) was added to this buffer (150 ml). The mixture was flushed with nitrogen for 5 min. The sample of flour (4 g) or lyophilized dough (4 g) was suspended and then vigorously stirred. [^{14}C]-GS-NPMI and [^{14}C]-Cys-NPMI (60 nmol each, with specific radioactivity 19 kBq/μmol) were added. Stirring was continued for 20 min in an atmosphere of nitrogen at room temperature. The insoluble material was filtered off. The soluble compounds with higher molecular masses were removed by ultrafiltration (Diaflo YM-10 membrane, Witten, Germany). The ultrafiltrate was freeze-dried.

The freeze-dried sample was dissolved in a freshly prepared mixture (6 ml) of 0.1*M* AA and methanol (9:1, v/v). The solution was pressed through a 0.45-μm filter (Schleicher & Schuell, Dassel, Germany) and subjected to gel chromatography. The effluent was collected (140–215 ml) (Fig. 1) and then lyophilized.

HPLC I. The lyophilisate was taken up in the mobile phase A (0.8 ml), and the solution obtained was pressed through a 0.45-μm filter (Millipore, Eschborn, Germany). The filtrate was separated in three HPLC runs (Fig. 2). The effluents containing C(1) and C(2) (23–30 ml) and G(1) and G(2) (36.5–41.5 ml) were separately collected and lyophilized. According to Smyth et al (1964), the NEMI derivative of Cys was separated by ion-exchange chromatography into two peaks that were suggested to be the diastereoisomers formed by the reaction of Cys and NEMI. By analogy with this result, we suggest that C(1) and C(2), as well as G(1) and G(2), are diastereomers of Cys-NPMI and GS-NPMI, respectively.

HPLC II. Each of the two lyophilisates was dissolved in mobile phase C (0.6 ml). Each solution was filtered and separated in two runs of HPLC II. The peaks C(1) and C(2) (Fig. 3a), as well as the peaks G(1) and G(2) (Fig. 3b), were individually collected in vials used for liquid scintillation analysis and freeze-dried. The area of each of the four peaks was determined by electronic integration. The amounts of Cys-NPMI in the vials containing C(1) and C(2) and the amounts of GS-NPMI in the vials containing G(1) and G(2) were calculated from the data obtained by HPLC II of definite amounts of Cys-NPMI and

GS-NPMI.

Liquid scintillation analysis of each of the four lyophilisates and the calculation of the amounts of the thiols were performed as reported for GSH (Sarwin et al 1992a).

IDA of GSS and CysSS

The buffer (pH 4.5) containing citric acid and HCl was prepared as described above.

A suspension of wheat flour (2 g) or crude glutenin (150 mg) in water (80 ml) containing 6 mg of DTE was flushed with nitrogen and stirred for 10 min. A solution of NPMI (440 mg) in acetone and buffer (pH 4.5, 1:4 v/v, 80 ml) and the internal standards [^{14}C]-GS-NPMI and [^{14}C]-Cys-NPMI (60 nmol each, with specific radioactivity 19 kBq/μmol) were added to the suspension and then stirred for 20 min in an atmosphere of nitrogen. After filtration and ultrafiltration, the IDA was carried out as described above.

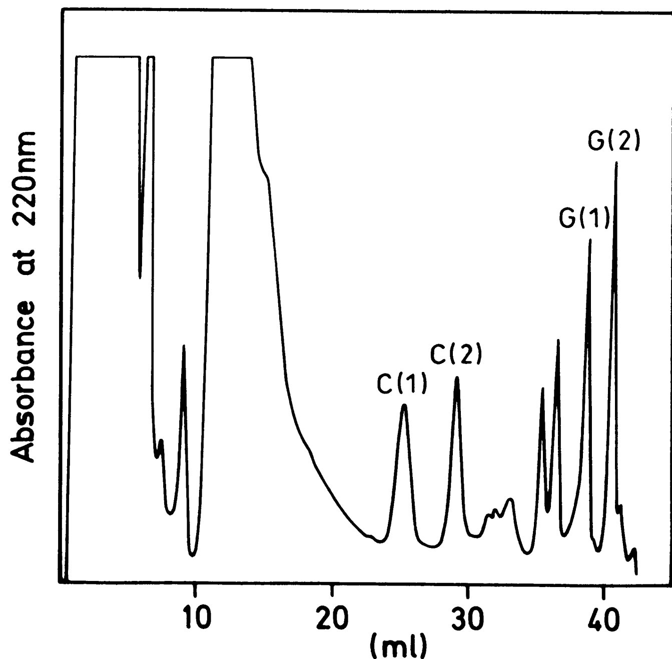

Fig. 2. High-performance liquid chromatography (system I) of the fraction obtained from Fig. 1. Subfractions C(1)-C(2) and G(1)-G(2) were individually collected.

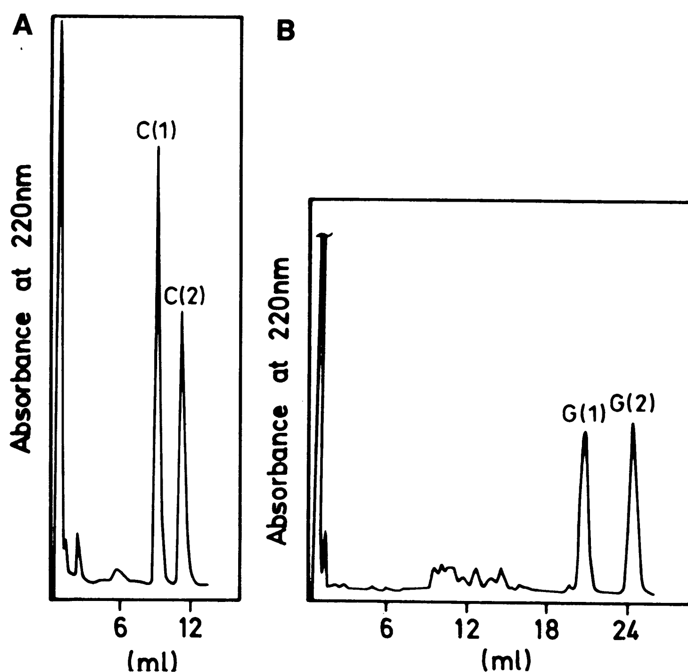

Fig. 3. High-performance liquid chromatography (system II) of the subfractions obtained from Fig. 2. **A,** C(1)-C(2). **B,** G(1)-G(2).

RESULTS

Examination of the Method

To check the accuracy of this new method, known amounts of Cys were added to a flour of the cultivar Maris Huntsman containing 81 μmol per gram of GSH (Sarwin et al 1992a). The results in Table I show that 99–104% of the Cys was recovered.

In a second experiment, GSH was measured in two flour samples by using the new method. The GSH levels of the two samples had been determined previously by derivatization with NEMI (Sarwin et al 1992a). Table II indicates that the GSH levels found by the two methods differed $<10\%$.

On the basis of the results shown in Tables I and II, it was concluded that the new method was sufficiently precise for the quantification of GSH and Cys in cereals.

GSH and Cys in Flours and Bran

As summarized in Table III, the GSH levels of six flours of similar extraction grade differed between 8 and 35 nmol/g. Flour A was the lowest in both GSH and Cys. Kraka was the highest in GSH. In three flours (Gambrinus, Bussard, and Kraka), the concentration of GSH surpassed that of Cys. It varied between 1.8–1 (Gambrinus and Bussard) and 3.2–1 (Kraka). In the remaining three flours, the molar ratio of the two SH compounds was nearly 1. In the flour samples, the levels of Cys were much lower

TABLE I
Recovery of Free Cysteine in the Isotope Dilution Assay

Cysteine, nmol/g		
Added[a]	Found[b]	Recovered, %
0	9 ± 0.7	...
30	39 ± 2.0	99
60	72 ± 2.5	104

[a] Cysteine dissolved in water (2 ml) was added during the first minute of extraction. (Flour: Maris Huntsman; ash content: 0.68 wt%).
[b] Means ± standard deviations of two determinations.

TABLE II
Determination of Glutathion in Two Wheat Flours by Derivatization with *N*-ethylmaleimide (NEMI) and *N*-phenylmaleimide (NPMI)

	GSH, nmol/g[a,b]	
Sample	NEMI	NPMI
Commercial flour B, type 550	30 ± 1.2	32 ± 2.2
Flour from CWRS, type 550	14 ± 0.1	13 ± 1.2

[a] Amounts related to dry mass.
[b] Mean values of two determinations.

TABLE III
Levels of Glutathion (GSH) and Cysteine (Cys) in Wheat Flours and Bran

Wheat Cultivar	Ash Type	GSH[a,b] (nmol/g)	Cys[a,b] (nmol/g)
Flour			
A, cultivar unknown	550	8 ± 0.8	8 ± 0.1
Gambrinus	550	27 ± 0.6	15 ± 0.2
Bussard	550	27 ± 1.2	15 ± 0.6
Kraka	550	35 ± 0.7	11 ± 0.6
Columbus	550	16 ± 0.6	13 ± 1.0
CWRS	0.54[c]	13 ± 1.2	11 ± 0.6
Kanzler	0.62[c]	35 ± 2.5	8 ± 0.8
Bran			
Kanzler		190 ± 12	12 ± 0.6

[a] Amounts related to dry mass.
[b] Mean values of two determinations.
[c] Ash in % by weight.

than the 59–86 nmol/g levels reported by Ewart (1990), who determined Cys by amino acid analysis in diffusates obtained after dialysis of flour suspensions at 4°C for 24 hr.

A comparison of the flour and bran of the Kanzler wheat (Table III) indicated a 5.6-fold higher level of GSH in the bran. This higher level agreed with the findings (Fahey et al 1980) that GSH is preferentially located in the germ component of the bran. The bran contained only 50% more Cys than the flour (Table III).

GSS and CysSS were determined in three flours (Table IV) and in two samples of crude glutenin (Table V).

In DNS and Maris Huntsman, the sum of GSSG and protein-glutathione mixed disulfide was ~65% of the total glutathione; it was 80% in the flour of the Kanzler (Table IV). CysSS was lower than GSS in the three flours; also, the proportion of Cys in CysSS was lower than that of GSH in GSS.

The levels of GSS and CysSS were higher in the crude glutenin of the Bussard than in Flour A (Table V). However, in this sample of crude glutenin the sum of GSS and CysSS was also only a small fraction (0.8 mol%) of the peptide-bound Cys.

GSH and Cys were assayed in two flour-water doughs that were kneaded for 3 min with, and without, AA stereosiomers.

Table VI shows that kneading the doughs without additions lowered the concentrations of GSH by 56 and 50% in DNS and Kanzler, respectively. In contrast, the levels of Cys increased to those of GSH. The increase of Cys partially compensated for the loss of GSH; therefore, in the doughs of DNS and Kanzler, only 24 and 17%, respectively, of the total amounts of the two low molecular thiols disappeared during kneading.

Adding L-*threo*-AA accelerated the loss of GSH. Only 20 and 26% of the GSH levels in the flours of DNS and Kanzler, respectively, were found in the corresponding doughs. The increase of Cys was also delayed more than it was in doughs without additions. The total levels of GSH and Cys were reduced to 43 and 53% in DNS and Kanzler, respectively, after adding L-*threo*-AA.

The levels of GSH and Cys of the doughs with D-*threo*-AA were nearly equal to those without additions (Table VI). This result indicates that, unlike L-*threo*-AA, D-*threo*-AA did not significantly affect the reactions of the two thiols.

TABLE IV
Glutathione (GSH), Cysteine (Cys), Total Glutathione (GSS), and Total Cysteine (CysSS) Contents (nmol/g) of Wheat Flours[a,b]

Cultivar	Ash (% by weight)	GSH	GSS	Cys	CysSS
DNS	0.78	100 ± 2.2[c]	279 ± 6.6	13 ± 1.6	159 ± 1.7
Maris Huntsman	0.68	81 ± 1.4[c]	232 ± 1.0	9 ± 0.7	145 ± 3.9
Kanzler	0.62	35 ± 2.5	180 ± 6.4	8 ± 0.8	118 ± 7.0

[a] Amounts related to dry mass.
[b] Mean values for two determinations.
[c] Values obtained after derivatization with *N*-ethylmaleimide (Sarwin et al 1992a).

TABLE V
Comparison of Total Glutathione (GSS) and Total Cysteine (CysSS) with the Peptide-Bound Cysteine (Cys) in Crude Glutenins (μmol per gram of protein)[a]

Flour	GSS	CysSS	Peptide Bound Cys[b]
A	0.23	0.14	183
Bussard	0.84	0.58	188

[a] Mean value of two assays.
[b] Concentration of peptide bound Cys determined by amino acid analysis after acid hydrolysis of the sample (Wieser et al 1987).

TABLE VI
Effect of L-*threo* Ascorbic Acid (AA) and D-*erythro*-AA on the Concentrations of Glutathion (GSH) and Cysteine (Cys) in Flour-Water Doughs[a,b]

Sample	GSH, nmol/g	Cys, nmol/g
Wheat cultivar DNS (0.78 % by weight ash)		
Flour	100 ± 2.2	13 ± 1.6
Dough without additions	44 ± 0.8	42 ± 2.5
Dough with L-*threo*-AA[c]	20 ± 0.8	28 ± 1.0
Dough with D-*erythro*-AA[c]	39 ± 0.6	41 ± 2.1
Wheat cultivar Kanzler (0.62 % by weight ash)		
Flour	35 ± 2.5	8 ± 0.8
Dough without additions	17 ± 1.7	18 ± 0.6
Dough with L-*threo*-AA[c]	9 ± 2.6	11 ± 0.5
Dough with D-*erythro*-AA[c]	17 ± 1.2	22 ± 0.5

[a] Amounts related to dry mass.
[b] Mean values for two determinations.
[c] Amount added: 0.17 μmol per gram of flour.

DISCUSSION

The SH-SS interchange reactions summarized in Figure 4 are proposed for an interpretation of the results.

Interchange reactions of gluten proteins with both GSH (Fig. 4, line 1) and Cys (Fig. 4, line 4) were detected in doughs after adding corresponding thiols labeled with the sulphur isotope-35 (Stewart and Mauritzen 1966, Mauritzen 1967, Kuninori and Sullivan 1968, Lee and Lai 1968). It is suggested (Goldstein 1957, Mecham 1959, Stewart and Mauritzen 1966, Mauritzen 1967) that these reactions with intermolecular SS bonds depolymerize gluten proteins, leading to a decrease in dough strength.

The concentrations of Cys in the two flours were much lower than those of GSH, but they increased during the kneading period and reached the level to which GSH had fallen. As the total amount of CysSS was much higher than that of free Cys in the flours of DNS and Kanzler, the interchange reactions of GSH with protein-Cys mixed disulfides (Fig. 4, line 2) and cystine (Fig. 4, line 3) might explain the increase of Cys during dough mixing. In addition, the balance in the GSH and Cys concentrations in dough is presumably stabilized by the reactions of Cys with protein-glutathione mixed disulfides (Fig. 4, line 5) and oxidized glutathione (Fig. 4, line 6).

As shown by several authors (Elkassabany et al 1980, Nicholas et al 1980), AA stereoisomers added to doughs are oxidized very rapidly to the corresponding DHAA (Fig. 4, line 7). Results indicate that adding L-*threo*-AA reduces the concentration of GSH in the dough and inhibits the increase of Cys. The decrease in GSH is due to the activity of the enzyme GSH-DH, which oxidizes GSH with L-*threo*-DHAA as cosubstrate (Fig. 4, line 8).

Most of SH-SS interchange in gluten with low molecular SH compounds (Fig. 4, line 1) takes place in the first few minutes of dough mixing (Stewart and Mauritzen 1966, Mauritzen 1967). However, we suggest that the enzymatic oxidation of endogenous GSH to GSSG proceeds much faster than the interchange reaction with the gluten proteins, protein-Cys mixed disulfides, or cystine (Fig. 4, lines 1–3). This suggestion is in accord with the finding that the release of Cys in the reactions (Fig. 4, lines 2 and 3) is partially inhibited in the doughs with L-*threo*-AA. The rapid enzymatic oxidation of endogenous GSH to GSSG (Fig. 4, line 8) increases dough strength because GSSG in the low levels formed in dough is rheologically inactive (Sarwin et al 1992b).

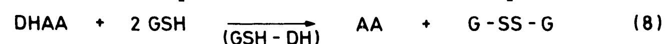

Fig. 4. Proposed reactions of glutathione and cysteine in doughs with and without ascorbic acid stereoisomers. See Discussion.

As D-*erythro*-DHAA is reduced more slowly by GSH-DH than by L-*threo*-DHAA (Walther and Grosch 1987), the GSH in the wheat flour is not oxidized fast enough to inhibit the interchange reactions (Fig. 4, lines 1-3). Therefore, the levels of GSH and Cys in the doughs are comparable to those of the doughs without additions.

CONCLUSION

This work confirms the hypothesis that the improver action of L-*threo*-AA is caused by a rapid enzymatic oxidation of the GSH in the wheat flour to the rheologically inactive GSSG. Consequently, the interchange reactions of GSH with both protein-Cys mixed disulfides and with intermolecular SS bonds of gluten proteins are at least partially inhibited during dough mixing.

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