JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 1263–1269 1263
DOI:10.1021/jf903228x

Functional Characteristics of Egg White Proteins within Wheat, Rye, and Germinated-Rye Sourdoughs

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Egg white (EW) proteins are functional proteins, which possess certain biological activities (antimicrobial, antigenic, and peptidase-inhibitory) that may influence the food processing or vice versa can be affected by processing. This study investigated the behavior of EW proteins within sourdough systems with respect to proteolysis and fermentation parameters, and the ability of EW to build foam structures with sourdoughs. Of the EW proteins, ovotransferrin was hydrolyzed in all sourdoughs (wheat, rye, and germinated-rye), whereas the breakdown of ovalbumin was specific for germinated-rye sourdoughs, with the cysteine endopeptidases being responsible for the hydrolysis. The presence of EW in sourdough fermentations had no influence on the prolamin hydrolysis or the growth of starter culture, indicating that the peptidase-inhibitory and antimicrobial properties of EW play no important role in sourdoughs. EW foams, however, appeared as potential structure builders in sourdough applications and could serve as alternative structural agents in the production of baked goods with low gluten content.

KEYWORDS: Egg white; sourdough; proteolysis; ovalbumin; cysteine endopeptidases; foaming; gluten-free

INTRODUCTION

Egg white (EW) is widely used in food applications both in domestic and industrial cooking. EW has a high nutritional value, a diverse biological activity, and unique technological properties. Technological properties of EW include its ability to foam, gelatinize, emulsify, and bind water. These are mainly due to the proteins of EW, which are the major macromolecule component of EW and account for approximately 10% of its fresh weight. The main proteins of EW are ovalbumin (54%), ovotransferrin (12−13%), ovomucoid (11%), and lysozyme (3.5%) (*1*).

The biological functions of EW include antimicrobial, inhibitory, and allergenic activities. Of the EW proteins, lysozyme, ovomucin, ovalbumin, and ovomucoid are allergenic (*2*), whereas ovalbumin, ovotransferrin, and lysozyme have antimicrobial activities. Tryptic and chymotryptic peptides of ovalbumin showed antimicrobial activity especially against *Bacillus subtilis* (*3*). Ovotransferrin and lysozyme possess broader antimicrobial activities as ovotransferrin binds iron that is essential for microbial growth (*4*), whereas lysozyme hydrolyzes cell walls of Gram-positive bacteria (*5*). EW also contains proteins that inhibit endopeptidases. Ovocystatin inhibits cysteine endopeptidases, whereas ovomucoid and ovoinhibitor inhibit serine endopeptidases (*4*, *6*). Ovomacroglobulin inhibits enzymes from all four endopeptidase classes (*4*, *7*). However, despite the routine use of EW in baking applications, its inhibitory activity against flour peptidases in dough systems remains unclear.

The most important technological feature of EW proteins is probably their ability to foam. Foaming is a phenomenon where whipping, stirring, shaking, or bubbling incorporates air into a protein solution. Electrostatic interactions between proteins affect the foam stability and foaming capacity, the key parameters describing foam behavior. Generally, foam behavior improves when the pH of the system equals the isoelectric point of proteins present in the system. Of the major EW proteins, ovalbumin, ovomucoid, and ovomucin have isoelectric points at acidic conditions, whereas ovotransferrin and lysozyme have those at neutral and basic pH, respectively. Hammershoj et al. (*8*) showed that the EW foam behavior improved at acidic pH of 4.8 compared to neutral or basic pH conditions. It thus seems evident that mildly acidic conditions could also favor the foaming behavior of EW in food systems.

Sourdough fermentation is a prebaking process that provides mild acidic conditions. Sourdoughs are used in baking mainly to improve the flavor and shelf life of bread. For sourdoughs, it is characteristic that during acidification some protein degradation occurs. Cereal enzymes, derived from flours, are responsible for the enzymatic peptide bond cleavage, whereas the thiol-mediated reducing activity affects the depolymerization of polymeric proteins (*9*). A recent study showed that the redox activity of the starter culture also strongly affected the degradation of EW proteins in fermenting sourdoughs (*10*). In addition, sourdoughs prepared from germinated wheat or rye might turn out as convenient bread improvers in baking that produces bread with

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Published on Web 12/31/2009

low gluten content (*11*, *12*). In such gluten-less applications, alternative ways to retain gas and to form continuous networks are of interest. Exploiting the functionality of EW proteins could also serve as an option to build up structure in sourdough based low-gluten baking.

The present study investigated the effects of EW proteins on sourdough fermentation and, vice versa, whether the addition of EW inhibits the hydrolysis of cereal proteins in sourdoughs and to what extent the EW proteins undergo hydrolysis in different sourdough systems. The aim was also to study the foaming behavior of EW with sourdoughs and to test whether EW may serve as a structural component in low-gluten sourdough baking.

MATERIALS AND METHODS

Raw Materials. Sunnuntai baker's wheat flour (0.7% ash) and Sunnuntai whole grain rye flour (1.8% ash) (Raisio Oyj, Raisio, Finland) were purchased from a supermarket. Laihian Mallas Oy (Laihia, Finland) provided the germinated rye grains. The grains were milled with a Retsch ZM 200 GWB ultracentrifugal mill (Retsch GmbH, Haan, Germany) using a 0.5 mm sieve. The starter used for sourdough fermentation was *Lactobacillus brevis* (Florapan L 62, Lallemand SA, Blagnac-Cedex, France). Scanegg Suomi Oy (Piispanristi, Finland) provided the EW powder.

Preparation of Sourdough and Determination of Fermentation Parameters. Wheat sourdough (WSD), rye sourdough (RSD), and germinated-rye sourdough (GRSD) were each prepared in glass jars with or without EW by mixing 100 g of flour, 150 g of water, and 20 mg of dry starter, and for the EW containing sourdoughs, 5 g of the EW powder was added. The sourdoughs were fermented at 34 °C for 23 h. Total titratable acidity (TTA) and pH values of sourdoughs were measured at the beginning and at the end of the fermentation; 10 g of sourdough was suspended in 90 mL of water and mixed with a bar blender for 20 s, and the pH was measured and TTA determined by titrating the mixture with 0.1 N NaOH to pH 8.5, and the volume of NaOH (mL) consumed was recorded as the TTA-value. Cell counts of viable lactic acid bacteria were determined on MRS agar plates under anaerobic conditions (37 °C, 72 h). Sourdoughs were freeze-dried for further analyses.

Determination of Protein Hydrolysis. *SDS−PAGE (13).* The soluble proteins were extracted from a sample of 0.1 g with 1.0 mL of 100 mM sodium phosphate buffer (pH 8) by shaking continuously at 5 °C for 60 min. After centrifugation (10000*g*, 10 min), 50 μL of the supernatant was mixed with 50 μL of SDS−PAGE sample buffer containing 2% dithiothreithol (DTT), boiled for 2 min, and analyzed with NuPAGE Novex Bis-Tris 4−12% mini gels (Invitrogen, CA, USA). For the determination of residual protein, 1 mL of the SDS−PAGE sample buffer with 1% DTT was added onto the pellet and extracted with continuous shaking at 55 °C for 30 min, centrifuged, and analyzed.

Amino Nitrogen. The soluble extracts of sourdoughs were analyzed with a ninhydrin method (*14*). Two hundred microliters of diluted sample was mixed with 100 μL of ninhydrin reagent, incubated at 100 °C for 16 min, and cooled down at room temperature for 20 min, and after adding 500 μL of the dilution solution (0.2% KIO_3 in 40% ethanol), the absorbance was read at 570 nm.

Quantification of Egg White Proteins. Ridascreen Fast Ei/Egg Protein sandwich immunoassay (R-Biopharm AG, Darmstadt, Germany) determined the contents of EW proteins in EW supplemented sourdoughs. Of each EW containing sourdough, 0.25 g was mixed with 5 mL of extraction buffer (60 °C), incubated at 60 °C for 10 min, and centrifuged (3200*g*, 10 min, 4 °C). The supernatants were filtered with Millex PVDF 0.45 μm pore size filters (Millipore, Bedford, USA), and three different dilutions of the filtrates were assayed according to the manufacturer's instructions in duplicate. Briefly, 100 μL of diluted samples was added to the antibody coated microtiter plate wells. After 10 min of incubation at room temperature, the samples were poured out and the wells washed thrice with 250 μL of the washing buffer. One hundred microliters of the diluted enzyme conjugate solution was added and the mixture incubated for 10 min and washed as described above. After an addition of 100 μL of the substrate−chromogen solution, followed by incubation in the dark (10 min at room temperature), the reaction was terminated by adding 100 μL of the stop solution to each well. The absorbance was measured at 450 nm and results calculated on the basis of the standards provided.

Prolamin Quantification. Ridascreen gliadin sandwich immunoassay (R-Biopharm) determined the prolamin contents of sourdoughs. Sourdough samples (125 mg) were extracted with 1.25 mL of the cocktail solution and shaken at 50 °C for 40 min, cooled down, and mixed with 3.75 mL of 80% ethanol, incubated for 1 h at room temperature, and centrifuged (3200*g*, 10 min). The diluted supernatants were analyzed according to the manufacturer's instructions similar to that described above; the only difference to the EW procedure was the use of an incubation time of 30 min and a separate addition of substrate and chromogen solutions. The results were not multiplied by factor 2 because that would overestimate the prolamin contents especially of the samples taken at the end of the sourdough fermentation (when most of the prolamins are ethanol-soluble).

In Vitro Hydrolysis of Egg White Proteins with Enzymes from Germinated Rye. EW hydrolysis by a crude enzyme extract of germinated rye in the presence of endopeptidase-class specific inhibitors determined the endopeptidases that hydrolyzed the EW proteins in GRSD. Enzymes were extracted from 30 g of germinated-rye flour with 200 mL of 50 mM sodium acetate (pH 5.0) containing 0.1 mM ethylenedinitrilotetraacetic acid (EDTA) and 2 mM L-cysteine hydrochloride at 5 °C with continuous shaking for 60 min, and centrifuged (12000*g*, 20 min, 5 °C). The supernatant was the crude enzyme extract.

In order to hydrolyze EW proteins in vitro, 300 μL of 1% EW powder suspension (w/v), dissolved in 100 mM sodium acetate at pH 3.5 and containing 0.1% DTT, was mixed with 200 μL of the crude enzyme extract. When class-specific endopeptidase inhibitors [pepstatin A (PEP-A stock solution of 2 mM in MeOH), epoxysuccinyl-L-leucylamido-(4-guanidino) butane (E-64, 1 mM in H_2O), 1,10-phenanthroline (O-FEN, 100 mM in MeOH), phenylmethylsulfonyl fluoride (PMSF, 500 mM in MeOH)] were used, they were added to the enzyme extract 5 min before initiating the reaction. The final reaction concentrations for PEP-A, E-64, O-FEN, and PMSF were 20 μM, 10 μM, 1 mM, and 10 mM, respectively. In addition, selected combinations of the individual inhibitors were tested. The reaction took place at 40 °C for 3 h and was terminated by adding 500 μL of SDS−PAGE sample solution containing 2% DTT and boiling for 3 min. The samples were analyzed by SDS−PAGE using the NuPAGE NovexBis-Tris mini gels (Invitrogen).

Foaming and Baking Experiments. A 10% (w/v) EW suspension prepared in distilled water was whipped with a Braun Multimix mixer (Braun Espanola SA, Barcelona, Spain) first at the low speed for 2 min and then at high speed for 3 min. The whipped EW was mixed with ripe sourdough at high speed for 2 min. The foam volume (the total volume subtracted with the volume of liquid part) was recorded immediately after the mixing and after 20 min of standing. Different proportions of EW suspension (w-%) were tested: 0%; 12.5%; 25%; 50%; 75%; 87.5%. In addition, because of the low foaming capacity of GRSD, the effect of mixing time was determined by mixing 50 g of GRSD with 50 g of a previously foamed 10% EW suspension. After adding the GRSD to the EW foam, the suspension was mixed for 3 min, 2 min, 1 min, 30 s, or 15 s at high speed. In addition, gentle mixing was done with a spoon. Furthermore, the influence of EW foam in starch-based sourdough baking was tested. Fifty grams of the foamed 10% EW was mixed (30 s) with 50 g of sourdough, 75 g of potato starch, and 2.6 g of baking powder; the doughs were prepared either without or with 1 g of salt. The doughs were baked at 200 °C for 10 min. When the EW was excluded, we used water to replace it in the recipe. After cooling, the volumes of the bread loaves were determined with a rapeseed displacement method and the crust color examined visually.

Statistical Methods. The statistical significance between the concentration of amino nitrogen, egg white protein, and prolamin in the beginning and at the end of the fermentations was tested with paired *t* tests. Factorial design considered flour type, fermentation and egg white addition (amino nitrogen, EW protein, and prolamin), or egg white addition and salt addition (baking test) as factors using duplicate samples. Analysis of variance was performed using SAS System for Windows V7 Software (SAS Institute Inc., Cary, NC, USA). The Duncan's multiple range test determined variable effects. Results were considered significant at the 95% confidence level.

Table 1. Fermentation Parameters: pH, Total Titratable Acidity (TTA), Cell Count, and Amino Nitrogen Values of Sourdoughs[a]

	pH		TTA		amino nitrogen (mg/kg)[b]		cell count (cfu/g)
	0 h	23 h	0 h	23 h	0 h	23 h	23 h
WSD	6.0	3.3	1.8	12.4	160	420	2.2×10^8
WSD + EW	6.2	3.4	1.9	13.4	220	480	6.1×10^8
RSD	6.4	3.4	2.5	22.6	340	1040	1.5×10^9
RSD + EW	6.4	3.5	2.5	22.7	340	1130	1.1×10^9
GRSD	5.9	3.6	4.4	26.0	1030	2580	2.4×10^9
GRSD + EW	6.0	3.5	4.3	26.8	900	3140	2.7×10^9

[a] The values are mean values from duplicate analyses of two independent fermentations. [b] Within each sourdough, the increase of amino nitrogen concentration was significant during the 23 h fermentation (paired *t*-test $p < 0.05$). Factorial analysis showed that flour type (wheat, rye, and germinated rye) and fermentation significantly influenced the amino nitrogen concentrations, whereas the presence of EW did not.

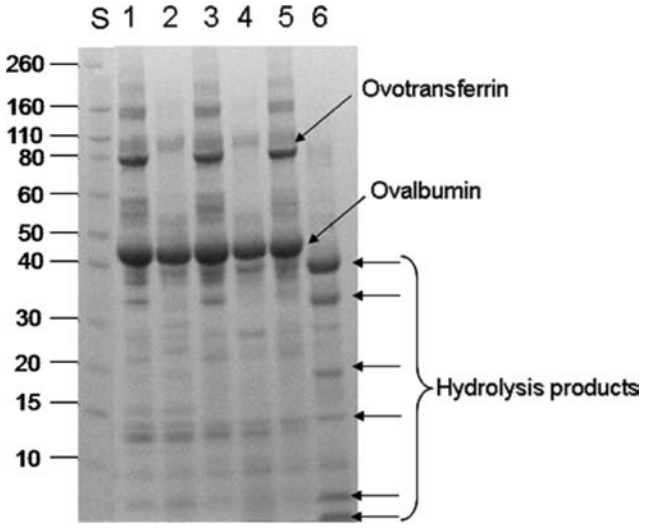

Figure 1. SDS−PAGE analysis of soluble proteins extracted from sourdoughs supplemented with egg white (EW). S, the standard protein marker; 1, WSD + EW 0 h; 2, WSD + EW 23 h; 3, RSD + EW 0 h; 4, RSD + EW 23 h; 5, GRSD + EW 0 h; 6, GRSD + EW 23 h. Molecular weights (kD) of the marker proteins are given on the left.

RESULTS

Sourdough Fermentation Parameters. The fermentation parameters showed that the sourdoughs underwent normal acidification and that the addition of EW had no major effect on pH, TTA, or cell counts (**Table 1**). The increase of amino nitrogen content during each of the sourdough fermentation was significant. Moreover, the flour type influenced the amino nitrogen levels, whereas the addition of EW had no significant influence. Thus, the enzyme activity is the limiting factor in the proteolysis taking place in sourdoughs because adding a protein substrate failed to increase the amino nitrogen content of sourdoughs (**Table 1**).

Egg White Proteins in Sourdoughs. SDS−PAGE patterns of soluble sourdough proteins showed that some hydrolysis of EW proteins occurred during sourdough fermentations (**Figure 1**). Ovotransferrin hydrolysis was quantitative in all three sourdoughs, whereas ovalbumin underwent no visible changes during the WSD and RSD fermentations. Ovalbumin, however, degraded during GRSD fermentation, and this evidently produced low molecular weight (LMW) hydrolysis products (**Figure 1**). No EW proteins were detectable in the residual protein fraction, which confirmed that no solubility shift of EW proteins to this fraction had occurred (data not shown). The level of EW proteins in sourdoughs was quantified with an immunoassay (**Table 2**). Percent amounts of degraded EW proteins were 48%, 54%, and 86% in WSD, RSD and GRSD, respectively. The hydrolysis of egg white proteins was significant in all sourdough types, and the extent of hydrolysis was influenced by the flour type.

Table 2. Egg White (EW) Protein Content (g/kg) of Sourdoughs Supplemented with EW

	EW protein concentration[a] (g/kg)	
	before fermentation	after fermentation
WSD + EW	98 ± 26	51 ± 12 (−48%)
RSD + EW	90 ± 13	41 ± 8 (−54%)
GRSD + EW	66 ± 4	9 ± 7 (−86%)

[a] Within each sourdough, the decrease of EW protein concentration was significant during the 23 h fermentation (paired *t*-test $p < 0.05$). Factorial analysis showed that flour type (wheat, rye, and germinated rye) and fermentation significantly ($p < 0.05$) influenced the EW protein concentrations.

Table 3. Prolamin Contents (g/kg) of Sourdoughs and EW Containing Sourdoughs before and after Fermentation

	prolamin concentration[a] (g/kg)	
	without EW	with EW
WSD 0 h	51 ± 9	54 ± 13
WSD 23 h	48 ± 13	46 ± 9
RSD 0 h	85 ± 7	81 ± 14
RSD 23 h	92 ± 13	91 ± 10
GRSD 0 h	41 ± 6	50 ± 29
GRSD 23 h	0.45 ± 0.23	0.45 ± 0.04

[a] In GRSD, the decrease in prolamin concentration was significant during the 23 h fermentation. Factorial analysis showed that flour type (wheat, rye, and germinated rye) significantly ($p < 0.05$) influenced the prolamin concentrations, whereas the presence of EW did not.

Influence of Egg White on Prolamin Hydrolysis in Sourdoughs. A prolamin specific immunoassay was used for the quantification of prolamins in sourdough samples (**Table 3**). No significant changes occurred in prolamin levels during RSD or WSD fermentation. Generally, it is, however, known that hydrolysis of prolamins occurs in wheat and rye sourdoughs. The prolamin assay used in the present study detects prolamin fragments that possess at least two binding sites for the R5 antibody. Thus, no decrease in prolamin concentration is necessarily detectable even if some hydrolysis of prolamins to large polypeptides would occur. Moreover, the solubility of prolamins increases during acidification, which may have increased prolamin concentration in sample extracts. Nevertheless, the prolamin concentration in sourdoughs prepared from germinated rye decreased by ~99%, which indicates the highly extensive breakdown of prolamins in GRSD. The presence of egg white in sourdoughs had no influence on the prolamin levels. Overall, these findings indicate that despite the fact that EW proteins are known to inhibit a wide range of endopeptidases they do not hinder the activity of cereal endopeptidases in sourdoughs.

In Vitro Hydrolysis of Egg White Proteins with Germinated-Rye Peptidases. Ovotransferrin and ovalbumin degraded during in vitro hydrolysis by germinated-rye peptidases (**Figure 2**). Ovotransferrin hydrolysis occurred during the first 60 min and was rapid compared to ovalbumin hydrolysis that was completed after 6 h of hydrolysis (**Figure 2**). The peptidases that hydrolyzed EW proteins in GRSD were classified by using class-specific endopeptidase inhibitors and their cocktails in the reaction mixtures. The inhibitor combination of PEP-A (an aspartic endopeptidase inhibitor) and E-64 (a cysteine endopeptidase

inhibitor), as well as the combination of all four tested inhibitors clearly inhibited the hydrolysis of ovotransferrin (**Figure 3**, left side gel, up−down arrows). In the case of ovalbumin, the specificity of hydrolysis pattern appeared highly more specific; as for the tested inhibitors, only the presence of the cysteine endopeptidase inhibitor, E-64, in the reaction mixtures totally prevented the breakdown of ovalbumin (**Figure 3**, right side gel, up−down arrows), whereas other inhibitors had no apparent effect on its breakdown.

Foaming Experiments with Egg White and Sourdoughs. Sourdoughs that were supplemented and fermented with EW had no improved foaming properties compared to those of their EW-free counterparts, which indicated that the EW lost its foaming ability during fermentation (data not shown). Therefore, the foaming experiments were conducted by whipping first the EW, after which the ripe sourdough was added. The foaming capacity and foam stability of 200 g of 10% EW suspension itself were 190 and 175 mL, respectively. Foam volumes of mixtures with different amounts of 10% EW suspension and each sourdough were determined immediately after whipping and after 20 min of standing (**Figure 4**). Overall, the best foam properties with the used protocol were obtained when equal amounts of EW and WSD or EW and RSD were used. GRSD had poor foaming properties in the tested setup compared to those of RSD and WSD (**Figure 4**). To overcome this, the effect of shortened mixing times on foaming properties of GRSD and EW combination (1:1) was investigated. The foam volume increased with shortened mixing times, and both the foam capacity and stability remained good after 15 s of mixing (**Figure 5**). Gentle mixing with a spoon resulted in foam with the highest volume but decreased stability compared to those of the 15 s mixing.

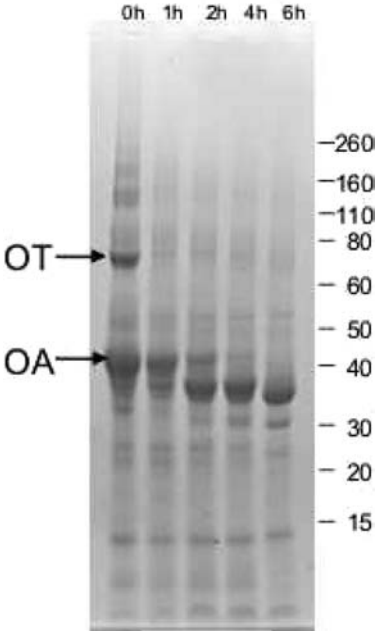

Figure 2. In vitro hydrolysis of EW proteins with GRSD enzymes. The protein bands of ovotransferrin (OT) and ovalbumin (OA) are indicated left of the gel and the migration of standard proteins and respective molecular weights (kD) on the right. Hydrolysis time is indicated above the gel.

Egg White in Sourdough Baking. The baking experiment showed that the use of EW foam built up the structure of the baked goods as a substantial increase in the loaf volumes was observable (**Table 4**). When water replaced EW in the recipes, the doughs retained no gas during baking, which resulted in a flat surface, whereas the loaves with the EW foam retained gas and built up structure (**Figure 6**) and had increased specific volumes (**Table 4**). Statistical analysis (Duncan's multiple range test) verified that the use of EW foam in the recipes significantly increased the volumes of baked goods, whereas the addition of salt had no effect. Furthermore, while the WSD containing products remained pale, the use of GRSD brought in dark color to the crust of the baked products.

DISCUSSION

This study investigated the behavior of EW proteins within sourdough systems. The proteolytic degradation of EW proteins by cereal enzymes and the influence of EW proteins on prolamin hydrolysis in sourdoughs prepared from wheat, rye, or germinated rye were under investigation. In addition, the behavior of EW foams with sourdoughs and in a low-gluten baking procedure was tested.

The EW proteins underwent degradation during sourdough fermentations, and the extent by which the degradation occurred varied among the studied sourdough types and, thus, was

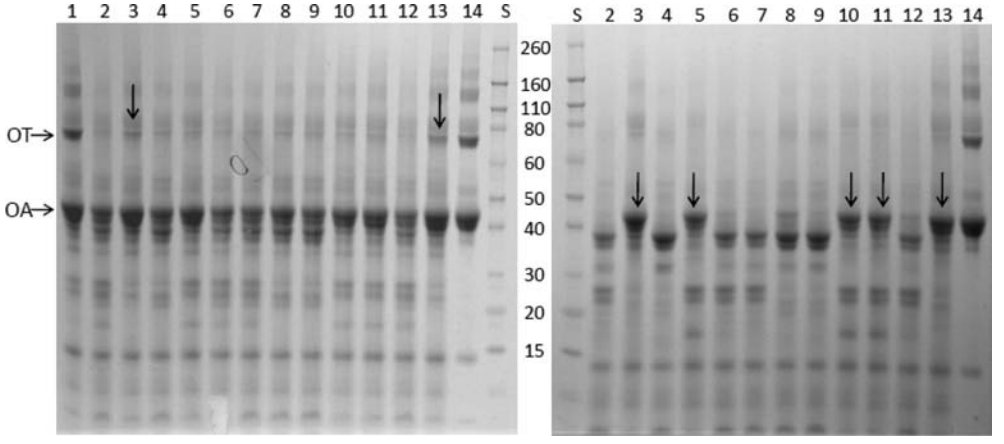

Figure 3. Effect of endopeptidase inhibitors on the in vitro hydrolysis of EW proteins with enzymes extracted from germinated rye (GRENZ). The mixtures were incubated at 40 °C either for 1 h (left side gel) or 6 h (right side gel). 1, EW + GRENZ (0 h); 2, EW + GRENZ; 3, EW + GRENZ + PEP-A + E-64; 4, EW + GRENZ + PEP-A; 5, EW + GRENZ + E-64; 6, EW + GRENZ + O-FEN; 7, EW + GRENZ + PMSF; 8, EW + GRENZ + PEP-A + O-FEN; 9, EW + GRENZ + PEP-A + PMSF; 10, EW + GRENZ + E-64 + O-FEN; 11, EW + GRENZ + E-64 + PMSF; 12, EW + GRENZ + PMSF + O-FEN; 13, EW + GRENZ + PEP-A + E-64 + PMSF + O-FEN; 14, EW incubated alone for 6 h under reaction conditions. Molecular weights of standard proteins (kD) are indicated between the gels. OT, ovotransferrin; OA, ovalbumin.

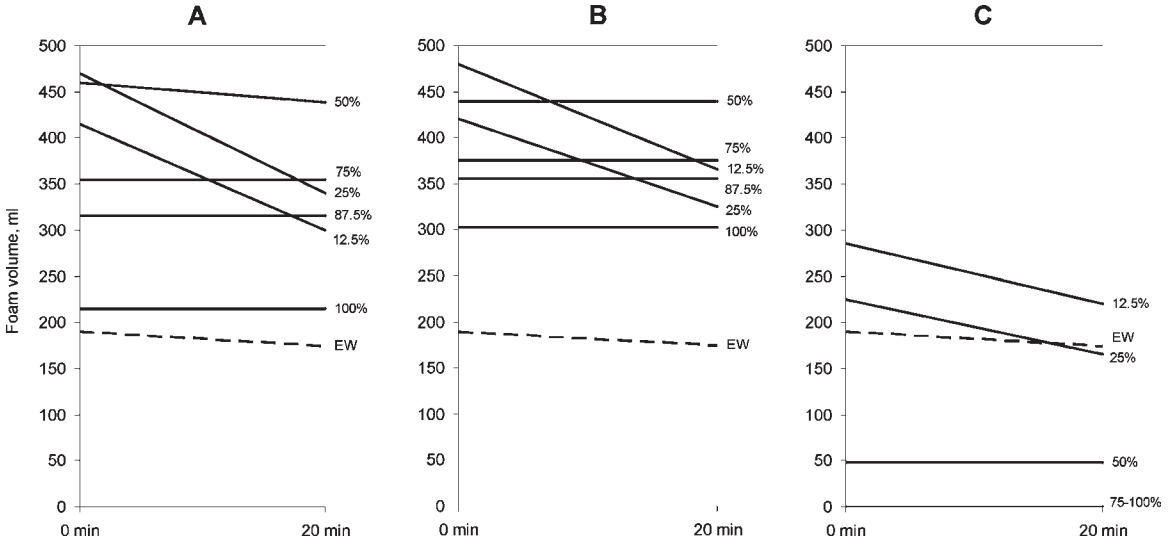

Figure 4. Foaming properties of sourdough and egg white mixtures (**A**, WSD; **B**, RSD; **C**, GRSD). The proportion of sourdough used is indicated on the right side of charts. The results represent duplicate analyses of two independent experiment sets (experimental error <10%).

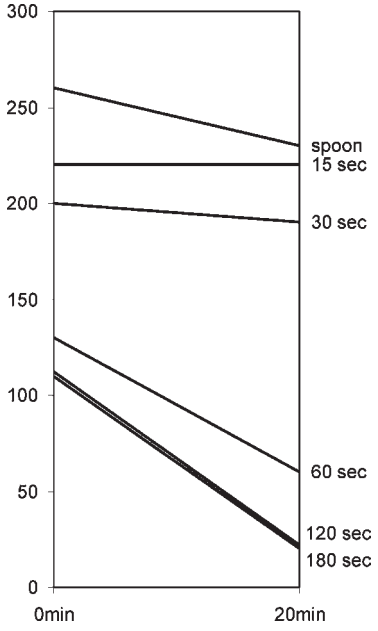

Figure 5. Foam volumes of GRSD and EW foam suspensions mixed for different times or with spoon (time indicated on the right). The values for 0 min are the total volumes of the mixture immediately after mixing, and the values for 20 min were derived by subtracting the volume of the separated liquid phase from the total volume. The results represent duplicate analyses of two independent experimental sets (experimental error <10%).

Table 4. Volumes and Specific Volumes of Baked Goods

	volume (mL)		specific volume (mL/g)	
sourdoughs	without EW	with EW[a]	without EW	with EW
WSD	220	220	1.6	1.7
WSD + salt	200	270	1.5	2.0
RSD	195	260	1.4	2.0
RSD + salt	160	300	1.2	2.4
GRSD	150	215	1.2	1.6
GRSD + salt	180	215	1.4	1.6

[a] Factorial analysis showed that the use of egg white foam in baking significantly increased the volume, whereas the addition of salt did not.

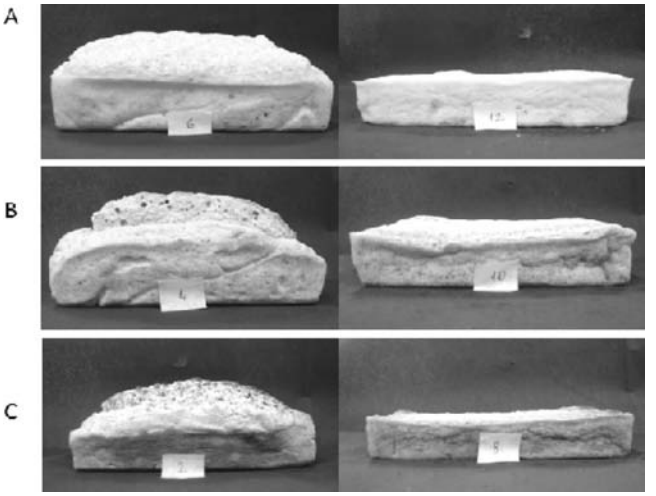

Figure 6. Baked goods made from different sourdoughs with egg white (left side) or without egg white (right side). (**A**) WSD + EW (left side), WSD (right side); (**B**) RSD + EW, RSD; (**C**) GRSD + EW, GRSD.

dependent on the flour used as a raw material. At the end of the wheat, rye, and germinated-rye sourdough fermentations, the concentrations of EW proteins were 52%, 46%, and 14% of that present in the beginning of fermentation, respectively. Extensive proteolysis may generally eliminate the antigenic nature of allergenic proteins, but in the present work, the degradation of EW proteins in sourdoughs was only moderate. In WSD and RSD, ovotransferrin hydrolysis was quantitative, but no degradation of ovalbumin, for instance, was detectable. Aspartic endopeptidases are the predominant peptidases in wheat and rye flour and, thus, most probably were responsible for ovotransferrin hydrolysis as this was previously shown to occur in WSD (*10*). A previous finding showing that ovalbumin was only slowly digested by pepsin even at relatively high enzyme concentrations (*15*) explains the resistance of ovalbumin to aspartic endopeptidase mediated hydrolysis in WSD and RSD. In GRSD, however, quantitative hydrolysis of ovalbumin also occurred, and the in vitro hydrolysis experiments showed that cysteine endopeptidases were responsible for this. The ovalbumin hydrolysis produced hydrolysis products with molecular weight up to 40 kD. Previously, it was shown that despite substantial proteolytic breakdown, the hydrolysis products of ovalbumin still

remained substantially antigenic (*15*). It is, thus, predictable that ovalbumin, despite clear degradation during GRSD, still remained antigenic, especially because some of the hydrolysis products formed were relatively high in their molecular size.

Nevertheless, the extent by which the hydrolysis of EW proteins in different sourdoughs took place occurred in a logical order because whole grain rye has a higher proteolytic activity compared to that of wheat endosperm flour, whereas germinated rye has a higher and a more diverse proteolytic activity compared to that of native rye.

Proteolytic activity of germinated rye is predominantly a combination of aspartic and cysteine endopeptidase activities (*16*). The in vitro hydrolysis experiments clearly showed that the cysteine endopeptidases of germinated rye hydrolyzed ovalbumin, whereas the other endopeptidases did not. This indicates that ovalbumin degradation in a sourdough environment is highly specific and dependent on the presence of cysteine endopeptidases. Preliminary experiments (data not shown) also made it clear that hydrolysis only took place under reductive conditions. Reductive conditions may accelerate the cysteine protease mediated ovalbumin hydrolysis with a 2-fold mechanism: first, the unraveling of disulfide bonds and, thus, the compact structure of ovalbumin, make it a more susceptible substrate for proteolytic enzymes, and second, reducing conditions increase the activity of thiol dependent cysteine endopeptidases. Saxena and Tayyab (*7*) classified endopeptidases into three groups based on their hydrolytic activity against ovalbumin. Bromelain, a cysteine endopeptidase, exhibited a specific digestion pattern after 5 h of hydrolysis, which was probably similar to the finding made in the present study with germinated-rye cysteine endopeptidase activity. In the present study, ovotransferrin was clearly more sensitive to proteolytic breakdown compared to ovalbumin as the use of inhibitor cocktails containing inhibitors for both aspartic and cysteine endopeptidases or for all four main endopeptidase classes elicited only a partial inhibition in the breakdown of ovotransferrin. An explanation for this may be the sensitiveness of ovotransferrin to reductive conditions (*17*).

EW contains a number of inhibitors that widely inhibit endopeptidases of different classes. It was therefore presumable that this inhibitory activity would reflect in the prolamin degradation that took place during sourdough fermentation. However, the addition of EW in sourdough fermentations had no effect on the final prolamin concentrations of sourdoughs. This may be due to the reductive conditions present in sourdoughs that might hinder the inhibitory activity of EW derived endopeptidase inhibitors. For instance, ovomacroglobulin that in its native form inhibited aspartic endopeptidases, pepsin and chymosin, lost its inhibitory activity at reducing conditions (*18*).

The present study showed that a convenient way to form foams containing both EW and sourdough was to first whip egg whites into foam and then mix in the fermented sourdough. Foams prepared from WSD and RSD showed good foaming properties when a 3 min mixing time and equal weights of foamed 10% EW suspension and sourdough were used (**Figure 4A** and **B**). Combining GRSD with EW foam did not show that good behavior in the first place, but shortening the mixing time to 30 s or less produced more stabile foam structures (**Figure 5**). Protein hydrolysates containing low molecular weight peptides are known to effectively decrease the stability of protein foams (*19*, *20*). This could explain the destabilizing effect that GRSD showed on egg white foam in the present study since GRSD obviously contains high amounts of low molecular weight peptides that may destabilize the liquid film structures of bubbles in the egg white foam.

In conclusion, the EW proteins underwent hydrolysis in sourdoughs. The hydrolysis, however, albeit being substantial, likely did not remove the antigenic nature of EW proteins. Ovalbumin broke down only in germinated-rye sourdoughs, whereas ovotransferrin underwent degradation in all studied sourdoughs. Inhibitor experiments showed that cysteine endopeptidases of germinated rye were responsible for ovalbumin hydrolysis. Despite their well-known ability to inhibit endopeptidases, the EW proteins showed no inhibitory effect on prolamin hydrolysis that occurred in sourdoughs. Finally, this study showed that the use of EW foam substantially improved the structural quality of model baked goods which were prepared from sourdough and potato starch. The use of EW foam in the starch based sourdough baking recipes could serve as an alternative way to build up structure in low-gluten bread making applications.

ABBREVIATIONS USED

DTT, dithiothreithol; E-64, epoxysuccinyl-L-leucylamido-(4-guanidino) butane (a cysteine endopeptidase inhibitor); EW, egg white; GRENZ, germinated-rye enzyme extract; GRSD, germinated-rye sourdough; HMW, high molecular weight; LMW, low molecular weight; O-FEN, 1,10-phenanthroline (a metalloendopeptidase inhibitor); PEP-A, pepstatin A (an aspartic endopeptidase inhibitor); PMSF, phenylmethylsulfonyl fluoride (a serine endopeptidase inhibitor); RSD, rye sourdough; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TTA, total titratable acidity; WSD, wheat sourdough.

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Received for review September 12, 2009. Revised manuscript received December 15, 2009. Accepted December 17, 2009.