

# Modifications of Wheat Proteins Due to Flour Chlorination

M. P. DUVIAU,<sup>1</sup> H. YAMAMOTO,<sup>2,3</sup> P. K. W. NG,<sup>2</sup> and K. KOBREHEL<sup>1,4</sup>

## ABSTRACT

Cereal Chem. 73(4):490-494

The extractability of wheat flour proteins underwent considerable changes as the result of chlorination, especially as detected under different conditions for sequential protein extraction. Compared to controls, changes in protein extractability were greater when flours were chlorinated to pH 4.3 than to pH 4.8. Gliadins seemed to be more affected than glutenins. Low molecular weight proteins, whose molecular mass ranged

between 9 and 15 kDa, were also considerably affected, but to a varying extent. While free -SH groups decreased slightly in treated samples, results suggested that SS bonds did not seem to be affected under chlorination, and their accessibility to thioredoxin and to dithiothreitol also remained unchanged.

The chlorination process is commonly used to improve the cake-baking quality of soft wheat flour. The improving effect of chlorination has been ascribed primarily to the modification of starch characteristics (Sollars 1958, Gough et al 1978, Johnson et al 1980, Varriano-Marston 1985). However, other flour components such as gluten proteins also undergo considerable changes that affect the treated flour quality (Sollars 1958). In addition, studies on proteins after chlorination have shown that the amount of water-extractable proteins increased with chlorine treatment (Kissel 1971), while the amount of proteins extractable in acetic acid decreased slightly with increasing levels of chlorination (Tsen et al 1971). According to model experiments of chlorination, nonwheat water-soluble proteins became more hydrophobic, suggesting that the lipophilic properties of chlorinated starch resulted from the lipophilic protein film on the starch granule (Seguchi 1985). The effects of free (hexane-extracted) lipids to baking performance due to flour chlorination was also reported (Kissel et al 1979). More recently, a study showed the combined effects of protein and fatty acid contents on improving baking quality in chlorinated soft wheat flour (Conforty et al 1993).

On the basis of reported results, in general agreement with earlier conclusions (Gough et al 1978), it seems reasonable to assume that the improvement of cake flour under chlorination is the result of an overall effect of chlorination on most of the components of flour. However, it also appears clear that the effect of chlorination on flour components is still not thoroughly understood and, in view of a possible replacement of the chlorination process in the cake-making industry, there is clearly a need for further studies to provide a more thorough understanding of all the changes that are taking place under flour chlorination.

In the present article, we report the first part of our investigations regarding some modifications of wheat protein characteristics due to flour chlorination.

## MATERIALS AND METHODS

### Wheat Samples

Four soft wheat cultivars were used in the present study: Lewjain (soft white winter from Washington), Tres (soft white

club from Washington), Frankenmuth (soft white winter from Michigan), and Dynasty (soft red winter from Ohio). These cultivars cover all classes of soft wheats produced in the United States. The samples were milled on a Miag-Multomat mill to obtain patent flours at a 45% extraction rate.

### Chemicals and Reagents

Monobromobimane (mBBr), TEMED, and NADPH were purchased from Sigma Chemical Co. (St. Louis, MO). *Escherichia coli* thioredoxin and NADP-thioredoxin reductase (NTR) were kindly provided by B. B. Buchanan (Plant Biology, University of California, Berkeley). Acrylamide and bisacrylamide were obtained from BDH (Poole, England), and ammonium persulfate from Merck Chemical Co. (Darmstadt, Germany). All other chemicals were obtained from commercial sources and were of the highest quality available.

### Flour Chlorination

Flour samples were chlorinated to pH 4.8 and 4.3 by adding Cl<sub>2</sub> to the samples under the conditions described by Kissel and Marshall (1972). A portion of each flour was set aside as an unchlorinated control.

### Total Protein Extraction

For extracting total flour proteins, 80 mg of Na-tetradecanoate and 8 ml of distilled water were added to 1 g of flour sample. The mixture was stirred for 2 hr and then centrifuged at room temperature for 30 min at 27,000 × g.

### Sequential Protein Extraction

For sequential extraction of flour proteins, either a modified Osborne procedure (for cvs. Tres and Frankenmuth) or the method described by Kobrehel (1980) (for all cultivars) was used.

The modified Osborne procedure was performed as follows. Albumins and globulins were extracted together with 0.5M NaCl at 4°C and gliadins were extracted with 70% ethanol at room temperature. Three successive extractions were made with both solvents; 5 ml of solvent per 1 g of flour was used for each extraction. For the first extractions, samples were stirred for 1 hr. For the second and third extractions, samples were stirred for 30 min each. After the extraction of the albumin-globulin fraction, residues were washed by suspending the residues in 5 ml of water, centrifuged, and the supernatants discarded. Glutenins were extracted by suspending the residues with 250 mg of Na-tetradecanoate in 15 ml of water. Samples were stirred for 3 hr at room temperature, then centrifuged. In addition, a fourth extraction was made on the final residue in the presence of a reducing agent (8 ml of 0.2% 2-ME). All centrifugations were run for 30 min at 4°C, 27,000 × g.

<sup>1</sup>Laboratoire de Biochimie et Biologie Moléculaire des Céréales, INRA, 2, Place Viala 34060 Montpellier-Cedex, France.

<sup>2</sup>Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824.

<sup>3</sup>Present address: Yamazaki Baking Co., Ltd., 3-15-6, Chitose, Sumida-ku, Tokyo, 130, Japan.

<sup>4</sup>Corresponding author. E-mail: Kobrehel@ENSAM.inra.fr

In Kobrehel's sequential extraction method, proteins were extracted in Na-salts of fatty acids (detergent). In the initial extraction, 10 mg of Na-tetradecanoate and 8 ml of water were added to 1 g of flour; the mixture was stirred for 2 hr and centrifuged for 30 min at  $27,000 \times g$  at room temperature. Protein fraction I and residue I were obtained. Then, an additional 5 mg of Na-tetradecanoate and 8 ml of water were added to residue I, and extracted as before to obtain fraction II and residue II. A third and fourth extraction used 10 mg of Na-tetradecanoate, added to residue II and then to residue III, and the extractions performed as above to obtain fractions III and IV, and residues III and IV. Finally, in the fifth extraction, instead of water, 8 ml of 0.2% 2-ME was used to obtain fraction V and residue V.

### Protein Determination

Protein content in the flours was determined by the micro-Kjeldahl method (AACC 1995) and protein content for extracts was determined either by the Bradford method, or when this method could not be used (extractions made with 70% ethanol or with Na-tetradecanoate), by measuring absorption at 280 nm by using bovine serum albumin as a standard. All determinations were duplicated.

### mBBR Fluorescent Labeling of Proteins

During the one-step extraction of proteins with Na-tetradecanoate, as described above, the direct labeling of the free SH groups with mBBR was performed by the method of Crawford et al (1989) as modified by Kobrehel et al (1991). The conditions of thioredoxin treatments were those described by Kobrehel et al (1992).

### Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

Nonreduced or reduced protein fractions were applied to the gels (4% stacking, 17.5% separating) and the electrophoresis was performed for 16 hr at a constant current (Ng and Bushuk 1987).

## RESULTS AND DISCUSSION

### Total Flour Protein Extractions

Storage proteins, including glutenins, are readily solubilized in the presence of Na-salts of fatty acids (Kobrehel and Bushuk 1977, 1978). Given this solubility, the use of Na-salts of fatty acids for extracting total flour proteins extracted up to 90–95% of the proteins in the flour in one step (Kobrehel 1980, Fliedel and Kobrehel 1985). In the present study, using these extraction conditions, total proteins were extracted from unchlorinated and chlorinated flour samples of four cultivars. The total proteins were then analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under both reducing and nonreducing conditions. There were essentially no differences among the unchlorinated and the chlorinated samples (data not shown, but results were obtained by using similar protein extraction conditions, except that free SH groups were labeled during protein solubilization). Accordingly, chlorination seemed to have no detectable influence on the extractability of total flour proteins in a high concentration of Na-tetradecanoate (80 mg to 1 g of flour in 8 ml of water). An earlier

study suggested the possible cleavage of peptide bonds under chlorination (Tsen et al 1971). However, our results did not confirm the occurrence of such an effect, which would have resulted in noticeable qualitative changes between the electrophoretic patterns of the unchlorinated and chlorinated samples.

### Sequential Protein Extractions

**Modified Osborne method.** The extractability of the albumin-globulin fraction decreased with increasing chlorination (Table I). Conversely, the extractability of gliadins tended to increase with chlorination. The treatments had less evident effect regarding glutenins. Differences in the extractability of all protein fractions were also found between samples chlorinated to pH 4.3 or to pH 4.8. When, after the extraction with NaCl, the residues were washed sequentially several times with water, some of the gliadins became extractable, especially in the chlorinated samples (results not shown).

The composition of protein groups obtained from the modified Osborne method were also compared using SDS-PAGE. Both reducing and nonreducing conditions of SDS-PAGE were used. In either case, mostly quantitative differences (change in the intensity of the protein bands) were found between chlorinated samples and the respective controls. The electrophoretic patterns for cv. Tres, under nonreducing conditions, illustrate these results (Fig. 1). The most considerable decrease in the intensity of protein bands was observed for the albumin-globulin fraction in the treated samples. These results suggested the establishment of interactions between these, mostly cysteine-rich proteins (Barber et al 1986, Joudrier et al 1995) and other proteins or other flour components under chlorination. However, some of these unextracted proteins were recovered in the wash with water subsequent to the extraction with NaCl (Fig. 1). No considerable changes were noticed with gliadins and glutenins.

**Sequential extraction with Na-tetradecanoate.** Earlier results showed that with the sequential method of protein extraction using Na-tetradecanoate, and depending on the amount of detergent used at each extraction step, the protein groups, as defined in the classification by Osborne, are separated into several subfractions (Kobrehel 1980). Our results indicated that chlorination modified the extractability of the different protein groups. Differences were observed between the extractability of the proteins of chlorinated

TABLE I  
Sequentially Extracted Proteins<sup>a</sup> from cv. Tres  
Using a Modified Osborne Method

Treatments	NaCl 0.5M	Ethanol 70%	Na-Tetradecanoate 250 mg/g of flour	2-ME <sup>b</sup> 0.2%	Total Proteins Extracted
Control	12	43	25	7	87
pH 4.8	9	48	27	5	89
pH 4.3	6	50	25	5	86

<sup>a</sup> % of total protein content.

<sup>b</sup> 2-Mercaptoethanol.

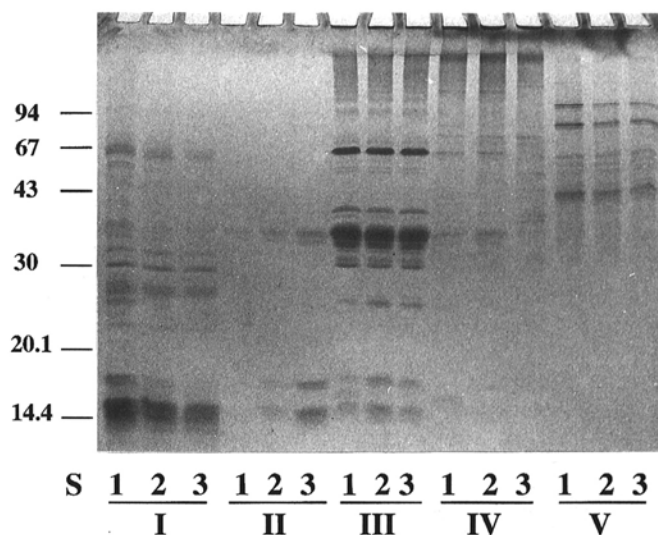


Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Sequentially extracted (nonreduced) proteins of cv. Tres according to a modified Osborne method. I: albumins and globulins; II: wash with water; III: gliadins; IV: glutenins; V: residual proteins extracted with 2-mercaptoethanol. 1: Control; 2: flour chlorinated to pH 4.8; 3: flour chlorinated to pH 4.3. S = molecular weight standards (kDa).

and unchlorinated samples at each extraction step. The determination of the protein content in the extracts showed that, as in the case of the Osborne method, in the first fraction less protein was extracted from chlorinated samples than from the unchlorinated samples. Additionally, samples chlorinated to pH 4.3 had, in general, lower protein amounts extracted than samples chlorinated to pH 4.8 (Table II). At the second extraction step, fewer differences were found between treated and untreated samples on the one hand, and between samples chlorinated to pH 4.8 or samples chlorinated to pH 4.3 on the other hand.

Differences among chlorinated and unchlorinated samples were not only quantitative but also qualitative. This can be seen from the electrophoregrams of the protein fractions extracted by the Na-tetradecanoate sequential method, as illustrated for cv. Tres (Fig. 2). In the first two extraction steps, the intensity of most of the protein bands decreased in the chlorinated samples. Proteins not extracted in these fractions were, however, recovered in the further extraction steps.

In the first extraction step, most metabolic proteins (albumins and globulins) were extracted by using 10 mg of Na-tetradecanoate per 1 g of flour. As with the modified Osborne method, differences appeared in the extractable quantities of these proteins between the unchlorinated and the respective chlorinated samples (proteins were less extractable from the chlorinated samples) (Figs. 1 and 2, see also Tables I and II).

The effect of chlorination was very clearly seen in the second fraction, extracted with an additional 5 mg of Na-tetradecanoate. The electrophoretic patterns (Fig. 2) revealed that this fraction contained most of the gliadins. These proteins were less extractable from the chlorinated samples than from the unchlorinated samples, and increasing chlorination (pH 4.3) appeared to decrease their extractability. Most of the gliadins and glutenins from the chlorinated samples were not extractable in the second fraction but were extracted during the third extraction with an additional amount of Na-tetradecanoate. Conversely, the third fractions of the unchlorinated samples contained lower amounts of protein, especially gliadins, than did the third fractions of the chlorinated samples (Fig. 2). Chlorination did not seem to have much effect on the extractability of high molecular weight glutenins.

The extractability of cysteine-rich proteins was also considerably affected by chlorination. Generally they were less extractable from the chlorinated samples. However, chlorination did not have the same effect on all of these proteins whose molecular mass ranged mostly from 9 to 15 kDa. In particular, one of these proteins at  $\approx 14$  kDa was never detected in the chlorinated samples

(Fig. 2A lanes III-1 and IV-1, band indicated with arrow for the latter) even with the use of 2-ME as a reducing agent.

Changes in the extractability of protein groups due to chlorination of the flours were found in all flour samples studied. Results also showed some, rather slight, quantitative differences among cultivars. At the present stage of our study, however, it is not possible to relate these differences to any characteristics of the flour samples used.

The conditions of the sequential extraction procedure used extracted most of the proteins in the first three fractions, beyond which additional amounts of Na-tetradecanoate were not very efficient. However, residue V still contained between 8 and 20% of the total proteins (Table II). These residual proteins were recovered by using a reducing agent. The electrophoretic patterns showed that these proteins were composed mostly of glutenins and some gliadins (Fig. 2). While composition of the residual proteins of the unchlorinated samples was similar to that of the chlorinated samples (fraction V in Fig. 2), results showed considerable quantitative differences among cultivars (Table II). For instance, Tres and Dynasty had increased amounts of residual proteins in the chlorinated samples. Accordingly, residual protein bands from chlorinated samples were of higher intensity than those of proteins from the control (Fig. 2). Conversely, no such differences were found for Frankenmuth and Lewjain (Table II, figures not shown).

TABLE II  
Sequentially Extracted Proteins<sup>a</sup> with Na-Tetradecanoate

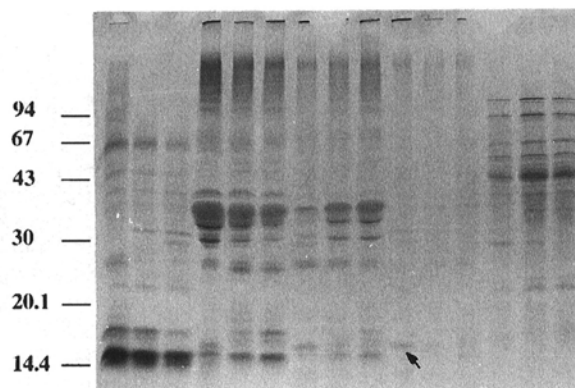
Cultivars	Treatments	Successively Added Na-Tetradecanoate <sup>b</sup> (mg/g of flour/8 ml of water)				Residue IV Plus 0.2% 2-ME <sup>c</sup>
		10	5	10	10	
Tres	Control	17	46	11	7	9
	pH 4.8	8	44	23	4	12
	pH 4.3	7	35	24	9	14
Frankenmuth	Control	19	27	21	5	18
	pH 4.8	8	40	16	6	20
	pH 4.3	7	32	27	5	19
Lewjain	Control	15	40	18	4	13
	pH 4.8	13	37	24	4	12
	pH 4.3	4	19	56	3	8
Dynasty	Control	26	40	7	6	11
	pH 4.8	14	42	13	5	15
	pH 4.3	8	46	19	4	12

<sup>a</sup> % of total protein content.

<sup>b</sup> After the first extraction, Na-tetradecanoate was added to residue I, then to residue II, then to residue III.

<sup>c</sup> 2-Mercaptoethanol.

A



B

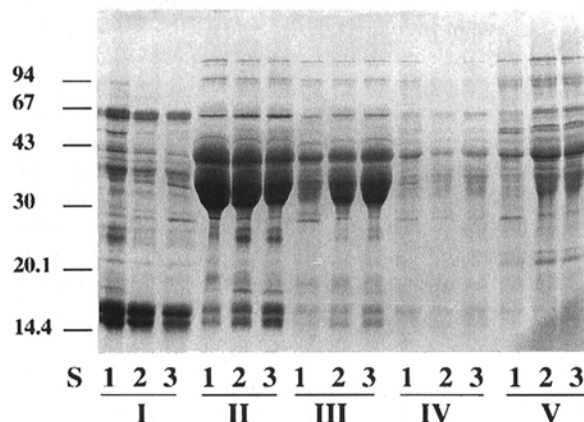


Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Sequentially extracted proteins of cv. Tres with Na-tetradecanoate. A, Unreduced proteins. B, Proteins reduced with 2-mercaptoethanol. I, II, III, and IV: proteins extracted with 10 mg, with an additional 5 mg, and with yet additional 10 and 10 mg amounts of Na-tetradecanoate, respectively (the additional amounts of detergent were added to the preceding residue). V: residual proteins extracted with 2-mercaptoethanol. 1: Control; 2: flours chlorinated to pH 4.8; 3: flours chlorinated to pH 4.3. S = molecular weight standards (kDa).

### Chlorination Effect on the SH and SS Groups of the Proteins

By labeling the SH groups with mBBr, protein fractions containing free SH groups can be visualized under UV light after electrophoretic separation of the proteins. In the present study, free SH groups were labeled using a direct mBBr method (Kobrehel et al 1992). In this method, free SH groups of the proteins are derivatized as soon as proteins are solubilized, thus avoiding oxidation of SH groups during extraction. Note that with the mBBr labeling method, the intensity of the fluorescent bands detected after the electrophoretic separation of the proteins is proportional to the amount of free SH groups in the corresponding protein fractions (Kobrehel et al 1991).

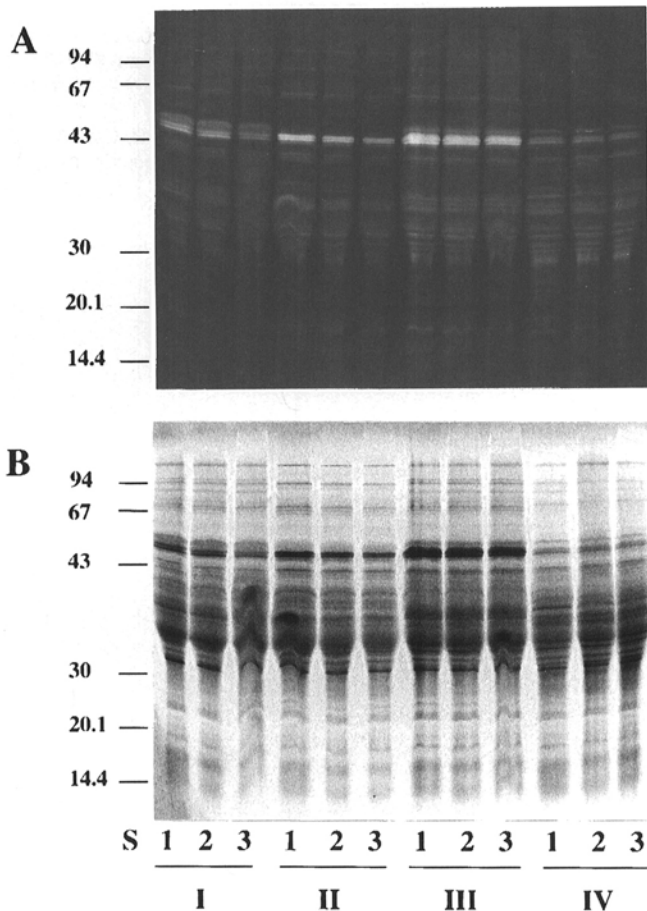
In these experiments, proteins were extracted in a high concentration of Na-tetradecanoate (80 mg per 1 g of flour) and, under these conditions, all the flour protein fractions are present in the extracts. The levels of free SH groups in the unchlorinated flours were low, which confirms the results reported on the redox state of wheat proteins by Kobrehel et al (1992) and Wong et al (1993). Among the four cultivars, the intensity of fluorescent bands decreased only slightly in the chlorinated samples (Fig. 3A), denoting a slight decrease of free SH groups, which were a bit higher at pH 4.3 than at pH 4.8. This is in general agreement with earlier reports by Tsen et al (1971) that chlorination seemed to have little effect on the SH groups.

The labeling of SH groups was also performed after the reduction of the SS groups by thioredoxin, a physiological reductant of

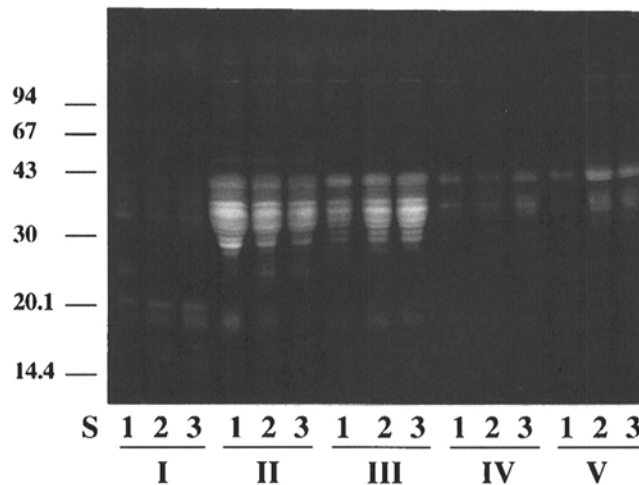
cystine-rich proteins (Kobrehel et al 1991), glutenins, and gliadins (Kobrehel et al 1992, Wong et al 1993). It targets more specifically the intramolecular SS bonds (Shin et al 1993). For these experiments, proteins of the four cultivars were extracted under the conditions of the sequential extraction procedure with Na-tetradecanoate. Results require the same comments as those made regarding protein extractability under conditions of the sequential extraction procedure with Na-tetradecanoate: specifically, that differences in the fluorescent intensities of protein bands between chlorinated and unchlorinated samples (Fig. 4) were due to differences in protein extractability. On the other hand, results suggested that SS groups did not seem to be affected, and that the accessibility of SS bonds to thioredoxin did not change due to the chlorination of the samples. When the labeling of SH groups was performed after the reduction of SS bonds with dithiothreitol (results not shown), the differences observed between samples seemed to be due to the differences in protein extractability. Thus, in this respect, results lead to conclusions similar to those obtained by using thioredoxin, although the extent of protein reduction under the two reducing conditions was different. All these results suggested that SS groups did not seem to be affected by chlorination.

### CONCLUSIONS

Under sequential extraction conditions, wheat flour chlorination modified the extractability of proteins. However, in the presence of strong extractant, such as a high concentration of detergent, these modifications could not be evidenced. Modifications in the extractability of flour proteins suggest that the capability of proteins to interact with themselves and with other flour components is changed under chlorination. However, only noncovalent interactions are affected by chlorination; protein SS bonds do not seem to be involved. Nevertheless, the modifications to protein properties may be sufficient to produce considerable effect during the technological transformation of flours, especially for cake baking. Technological experiments are needed to link the modifications occurring in proteins under chlorination to the modifications of the technological quality of the treated flour samples. However, investigations regarding the modifications to protein-starch, protein-lipid, and protein-protein interactions are probably the most needed for a better understanding of the effect of chlorination on wheat flour.



**Fig. 3.** Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Total proteins extracted with Na-tetradecanoate. (1 g of flour, 80 mg of detergent, 8 ml of distilled water). Free SH groups were labeled with monobromobimane (mBBr) during protein solubilization. **A**, mBBr labeled proteins. **B**, Coomassie blue stained proteins. I, II, III, and IV are cvs. Lewjain, Tres, Frankenmuth, and Dynasty, respectively. 1: Control; 2: flours chlorinated to pH 4.8; 3: flours chlorinated to pH 4.3. S = molecular weight standards (kDa).



**Fig. 4.** Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Legends are as for Fig. 2B, except that here extracted proteins were reduced by thioredoxin and then free -SH groups were labeled with mBBr. S = molecular weight standards (kDa).

## ACKNOWLEDGMENTS

We thank P. L. Finney (USDA-ARS Soft Wheat Quality Laboratory, Wooster, OH) for use of the milling facility and chlorination of the flour samples. We also thank S. T. Worthington for assistance with milling. Partial support of this research from Michigan Agricultural Experiment Station and from Yamazaki Baking Co., Ltd. of Japan, is gratefully acknowledged.

## LITERATURE CITED

- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1995. Approved Methods of the AACCC, 9th ed. Method 46-13. The Association: St. Paul, MN.
- BARBER, D., SANCHEZ-MONGE, R., GARCIA-OLMEDO, F., SALCEDO, G. and MENDEZ E. 1986. Evolutionary implications of sequential homologies among the members of the trypsin/ $\alpha$ -amylase inhibitor family (CM-proteins) in wheat and barley. *Biochim. Biophys. Acta* 873:147-151.
- CONFORTI, F. D., JOHNSON, J.M., and ALLEY, M. 1993 The effects of nitrogen fertilization and chlorination on the protein and fatty acid contents of soft red winter flour, and their influence on baking quality of angel food cake. *J. Cereal Sci.* 18:187-195.
- CRAWFORD, N. A., DROUX, M., KOSOWER, N. S., and BUCHANAN, B. B. 1989. Evidence for function of the ferredoxin/thioredoxin system in the reductive activation of target enzymes of isolated intact chloroplasts. *Arch. Biochem. Biophys.* 271:223-239.
- FLIEDEL, G., and KOBREHEL, K. 1985. Studies on sorghum proteins. 1. Solubilizations of proteins with soaps. *J. Agric. Food Chem.* 33:303-308.
- GOUGH, B. M., WHITEHOUSE, M. E., and GREENWOOD, C. T. 1978. The role and function of chlorine in the preparation of high-ratio cake flour. *CRC Crit. Rev. Food Sci. Nutr.* 11:91-113.
- JOHNSON, A. C., HOSENEY, R. C., and GHAI, K. 1980. Chlorine treatment of cake flours. V. Oxidation of starch. *Cereal Chem.* 57:94-96.
- JOUDRIER, P., LULLIEN-PELLERIN, V., ALARY, R., GROSSET, J., GUIRAO, A., and GAUTIERM, F. 1995. Characterization of the *Triticum durum* Desf. Chloroform-methanol-soluble protein family. *DNA Sequence* 5:153-162.
- KISSELL, L. T. 1971. Chlorination and water-solubles content in flours of soft wheat varieties. *Cereal Chem.* 48:102-108.
- KISSELL, L. T., and MARSHALL, B. D. 1972. Design and construction of a reactor for gaseous-treatment of flour. *Cereal Sci. Today* 17:152-155.
- KISSELL, L. T., DONELSON, J. R., and CLEMENTS, R. L. 1979. Functionality in white layer cake of lipids from untreated and chlorinated patent flours. I. Effect of free lipids. *Cereal Chem.* 56:11-14.
- KOBREHEL, K. 1980. Extraction of wheat proteins with salts of fatty acids and their electrophoretic characterization. *Ann. Technol. Agric.* 29:125-132.
- KOBREHEL, K., and BUSHUK, W. 1977. Studies of glutenin. X. Effect of fatty acids and their sodium salts on solubility in water. *Cereal Chem.* 54:833-839.
- KOBREHEL, K., and BUSHUK, W. 1978. Studies of glutenin. XI. Note on glutenin solubilization with surfactants in water. *Cereal Chem.* 55:1060-1064.
- KOBREHEL, K., YEE, B. C., and BUCHANAN, B. B. 1991. Role of the NADP/thioredoxin system in the reduction of  $\alpha$ -amylase and trypsin inhibitor proteins. *J. Biol. Chem.* 266(24):16135-16140.
- KOBREHEL, K., WONG, J. H., BALOGH, A., KISS, F., YEE, B. C., and BUCHANAN, B. B. 1992. Specific reduction of wheat storage proteins by thioredoxin *h*. *Plant Physiol.* 99:919-924.
- NG, P. K. W., and BUSHUK, W. 1987. Glutenin of Marquis wheat as a reference for estimating molecular weights of glutenin subunits by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Cereal Chem.* 64:324-327.
- SEGUCHI, M. 1985. Model experiments on hydrophobicity of chlorinated starch and hydrophobicity of chlorinated surface protein. *Cereal Chem.* 62:166-169.
- SHIN, S., WONG, J. H., KOBREHEL, K., and BUCHANAN, B. B. 1993. Reduction of castor seed 2S protein by thioredoxin. *Planta* 189:557-560.
- SOLLARS, W. F. 1958. Cake and cookie flour fractions affected by chlorine bleaching. *Cereal Chem.* 35:100-115.
- TSEN, C. C., KULP, K., and DALY, C. J. 1971. Effect of chlorine on flour proteins, dough properties and cake quality. *Cereal Chem.* 48:247-255.
- VARRIANO-MARSTON, E. 1985. Flour chlorination: New thoughts on an old topic. *Cereal Foods World* 30:339-343.
- WONG, J. H., KOBREHEL, K., NIMBONA, C., YEE, B. C., BALOGH A., KISS, F., and BUCHANAN, B. B. 1993. Thioredoxin and bread wheat proteins. *Cereal Chem.* 70:113-114.

[Received June 26, 1995. Accepted March 28, 1996.]