

Effects of Commercial Hydrolytic Enzyme Additives on Japanese-Style Sponge and Dough Bread Properties and Processing Characteristics

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ABSTRACT

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The effects of increasing levels of eight commercial fungal enzymes enriched in four types of activity (α -amylase, protease, xylanase, or cellulase) on Japanese-style sponge and dough bread quality and processing characteristics have been studied using a Canadian red spring wheat straight-grade flour. At optimum levels, the enriched α -amylases, xylanases, and cellulases increased loaf volume and bread score and reduced crumb firmness, while the proteases only reduced crumb firmness. For α -amylases, xylanases, and cellulases, optimum levels for crumb firmness were obtained at higher levels of addition than for loaf volume and bread score. At high levels of addition, all four enriched enzyme types reduced loaf volume and bread score and increased crumb firmness relative to

optimum levels, with the proteases showing the most dramatic effects. α -Amylases and cellulases had little impact on dough mixing requirements, while xylanases increased and proteases greatly reduced mixing requirements. All enzymes at optimum levels reduced sheeting work requirements, resulting in softer more pliable dough. Optimum bread properties for α -amylases, xylanases, and cellulases were attained within a relatively narrow range of dough sheeting work values. This similarity in response suggests a dominant common nonspecific mechanism for their improver action, which is most likely related to water release and the resulting impact on physical dough properties.

Hydrolytic enzymes are used widely in the baking industry to improve dough-handling properties and enhance bread quality. The most commonly used of these enzymes are α -amylases, with the fungal form being the most popular. The preference for fungal α -amylase can be attributed to its lower denaturation temperature relative to plant and bacterial α -amylases. This property essentially eliminates the occurrence of gummy crumb associated with the production of dextrans from hydrolysis of gelatinized starch during the early baking stage without affecting the production of fermentable sugars (Ranum and DeStefanis 1990). Proteases have been widely used to reduce mixing time and eliminate “bucky” handling properties associated with overly strong flours. More recently, xylanases (hemi-cellulases), which hydrolyze pentosans, and cellulases (β -glucanases), which hydrolyze complex cell wall carbohydrates, have been introduced. These enzymes also improve handling properties and bread quality, including crumb softness (Kulp 1968; Rouau et al 1994; Martínez-Anaya and Jiménez 1997a; Monfort et al 1997; Si 1997; Harada et al 2000).

The improver effects of these hydrolytic enzymes at optimum levels have been attributed to both common nonspecific and specific effects. The former effect appears to be associated with improved (softer) dough-handling characteristics, improved oven spring, and softer crumb due to the hydrolytic release of water from the respective polymeric substrates (Navickis et al 1982; Kulp 1993; Rouau et al 1994; Martínez-Anaya and Jiménez 1997b; Harada et al 2000). The latter effect has been associated with interactions between the enzyme-specific hydrolysis reaction products and other dough or bread components, resulting in improved processing and bread characteristics (Kulp 1968; D’Appolonia 1980; Martin and Hoseney 1991; Biliaderis et al 1995; Bombara et al 1997). The relative importance of these effects is currently not well understood owing to a lack of studies comparing the performance of different types and sources of hydrolytic enzymes (Ranum and DeStefanis 1990; Rouau et al 1994; Martínez-Anaya and Jiménez 1997a; Si 1997) and the confounding effects of other factors such as baking process and flour characteristics that strongly influence response (McDonald 1969; Cauvain and Chamberlain 1988; Rouau et al 1994).

In a recent study using a high-quality Canadian bread wheat flour (Harada et al 2000), we provided evidence suggesting that for no-time dough, a nonspecific mechanism involving water release as that described above was mainly responsible for the bread-improving properties of hydrolytic enzymes. All four commercial enzyme types enriched in α -amylase, protease, xylanase, and cellulase activity were equally effective in improving bread quality characteristics at optimum levels. Furthermore, optimum bread properties with all four enriched enzyme types were obtained when dough sheeting work showed values within a relatively narrow range, presumably corresponding to optimum dough-handling properties.

In this study, we report on the effects of the same commercial hydrolytic enzymes on the processing and bread properties of sponge and dough bread prepared from a straight-grade Canadian Western Red Spring (CWRS) wheat flour using ingredients and processing conditions similar to those used in Japanese bakeries. The use of a similar flour and similar enzyme levels compared with the work cited above (Harada et al 2000) also allowed an evaluation of the importance of baking processes in determining the impact of these commercial enzymes.

MATERIALS AND METHODS

Flour and Enzyme Samples

Straight-grade flour was prepared from a No. 1 CWRS wheat on the GRL pilot mill. The flour had a protein content of 12.2%, ash of 0.51%, farinograph dough development time of 4.50 min, and a farinograph absorption of 62.4% (all data corrected to 14.0% flour moisture basis).

Commercial enzymes were supplied by Enzyme Development Corporation, New York, NY, and by Amano Enzyme USA Co., Ltd., Troy, VA. Descriptions of the enzymes including side activities, which were provided by the manufacturers, were given in a previous publication (Harada et al 2000). Enzymes were mixed thoroughly with a base flour and the appropriate amount of this mixture was added to the test flour to give the desired activity based on activities provided by the manufacturer. To protect the company enzyme source, a number code was used for each pair of enzyme types. Activities for the α -amylases are reported as Sandstedt-Keen-Blish (SKB) units and for proteases as hemoglobin units (HUT). Activities reported for xylanases and cellulases varied depending on the supplier. For xylanases, activity units on birch xylan substrate (BXU) or activity units on xylan substrate (u) are reported. For cellulases, cellulase units on hydroxyethyl cellulose (ECU) or carboxymethyl-cellulose hydrolyzing activity

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units (CMC-ase) are given. Further details for the enzyme assays are available from the manufacturers.

Baking Procedures

The sponge and dough baking procedure was performed as previously described (Yamada and Preston 1994). Sponge ingredients included flour (70 g, 14.0% moisture basis), fresh compressed yeast (2.0 g), salt (0.15 g), ammonium phosphate (0.1 g), ascorbic acid (40 ppm), 60°L malt syrup (0.2 g), water (prorated optimum absorption – 2 mL as assessed by an experienced baker at panning), and enzyme additive (variable, see below). Sponge ingredients were mixed in a GRL 200 mixer at 135 rpm for 2.5 min (27°C) then fermented for 4.5 hr at 27°C (90% rh) in greased, glazed crockery bowls in a fermentation cabinet. The sponge and remaining ingredients including flour (30 g), salt (2.25 g), sucrose (5.0 g), shortening (3.0 g), skim milk powder (2.0 g), malt syrup (0.1 g), and water (prorated optimum absorption plus 2 mL) were mixed to 10% past peak consistency in the same mixer at 135 rpm at 30°C. After a 15-min rest period, the dough was punched lightly seven times, rounded by hand, and given an intermediate proof of 15 min (30°C). Dough was then sheeted three times (8.7, 4.8, then 3.2 mm), molded on the GRL molder, panned and then proofed for 70 min at 38°C (83% rh). Baking was performed in heat-sink ovens for 24 min at 195°C as previously described (Kilborn et al 1990).

Measurement of Dough and Bread Properties

Mixing time (to peak consistency) and mixing energy to peak at the dough stage were obtained using a GRL Whr meter attached to the GRL 200 mixer (Kilborn 1979). Dough sheeting energy (sum of 2nd and 3rd sheetings) was obtained by means of a force transducer attached to the arm of the sheeter as described in (Kilborn and Preston 1982) except that after A/D conversion and amplification the digitized signal was fed to a 486 microcomputer. Signal processing and integration of the resulting force-time curve to obtain sheeting energy was done using Labtech Notebook v. 7.2.1 for DOS (Labtech, Wilmington, MA). Bread volume, crust appearance, crumb color and texture, and total bread score were assessed by an experienced baker as described previously (Preston et al 1982). Crumb firmness was determined 24 hr after baking by measuring compression force at 50% deformation for three stacked bread slices (38 mm total height) using the GRL compression tester (Kilborn et al 1983). All loaves were stored at room temperature in plastic bags and sliced just before compression.

Experimental Design for Testing Enzymes

For each experiment, enzyme was added at nine different levels including a control (0%) using a randomized block design. Three blocks, baked on different days, were used to obtain mixing energy, mixing time, total sheeting work, loaf volume, crumb and crust characteristics, bread score, and crumb firmness after 24 hr. Results

were analyzed by analysis of variance (ANOVA) using SAS v. 6.11 (SAS Institute, Cary, NC). Appropriate means were compared for significance at the 5% level using Duncan's multiple range test.

RESULTS

Preliminary studies were conducted with the same range of enzyme levels used in a previous study where a no-time dough process was assessed (Harada et al 2000). In keeping with Japanese commercial practice, enzymes were added at the sponge stage. Dough absorption was maintained at the optimum (64%) value obtained for the control (no enzyme addition). At the highest levels of addition, dough treated with the commercial enzymes enriched in α -amylase, xylanase, and cellulase activity was softer, as assessed by dough feel at panning, while the higher levels of protease gave very slack and sticky dough that was very difficult to handle. Maximum protease levels were therefore reduced to 1,000 HUT units (from 3,000 HUT), while the previous no-time dough maximum levels were maintained for the other enzymes.

It should be noted that there was some side activity in all the commercial enzymes used as outlined in the previous no-time baking study cited above (Harada et al 2000). Thus, reference to a particular enzyme type in the remainder of the discussion does not necessarily imply that the effects can be totally associated with that primary activity. To reduce the influence of side activity on the interpretation of results, two different commercial enzyme sources with differing degrees of side activities were used for each enzyme type.

Effects of Enzyme Level on Loaf Volume, Bread Score, and Crumb Firmness

ANOVA results (Table I) showed that enzyme level had significant ($P < 0.05$) effects on sponge and dough bread volume, score and crumb firmness after 24 hr storage at room temperature for most of the enzyme types. The exceptions included xylanase1, where level had no significant overall effect on loaf volume and crumb firmness, and protease1, where level had no significant overall effect on crumb firmness.

Figures 1–3 show the impact of increasing enzyme levels on bread volume, bread score, and crumb firmness, respectively. For loaf volume, initial optimum values with increasing enzyme level (beyond which there was no significant increase) for the two α -amylases were attained at levels of 100–200 SKB units, while much higher values had little further impact. A similar trend was evident with bread score at lower levels of addition, but at higher values (1,200 and 2,000 SKB units), significant reductions in score were evident relative to both optimum and control values. These reductions could be primarily attributed to deterioration in loaf appearance and crumb structure scores (data not shown). No significant increases in loaf volume or bread score were apparent with increasing levels of protease1 relative to the control (no

TABLE I
ANOVA^a and CR^b of Effects of Enzyme Level on Dough Processing and Bread Properties for Sponge and Dough Bread Produced from a Canadian Straight-Grade Flour

Enzyme ^c	Loaf Volume (cm ³)	Bread Score (units)	Crumb Firmness (Nm ²)	Mixing Time (min)	Sheeting Work (Whr/kg)
Amylase1	0.008 (47)	0.0001 (8.3)	0.02 (542)	ns ^d (0.48)	0.0004 (0.017)
Amylase2	0.009 (37)	0.004 (10.8)	0.05 (850)	ns (0.61)	0.0006 (0.030)
Protease1	0.0005 (36)	0.0001 (11.4)	ns (828)	0.0001 (0.54)	0.0001 (0.028)
Protease2	0.0008 (58)	0.0001 (15.6)	0.02 (1,119)	0.0001 (0.70)	0.0001 (0.028)
Xylanase1	ns (59)	0.04 (12.8)	ns (590)	0.0001 (0.49)	0.0006 (0.024)
Xylanase2	0.02 (69)	0.0001 (11.0)	0.002 (611)	0.005 (0.84)	0.0001 (0.017)
Cellulase1	0.01 (67)	0.0002 (7.5)	0.008 (470)	ns (0.41)	0.0001 (0.017)
Cellulase2	0.02 (76)	0.0001 (10.2)	0.03 (715)	ns (0.91)	0.0001 (0.025)

^a ANOVA values ($P <$) followed by Duncan's Critical Range (CR).

^b CR values (difference between values for $P < 0.05$) in parentheses.

^c Number 1 or 2 following enzyme abbreviation is coding for company source.

^d Nonsignificant ($P > 0.05$).

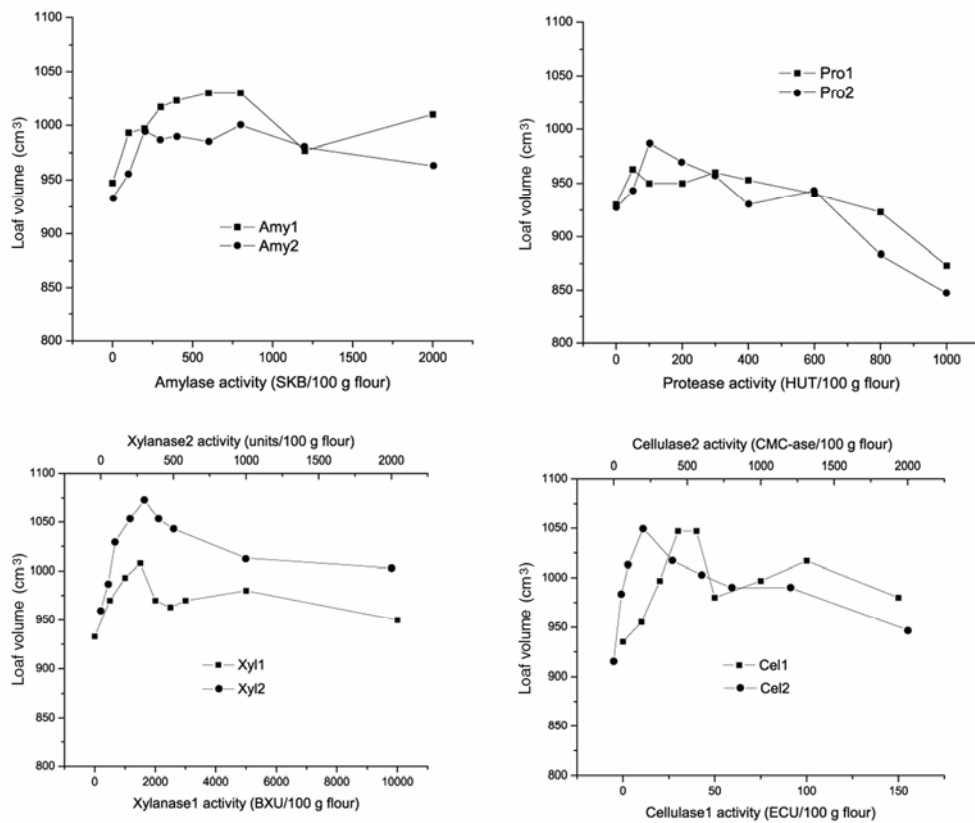


Fig. 1. Effects of increasing levels of commercial hydrolytic enzymes on sponge and dough bread loaf volume. See Table I for ANOVA and Duncan's critical range (CR) values for $P < 0.05$.

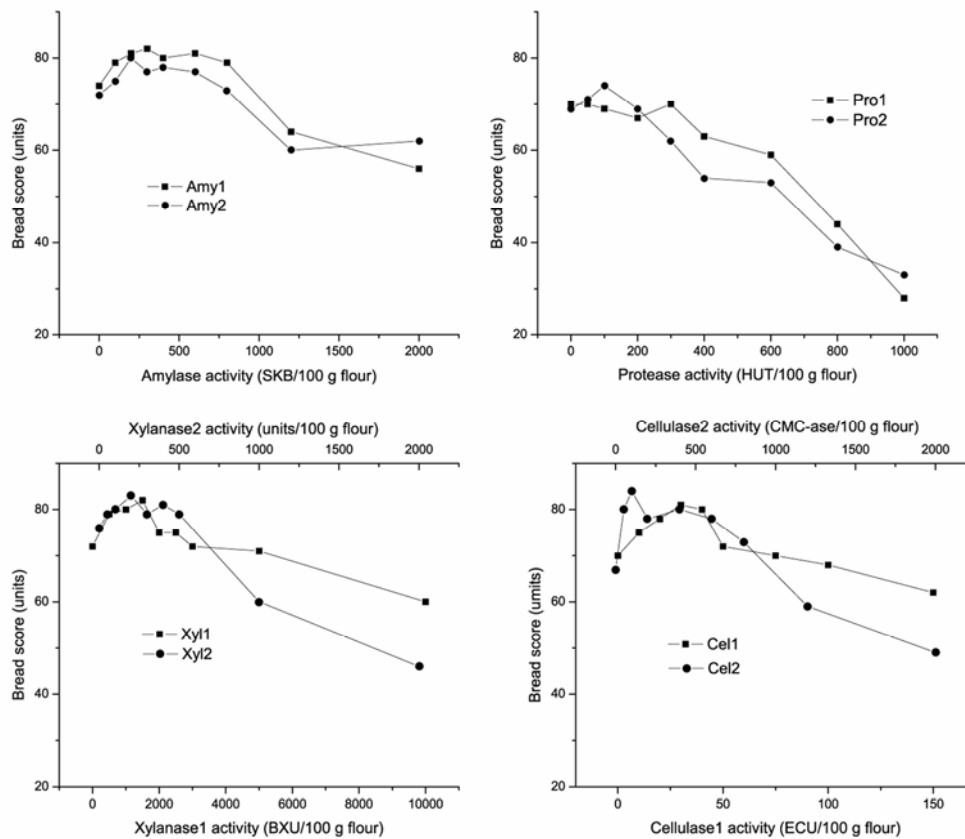


Fig. 2. Effects of increasing levels of commercial hydrolytic enzymes on sponge and dough bread score. See Table I for ANOVA and Duncan's critical range (CR) values for $P < 0.05$.

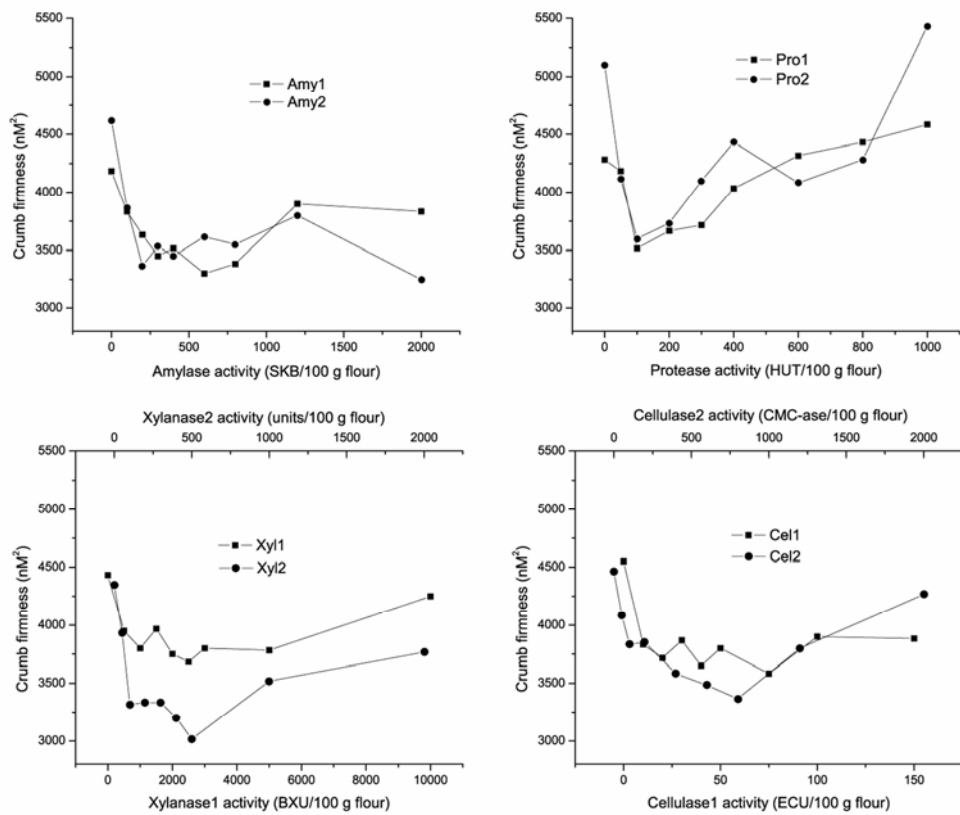


Fig. 3. Effects of increasing levels of commercial hydrolytic enzymes on sponge and dough bread crumb firmness. See Table I for ANOVA and Duncan's critical range (CR) values for $P < 0.05$.

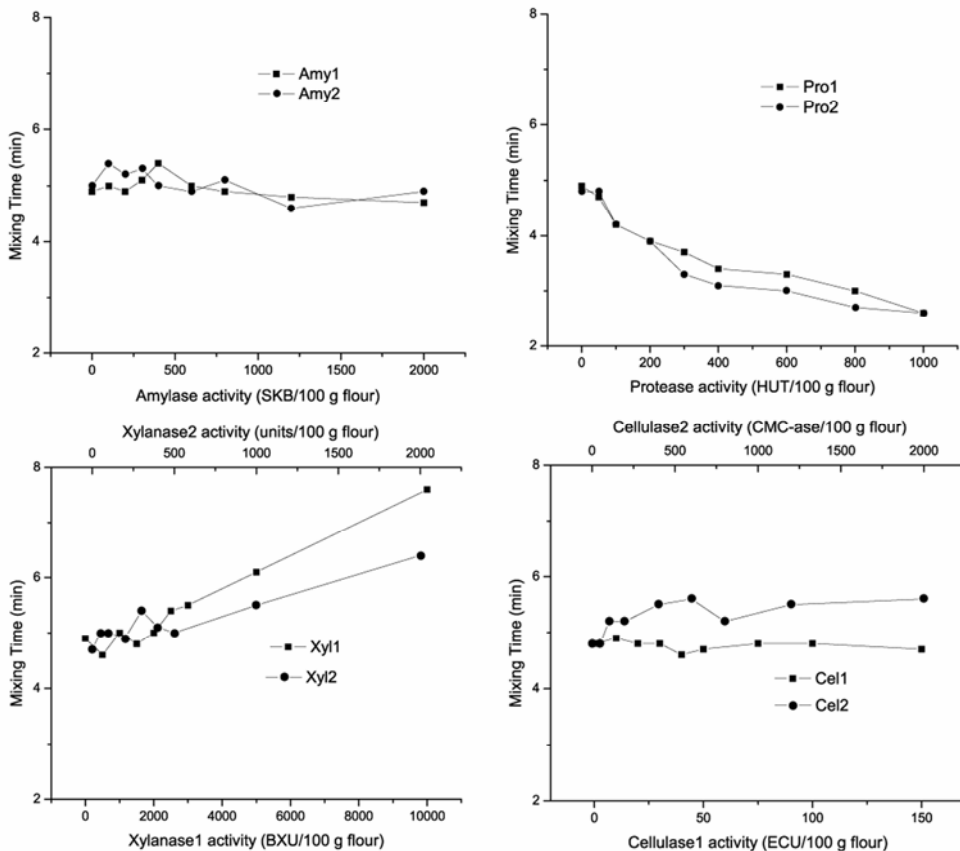


Fig. 4. Effects of increasing levels of commercial hydrolytic enzymes on sponge and dough bread mixing time. See Table I for ANOVA and Duncan's critical range (CR) values for $P < 0.05$.

enzyme added). For protease2, only the 100 HUT level of addition showed a significant ($P < 0.05$) increase in bread volume compared with the control. As with protease1, no significant increase in bread score was achieved at any level of protease2 addition. At higher levels of addition of either protease, significant decreases in bread volume and score were clearly evident relative to the control.

Bread volume and score showed a strong trend to higher values with increasing levels of either xylanase, followed by decreasing values at the highest levels. Although the overall effect of xylanase1 on bread volume was not significant (Table I), addition of levels of 1,000 and 1,500 BXU/100 g of flour gave significant increases in this parameter relative to the control. For xylanase2, significant increases in volume relative to the control were attained at levels ranging from 100 to 500 units/100 g of flour. For bread score, only the highest levels of xylanase2 were significantly different (lower) than the control. Results with cellulases were more definitive. Significant increases in both bread volume and score were evident with increasing levels of both cellulases with optimum values attained at levels of 30–40 ECU units for cellulase1 and 50–600 CMC units for cellulase2. At higher levels, significant decreases in both volume and score were evident for both cellulases relative to optimum values.

The effect of excess cellulase was most evident with bread score because, in contrast to volume, values were significantly lower than the control. In addition to decreased volume, these rapid decreases in bread scores could be attributed to deterioration in loaf appearance and crumb structure scores (data not shown).

Crumb firmness showed significant overall decreases relative to the control with six of the eight enzymes tested (Table I, Fig. 3). Both amylases showed a significant crumb softening effect relative to the control over the same range where bread volume and score attained the highest values. At the highest levels of amylase1, firmness increased to values not significantly different than the control, while at the highest levels of amylase2, firmness was still significantly lower than the control. For both proteases, there was a trend to softer crumb with increasing levels of enzyme followed by an increase in firmness at the highest level. However, for protease1, no individual values were significantly different than the control. For protease2, levels of addition of 100–200 HUT

units were significantly lower than the control, even though no improvement in bread volume or bread score was evident. Addition of increasing levels of the two xylanases showed the same trend as the proteases. Softer crumb was generally associated with higher bread volumes and scores. At the highest levels of addition, crumb was still generally softer ($P < 0.05$) than the corresponding controls. For cellulase1, all levels of enzyme addition resulted in significantly reduced crumb firmness, while for cellulase2, enzyme addition levels of 400–1,200 CMC units gave significantly softer crumb relative to the control.

Effects of Enzyme Level on Mixing and Sheeting Properties

The effects of increasing enzyme levels on mixing time and sheeting properties of the Canadian straight-grade CWRS flour dough are shown in Table I (ANOVA) and in Figs. 4 and 5. ANOVA results showed that increasing levels of amylases and cellulases had no significant effect on mixing time, while highly significant ($P < 0.01$) effects were evident for both proteases and both xylanases. For both proteases, increasing levels of enzyme addition resulted in significantly reduced mixing time (Fig. 4). In contrast, both xylanases caused significant increases in mixing time with increasing enzyme levels. Mixing energy requirements showed the same trend as mixing time for all enzymes except xylanase2, where no significant increase was evident with increasing enzyme level (data not shown).

ANOVA showed that all enzymes had highly significant effects ($P < 0.001$) on the amount of work required to sheet the dough before rounding. Increasing levels of all enzymes resulted in significant decreases in sheeting work (Fig. 5). The most profound effects were evident for the two protease enzymes where sheeting work dropped by a factor of >10 from the control to the highest enzyme levels. Very low sheeting work values were also evident at the highest levels of addition of cellulase2 and xylanase2. These low levels were associated with poor baking quality (low volumes and scores). A narrow range of sheeting energy (0.070–0.084) was associated with the highest values for bread volume and score from the three types of enriched enzymes (amylases, xylanases, and cellulases) that significantly improved baking performance relative to the control.

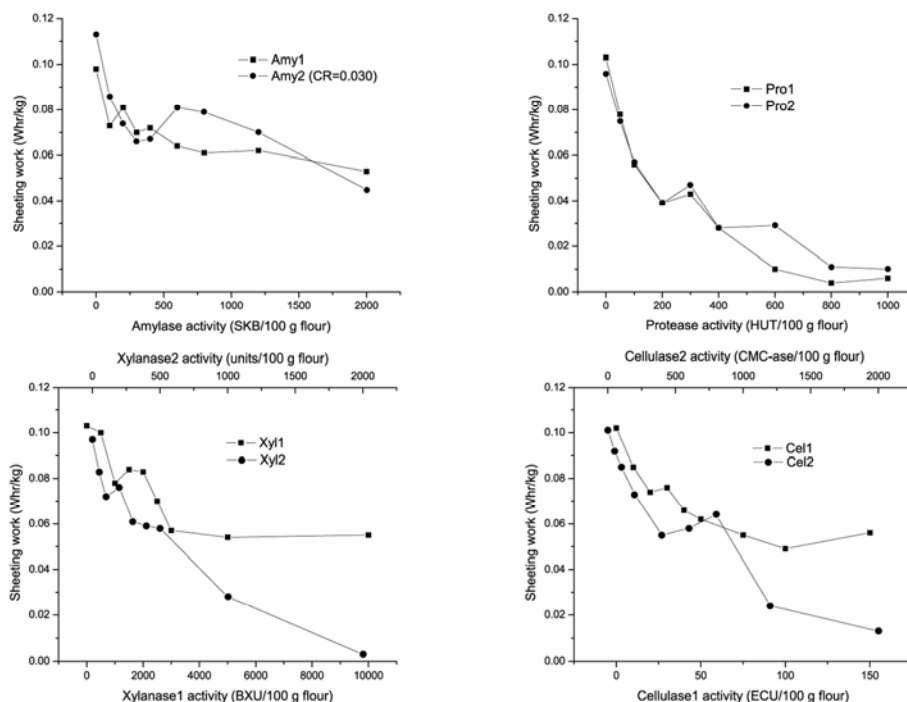


Fig. 5. Effects of increasing levels of commercial hydrolytic enzymes on sponge and dough bread sheeting work. See Table I for ANOVA and Duncan's critical range (CR) values for $P < 0.05$.

DISCUSSION

This study demonstrates that addition of commercial hydrolytic enzymes enriched in fungal α -amylase, xylanase, and cellulase activity during the sponge stage can lead to improved Japanese style sponge and dough bread quality through increased bread volume and score and softer crumb texture. Although some differences were apparent in response among and between these commercial enzyme types, overall these enzyme types appeared to show a similar degree of effectiveness at optimum levels and similar responses (tolerances) to addition of higher activity levels. Previous studies using sponge and dough processing conditions have shown that addition of α -amylases (Maeda et al 2003) or various types of pentosanases can increase bread volume and improve crumb softness. Other studies using straight or no-time dough processing conditions have also demonstrated the positive effects of hydrolytic enzymes on bread volume, bread score, and bread texture for α -amylases (Cauvain and Chamberlain 1988; Bajwa 1990; Martinez-Anaya and Jiménez 1997a; Si 1997), pentosanases/xylanases (McCleary 1986; Martinez-Anaya and Jiménez 1997a; Monfort et al 1997; Si 1997; Courtin et al 2001; Rouau et al 1994) and cellulases (Harada et al 2000).

Comparison studies among different types of hydrolytic enzymes are limited. Martinez-Anaya and Jiménez (1997a) and Si (1997) showed that addition of appropriate levels of commercial α -amylase and pentosanase/xylanase preparations improved straight-dough process bread volume and crumb softness and reduced staling rate, but that the response was dependent on both enzyme type and source. More recently, Harada et al (2000) compared the performance of essentially the same flour and the same commercial enzymes tested in the present study using a no-time (Canadian short process) baking procedure. The general response of bread volume and score and crumb softness to increasing levels of commercial enriched fungal α -amylases, xylanases, and cellulases were very similar to the present study, indicating a lack of response to baking procedure. However, different responses were apparent in terms of the impact of commercial enriched proteases. For both processes, low protease levels improved crumb softness. The positive impact of proteases at lower levels on bread volume and score were clearly evident with the no-time Canadian short process, but no improvement in volume and score was evident at lower protease levels with the sponge and dough process, while at higher levels, large decreases in bread score occurred. The lack of a positive response in the present study is likely related to the weakening of the gluten protein network through the action of the proteases during the sponge fermentation stage (McDonald 1969; Kruger 1971). This is consistent with the significant decrease in mixing time with increasing levels of protease as shown in Fig. 4. No decrease was evident with the other enzyme types and, in fact, xylanases significantly increased mixing time at the highest levels of addition. The latter effect may be related to excessive water release during sponge fermentation by the action of the xylanases on pentosans, which have very high water absorption capacity (Jelaca and Hlynka 1971). Previous studies have shown increasing water content can increase mixing requirements with higher speed mixers (Larsen and Greenwood 1991). We noted that at the higher levels of xylanases, the initial phase of mixing was extended (data not shown), which could be an indication of a longer dough hydration phase due to excess water.

As shown in a previous study with no-time dough (Harada et al 2000), the similarity of the response of sponge and dough bread volume and score and crumb softness to increasing levels of the commercial enriched α -amylases, xylanases, and cellulases and the narrow range of sheeting values over which optimum bread properties were achieved suggests a common mechanism. This mechanism likely involves the release of water through the action of the hydrolytic enzymes on their corresponding substrates, leading to softer dough with better machining properties and improved

oven spring, resulting in increased volume and bread score (Navickis et al 1982; Kulp 1993; Rouau et al 1994; Martínez-Anaya and Jiménez 1997b; Si 1997). Improvement in crumb softness would also result from increased bread volume (Si 1997) plus the increased availability of water that can associate with the major crumb components such as starch, gluten, and pentosans (He and Hosney 1990; Biliaderis et al 1995). The water released by the hydrolases after sheeting and molding (during proofing and the early baking stage) may be particularly important in these improvements because they would not negatively affect dough machinability through excess water release that causes overly soft and soft sticky dough (Harada et al 2000).

The conclusions drawn from this study must be tempered by the fact that commercially enriched rather than highly pure enzymes were used. As noted earlier, the impact of side activities could influence results. Thus studies with highly purified enzymes are needed to further assess the relative influence of specific versus nonspecific effects of hydrolytic enzymes on processing and bread characteristics.

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