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Characterization of Sulfur Speciation in Low Molecular Weight Subunits of Glutenin after Reoxidation with Potassium Iodate and Potassium Bromate at Different pH Values Using X-ray Absorption Near-Edge Structure (XANES) Spectroscopy

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Sulfur speciation in low molecular weight (LMW) subunits of glutenin after reoxidation with potassium iodate and potassium bromate at different pH values, aged subunits of glutenin as well as gluten, and gliadin have been investigated in situ by S K-edge X-ray absorption near-edge structure (XANES) spectroscopy. XANES spectra were analyzed quantitatively using a least-squares fitting routine to provide relative percentage contribution of different sulfur species occurring in the samples. Using potassium iodate and potassium bromate for reoxidation of reduced LMW subunits of glutenin led not only to disulfide states but also to higher oxidation states (sulfoxide state, sulfonic acid state). Strongest oxidation occurred at low pH values. Higher oxidation states were also predominantly detected in the aged subunits of glutenin, whereas the disulfide state was the main sulfur species in gluten and gliadin samples. The results showed that the oxidation state of sulfur prior to oxidation (thiol, disulfide) strongly influences sulfur speciation after oxidation. The choice of the oxidizing reagent seems to be of minor importance.

KEYWORDS: LMW subunits of glutenin; X-ray absorption near-edge structure (XANES) spectroscopy; quantitative analysis; sulfur K-edge; wheat gluten

INTRODUCTION

Gluten proteins mainly determine the functional properties of doughs and the baking quality of wheat. They have been classified into sulfur-rich, sulfur-poor, and high molecular weight (HMW) groups by their molecular weight and amino acid sequences (*1*). The α- and γ-gliadins and the low molecular weight (LMW) subunits of glutenin (B- and C-types) form the first group (sulfur-rich) and the ω-gliadins and the D-type LMW subunits of glutenin the second group (sulfur-poor) (*1*, *2*).

Cysteine-containing components are able to form HMW aggregates linked by intermolecular disulfide bonds and have thus a key role for functionality of doughs. Whereas ∼95% of the cysteine residues of gluten protein compounds occur as inter- or intramolecular disulfides in freshly prepared flour, only 5% appear in the thiol form (*2*, *3*). Sulfur in the thiol form might catalyze thiol−disulfide exchange reactions during dough mixing. In the gluten network, elasticity is determined by intermolecular disulfide bonds within the glutenins, whereas viscosity is determined by the monomeric gliadin fraction, containing exclusively intramolecular disulfide bonds. In particular, the number and the amount of subunits belonging to the LMW group (B- and C-type LMW subunits of glutenin) are correlated significantly with the extensibility of doughs as shown by extensograph measurements (*4*). The influence of sulfur fertilization on bread-making quality was published recently showing that application of sulfur reduced the dough resistance and increased the extensibility (*5*).

The total cysteine content of gluten protein components can be determined by amino acid analysis, for example, after oxidation with performic acid, but a distinction between cysteine and cystine is not possible by this method (*6*). Free SH and SS

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groups can be determined spectrophotometrically prior to and after reduction according to the methods of Ellman (*7*) and Henschen (*8*). The identification and localization of cysteine and cystine residues are, however, only possible after labeling of SH groups and subsequent partial enzymatic hydrolysis of gluten proteins and sequence analysis of the relevant peptides (*6*, *9*).

The most important disadvantage of all these methods is the limited information about the oxidation state of sulfur as only the SH and SS forms can be determined and structural changes may have occurred. Therefore, X-ray absorption near-edge structure (XANES) spectroscopy at the sulfur K-edge—as an in situ method—was applied to characterize the speciation of sulfur in gluten proteins on the basis of native samples. XANES spectroscopy at the sulfur K-edge using synchrotron radiation is an excellent tool to investigate the speciation of sulfur in biological samples (*10*−*13*). A more detailed insight into the method (including theoretical and technical details) and a topical overview over the broad range of scientific questions of biological, environmental, and agricultural research that were recently tackled and investigated using X-ray absorption spectroscopy are provided by Prange and Modrow (*14*).

A main advantage of XANES spectroscopy is that the method is nondestructive and measurements can be conducted in situ. It allows the determination of the valence of an excited atom and of the electronegativity of neighboring atoms (*15*). Because ab initio calculations of the XANES region are complicated and reliable results are still difficult to obtain, structural information is often deduced by a "fingerprint method". The near-edge spectrum of the sample to be analyzed is compared with those of suitable model compounds yielding "qualitative information". The successful application of XANES spectroscopy as a fingerprint method has been demonstrated (*16*, *17*). However, to describe spectral features quantitatively, a "quantitative analysis" can also be performed. The fact that only the local environment of the absorbing atoms is probed implies that the spectrum of a mixture of substances A and B can be composed from the spectra A and B measured separately. This additivity is the basis for the quantitative analysis of XANES spectra, which means the decomposition of a sum spectrum into its components. A "quality function" defined by the difference between experimental data and a linear combination of spectra contained in a basis set can be minimized to achieve the decomposition of the sum spectrum (e.g., refs *12* and *18*). A detailed verification and successful application of the quantitative analysis can be found, for example, in refs *12* and *19* using defined mixtures of different sulfur substances.

By using XANES spectroscopy as a fingerprint method and by comparing the obtained spectra with those of reference compounds, we recently found evidence for the presence of higher oxidation states (sulfoxide, sulfonate) in LMW and HMW subunits of glutenin reoxidized with potassium bromate at pH 2 and in glutenin subunits stored under ambient air and temperature conditions (*20*). Potassium bromate and potassium iodate, which have a positive effect on the dough structure after mixing, have been widely used for breadmaking, especially in the United States and the United Kingdom (*21*−*23*). It is accepted that these oxidizing agents promote the oxidation of cysteine to the disulfide state (*24*, *25*). The presence of oxidation states higher than disulfide, however, is not in accordance with the traditional point of view (*26*). To clarify the occurrence of higher oxidation states and their amounts in reoxidized subunits of glutenin by potassium bromate and potassium iodate in subordination to different pH values, the present study aimed at providing a quantitative description of the sulfur speciation using a least-squares fitting routine to determine possible contributions of different reference compounds to the XANES spectra.

MATERIALS AND METHODS

LMW and HMW Subunits of Glutenin. LMW and HMW subunits of glutenin were extracted from the cultivar Rektor using a modified method according to Melas et al. (*27*). The residue (glutenins) was extracted stepwise with 50% (v/v) aqueous 2-propanol containing Tris-HCl (0.08 mol/L, pH 8) and dithiothreitol (1.0%) under nitrogen at 60 °C after removal of albumins, globulins, and gliadins with 60% (v/v) aqueous ethanol. After centrifugation, HMW subunits of glutenin were precipitated by the addition of acetone to a final concentration of 40% (v/v), whereas LMW subunits of glutenin remained in solution. All fractions were dialyzed against nitrogen-saturated acetic acid (0.01 mol/L) and freeze-dried. LMW subunits of glutenin were reoxidized with potassium bromate ($KBrO_3$) and potassium iodate (KIO_3), respectively, at different pH values (pH 2, 4, 6, and 8) according to the method of Antes and Wieser (*28*). Furthermore, stored LMW and HMW subunits of glutenin (stored for 2 years under ambient air and temperature conditions) as well as commercial gluten and commercial gliadin (Sigma, Deisenhofen, Germany) were analyzed. To prepare the samples for XANES measurements, 20 mg of freeze-dried subunits of glutenin, gluten and gliadin, respectively, was pressed to a thin homogeneous film.

X-ray Absorption Spectroscopy—Experimental. The XANES spectra were recorded at beamline BN3 using synchrotron radiation of the electron stretcher accelerator (ELSA) of the Institute of Physics, Bonn, Germany (*29*). The storage ring was operated at an energy of 2.3 GeV with electron currents between 70 and 20 mA. The synchrotron radiation was monochromatized by a modified Lemonnier-type double-crystal monochromator (*30*) equipped with InSb (111) crystals. The monochromatic flux rate per second was some 10^9 photons (at 50 mA). The measurements were performed in transmission mode with ionization chambers (filled with 60 mbar of air), measuring the beam intensities in front of and behind the sample. For energy calibration of the spectra, the S K-edge XANES spectrum of zinc sulfate was used as a "secondary standard". The maximum of the first resonance (so-called white line) was set to an energy of 2481.40 eV. According to the step width, this value is reproducible to ±0.09 eV (±1 step). Spectra were scanned with step widths of 0.6 eV in the pre-edge region between 2450 and 2460 eV, 0.09 eV between 2460 and 2490, and 0.2 eV between 2490 and 2510 eV according to the spectral features of interest and with an integration time of 1000 ms per point. Further details of the experimental setup have been published previously (*31*). A linear background determined in the pre-edge region was subtracted from the raw data to correct for the absorption from higher shells and from supporting materials. Spectra were normalized at 2510 eV, where the variation of the absorption cross section is small.

Reference Compounds. Glutathione (γ-L-glutamyl-L-cysteinylglycine; oxidized and reduced forms), methionine, dimethyl sulfoxide, cysteic acid, and zinc sulfate were used as reference compounds. These compounds were of reagent grade, purchased from Sigma, and used as received. Solids were ground to fine powder of ∼1 μm particle size and put homogeneously on a self-adhesive Kapton film available from CWC Klebetechnik (Frankenthal, Germany). Dimethyl sulfoxide was pipetted on a sulfur-free filter paper for measurements. The thickness of the samples was optimized in order to avoid possible thickness as well as pinhole effects.

Quantitative Analysis of XANES Spectra. To analyze the XANES spectra quantitatively, the interactive fitting and plotting package Mn-Fit 4.04/15 (available at http://zina06.physik.uni-bonn.de/∼brock/mn_fit.html) was used. Mn-Fit 4.04/15 uses the minimization tool "MINUIT", which is part of the CERNlib available at CERN-European Organization for Nuclear Research (http://wwwinfo.cern.ch/asdoc/minuit/node2.html) to fit histograms or data. MINUIT provides tools

Table 1. Results of Fitting the Sulfur K-Edge XANES Spectra of Different LMW and HMW Subunits of Glutenin, Gluten, and Gliadin (cf. **Figures 4**, **6**, and **7**) to the Sum of Different Reference Spectra (cf. **Figure 2**)[a]

	percentage contribution of sulfur speciation					
	C–S–H (GSH[b])	C–S–S–C (GSSG[b])	C–S–C (methionine)	C–SO–C (dimethyl sulfoxide)	C–SO_3–C (cysteic acid)	SO_4^{2-} (zinc sulfate)
LMW[c] (bromate, pH2)	4	39	7	10	39	–
LMW[c] (bromate, pH 4)	17	53	–	2	27	–
LMW[c] (bromate, pH 6)	19	60	–	2	18	–
LMWc (bromate, pH 8)	14	43	36	–	5	–
LMW[c] (iodate, pH 2)	21	44	–	1	33	–
LMW[c] (iodate, pH 4)	22	44	–	1	33	–
LMW[c] (iodate, pH 6)	32	55	–	–	13	–
LMW[c] (iodate, pH 8)	14	49	32	–	4	–
LMW[c] (reduced)	40	7	53	–	–	
HMW[d] (aged)	–	5	9	6	79	–
LMW[c] (aged)	–	4	11	8	76	–
gluten	12	65	24	–	–	–
gliadin	17	71	13	–	–	–

[a] Reference compounds for the different sulfur species are shown in parentheses. Error: ≤±10%; –, contribution <0.1 %. [b] GSH, reduced glutathione; GSSG, oxidized glutathione. [c] LMW subunits of glutenin. [d] HMW subunits of glutenin.

to find the minimum value of a multiparameter function and to analyze the shape of the function around the minimum. In this study, we used a set of six reference spectra (reduced and oxidized glutathione, methionine, dimethyl sulfoxide, cysteic acid, and zinc sulfate) and a χ^2 criterium to find the linear combination of these spectra that reproduces the XANES spectrum of the LMW subunits with the highest probability. Nonstatistical errors may occur from deviations in energy calibration, an incomplete set of reference spectra, or reference spectra that do not monitor exactly the local environment of sulfur. The errors of the percentage contributions of sulfur (**Table 1**) can be estimated as <±10% (absolute value) (*19*, *12*). As an example, we provide in **Figure 1** χ^2 plots (i.e., the change of the goodness-of-fit parameter with a change of the parameter value) and a contour plot for reduced glutathione and methionine (i.e., the characterization of the parameter space within which a change in the χ^2 value of ≤0.1 is induced by variation of this pair of parameters while allowing for reoptimization of the remaining variables) as additional statistical information for the spectrum of LMW subunits of glutenin (reduced, without reoxidation) (cf. **Figures 4e**, **5**, and **6e**). This is a critical case, because potential linear dependence in the set of reference spectra affects mainly the references that play an important role in this fit.

RESULTS AND DISCUSSION

Following our earlier investigations probing the speciation of sulfur in different gluten proteins (gliadin and LMW and HMW subunits of glutenin) and stored glutenin subunits using XANES fingerprints (*20*), it was one goal of this study to investigate the influence of the oxidizing agents potassium bromate and potassium iodate, respectively, on the speciation of sulfur in LMW subunits of glutenin that were reoxidized at different pH values (pH 2, 4, 6, and 8). A second goal was to analyze the XANES spectra quantitatively to obtain the relative percentage contribution of different sulfur species (see above, **Table 1**). First, a broad variety of reference compounds (biologically important compounds, e.g., cysteine and cystine or different sulfonates) for sulfur in different speciations was measured, and their respective relevance was tested in different combinations for the quantitative analysis. The S K-edge XANES spectra of compounds that seem to be representative for the system "gluten proteins" and yield the best fits (reduced glutathione, oxidized glutathione, methionine, dimethyl sulfoxide, cysteic acid, and zinc sulfate) were used for fitting the S K-edge XANES spectra of the different samples to specify the forms of sulfur in a quantitative manner. The S K-edge XANES spectra of these compounds are shown in **Figure 2**. In general, it should be noted that these reference compounds are used only as representatives for a given class of an atomic environment of the sulfur atom. For instance, zinc sulfate was used as reference compound. Obviously, zinc sulfate crystals are not present in the gluten proteins. However, this compound was used as a proxy for sulfate anions, which indicates the presence of sulfate in general. It should be noted that this rough approach can induce problems, as the sensitivity of the S K-XANES to the exact nature of the surrounding chemical environment is extremely high, as discussed in more detail by Chauvistré et al. (*32*) and von Busch et al. (*33*). Interestingly, a set of reference compounds including cysteine and cystine instead of reduced and oxidized glutathione yields considerably worse agreements (data not shown), although both groups have similar atomic environments in the direct vicinity of the sulfur atom. Due to the considerable sensitivity of S K-edge XANES spectra with respect to higher coordination shells (*32*, *33*), the corresponding XANES spectra show significant differences from each other not only in the area of the shape resonances (≈2474−2480 eV) but also with respect to splitting and relative amplitudes of the transitions, which contribute to the white line, as demonstrated in the spectra of the oxidized species (**Figure 3**). In fact, the improved fitting result that is obtained when using reduced and oxidized glutathione can be correlated to the fact that glutathione is a tripeptide and thus resembles the sulfur-containing structures which are likely to be encountered in the gluten network more than a "single amino acid" possibly can. Still, this compound might not represent an optimized model of the encountered structures, as in all cases in which the "thioether (C−S−C) species" becomes dominant; that is, when a small deviation from the actual structure can induce a significant deterioration of the χ^2 value, an additional contribution of the methionine spectrum is observed in the fit (**Table 1**).

Reoxidation of LMW Subunits of Glutenin with Potassium Bromate. In **Figure 4** the S K-edge XANES spectra of LMW subunits of glutenin reoxidized with potassium bromate at different pH values (a−d) and the S K-edge XANES spectrum of LMW subunits of glutenin (without reoxidation) (e) are shown together with their corresponding MINUIT fits; in **Figure 5** residual of the fit and the respective accordingly weighted reference spectra are shown for the example of LMW subunits of glutenin (without reoxidation). Whereas it is *prima specie*

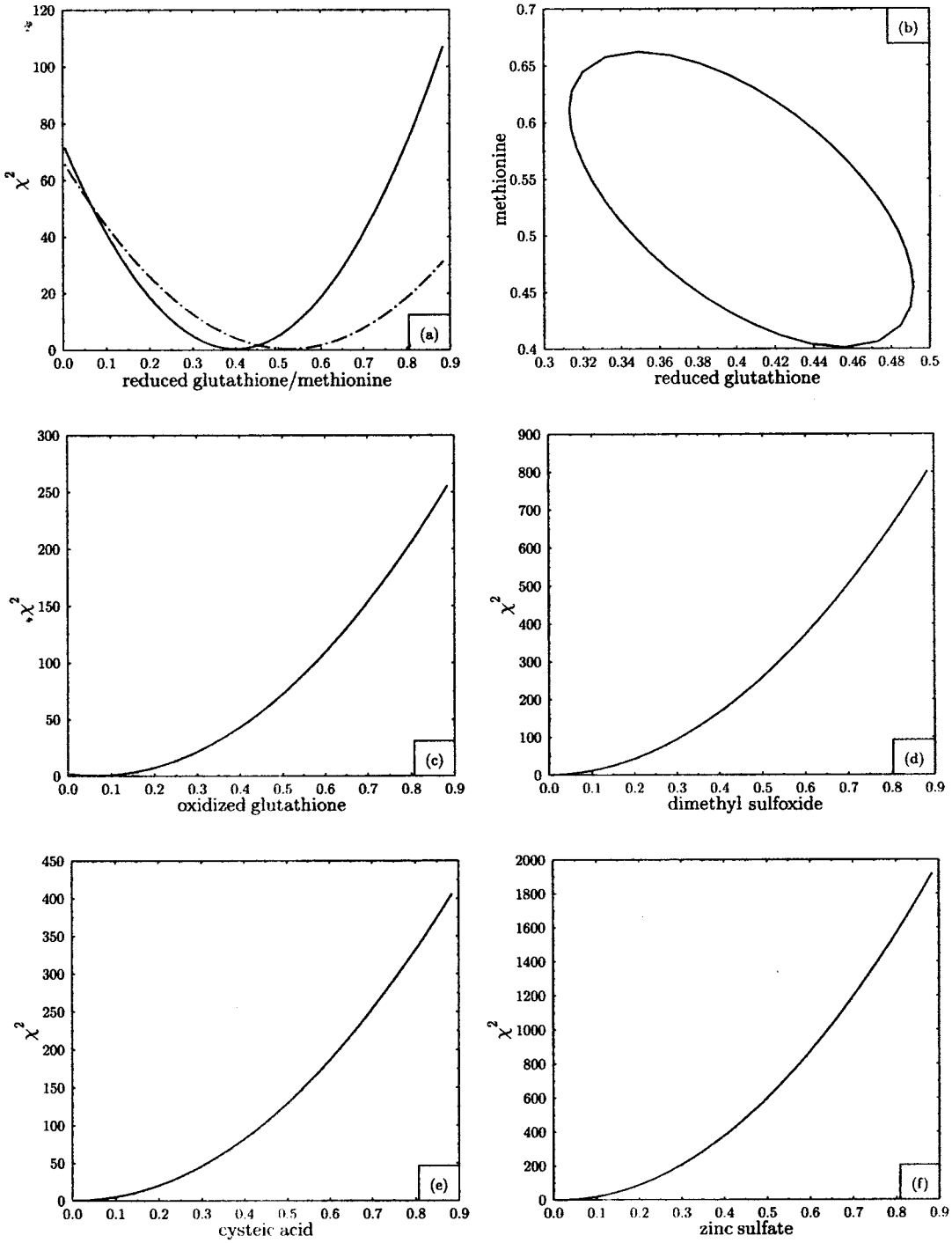

Figure 1. Statistical information on the fit of LMW subunits of glutenin (without reoxidation): (**a**) χ^2-fit of reduced glutathione (solid line) and methionine (dash-dot line); (**b**) contour plot of these parameters as discussed under Quantitative analysis of XANES Spectra; (**c**) χ^2-fit of oxidized glutathione; (**d**) χ^2-fit of dimethyl sulfoxide; (**e**) χ^2-fit of cysteic acid; (f) χ^2-fit of zinc sulfate.

evident from the small width and energy position of the corresponding white line that in LMW subunits of glutenin which were not reoxidized hardly any disulfides (disulfide bridges) are present, such a straightforward approach fails, for example, for the spectra measured at pH 4, 6, and 8 (**Figure 4a**−**c**). However, using the quantitative analysis, it is possible to extract detailed information on the relative contributions of mono- and disulfides, respectively. As assumed above, sulfur in LMW subunits of glutenin, which were not reoxidized, occurs predominantly in a "reduced state" (≈93%) and to only a minor extent in a disulfide state (≈7%) (**Table 1**). Other oxidation states, higher than the disulfide state, were not detected. Using potassium bromate for reoxidation of LMWs, however, leads to significantly higher percentages of sulfur in the disulfide state (≈39−60%). At pH 6, which is the pH present under usual baking conditions, reoxidation with potassium bromate was most effective, resulting in ≈60% of sulfur in the disulfide state. Furthermore, reoxidation led to oxidation of sulfur higher than the disulfide state. Sulfur in the sulfonic acid state could be observed at all pH values, whereas the sulfoxide state was detected in only the LMW subunits reoxidized at pH 2. The percentage of sulfur in the sulfonic acid state was ≈39% at

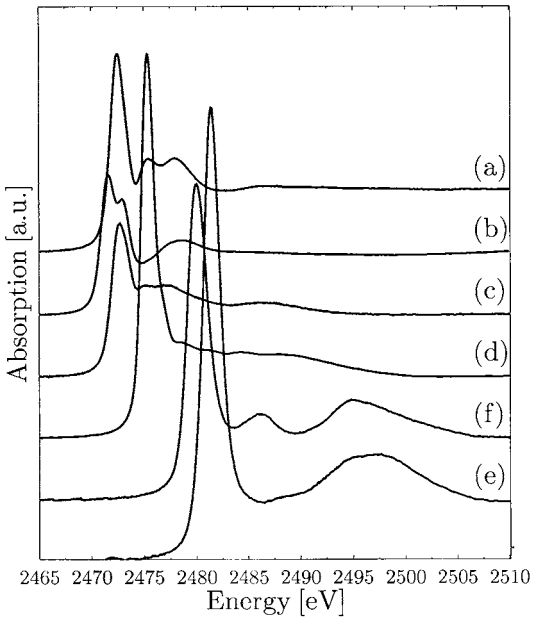

Figure 2. Sulfur K-edge XANES spectra of the reference compounds that were used for fitting XANES spectra (**Figures 4**, **6**, and **7**): (**a**) glutathione (reduced form); (**b**) glutathione (oxidized form); (**c**) methionine; (**d**) dimethyl sulfoxide; (**e**) cysteic acid; (**f**) zinc sulfate.

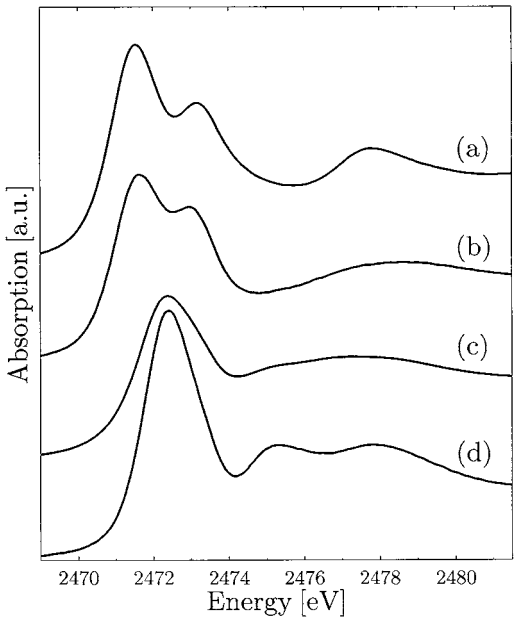

Figure 3. Sulfur K-edge XANES spectra of the reference compounds: (**a**) cystine; (**b**) glutathione (oxidized); (**c**) glutathione (reduced); (**d**) cysteine.

pH 2, ≈27% at pH 4, ≈18% at pH 6, and ≈5% at pH 8, thus showing strongest oxidation at low pH. The higher oxidation states most probably result from the oxidation of cysteine or cystine contributions of the complex gluten network. Sulfur in methionine would be oxidized to the sulfone state, which was neither observed in the spectra directly nor indicated by the quantitative analysis of the spectra when "sulfone" was included in the set of reference spectra, which supports our earlier findings (*20*).

The S K-edge XANES spectra of HMW subunits of glutenin reoxidized with potassium bromate at different pH values (spectra not shown) show the same features as those of LMW

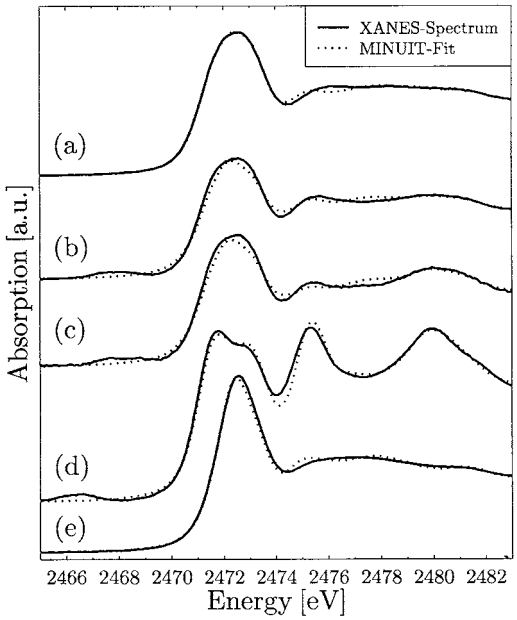

Figure 4. Sulfur K-edge XANES spectra of LMW subunits of glutenin: (**a**) reoxidized with potassium bromate at pH 8; (**b**) reoxidized with potassium bromate at pH 6; (**c**) reoxidized with potassium bromate at pH 4; (**d**) reoxidized with potassium bromate at pH 2; (**e**) LMW glutenin (reduced) and corresponding MINUIT fits.

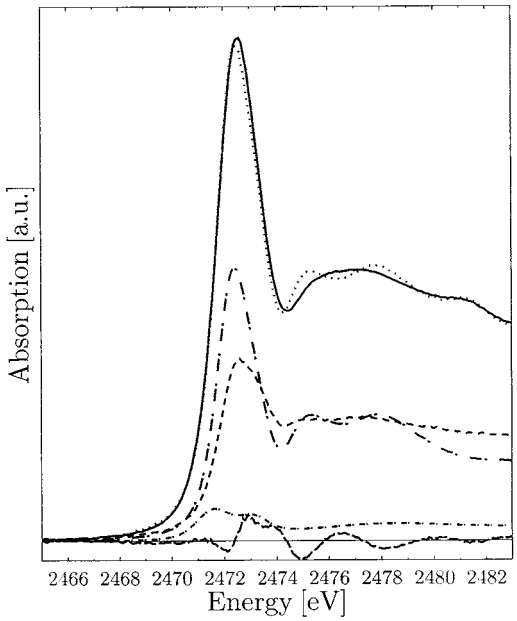

Figure 5. Sulfur K-edge XANES spectra of LMW subunits of glutenin (reduced, without reoxidation) (solid line), corresponding MINUIT fit (dot line), residuum (long dash line), weighted spectra of glutathione (reduced) (long dash and dot line), methionine (short dash line), and glutathione (oxidized) (short dash and dot line).

subunits of glutenin. However, the HMW spectra are weaker and noisier because of the lower sulfur content in HMW subunits of glutenin [cf., e.g., S K-edge XANES spectra of aged HMW (**Figure 7a**) and aged LMW subunits of glutenin (**Figure 7**)].

Reoxidation of LMW Subunits of Glutenin with Potassium Iodate. Corresponding to results shown in **Figure 4**, **Figure 6** shows the S K-edge XANES spectra of LMW subunits of glutenin reoxidized with potassium iodate at different pH values (a−d) and the S K-edge XANES spectrum of LMW subunits

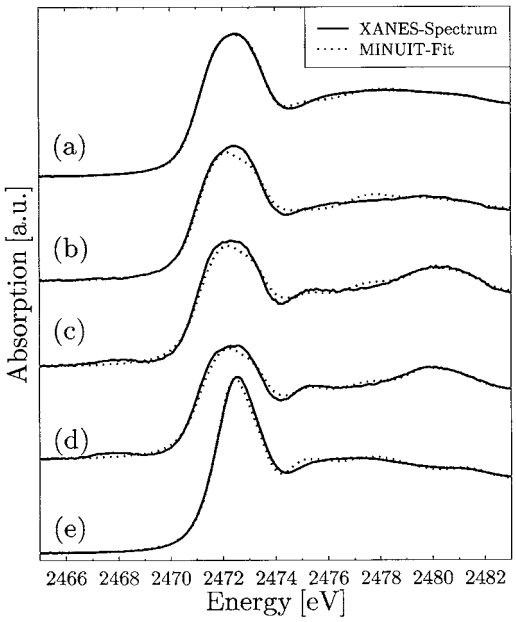

Figure 6. Sulfur K-edge XANES spectra of LMW subunits of glutenin: (**a**) reoxidized with potassium iodate at pH 8; (**b**) reoxidized with potassium iodate at pH 6; (**c**) reoxidized with potassium iodate at pH 4; (**d**) reoxidized with potassium iodate at pH 2; (**e**) LMW glutenin (reduced) and corresponding MINUIT fits.

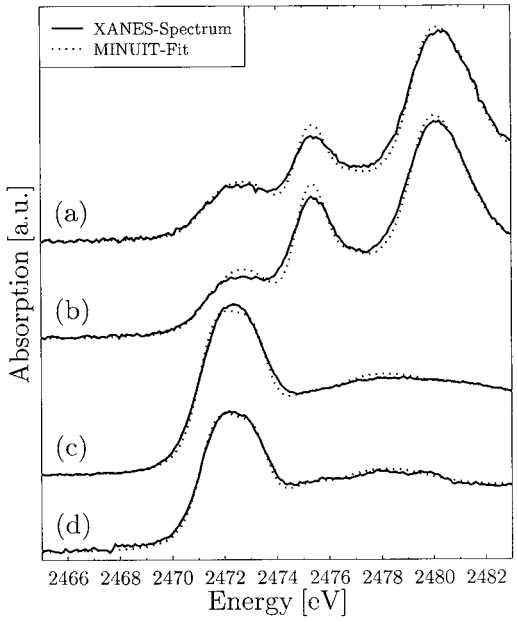

Figure 7. Sulfur K-edge XANES spectra of (**a**) aged HMW subunits of glutenin, (**b**) aged LMW subunits of glutenin (stored for 2 years under ambient air and temperature conditions), (**c**) gluten, and (**d**) gliadin and corresponding MINUIT fits.

of glutenin (without reoxidation) (e) together with their corresponding MINUIT fits. The results of the quantitative analysis are shown in **Table 1**. Reoxidation of LMW subunits with potassium iodate at the different pH values led to similar results as in the samples reoxidized with potassium bromate. However, when reoxidizing LMW subunits of glutenin, potassium iodate seems to be a weaker oxidizing agent than potassium bromate as shown by slightly lower quantities of sulfur in the sulfonic acid state, despite the higher velocity of oxidation in comparison with potassium bromate (*28*). Furthermore, oxidation to the sulfoxide state could not be detected. Interestingly, at pH 6 reoxidation with potassium iodate was also most effective, resulting in ≈55% of sulfur in the disulfide state.

Sulfur Speciation in Aged Subunits of Glutenin and Gluten Proteins. The S K-edge XANES spectra and their corresponding MINUIT fits of aged LMW (a) and aged HMW (b) subunits of glutenin (stored for 2 years under ambient air and temperature conditions) as well as those of gluten (c) and gliadin (d) are shown in **Figure 7**. As discussed earlier (*20*), air can have a remarkable effect on sulfur speciation of gluten proteins, leading to higher oxidation states detectable by XANES spectroscopy. The quantitative analysis (**Table 1**) proves this finding, indicating that ≈76−79% of sulfur is present in the sulfonic acid state and ≈6−8% in the sulfoxide state. It can be thus assumed that exposure of reduced glutenin subunits to air for long periods induces oxidation of thiol groups mainly to the sulfonic acid state; that is, cysteine is oxidized to cysteic acid and can therefore no longer participate in thiol−disulfide interchange reactions. However, it is not appropriate to make the same suggestion for stored flour, as only a minor portion of the cysteine residues is present in the thiol form (*2*, *3*).

The S K-edge XANES spectrum of aged HMW subunits is weaker and noisier in comparison with the spectrum of aged LMW subunits of glutenin, because of lower sulfur contents in the HMW subunits of glutenin (**Figure 7a**,**b**). Fitting of these spectra, however, yields comparable results for both. Correspondingly, reoxidation of reduced HMW and LMW subunits with potassium bromate and potassium iodate in solution showed no significant difference in the concentration of thiol groups after complete reoxidation (*28*).

The results of fitting the spectra of gluten and gliadin showed that sulfur is predominantly present in the disulfide state (≈65−71%) and at minor percentages in the reduced (C−S−H) and the thioether (C−S−C) states (≈30−36%) (**Table 1**). In fact, the increased width of the white line of the spectra shown in **Figure 7c**,**d** in comparison with the one in the reduced LMW subunits of glutenin (**Figures 4e**, **5**, and **6e**) indicates clearly that an additional transition contributes significantly to the S K-edge XANES spectra at an energy which is in the typical regimen of the S−S π^* excitation (*17*, *31*). This finding was already gained in our earlier study using XANES fingerprints (*20*) but could not be interpreted definitely and set in relation to chemical analysis of the amount of disulfides and thiol in flour as reported by Grosch and Wieser (*3*). Interpreting the spectra quantitatively using the fitting and plotting software MnFit 4.04/15, a significant amount of disulfide bridges (see above) in gluten proteins was detected, which is in good accordance with the chemical analysis of flours (*2*, *3*). It seems that reduced HMW and LMW subunits of glutenin stored under ambient air for a long time behave differently in comparison with gluten proteins and flours. An explanation might be the different amount and exposition of the cysteine residues present in HMW and LMW subunits of glutenin.

Quantitative XANES spectroscopy is a very suitable method to describe sulfur speciation in gluten proteins on the basis of native samples. The quantitative analysis of the XANES spectra proved the presence of higher oxidation states in glutenin subunits reoxidized with potassium bromate and potassium iodate, respectively, in dependence of different pH values and in aged subunits of glutenin. A relative percentage contribution of sulfur species for these samples as well as for gluten and gliadin was provided for the first time. To improve the quantitative analysis of XANES spectra of gluten proteins, tailor-

made reference substances, for example, peptides representing typical gluten structures, have to be used in further studies.

It has to be taken into consideration that the phenomena observed for the reoxidation of reduced, isolated glutenin subunits cannot be assumed for native flour or dough, which contain only a low amount of free thiol. Obviously, the presence of the disulfide state at the beginning of oxidation makes conversion to higher oxidation states more difficult. This is supported by the fact that in gluten and gliadin samples, which can be regarded as model substances for the situation in doughs, sulfur is predominantly present in the disulfide state. In contrast, oxidation starting from the thiol state primarily proceeds to oxidation states higher than disulfide. Reoxidation of reduced HMW and LMW subunits of glutenin might be regarded as an indication for this hypothesis. The choice of the oxidizing reagent (potassium bromate, potassium iodate, oxygen) seems to be less important than the oxidation state of sulfur (thiol, disulfide) prior to oxidation.

ABBREVIATIONS USED

XANES, X-ray absorption near-edge structure; LMW, low molecular weight; HMW, high molecular weight.

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