Change in Sulfhydryl-Disulfide Status of Wheat Proteins During Conditioning and Milling

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ABSTRACT

The sulfhydryl-disulfide status of wheat kernel proteins underwent modifications during the conditioning process performed before milling. Wheat conditioned 12 hr to a moisture content of 16% at room temperature resulted in a significant increase of available sulfhydryls in the proteins of all parts of the kernel: endosperm, embryo, and bran, including both metabolic and storage proteins of the endosperm. The sulfhydryl groups generated during conditioning remained reduced even after lengthy storage of the flour at 4°C. The results imply that the technological quality of wheat flour may be considerably influenced by the conditioning process used before milling, as well as by subsequent storage conditions.

A number of conditioning processes have been developed for the industrial milling of wheat (Ziegler and Greer 1971, Willm and Jollet 1994). The role of conditioning, the central feature of which is the addition of water to grain, is to modify the wheat kernel so that milling can be performed under optimal conditions (Bass 1988). Water is added, usually to obtain a moisture content of ≈16%, and, after storage, which generally lasts several hours, additional water is often added before milling (Willm and Jollet 1994). The optimal amount of water, as well as tempering time, differs also according to characteristics of the grain (Moss 1973, Bass 1988, Dexter and Martin 1993). When used, heat is also an important factor, with the mode of application varying as to the process (Ziegler and Greer 1971, Willm and Jollet 1994). Conditioning influences not only milling quality (Hook et al 1982a,b; Bolling 1987) but also the technological quality of the final flour product (Ziegler and Greer 1971, Cretois 1990).

The primary aim of conditioning is to change the mechanical characteristics of the different tissues of the kernel, and thereby improve the separability of the endosperm from the outer layers of the grain, notably the bran. The addition of water also triggers a number of biochemical events in the kernel, thereby modifying characteristics of its components. These modifications can be amplified by increasing the temperature and the moisture content.

The recent finding that disulfide groups of endosperm proteins become reduced during germination (Kobrehel et al 1992) raises the question as to whether changes of this type take place during conditioning. Here we report results which show that the sulfhydryl content of kernel proteins, including storage proteins, undergoes significant increase during conditioning. In view of the role generally attributed to sulfhydryls and disulfides in the processing of flour into bread, the observed changes may influence the quality of the final product. Thus, the way in which wheat is conditioned may play a more important role in determining the technological quality of flour than previously thought.

MATERIALS AND METHODS

Plant Materials and Chemicals

A French wheat cultivar (*Triticum aestivum* L., cv. Arbon) was used in this study. Monobromobimane (mBBr) was obtained from Sigma Chemical Co. (St. Louis, MO), acrylamide and bisacrylamide from BDH (Poole, England), TEMED from Kodak, Inc. (Rochester, NY) and ammonium persulfate from Merck Chemical Co. (Darmstadt, Germany). All other chemicals were obtained from commercial sources and were of the highest quality available.

Conditioning and Milling

Grain was conditioned at room temperature. After adding water to a moisture content of 16%, samples were mixed and then stored for 12 hr. Just before milling, the moisture content of the samples was increased to 16.5%. Grain was milled in a Buhler pilot mill under standard conditions. Four flour streams were collected. Analyses were performed either on the resultant flour or on conditioned grain from which the embryo and the endosperm had been manually separated.

Extraction and mBBr Fluorescent Labeling of Proteins

Total protein was extracted from wheat kernels in the presence of Na-tetradecanoate (80 mg/g) in distilled water (8 ml/g) as previously described (Kobrehel et al 1992). For direct labeling of available sulfhydryl groups, mBBr was added at the beginning of the extraction, (final concentration, 100 nmol). Samples were extracted at room temperature for 2 hr and then centrifuged at 27,000 × *g* for 10 min. Sulfhydryl groups were fluorescently labeled by the method of Crawford et al (1989) as modified by Kobrehel et al (1991). To the mBBr-labeled protein extracts, 10 ml of 10% sodium dodecyl sulfate (SDS) and 10 µl of 100 m*M* 2-mercaptoethanol were added to derivatize excess mBBr. The labeled samples were then applied to gels for electrophoretic analysis.

SDS-Polyacrylamide Gel Electrophoresis Analysis

SDS-polyacrylamide gel electrophoresis (PAGE) of the crude protein extracts and mBBr-derivatized proteins was performed at pH 8.5 in 10% gels of 1.5 mm thickness that were developed for ≈14 hr at a constant current of 8A (Laemmli 1970). Electrophoresis was terminated when the solvent front, indicated by a bromophenyl blue tracking dye, migrated to ≈1 cm from the bottom of the gel.

Protein Fixing and Fluorescence Photography

Following electrophoresis, gels were placed in 12% (w/v) trichloroacetic acid for 30 min to fix the proteins and then transferred to a solution of 40% ethanol and 10% acetic acid for 4–10 hr to remove excess mBBr (Kobrehel et al 1992). The fluorescence of protein-bound mBBr was visualized by placing gels on a light box fitted with a UV light source (365 nm), and photographed with Polaroid positive-negative film, type 55, through a

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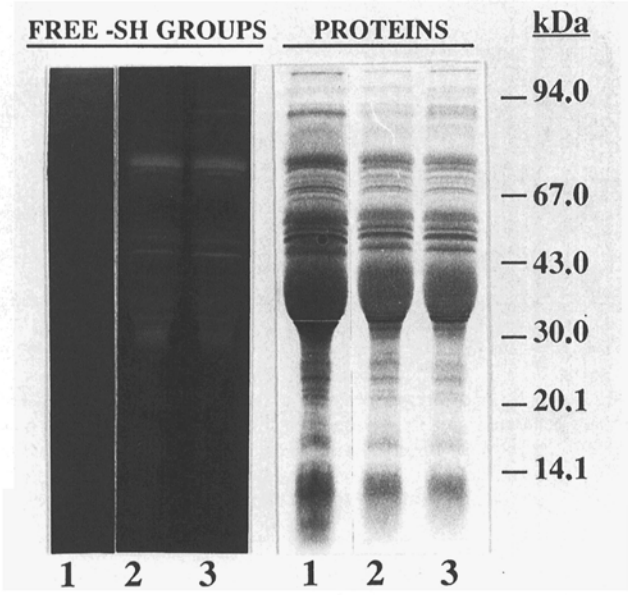

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of proteins extracted with Na-tetradecanoate. Proteins were labeled with mBBr and then were stained with Coomassie blue. Lane 1: total proteins of dry kernels; 2: proteins extracted from freshly milled flour; 3: proteins extracted from flour stored for three months. The relative extent reduction for treatments 1–3 was 0, 44, and 100%, respectively.

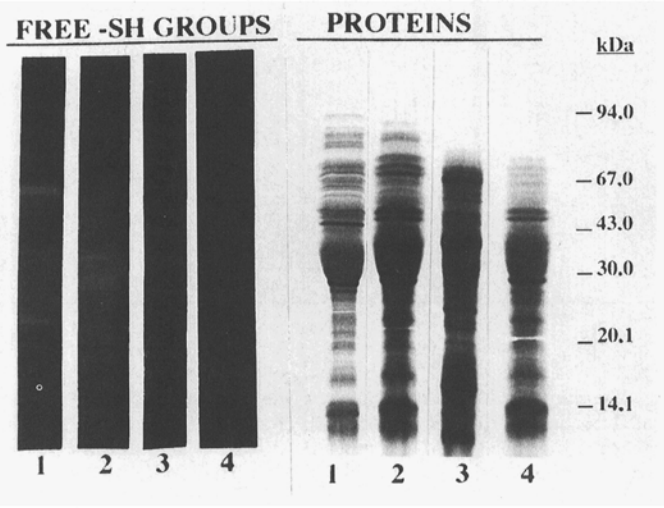

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of kernel proteins extracted with Na-tetradecanoate. Proteins were labeled with mBBr and then stained with Coomassie blue. Lane 1: proteins extracted from whole dry kernels; 2: from the flour of dry kernels; 3: from the embryo of dry kernels; 4: from the bran of dry kernels. The extent reduction for all treatments was too low for reliable scanning measurements.

yellow Wratten gelatin filter N 8 (cutoff = 460 nm) with an exposure time of 4–6 sec (Crawford et al 1989, Kobrehel et al 1991).

Protein Staining and Photography of Gels

After SDS-PAGE, both mBBr labeled and unlabeled proteins were stained for 2 hr with 0.1% (w/v) Coomassie brilliant blue R-250 in a solution of 10% acetic acid and 25% isopropanol. After destaining overnight in 10% acetic acid, gels were photographed with black and white film (Ilford PAN F iso 50/18). The negatives of the fluorescence gels were scanned with a laser densitometer (Pharmacia-LKB Ultrascan XL). Fluorescence was quantitated by integrating the area under all of the peaks (Kobrehel et al 1991).

RESULTS

Change in Sulfhydryl Content during Conditioning and Milling

Previous results have shown that the content of sulfhydryl groups of the major proteins in mature dry grain of different wheat cultivars was low (Kobrehel et al 1992, Gobin et al 1993). A follow up study in our laboratory, based on flour samples of different origins showed that the level of available sulfhydryl groups (accessible to mBBr without reduction or denaturation of the proteins) was higher in flour than in the parent kernel endosperm.

A study was initiated to explain this difference. The level of available sulfhydryl groups was determined sequentially in proteins extracted from mature dry kernels, freshly milled flour and flour stored for three months at 4°C. To avoid possible oxidation of the sulfhydryl groups during protein extraction, labeling was accomplished by the direct mBBr method described above. Under these conditions, free sulfhydryl groups are derivatized as the proteins enter solution (Gobin et al 1993). It should be noted that, with this labeling protocol, the intensity of each fluorescent protein band is proportional to the content of accessible sulfhydryl groups following electrophoretic separation (Kobrehel et al 1991, O'Keefe 1994). To our knowledge, a study of this nature has not previously been performed on wheat.

The results show that when tested in either fresh or stored flour, the proteins contained a considerably higher level of sulfhydryl groups than did the parent fraction obtained from the dry kernel (Fig. 1). The extract, which consistently contained about 80% of the total flour protein, consisted of the major protein groups (Fig. 1). The results are in accord with earlier amperometric titration studies in which free sulfhydryl groups were detected in the water soluble fraction of freshly milled flour (Yoneyama et al 1970a,b). Similar overall minor differences were observed in the redox status of the sulfhydryl-disulfide groups of fresh flour and samples stored for three months. However, since the focus of this study was on the change in the status of sulfhydryl groups during conditioning and milling, the influence of storage time was not systematically investigated.

Milling of Dry Grains

When wheat grain is crushed into small particles during milling, its contents are exposed to oxygen. Moreover, in concert with passage through the rolls, the kernels and derived particles undergo heat shock (Ziegler et al 1971, Hook et al 1982a). As a consequence of these changes, one would expect to see an oxidation rather than a reduction of flour components as has, for example, been documented for unsaturated lipids (Clayton and Morrison 1972, Bellenger and Godon 1972, Berger 1982). Milling experiments performed with dry grains, i.e., without prior conditioning by adding water, were in accord with this conclusion. As determined by mBBr/SDS-PAGE analysis, no reduction of disulfides took place during milling, indicating that neither the mechanical disruption nor the heat generated by the rolls is capable of reducing disulfide groups. In this case, the proteins recovered in each of the four flour streams remained in the same oxidized state as in the mature, dry kernel (Fig. 2). On the basis of these results, it was concluded that the reduction of the protein disulfides occurred before the milling step, i.e., during conditioning.

Change in Sulfhydryl Content during Conditioning

In the these experiments, water was added as for grain conditioned for pilot milling. After 12 hr of conditioning, the major parts of the kernel (bran, endosperm, embryo) were manually separated. Different components were extracted with Na-tetradecanoate and the sulfhydryl groups of the proteins were labeled with mBBr. The results showed that the effect of conditioning is similar to that observed in the early stages of germination (Kobrehel et al 1992). That is, specific disulfide groups of proteins of the different parts of the kernel (endosperm, embryo, and bran) became reduced

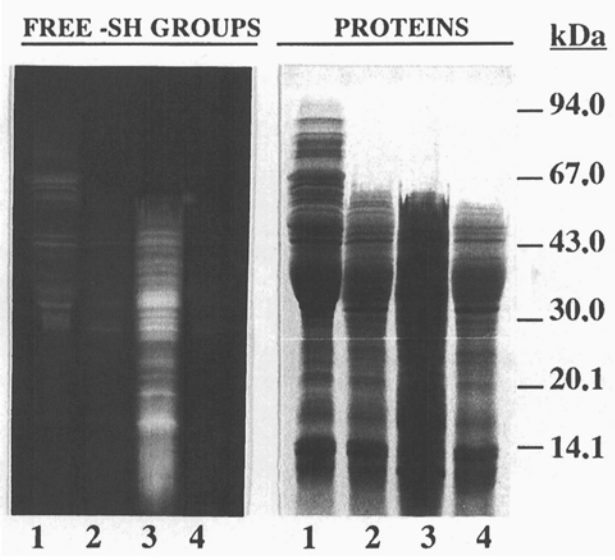

Fig. 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of kernel proteins extracted with Na-tetradecanoate after conditioning. Available sulfhydryl groups of the proteins were labeled with mBBr, then proteins were stained with Coomassie blue. Lane 1: proteins extracted from whole kernels; 2: flour; 3: embryo; 4: bran. The relative extent reduction for treatments 1–4 was 44, 23, 100, and 20%, respectively.

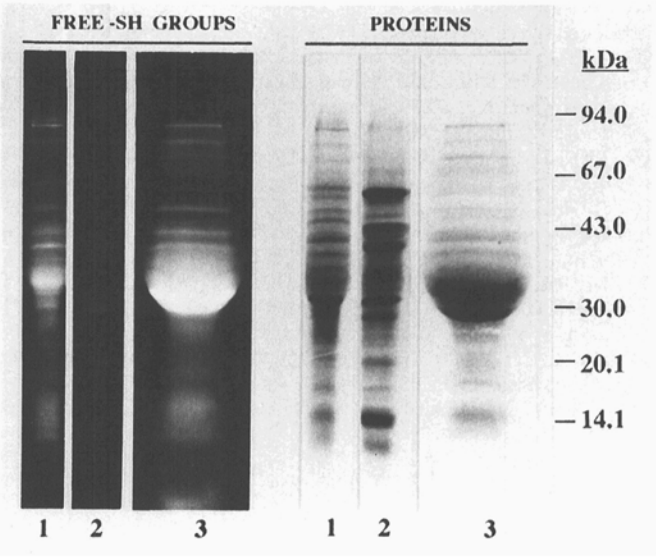

Fig. 4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of proteins fractions from whole, conditioned kernels. The free sulfhydryl groups of the proteins were labeled with mBBr, then proteins were stained with Coomassie blue. Lane 1: total proteins extracted with Na-tetradecanoate; 2: albumin-globulin fraction; 3: storage proteins (gliadin plus glutenin). The relative extent reduction for treatments 1–3 was 83, 0, and 100%, respectively.

(Fig. 3). An especially high rate of reduction was observed for the embryo, perhaps owing to its high metabolic activity. Figure 3 illustrates also the differences in protein composition between bran, embryo, and endosperm.

These findings suggest that different proteins of the endosperm undergo reduction during conditioning (Fig. 1). Results obtained by separating the protein fractions confirmed that gliadins, as well as the high and low molecular weight glutenins, became partially reduced during conditioning (Fig. 4). The reduction of the storage proteins is of particular interest because of their well-recognized importance in the technological quality of wheat flour. Because of their known high reactivity, the available sulfhydryl groups contributed by conditioning could play a role in the processing of flour for bread and pasta making.

DISCUSSION AND CONCLUSIONS

The fragmentation of the endosperm that results from milling is accompanied by exposure of its constituents to air. Consequently, an oxidation of lipid components, such as unsaturated fatty acids, has depended on the conditions used for storing the milled products (Bellenger and Godon 1972, Clayton and Morrison 1972, Berger 1982). The present results show that, before milling, i.e., during conditioning, disulfide groups of proteins, including storage proteins of the endosperm, become reduced and remain reduced in the flour even after lengthy storage. How such a large fraction of the sulfhydryl groups generated during conditioning can remain reduced in stored flour is an open question. It is possible that lipids or other substances associate with wheat proteins and in this way play a protective role (Kobrehel and Sauvaire 1990).

The results of the present study also show that minor modification of the sulfhydryl-disulfide status of flour proteins occurred in samples stored at 4°C. Altering the conditions of storage, e.g., higher temperatures and longer storage time, could enhance these changes. Furthermore, the presence of even low levels of germ fragments may influence flour properties in view of the high level of sulfhydryl groups in embryo proteins after conditioning.

In light of the results presented here, investigations on the redox status of the sulfhydryl-disulfide groups of flour during storage could increase our understanding of the aging process. Further studies are also needed to determine possible varietal differences in the capacity of grain proteins to change during conditioning. The status of the disulfide groups in the storage proteins, especially individual glutenin subunits considered important to flour quality, is of special interest.

In conclusion, the present results demonstrate that the sulfhydryl content of proteins recovered in flour increases during conditioning and milling, in particular at the conditioning step. A better understanding of these changes could not only increase our understanding of breadmaking quality, but also open the door to enhancing the technological quality of flour by altering the milling or flour aging process.

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