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Abstract
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Effect of Enzymatic Tempering of Wheat Kernels on Milling and Baking Performance[1]

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ABSTRACT

This study examined the effect of cell-wall-degrading enzymes added to temper water on wheat milling performance and flour quality. An enzyme cocktail consisting of cellulase, xylanase, and pectinase and five independent variables (enzyme concentration, incubation time, incubation temperature, tempered wheat moisture content, and tempering water pH) were manipulated in a response surface methodology (RSM) central composite design. A single pure cultivar of hard red winter wheat was tempered under defined conditions and milled on a Ross experimental laboratory mill. Some treatment combinations affected flour yield from the break rolls more than that from the reduction rolls. However, a maximum for flour yield was not found in the range of parameters studied. Though treatments did not affect the optimum water absorption for breadmaking, enzyme-treated flours produced dough exhibiting shorter mixing times and slack and sticky textures compared with the control. Regardless of differences in mixing times, specific loaf volumes were not significantly different among treatments. Crumb firmness of bread baked with flour milled from enzyme-treated wheat was comparable to the control after 1 day but became firmer during storage up to 5 days.

Milling is a process by which cereals such as wheat are reduced in particle size to produce flour. Wheat milling consists of grain cleaning, tempering, grinding (break system and reduction system), and separation. The purpose of tempering is to toughen the bran so it can resist being broken into small pieces and to soften the endosperm to make it easier to grind. One of the main goals of tempering wheat before milling is to distribute water in the kernel as uniformly as possible. Tempering is considered a very important stage in the milling process from technical, flour quality, and economic points of view.

The starchy endosperm amounts to 81.4–84.1% (db) of the wheat kernel in hard red winter wheat (Hinton 1959). Despite the complexity of the conventional milling process, the normal commercial extraction rate is 70–77% (Jones and Ziegler 1964). In the last decade or so, efforts were made to research the various operations contributing to the milling process to determine methods of separating the bran from endosperm more easily and effectively. The Satake Company developed a mechanical wheat debranning process, but industrial adoption of the process was limited because of excessive energy requirements and questions about effectiveness in efficient bran removal (Forder 1997).

Wheat milling, specifically the creation of white flour by roll milling, has two disadvantages: 1) nutritional components present in nonendosperm tissues, which ideally should be recovered and included in the flour, are lost, and 2) a significant fraction of the endosperm is left attached to the bran. Low tempering moisture and hard grinding can increase flour extraction but result in undesirable quality parameters such as high ash content and dark color. An alternative is modifying the physical structure of the outer layers of wheat kernels to help in their removal. Enzyme-assisted tempering could, conceivably, accomplish this. However, uses of enzymes during tempering and their effects on efficacy and efficiency of the milling process have not yet been established.

Haros et al (2002) reported that wheat treated with enzymes such as cellulase, xylanase, and β-glucanase during tempering had a positive influence on quality of the final products, especially bread, with respect to volume, crumb, and firming rate (staling). Enzyme pretreatment was suggested to modify the initial structure of the starchy endosperm. This alternative method improved final bread quality and overcame enzyme distribution problems caused by nonuniform mixing and overdosage problems (slack and sticky dough) that occurred when enzymes were added directly to the flour or dough with other ingredients. However, the effect of enzymes on bran separation from endosperm was not investigated.

Uses of commercial cellulases in grain processing have been studied and are well established. Hirao et al (1963) studied starch recovery from cellulase pretreated cereals (rye, milo, corn, and barley). Also, Takahaski et al (1966) succeed in reducing the steeping time in corn wet milling by using cellulase. Al-Suaidy et al (1973) studied the effect of cellulase treatment on wheat milling, thinking that as hemicellulase and cellulase hydrolyze the bran layer, which is rich in cellulose and hemicellulose, the chemical composition of the bran layer might be modified. That would, in turn, change the physical properties of wheat kernels and the resultant milling behavior. Cellulase treatment disintegrated the aleurone layer cells as enzyme concentration increased. However, the effect was not enough to alter the milling properties. Benamrouche et al (2002) studied the effect of (1→4)-β-endoxylanase treatment on wheat bran and documented the degradation of the aleurone cell wall as a consequence of treatment; Saxena et al (1993) examined the effect of enzymatic pretreatment on pigeon pea grain milling; and Arora et al (2007) determined the optimum process parameters for the milling of enzymatically pretreated rice. The cellulase used in the latter study acted on the rice bran and cell walls, breaking them down. A lipase, activated along with the cellulase, degraded the oily outer bran layer that otherwise functioned as a barrier to water penetration. The combined actions of enzymes led to a reduction in cooking time.

Application of enzymes in temper water before wheat milling seems to have a number of beneficial effects. The objectives of this study were to determine the effect of enzymatic tempering of wheat kernels on bran separation using a response surface methodology (RSM) experimental design and the effects of enzyme application in tempering water on the milling and baking performance of wheat.

MATERIALS AND METHODS

Wheat Kernel

A pure cultivar sample of hard red winter wheat (2174) was procured from the Agronomy Department at Kansas State University (Manhattan, KS). The wheat kernel test weight was 60.3

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TABLE I
Variables and Levels in the Experimental Design[a]

Variables		Coded Levels				
		–2	–1	0	+1	+2
Enzyme concentration	Units[b]	0	60	120	180	240
	% (w/w)[c]	0	0.84	1.70	2.55	3.40
Incubation time	hr	6	9	12	15	18
Incubation temperature	°C	25	32.5	40	47.5	55
Target moisture content	%	16	18	20	22	24
Tempering water pH	pH	3	4	5	6	7

[a] 33 required tests including seven replicates in center.
[b] One unit is defined as the amount of enzyme that can hydrolyze the substrate and releases 15 mmol of reducing sugar in the supernatant.
[c] Added enzyme concentration based on the dry matter of the wheat kernels.

TABLE II
Experimental Design to Evaluate Enzyme Effect on Dough Characteristics and Baking Performance

	Enzyme Concentration[a]	Incubation Temperature	Tempering Water pH
1 (control)	–	Room temperature	Tap water
2	3%	50°C	pH 5
3	3%	Room temperature	Tap water
4	–	50°C	pH 5
5	–	50°C	Tap water
6	–	Room temperature	pH 5

[a] Tempering condition (tempered wheat moisture content 16%;incubation time 16 hr).
[b] Based on the wheat kernel dry matter (w/w %).

lb/bu. Wheat kernels were characterized using the single kernel characterization system (SKCS) model 4100 (Perten Instruments North America, Reno, NV). Single kernel weight, diameter, hardness, and moisture were 33.94 mg, 2.72 mm, 63.78 (hardness index), and 12.02%, respectively. Moisture content of the wheat was 12.36% (Approved Method 44-15A; AACC International 2000). The required amount of water to temper kernels was determined following a standard tempering table (AACC Approved Method 26-95).

Enzymes

Cellulase (Cellulase-5000 CMCase), xylanase (Xylanase-5000 X), and pectinase (LiquiSEB-RL PRESS) were obtained from Specialty Enzymes and Biochemicals (Chino, CA). An enzyme cocktail containing a combination of xylanase, cellulase, and pectinase was prepared in equal ratios based on enzyme activities. These amounts of enzymes were calibrated based on the amount of reducing sugar released from 1 g of destarched bran substrate when incubated for 2 hr at optimum temperature and pH (40°C, pH 6 for cellulose; 55°C, pH 5 for xylanase; and 45°C, pH 5 for pectinase). After enzyme incubation, the mixture was centrifuged at 10,000 × *g* for 10 min at room temperature, and the supernatant was used for the reducing sugar assay (Miller 1959). One enzyme unit was defined locally as the amount of enzyme that hydrolyzed the substrate and released 15 mmol of reducing sugar in the supernatant under the above reaction conditions. Hence, one unit of the enzymes cellulase, xylanase, and pectinase was 31.68 µg, 26.36 µg, and 127.94 µL, respectively, of the commercial preparations supplied.

Experimental Designs

The experiment was designed and conducted according to an RSM central composite design (Table I). A second-order design was employed using statistical software (Minitab 15, State College, PA) and conducted with five levels: high, medium high, medium, medium low, and low coded as –2, –1, 0, +1, and +2, respectively. Independent variables were enzyme concentration, incubation time, incubation temperature, tempering water pH, and tempered kernel moisture content. This permits the generation of a second-order multiple regression equation to simultaneously relate the dependent variables to all independent variables. The R^2 values measure the goodness of fit of the resulting model to the experimental data (Henika 1972). The results were analyzed using SAS software (v.9.1, SAS Institute, Cary, NC) at $P < 0.05$. Response surface analysis was used to estimate the model coefficient and perform a response surface regression (RSREG) procedure by SAS. The "ridge min" and "ridge max" options in the RSREG procedure were included to generate the ridge of maximum and minimum response of the dependent variables. The total required number of experiments was 33, including seven replicates at the center point. The experimental run order was randomized.

Wheat Preparation and Milling Process

Wheat (500 g) was tempered to the desired moisture content following the experimental design. The required amounts of water and enzymes were calculated and added to the wheat. Distilled water was mixed with hydrochloric acid (HCl) to adjust to pH 3, 4, 5, or 6. For pH 7, distilled water was used for tempering. The enzymes were dissolved completely before they were added to the wheat. Wheat samples were shaken by hand with water or enzyme solution in double-layer sealed plastic bags for 3 min and then incubated for the stipulated time and temperature in a force-draft oven. Because milling was to be done at 16% moisture content, wheat that was tempered to >16% was spread out on round sieves as a single layer and dried back to 16% moisture content at 32°C. Before milling, physical properties of the tempered kernels were measured using the SKCS. For grinding, the experimental laboratory mill (Ross, Oklahoma City, OK) was used in a flow that consisted of four breaks (BK), one sizing (SIZ), two tailings (T), and five reductions (M) (Posner et al 1997). The Ross mill was chosen in this study as it produced 17 product streams compared with the eight streams produced by the Buhler mill. Also, the Ross mill was controllable so as to maintain a constant break release across the various wheat treatments.

Milling and Flour Quality Parameters

The amounts of flour produced from the 13 streams were analyzed individually and then combined as patent, 1st clear, 2nd clear, and straight flours and analyzed with SAS to observe the effect of treatments on wheat flour yield. Flour obtained from each grinding was classified into patent and clear flours and then analyzed for flour quality and milling efficiency. Flour yield and quality parameters, namely flour protein, ash contents, and flour color, were determined following Approved Metods 46-30, 08-01, and 44-15A, respectively (AACC International 2000). For flour color, a reflectance color meter (model M-45-D, Agtron) was used in green light mode.

Dough Characterization and Test Baking

To compare the effects of enzymes on dough characteristics and breadmaking, it was necessary to simplify the experimental

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design in terms of the number of treatments. Only three factors, enzyme concentration (0 and 3% w/w based on dry matter, combination of cellulase, xylanase, and pectinase), incubation temperature (room temperature and 50°C), and tempering water pH (tap water and pH 5 solution), were varied during tempering, and the rest of the tempering conditions were maintained at 16% tempered wheat moisture content and 16 hr of incubation time. This experimental design is shown in Table II.

For dough testing, traditional empirical rheological methods including the mixograph (10-g flour bowl, National Manufacturing Division of TMCO, Lincoln, NE) and the Farinograph E (50-g flour bowl, C.W. Brabender, Duisburg, Germany) were conducted before the baking test to determine the optimum water absorption and mixing times and stabilities following Approved Methods 54-40A and 54-21, respectively (AACC International 2000)

Straight-dough pup loaves (100 g of flour) were baked following Approved Method 10-10B (AACC International 2000). Optimum mixograph water absorptions were 64% and mixing times varied at 4–6 min. Corrected water absorption and mixing time after a mixing time pretest were used for test baking. Proof heights and baked bread weights and volumes were measured. Loaf volumes were measured by the rapeseed displacement volume meter (AACC Approved Method 10-05). For the staling experiment, a texture analyser (Voland-Stevens LFRA, Hawthorne, NY) was used to measure the bread firmness using a bread slice 1-in. thick (25.4 mm) and penetration distance of 6 mm at 2 mm/sec speed (AACC Approved Method 74-09). Replicate loaves were baked for each treatment. Three slices were measured from each loaf, and the average for the six slices was taken for comparison purposes. The bread was stored in double plastic bags at room temperature for 1, 3, or 5 days before testing. The data obtained from the test bakings were analyzed with ANOVA using SAS software at $P < 0.05$.

TABLE III
Analyzed Significant Factors ($P < 0.05$) to Response Surface of Physical Characteristics of Tempered Wheat and Flour Protein and Ash Content

	Significant Factors[a]	R^2
Kernel physical characteristics		
Kernel weight	b, b*d, a*e	0.86
Kernel diameter	–	0.73
Kernel hardness	b	0.74
Flour qualities		
Clear flour ash	–	0.54
Patent flour ash	–	0.43
Clear flour protein	b, a*e	0.79
Patent flour protein	d, d*d	0.62
St. Flour color	–	0.71

[a] Enzyme concentration (a); incubation time (b); incubation temperature (c); tempering moisture content (d); tempering water pH (e); interaction between two treatments (*).

Sugar Analyses

The effects of enzymes and their activities on bran were assessed by an increase in total reducing sugars in the supernatant by the 3,5 dinitrosalicylic acid assay as described by Miller (1959) but were volumetrically modified to a total 1.5 mL of reactant. Dextrose was used as a standard.

RESULTS AND DISCUSSION

Treatment Effects on Tempered Wheat Kernel Physical Characteristics

Significant factors affecting the physical characteristics of the tempered wheat kernels are summarized in Table III. Enzyme incubation time and interactions between incubation time and moisture and between enzyme and pH were significant factors affecting single kernel weight after tempering, and the resulting RSM equation explained ≈86% of the variation in tempered kernel weight. Incubation time was the only significant factor affecting kernel hardness after tempering, and an RSM equation accounting for 74% of the variation in tempered kernel hardness

TABLE IV
Patent Flour Ash Content, Protein Content, and Flour Yield for Treatments Following RSM Central Composite Design

				Milling Product Yield (%)			
Test Run	Ash (%)	Protein (%)	Agtron Reading	Patent Flour	1st Clear	2nd Clear	Yield[a]
1	0.54	10.8	72	61.0	5.1	1.9	72.9
2	0.41	10.7	74	60.6	5.6	1.7	73.3
3	0.44	10.7	76	60.3	6.9	2.5	72.9
4	0.40	10.5	74	56.5	7.4	3.0	69.3
5	0.41	10.6	78	59.4	4.7	2.3	72.3
6	0.42	10.6	79	62.5	5.4	2.2	73.5
7	0.45	10.8	76	61.7	5.8	2.2	73.0
8	0.50	10.9	75	61.7	6.0	1.8	73.4
9	0.42	10.7	76	59.6	6.4	2.5	72.6
10	0.43	10.8	74	61.0	7.1	2.9	72.5
11	0.52	10.7	77	63.2	5.8	1.0	72.9
12	0.46	10.7	77	59.3	7.0	2.2	72.2
13	0.46	10.8	78	61.9	6.2	1.9	73.1
14	0.43	10.5	78	60.0	6.1	2.1	72.1
15	0.44	10.7	73	61.1	6.2	1.6	71.2
16	0.43	10.8	75	61.8	6.2	1.8	72.2
17	0.47	10.6	76	61.9	5.7	2.4	72.1
18	0.40	10.8	77	62.6	6.1	2.2	73.3
19	0.43	10.6	73	59.1	6.6	2.7	70.7
20	0.43	10.9	75	61.8	6.5	1.6	72.5
21	0.50	10.8	75	61.4	6.7	2.2	73.2
22	0.49	10.9	75	62.9	6.1	1.4	73.2
23	0.42	10.7	73	62.0	5.9	1.5	72.0
24	0.46	10.7	77	61.1	5.4	2.5	72.3
25	0.50	10.8	75	60.8	6.3	2.2	73.1
26	0.49	10.8	75	61.9	6.0	2.1	73.3
27[b]	0.43	10.6	77	60.9	6.2	2.4	72.1

[a] Based on the total product.
[b] Average of seven replicates.

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was obtained. There was no significant factor for tempered wheat diameter, and 73% of the variation was explained by the RSM equation. The predicted response surface was saddle shaped, thus only slight changes in physical characteristics were caused by the five variables as observed across the 33 data sets.

Effects on Product Yield

Each mill stream and final product yield of 33 tests is shown in Table IV, and significant factors affecting the response surface of product yield are summarized in Table V. The stationary points for all predicted response surfaces were at saddle points and did not show optimum condition. Most of the flours from the break, sizing, and tailing streams were affected by the treatments, whereas none of the flours from the reduction rolls were affected. The presence of enzyme alone and enzyme interactions with any other factors such as temperature, incubation time, and pH showed effects on the yields of 1BK, 2BK, 1T, bran, 1st clear, and straight-grade flours. Interactions among incubation time, temperature, moisture content, and pH had a significant influence on the response surface of the break flour yield. From the ridge analysis, pH change was the most obvious factor correlating with a production yield change. For the production of shorts, red dog, and germ, all factors but enzyme showed effects on decreasing or increasing the yield. The yield of 1st clear was well explained by the predicted response surface model with $R^2 = 0.91$. Ridge analysis showed that 1st clear flour yield increased from 6.2 to 6.6% when time, moisture, and pH varied from 12.00 to 12.75 hr, from 20.00 to 18.47%, and from 5.00 to 3.95, respectively. No change or very slight change was observed as a function of enzyme dose and temperature in this range. Like the 1st clear flour, the pattern of patent flour yield within the range of variables was estimated by ridge analysis and increased with an increase in enzyme, time, and temperature and a decrease in moisture and pH. Predicted product yield was determined by a model with 21 terms (equations not shown).

Effects on Flour Color and Flour Protein and Ash Contents

Agtron readings for the various treatments ranged from 72 to 79, 51 to 68, and 70 to 77 for patent, clear, and straight flour, respectively. There were no significant factors affecting flour color ($P < 0.05$), and the stationary point was, again, a saddle point. Further analysis (ridge analysis with radius 2.0) was required to see a pattern of color change for the variables within the given range. Ridge analysis showed that the flour Agtron color index increased slightly as the amount of enzyme and pH decreased and as the incubation time increased.

Significant factors and R^2 values for flour protein and ash contents are shown in Table III. The treatments were not statistically significant for the patent and clear flour ash contents with R^2 values of 0.43 and 0.54, respectively. Patent flour ash contents ranged from 0.382 to 0.532, and clear flour ash contents ranged from 0.569 to 1.010 for the treatments. For ash content in both flours, clear and patent, none of the factors appeared to be significant, and the result of ridge analysis of the predicted response surface showed that ash content increased as pH decreased with a slight change associated with the other treatments. Both the linear and the quadratic terms for tempering moisture content were the significant factors affecting the patent flour protein, whereas incubation time and the interaction between enzyme concentration and pH affected clear flour protein content. Patent flour protein content ranged from 10.4 to 10.9%, and clear flour protein content ranged from 10.1 to 12.3% across treatments. The resulting RSM equations explained 79.38 and 61.91% of the variation in patent and clear flour protein contents, respectively.

Test Baking

Different flours of the six tempering conditions listed in Table II showed that significantly higher protein contents of the flours resulted from the enzymatically tempered wheat samples (data not shown). Compared with the control, the straight-grade flour protein content was higher by 2.2% for enzymatically tempered wheat at 50°C with pH 5 water and higher by 2.9% for enzymatically treated wheat at room temperature with tap water. However, it could not be concluded that the increased protein is functional protein for breadmaking. The ash content did not increase and the extraction did not increase, so it does not appear that the protein increase was the result of scraping the aleurone layers. The applied enzyme amount (3% w/w based on dry matter of wheat kernels) could account for an increase in the protein level of flour from the enzyme-treated kernel. Farinograms showed that enzyme-treated flour seemed to require slightly higher absorption by 1% at 57% and broke down more rapidly after the peak time had been reached. This shorter stability of the doughs made from enzyme-treated flours reflected on the mixing times observed during test baking. The optimum mixing time for test baking was 3 min 30 sec for enzyme-treated flour. This was shorter than the mixing time of 4 min 15 sec for the untreated flour. After 4 min mixing, the flour from enzyme-treated wheat became sticky and slack.

The test baking results are shown in Table VI. There were significant differences in proof height and bread weight, but these differences did not result in differences in final bread volume. Any treatments applied during tempering did not make a difference in bread firmness after 24 hr. The bread produced with flour from kernels tempered at 50°C with tap water showed the lowest firmness after 3 and 5 days. Bread with only enzyme-treated flour showed the lowest in firmness after 1 day. However, enzyme treatment did not slow down the staling rate at 3 or 5 days, which does not agree with previous reports (Courtin and Delcour 2002).

CONCLUSIONS

Cell-wall-degrading enzymes cellulase, xylanase, and pectinase were used in this study to partially hydrolyze the bran fraction and release reducing sugars. When these enzymes were combined and added to tempering water under various conditions, no improvements in flour yields were observed. Flours milled from enzyme-treated tempered wheat contained significantly higher

TABLE V
Analyzed Significant Factors ($P < 0.05$) to Response Surface of Mill Streams and Final Product Yield

Milling Product	Significant Factors[a]	R^2
Mill stream		
First break flour	e*a, e*e	0.86
Second break flour	a, a*c, b*c, c*d, a*e	0.82
Third break flour	c*e	0.64
Fourth break flour	–	0.55
Sizing flour.	c	0.70
First middling flour	–	0.56
First tailing flour	a*c	0.71
Second middling flour	–	0.62
Third middling flour	–	0.57
Second tailing flour	–	0.68
Fourth middling flour	–	0.65
Fifth middling flour	–	0.75
Low grade flour	–	0.58
Final product		
Shorts, red dog, and germ	b, d, b*b, b*c, d*d, c*e	0.87
Bran	e*a, e*c	0.76
Patent flour	b, d*c	0.79
First clear flour	a, b, a*b, a*e, c*e, d*e, e*e	0.91
Second clear flour	–	0.61
Straight flour	b, a*a, a*c, b*d	0.87

[a] Enzyme concentration(a); incubation time (b); incubation temperature (c); tempering moisture content (d); tempering water pH (e); interaction between two treatments (*).

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TABLE VI
Baking Performances and Bread Firmness[a]

Treatment	Baking Performance			Load in Compression, g		
	Proof Height, cm	Bread Weight, g	Specific Vol, g/cm^3	1 Day	3 Days	5 Days
Control	6.05a,b	143.35a,b	5.250a	337.5a	714.0a	769.0b
pH 5[b]	6.10a,b	142.15a,b	5.394a	335.0a	623.5a,b	903.0a
50°C[c]	6.35a	143.65a,b	5.620a	328.5a	529.5b	740.5a
pH 5, 50°C	6.15a	144.35a	5.254a	334.0a	580.5a,b	891.0a
pH 5, 50°C, E[d]	5.60b	144.50a	5.091a	340.5a	601.5a,b	904.5a
E	6.20a	141.30b	5.586a	282.0a	548.0b	852.0a

[a] For a given test parameter, mean values ($n = 2$) followed by the same letter are not significantly different at $P < 0.05$.
[b] Tempering water at pH 5.
[c] Incubation temperature 50°C.
[d] Enzyme 3% w/w.

protein contents than flour from the nonenzyme-tempered wheat when the rest of the experimental conditions remained the same. Although treatments showed slight differences in mixing times between enzyme-treated and nonenzyme-treated flours, specific pup loaf volumes were not significantly different. However, the firmness of enzyme-treated bread was significantly higher than that of control bread after 5 days of storage.

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