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Influence of Thiol Metabolism of Lactobacilli on Egg White Proteins in Wheat Sourdoughs

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In wheat sourdoughs, the degradation of gluten proteins is favored by acidification and reducing conditions. This study aimed to determine the proteolytic degradation of egg white proteins in wheat sourdoughs acidified with lactobacilli differing in their thiol metabolism. Ovotransferrin was the only major egg white protein that degraded during sourdough fermentations. An extensive degradation of ovotransferrin required a heterofermentative lactobacilli starter, *Lactobacillus sanfranciscensis*, with glutathione reductase activity. Ovotransferrin was more resistant to breakdown when sourdoughs were acidified with homofermentative lactobacilli or a mutant strain of *L. sanfranciscensis* lacking the glutathione reductase. Its susceptibility to proteolysis in *L. sanfranciscensis* sourdoughs is thus attributable to thiol accumulation by *L. sanfranciscensis*, which apparently altered the structure of ovotransferrin through a reduction of disulfide bonds. Proteolytic degradation of ovotransferrin was attributable to wheat aspartic proteinases. In addition to the susceptibility to proteolysis, other functional properties of egg proteins may be influenced by thiol-exchange reactions.

KEYWORDS: Egg white; wheat sourdough; ovotransferrin; lactobacilli; thiol metabolism; proteolysis; aspartic proteinases

INTRODUCTION

Sourdough fermentation is a prebaking process that is used mainly in wheat and rye baking. The use of sourdoughs in breadmaking improves the shelf life and sensory properties of bread (*1*). The metabolic activity of heterofermentative lactobacilli in sourdough generally results in acidification and a decrease of the redox potential in sourdoughs. Acidification is effected by the formation of lactate and acetate in the carbohydrate metabolism of lactobacilli. The redox potential is decreased particularly by the glutathione reductase activity of heterofermentative lactobacilli, which results in the accumulation of thiols in wheat sourdoughs (*2, 3*).

The breakdown of gluten proteins in wheat sourdoughs is among the key features that affect wheat dough rheology and bread quality. Gluten breakdown is favored by acidification because the solubility of gluten proteins increases at low pH (*4*), and the aspartic proteases of wheat grain operate optimally under acidic conditions (*5, 6*).

The depolymerization of gluten requires reduced thiol compounds. The most abundant reducing agent in wheat flour is glutathione (GSH), which undergoes thiol/disulfide interchange reactions with gluten proteins to produce reduced gluten proteins and oxidized glutathione (GSSG) (*7*). The reduction of GSSG to GSH by heterofermentative lactobacilli maintains high GSH levels throughout the fermentation and contributes to the depolymerization of gluten. The gluten depolymerization increases the solubility of gluten proteins and thus favors their proteolytic breakdown (*8*). Homofermentative lactobacilli lack glutathione reductase activity in wheat sourdoughs (*2*).

The nutritional and sensory quality of a wide variety of baked goods is improved by supplementing their recipes with egg whites. Egg whites contain roughly 10% of protein with excellent nutritional quality and unique technological properties. Particularly the foaming properties of egg white proteins and their ability to form heat-induced gels are relevant for improved structure formation in food and confectionery applications. Furthermore, egg white proteins possess many biological activities (antimicrobial, immunomodulatory, etc.) that may offer benefits for human health (*9*). Egg whites are composed of at least 15 protein classes. The major egg white proteins are ovalbumin (54% of protein content, 45 kDa), ovotransferrin (12–13%, 78 kDa), ovomucoid (11%, 28 kDa), and lysozyme (3.5%, 14 kDa) (*10*). All major egg white proteins are stabilized by disulfide bonds in their native state. Ovalbumin contains one disulfide bond and four thiol groups. Ovotransferrin contains 15 disulfide bonds but no free thiols. Lysozyme contains four disulfide bonds, and ovomucoid has nine disulfides but no free thiols (*11*).

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Although eggs are used in baking applications in combination with sourdough fermentation, little information is available on the fate of egg white proteins during fermentation. It was therefore the aim of the present study to determine the proteolytic degradation of egg white proteins in wheat sourdoughs. The experimental design aimed to take into consideration the effects of acidification and thiol accumulation by using chemically acidified doughs and strains of lactobacilli that differ in their redox activities.

MATERIALS AND METHODS

Strains and Growth Conditions. This study used *Lactobacillus sakei* LS8 (Institute of Food Technology, University of Hohenheim, Stuttgart, Germany), *Lactobacillus sanfranciscensis* DSM20451, and its isogenic mutant strain *L. sanfranciscensis* DSM20451 Δ*gshR* lacking the glutathione reductase gene (*3*). The strains were grown in a modified de Man, Rogosa, Sharpe medium (mMRS) broth containing the following ingredients per liter: 10 g of maltose, 5 g of glucose, 5 g of fructose, 10 g of peptone, 5 g of yeast extract, 5 g of beef extract, 4 g of $K_2HPO_4 \cdot 3H_2O$, 2.6 g of KH_2PO_4, 3 g of NH_4Cl, 0.5 g of L-Cys $HCl \cdot H_2O$, 1 g of Tween 80, 0.05 g of $MnSO_4 \cdot H_2O$, 0.2 g of $MgSO_4 \cdot 7H_2O$; and 0.2 mg each of biotin, folic acid, nicotinic acid, pyridoxal phosphate, thiamin, riboflavin, cobalamin, and pantothenic acid. Additionally, 10 mg of erythromycin (Sigma, Oakville, Canada) was added for cultivation of the *gshR* mutant strain. The pH of media was adjusted to 6.2. For solid media, 15 g of agar was added. The strains were grown at 30 °C, and solid media were incubated under modified atmosphere (4% O_2, 20% CO_2, 76% N_2).

Sourdough Ingredients and Fermentations. Hen's eggs and wheat flour with 0.55% ash were obtained at a local supermarket. Egg whites were separated by hand from the yolks. To prepare the microbial inoculum, strains were subcultured twice in mMRS. Cells from 10 mL overnight culture were washed twice with tap water and resuspended in 1 mL of tap water. Doughs were prepared by mixing 100 g of flour, 100 mL of water, and 1 mL of microbial inoculum ($\sim 10^{10}$ cells). In the first stage of fermentation, doughs were incubated at 30 °C for 20 h. The pH value of a chemically acidified dough was adjusted with acetic acid and lactic acid (1:4 v/v) to pH 3.5. After the first stage of fermentation, 150 g of wheat flour and three egg whites (~120 g) were added, and these egg white supplemented doughs were further incubated for 24 h (the second stage). Fermentation samples were taken in the beginning (0 h), after the first stage (20 h), immediately after the addition of egg whites (20 h + EW), and after the second stage of fermentation (44 h). Fresh sourdough samples were used for cell count, pH, and thiol analyses and freeze-dried samples for amino nitrogen and SDS-PAGE analyses. Cell counts were determined, and the absence of contaminations was verified by observation of uniform colony morphology. The *gshR* mutant strain of *L. sanfranciscensis* was plated on mMRS and mMRS containing 10 mg L^{-1} erythromycin to verify that the deletion plasmid was maintained during fermentation.

Determination of Amino Nitrogen. The amino nitrogen contents of sourdough samples were measured using a ninhydrin method (*12*). Briefly, freeze-dried sourdough samples were extracted 1:10 (w/v) with 100 mM sodium phosphate, pH 8.0, for 1 h at room temperature and centrifuged (10000*g*, 10 min, Eppendorf Centrifuge 5417C, Eppendorf AG, Hamburg, Germany). Of each diluted supernatant, 200 μL was mixed with 100 μL of ninhydrin color reagent [5 g of Na_2HPO_4, 6 g of KH_2PO_4, 0.5 g of ninhydrin (Sigma, Oakville, Canada), and 0.3 g of fructose in 100 mL of distilled water, pH 6.7] and heated in a boiling water bath for 16 min. After 20 min of cooling at room temperature, 500 μL of the dilution solution (0.2% KIO_3 in 40% ethanol) was added and the absorbance at 570 nm was recorded. Glycine was used as a standard. Results are presented as means ± standard deviation of duplicate analyses each from two independent fermentations.

SDS-PAGE Analysis of Egg White Proteins. Sourdough samples were extracted 1:10 (w/v) with 1 M NaCl/200 mM Tris-HCl buffer, pH 8.0, at room temperature for 1 h. After centrifugation (10000*g*, 10 min, Allegra 25R, Beckman Coulter GmbH, Krefeld, Germany), 50 μL of the supernatant was mixed with 50 μL of SDS-PAGE sample buffer containing 2% dithiothreithol, heated for 3 min at 100 °C, and analyzed by SDS-PAGE (*13*) using 12% Tris-HCl SDS-PAGE gels (Bio-Rad Laboratories, Hercules, CA). Results shown are representative for analyses of independent fermentations.

Measurement of Thiol Groups. The contents of thiol groups in sourdough fermentations were analyzed in the SDS- and water-soluble fractions of sourdoughs. For the water-soluble extracts the sourdough samples were extracted 1:10 (w/v) with 100 mM sodium phosphate buffer, pH 8.0, at room temperature for 1 h. For the SDS-soluble fractions, sourdoughs were extracted as above but using 50 mM sodium phosphate buffer, pH 6.9, containing 1.5% SDS. Supernatants of centrifuged extracts were used for thiol analysis following the procedure of Antes and Wieser (*14*) that uses 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (*15*). The DTNB solution (Ellman's reagent) contained 40 mg of DTNB (Sigma, Oakville, Canada) in 10 mL of 500 mM sodium phosphate, pH 7.0. Of the sample supernatant, 300 μL was mixed with 600 μL of 50% *n*-propanol in 50 mM sodium phosphate buffer, pH 8.0, and an addition of 30 μL of Ellman's reagent started the reaction. The reaction mixtures were incubated in the dark for 30 min, and the absorbance was read at 412 nm. Glutathione was used as a standard. Results are presented as means ± standard deviation of duplicate analyses each from two independent fermentations. Statistical significances were evaluated with Student's *t* test using SigmaPlot software.

Preparation of Wheat Enzymes. In vitro hydrolysis experiments were carried out using freeze-dried egg whites or ovotransferrin as substrates and a wheat enzyme preparation (WEP) as a source of proteolytic enzymes. The WEP was prepared from a suspension of 30 g of wheat flour in 200 mL of 50 mM sodium acetate buffer, pH 5.0, containing 0.1 mM EDTA and 2 mM L-Cys·HCl. The suspension was extracted at 5 °C for 1 h with continuous stirring. Solids were removed by centrifugation (16000*g*, 4 °C, 20 min, Sorvall RC-5B, Du Pont Instruments, Wilmington, DE), and ammonium sulfate was added to supernatant to 30% saturation. The suspension was incubated at 0 °C for 5 h, and solids were removed by centrifugation (16000*g*, 4 °C, 20 min). The ammonium sulfate content of the supernatant was raised to 80%, and the suspension was incubated overnight at 0 °C. The precipitates were collected by centrifugation, resuspended in 50 mL of 5 mM sodium acetate, pH 5.0, and dialyzed (Spectra/Pro 3, Spectrum Laboratories, Rancho Dominguez, CA) overnight against an excess of 5 mM sodium acetate buffer to obtain the WEP as a retentate. The proteolytic acitivity of the WEP against acid-denatured hemoglobin was determined at pH 3.5. The hemoglobin substrate solution (2%) was prepared by dissolving 0.4 g of bovine hemoglobin (Sigma, Oakville, Canada) in deionized water and adjusting the pH of this solution to 1.7. After 10 min, the pH was adjusted to 3.5 with 0.5 M sodium acetate. The reaction mixture contained equal volumes of the hemoglobin solution, WEP, and 100 mM sodium acetate, pH 3.5. After 100 min of incubation at 40 °C, an addition of 3 volumes of 12% TCA terminated the reaction. The mixture was filtered and absorbance of the filtrate read at 280 nm. The activity of the WEP was 16 units. One unit increases the absorbance by 0.001 per minute under the assay conditions.

In Vitro Hydrolysis of Egg White Proteins and Ovotransferrin. Egg whites separated from hen's eggs were freeze-dried, finely ground, and dissolved in water to prepare a substrate solution with a dry matter content of 100 mg mL^{-1}. One hundred microliters of the egg white solution was incubated with 200 μL of the WEP and 300 μL of sodium acetate (100 mM, pH 3.5) at 40 °C for 3 h. The reaction was terminated by the addition of 600 μL of SDS-PAGE sample buffer containing 2% dithiothreithol and incubation in boiling water for 3 min. Hydrolysis of ovotransferrin was done with a similar procedure except the ovotransferrin solution contained 25 mg mL^{-1} of ovotransferrin (Sigma, Oakville, Canada) and the reaction mixture contained 30 μL of ovotransferrin solution, 200 μL of WEP, and 270 μL of acetate buffer. Hydrolysis times of ovotransferrin were 30 min, 60 min, and 6 h, and reactions were stopped as described above. A second set of experiments was performed in parallel with the addition of the aspartic proteinase inhibitor pepstatin A (Sigma, Oakville, Canada) to a final concentration of 20 μM. Results shown are representative for analyses of two independent experiments.

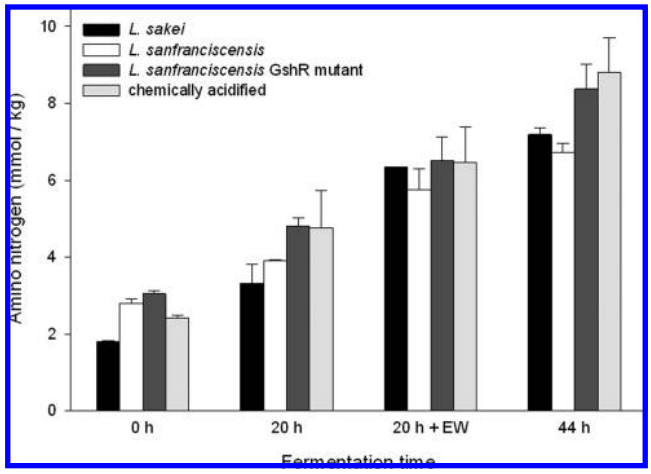

Figure 1. Amino nitrogen concentrations in sourdough samples. The measurements were carried with the albumin fraction (water-soluble, pH 8) using a ninhydrin method (*10*). Samples were taken before fermentation, after 20 h of fermentation, after the addition of egg whites (EW), and after 44 h of fermentation. Results shown are means ± standard deviation of duplicate analyses each of two independent fermentations.

RESULTS

General Sourdough Parameters. During the first stage of fermentation, doughs were fermented without egg whites for 20 h. After this, each of the sourdoughs was supplemented with egg whites (and wheat flour) and fermented for an additional 24 h (the second stage). Three wheat sourdough fermentations were carried out using single strains of lactobacilli as starters and compared to a chemically acidified dough as a control. The two-stage fermentation process was employed because the addition of egg whites to unfermented doughs inhibited the growth of lactic acid bacteria (data not shown). This inhibition is attributable to the egg white addition that increased the dough pH to a level above 6.6, which is the pH maximum for growth of *L. sanfranciscensis* (*16*). The presence of antimicrobial proteins such as lysozyme and/or ovotransferrin in fresh egg whites may additionally contribute to their inhibitory effect.

Egg whites had no inhibitory effect when they were added to ripe sourdough as the resulting pH was below 6.5 (data not shown). Accordingly, in all fermentations the pH lowered to 3.6 ± 0.2 and the cell counts at the end of the fermentation were in the range of (0.8–3.3) × 10^9 cfu g^{-1} (the cell counts of chemical control doughs were no more than 10^4 cfu g^{-1}, which is 1:100000 of the fermentation flora, demonstrating that microbes originating from flour and/or eggs had no effect on fermentations). The accumulation of amino nitrogen levels is shown in **Figure 1**. The development of amino nitrogen levels in the first stage was generally in agreement with earlier studies (*5, 17*). Egg white addition increased the amino nitrogen levels but did not accelerate the overall proteolytic activity in the wheat sourdoughs (**Figure 1**).

Degradation of Egg White Proteins in Wheat Sourdoughs. A specific degradation of egg white proteins occurred in different sourdoughs (**Figure 2**). An unidentified protein band with high molecular weight disappeared during the fermentation in all sourdoughs and the chemically acidified dough (**Figure 2**, uppermost horizontal arrow). Ovalbumin and ovomucoid were not hydrolyzed in any of the fermentations. Ovotransferrin hydrolysis was dependent on the fermentation flora (**Figure 2**, circles). Virtually complete hydrolysis of ovotransferrin was observed in dough fermented with *L. sanfranciscensis,* but ovotransferrin was more resistant to hydrolysis in doughs fermented with the glutathione reductase deficient mutant of *L. sanfranciscensis*. The intensity of the protein band corresponding to ovotransferrin was essentially unchanged in chemically acidified dough, and only a slight reduction in intensity was apparent in sourdough started with the homofermentative *L. sakei* (**Figure 2**). The SDS-PAGE sample size was adjusted to highlight the differences in ovotransferrin hydrolysis. SDS-PAGE analysis of SDS-soluble proteins from sourdough was carried out to verify that the disappearance of ovotransferrin from water-soluble sourdough fraction is not attributable to its association with water-insoluble gluten through disulfide bond interchange (data not shown).

Development of Thiol Levels in Sourdough Extracts. Determination of thiol levels aimed to confirm that the strain-specific hydrolysis of ovotransferrin is attributable to differences in the thiol levels of sourdoughs. In keeping with earlier observations in wheat sourdoughs (*2, 3*) *L. sanfranciscensis* ($p < 0.05$), but not its isogenic *gshR* mutant or *L. sakei*, increased total thiol levels during the first stage of fermentation (**Figure 3A**). The addition of egg whites substantially increased the thiol levels in the sourdough (**Figure 3A**). In *L. sanfranciscensis* sourdoughs, thiol levels increased by 1.8 μmol/g of dough during the 24 h fermentation after the addition of egg whites, whereas the corresponding increase was 1.0 μmol/g of dough or less in the other sourdoughs or chemically acidified dough (**Figure 3A**). An opposite trend was observed in the water-soluble sourdough fractions (**Figure 3B**). In sourdoughs fermented with *L. sanfranciscensis* and its *gshR* mutant strain, thiol levels in the water-soluble fraction decreased ($p < 0.09$) but remained essentially unchanged in *L. sakei* sourdough and chemically acidified dough. With *L. sanfranciscensis* and the mutant strain the decreases were 2.9 and 1.2 μmol/g of dough, respectively.

In Vitro Hydrolysis of Egg White Proteins and Ovotransferrin with Wheat Flour Enzymes. The activity of wheat flour proteases on egg white proteins was determined by incubating a wheat enzyme preparation (WEP) with freeze-dried egg whites at pH 3.5. In these reactions, ovotransferrin but not ovalbumin was hydrolyzed (**Figure 4**). Ovotransferrin hydrolysis was inhibited by the specific aspartic proteinase inhibitor pepstatin A, which confirmed that this protein is hydrolyzed by wheat aspartic proteases (**Figure 5**).

DISCUSSION

This study determined the effect of acidification and reduction on the fate of egg white proteins during sourdough fermentation. In wheat sourdoughs, egg white proteins are degraded predominantly by wheat aspartic proteases that exhibit a pH optimum of 3.5–4. Remarkably, our results demonstrated that egg white protein degradation in sourdoughs was highly specific and strongly influenced by thiol metabolism of lactobacilli. No ovotransferrin hydrolysis was observed in the chemically acidified dough, and ovotransferrin was extensively hydrolyzed only in sourdoughs fermented with heterofermentative lactobacilli that accumulate thiols in dough (*2, 3*). The higher resistance to proteolysis of ovotransferrin in dough fermented with the glutathione reductase mutant strain of *L. sanfranciscensis* and the homofermentative *L. sakei* confirmed the relevance of thiols for ovotransferrin degradation in dough and the relevance of the glutathione reductase activity of *L. sanfranciscensis* for redox reactions in sourdough (*3*). *L. sanfranciscensis* but not homofermentative lactobacilli increased the free thiol

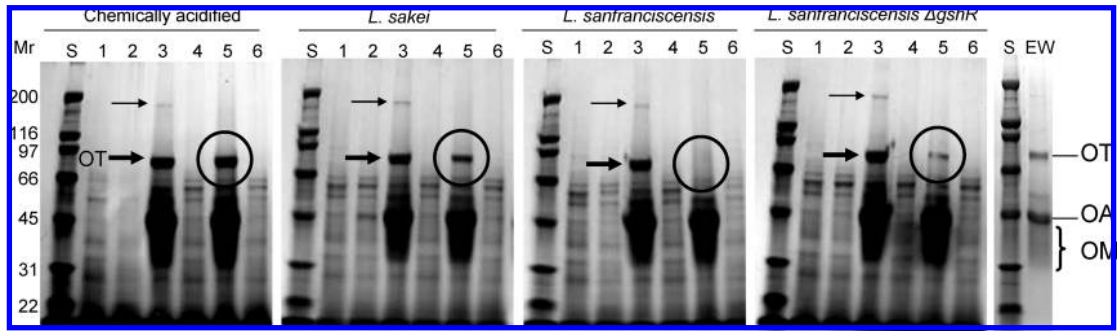

Figure 2. SDS-PAGE analysis of salt-soluble proteins extracted from sourdoughs. Lower arrow points out ovotransferrin protein after the addition of egg whites to sourdoughs. The circles point out the situation after the second stage of fermentation. Upper arrow points out a high molecular weight egg white protein that disappeared in all four fermentations. On the right, the migration of egg white proteins (EW) is shown, and the protein bands/areas corresponding to ovotransferrin (OT), ovalbumin (OA), and ovomucoid (OM) are indicated. Lanes: S, molecular weight marker proteins (M_r indicated on the left in kDa); 1, beginning of fermentation; 2, after the first stage (20 h) of fermentation; 3, after the addition of egg whites (20 h + EW); 4, control fermentation sample without egg whites (only flour added); 5, egg white supplemented sourdough after the second stage (44 h); 6, control fermentation without egg whites after the second stage; EW, proteins extracted from freeze-dried egg whites. Samples were loaded to represent equal dough weights. Results shown are representative for analyses of independent fermentations.

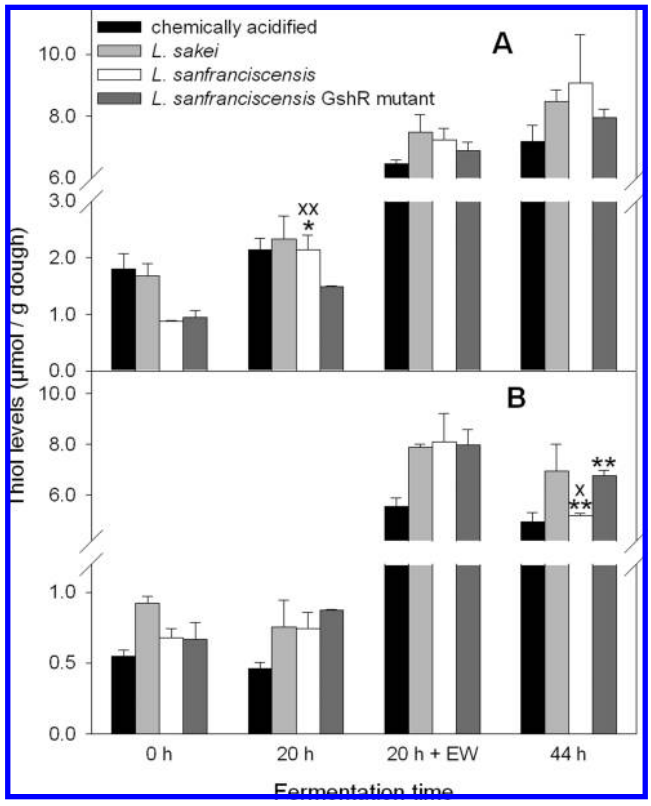

Figure 3. Thiol levels in SDS-soluble (**A**) and water-soluble (**B**) sourdough extracts. Samples were taken before fermentation, after 20 h of fermentation, after the addition of egg whites (20 h + EW), and after 44 h of fermentation. Results are presented as means ± standard deviation of duplicate analyses each of two independent fermentations. Statistically significant changes (Student's *t* test) within two sourdough fermentation stages (from 0 to 20 h and from 20 h + EW to 44 h) are marked with asterisks and between *L. sanfranciscensis* and the GshR mutant with '×' (×/∗, $p < 0.05$; ××/∗∗, $p < 0.09$). Statistical significance was evaluated with Student's *t* test using SigmaPlot software.

levels in wheat sourdough (*2*). Thermophilic heterofermentative lactobacilli such as *L. reuteri* and *L. pontis* accumulate thiols in dough through other enzymatic activities (*3*) but are expected to exert a comparable effect on ovotransferrin hydrolysis.

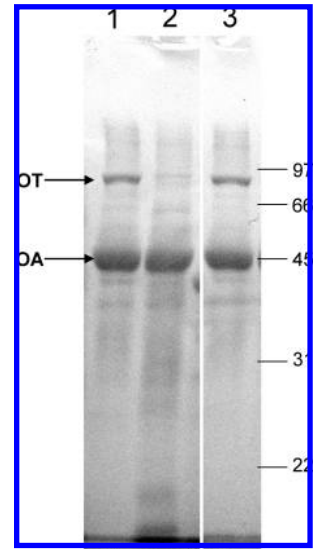

Figure 4. SDS-PAGE separation of egg white proteins (EW) before and after in vitro incubation with wheat enzyme preparation (WEP) at pH 3.5 (40 °C, 3 h). OT, ovotransferrin; OA, ovalbumin; lane 1, EW + WEP 0 h; lane 2, EW + WEP 3 h; lane 3, EW without WEP 3 h. The migration of the molecular weight marker and the corresponding molecular weight in kDa are indicated on the right. Results shown are representative for analyses of two independent experiments.

The use of the aspartic proteinase inhibitor pepstatin A confirmed that the hydrolysis of ovotransferrin in wheat sourdoughs was mainly attributable to wheat aspartic proteinases; aspartic proteinases are also responsible for the proteolysis in traditional wheat and rye sourdoughs (*5, 6, 18*), whereas lactic acid bacteria contribute to the peptide hydrolysis in sourdoughs in a strain-dependent manner (*19*). Generally, most egg white proteins are relatively resistant against hydrolysis with pepsin, an aspartic protease (*20*). Ovotransferrin, however, is an exception as it was degraded by pepsin under mildly acidic conditions (*20*). In the present study, ovotransferrin was degraded effectively by wheat aspartic proteinases, whereas no observable hydrolysis occurred with other egg white proteins.

Our finding confirms previous reports on the effect of redox agents on the biological activity of egg white proteins. Ovotransferrin, with 15 disulfide bonds in its native state, is highly sensitive to reducing agents and undergoes autolytic

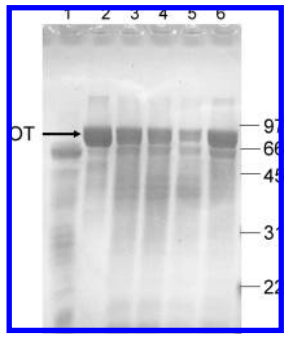

Figure 5. SDS-PAGE separation of ovotransferrin (OT, lane 2) before and after in vitro incubation (pH 3.5, 40 °C) with wheat enzyme preparation (WEP, lane 1) for 30 min (lane 3), 60 min (lane 4), 6 h (lane 5), and 6 h in the presence of 20 μM of the aspartic protease inhibitor pepstatin A (lane 6). The migration of the molecular weight marker and the corresponding molecular weight in kDa are indicated on the right. Results are representative for analyses of two independent experiments.

cleavage under reductive conditions (*21*). Ovomacroglobulin, an inhibitor of aspartic proteases, lost its inhibitory activity in the presence of a reducing agent (*22*). The susceptibility of ovotransferrin to proteolysis in sourdoughs acidified with heterofermentative lactobacilli is thus attributable to thiol accumulation by lactobacilli, which favors the reduction of intramolecular disulfide bonds in ovotransferrin. Additionally, reducing conditions may eliminate the inhibitory activity of ovomacroglobulin against wheat aspartic proteinases.

The starter cultures differed in their effect on thiol groups in SDS and water-soluble fractions of sourdoughs. *L. sanfranciscensis* but not *L. sakei* increased thiol levels in SDS extracts of sourdoughs, which contain gliadins and some of the glutenins in addition to low molecular weight thiols (*2, 8*). In contrast, thiol levels in the water-soluble fraction, in which prolamins are essentially absent, were strongly decreased by *L. sanfranciscensis* during the second stage of fermentation in the presence of egg whites. This apparent shift of thiols from the water-soluble fraction into the SDS-soluble fraction in *L. sanfranciscensis* doughs may be explained by an incorporation of thiol-containing hydrolysis products (peptides) into gluten proteins via disulfide exchange reactions.

In conclusion, this study demonstrated that ovotransferrin was the only major egg white protein that degraded during sourdough fermentation. Moreover, an extensive degradation of ovotransferrin required a heterofermentative lactobacilli starter, *L. sanfranciscensis*, with an active thiol metabolism. Thiol accumulation by lactobacilli apparently alters protein structure through a reduction of disulfide bonds and may increase their susceptibility to proteolysis. The study extends previous reports concerning the thiol metabolism of sourdough lactobacilli and gluten behavior (*2, 3*) to egg proteins and their behavior in sourdoughs. It adds to the body of evidence that protein functions in food fermentations (in addition to the effects of acidification and proteolysis) are modulated by the effect of lactobacilli on the redox state of fermenting systems. In addition to the susceptibility to proteolytic degradation, other properties of egg proteins related to their biological or technological function (e.g., allergenicity or foam formation) may be influenced by thiol-exchange reactions.

ABBREVIATIONS USED

GSH, glutathione (reduced); GSSG, glutathione (oxidized); *gshR*, glutathione reductase gene; GshR, glutathione reductase (enzyme); mMRS, modified de Man, Rogosa, Sharp medium; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); WEP, wheat enzyme preparation.

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