

Effects of Commercial Hydrolytic Enzyme Additives on Canadian Short Process Bread Properties and Processing Characteristics

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ABSTRACT

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The effects of increasing levels of eight commercial enzymes representing four types of fungal hydrolytic enzymes (α -amylases, proteases, xylanases, and cellulases) on Canadian short process (CSP) bread quality and processing characteristics were studied. Addition of all enzymes types at optimum levels resulted in increased loaf volume and bread score and softer crumb. All four types of enzymes appeared to be equally effective in improving bread properties compared with the controls. At high levels, greater tolerance to the addition of xylanases and cellulases compared with the addition of α -amylases and proteases was apparent. Mixing requirements increased with increasing levels of α -amylase but no change was

apparent with the other enzymes. Addition of all enzymes reduced sheeting work requirements, indicating a dough softening effect. Optimum bread properties for all enzymes were attained within a relatively narrow range of dough sheeting work values, which presumably correspond to optimum dough handling properties. The similarity in response of bread and sheeting characteristics at optimum levels of addition for all four enzyme types suggests a common nonspecific mechanism for improver action that is probably related to water release and the resulting impact on physical dough properties.

A variety of hydrolytic enzymes are used in the baking industry to improve dough handling properties and enhance bread quality. Both α -amylases and proteases have a long history of usage. Both enzymes can be used as dough softeners that lead to improved machining properties, higher loaf volume, and softer bread crumb (McDonald 1969, Cauvain and Chamberlain 1988, Ranum and DeStefanis 1990). α -Amylases, in conjunction with natural flour β -amylases, can also provide an important source of fermentable sugar for gas production in longer processes where natural or added sugar is depleted during bulk fermentation (Ranum and DeStefanis 1990). High levels of fungal α -amylase addition are also used extensively in no-time Chorleywood-type processes for additional loaf volume enhancement. This enhancement appears to be associated with prolonged dough expansion time during baking (Cauvain and Chamberlain 1988). Bacterial α -amylases, which retain activity after baking due to high temperature stability, have shown potential for increasing shelf life (Martínez-Anaya and Jiménez 1997a, Si 1997). Proteases that hydrolyze gluten proteins have been traditionally used to treat “bucky” dough resulting from overly strong (elastic) flours (McDonald 1969).

More recently, enzymes that hydrolyze pentosans such as xylanases (hemicellulases) and those that hydrolyze complex cell wall carbohydrates such as cellulases (β -glucanases) have been introduced. Manufacturers report that these enzymes are used to improve dough handling properties and enhance bread quality (*personal communication*). Studies with xylanases have confirmed their effectiveness in this regard (Kulp 1968, Rouau et al 1994, Martínez-Anaya and Jiménez 1997a, Monfort et al 1997, Si 1997). Xylanases also have been reported to extend shelf life by reducing the staling rate (Martínez-Anaya and Jiménez 1997a). Overall, they appear to be particularly effective in straight-dough processes (Rouau et al 1994).

The improver effects of hydrolytic enzymes have been attributed to both common nonspecific and enzyme-specific mechanisms. The former appears to be associated with the release of water due to hydrolysis of the respective polymeric substrates to smaller components with lower water binding capacity. At appropriate enzyme

levels, this results in softer dough with superior handling properties and the associated improvement in bread quality attributes (Navickis et al 1982, Kulp 1993, Rouau et al 1994, Martínez-Anaya and Jiménez 1997b). Enzyme specific mechanisms have also been proposed that involve interactions between the hydrolysis reaction products and other dough and bread components resulting in improved processing and bread quality characteristics (Kulp 1968, D’Appolonia 1980, Martin and Hosney 1991, Bombara et al 1997, Biliaderis et al 1995). At present, the relative importance of these mechanisms is not well known. In part, this is due to the lack of studies of both the relative performance of different types of hydrolytic enzymes (Rouau et al 1994, Si 1997) and the confounding effects of enzyme source (Ranum and DeStefanis 1990, Martínez-Anaya and Jiménez 1997a), baking process (Cauvain and Chamberlain 1988), and flour characteristics (McDonald 1969, Rouau et al 1994).

In Canada, the predominant baking process is the Canadian short process (CSP), a no-time dough involving the use of medium- to high-speed bar mixers combined with relatively high levels of ascorbic acid (≈ 50 – 100 ppm) and some azodicarbonamide (usually <10 ppm) to mechanically develop the dough. Although hydrolytic enzymes are used widely in the industry, no studies of the effects of these additives for this process have been reported. This study reports the effects of eight commercial fungal enzyme preparations representing the four major types of hydrolytic enzymes currently used (α -amylases, proteases, xylanases, and cellulases) on the processing and bread quality characteristics of a Canada Western Red Spring (CWRS) wheat flour using a laboratory-scale CSP procedure. Particular attention has been paid to the relative impact of the different enzyme types in terms of performance at optimum levels and tolerance to high levels of addition, as well as the relationship between the processing characteristics, particularly sheeting properties, and bread quality characteristics (loaf volume, bread score, crumb softness). This information has been used to try to assess the relative importance of mechanisms proposed to explain the positive benefits associated with addition of hydrolytic enzymes with respect to the CSP.

MATERIALS AND METHODS

Flour and Enzyme Samples

Straight-grade flour was prepared from a No. 1 CWRS wheat on the pilot mill of the Canadian International Grains Institute, Winnipeg (12.1% protein, 0.50% ash, 4.75 min farinograph dough development time, and 61.2% farinograph absorption; all data corrected to 14.0% flour moisture basis).

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Commercial enzymes were supplied by Enzyme Development Corporation (EDC), New York, NY, and by Amano Enzyme USA Co., Ltd., Troy, VA. Descriptions of the enzymes provided by the manufacturers are given in Table I. 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. Activity units reported for xylanases and cellulases varied depending on the supplier. α -Amylase activity was reported in Sandstedt-Keen-Blish (SKB) units; protease activity was reported in hemoglobin units (HUT); xylanase activity units on birch xylan substrate (BXU) or activity units on xylan substrate (u); cellulase units on hydroxyethyl cellulose (ECU) or carboxymethyl-cellulose hydrolyzing activity units (CMC-ase).

Baking Procedures

The CSP baking procedure was performed as previously described (Yamada and Preston 1992). Dough ingredients (percent flour weight basis) included flour (100 g, 14.0% moisture basis), fresh compressed yeast (3.0%), salt (2.4%), sucrose (4.0%), ammonium phosphate (0.1%), ascorbic acid (150 ppm), 60°L malt syrup (0.2%), shortening (3.0%), enzyme additive (variable), whey (4.0%) and optimum water (66% for all enzyme levels) as assessed by dough feel by an experienced baker at panning. Ingredients were mixed in a GRL 200 mixer at 165 rpm to 10% past peak consistency at 30°C. The dough was rested 15 min (30°C), 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, and 3.2 mm), molded on the GRL molder, panned, and then proofed for 70 min at 37.5°C (87% rh). Baking was performed in heat-sink ovens for 25 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 were obtained using a GRL watt hour meter attached to the GRL 200 mixer (Kilborn 1979). Dough sheeting energy (sum of second and third sheetings) was obtained by means of a force transducer attached to the arm of the sheeter as previously described (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 software (Labtech Notebook version 7.2.1 for DOS, Labtech, Wilmington, MA). Bread loaf volume, crust appearance, crumb color, crumb texture, and total bread score were assessed by an experienced baker as described previously (Preston et al 1982). Crumb firmness was deter-

mined 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%). A randomized block design was used to obtain results for analysis of variance (ANOVA) using SAS Release 6.11 (SAS Institute, Cary, NC). Appropriate means were compared for significance at the 5% level using Duncan's multiple range test. 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

The commercial hydrolytic fungal enzymes used in the study included α -amylases, proteases, xylanases (hemicellulases), and cellulases (Table I). Preliminary studies were conducted with each enzyme to determine approximate values for optimum levels of addition based on bread loaf volume and score. For the final studies, several levels below and above the initially determined optimum, as well as a 0% control and two higher levels, including a very high level ($\approx 10\times$ initial optimum) were used. At the highest levels, dough prepared with the α -amylases, cellulases, and xylanases was softer at panning than control dough as assessed by hand feel, while dough prepared with the highest level for the proteases was soft and slightly sticky. Dough absorption was maintained at 66% for all tests. This value was within the optimum range of absorption for all dough except the highest protease level as determined by dough feel at panning.

Effects of Enzymes on Loaf Volume and Bread Score

Addition of each of the enzymes tested had significant ($P \leq 0.05$) effects on loaf volume, bread score, and crumb firmness after 24 hr of storage at room temperature as determined by ANOVA (Table II). For both α -amylases, optimum visual bread quality

TABLE II
Effects of Enzyme Level on Dough Processing and Bread Properties^a

	Loaf Volume	Bread Score	Crumb Firmness	Mixing Time	Sheeting Work
Amylase1					
ANOVA	ns	0.0001	0.01	0.0001	0.0002
CR	69	9	599	0.7	0.025
Amylase2					
ANOVA	0.0003	0.0001	0.0001	0.0001	0.0009
CR	57	9	579	0.4	0.023
Protease1					
ANOVA	0.002	0.0001	0.01	ns	0.0001
CR	53	11	690	0.8	0.021
Protease2					
ANOVA	ns	0.0002	0.01	ns	0.01
CR	84	9	807	0.4	0.030
Xylanase1					
ANOVA	ns	0.0001	0.02	ns	0.0001
CR	125	10	728	0.5	0.028
Xylanase2					
ANOVA	0.0001	ns	0.0003	ns	0.01
CR	50	11	638	0.6	0.024
Cellulase1					
ANOVA	0.002	0.02	0.0002	ns	0.03
CR	61	12	553	0.6	0.019
Cellulase2					
ANOVA	0.0009	ns	0.0001	ns	0.04
CR	73	14	473	0.5	0.022

^a Analysis of variance (ANOVA), Duncan's critical range difference between values for $P < 0.05$ (CR), not significant (ns).

TABLE I
Description of Commercial Enzymes

Enzyme ^a	Supplier ^b	Fungal Source	Side Activities ^c
α -Amylase	EDC	<i>Aspergillus niger</i>	
α -Amylase	Amano	<i>Aspergillus oryzae</i>	Protease
Protease	EDC	<i>Aspergillus oryzae</i>	α -Amylase
Protease	Amano	<i>Aspergillus oryzae</i>	
Xylanase	EDC	<i>Trichoderma longibrachiatum</i>	Cellulase, β -glucanase, acid protease
Xylanase	Amano	<i>Aspergillus niger</i>	
Cellulase	EDC	<i>Trichoderma longibrachiatum</i>	Hemicellulase, β -glucanase, protease, amyloglucosidase
Cellulase	Amano	<i>Aspergillus niger</i>	

^a α -Amylase activity in Sandstedt-Keen-Blish (SKB) units; protease activity in hemoglobin units (HUT); xylanase activity units on birch xylan substrate (BXU) or activity units on xylan substrate (u); cellulase units on hydroxyethyl cellulose (ECU) or carboxymethyl-cellulose hydrolyzing activity units (CMC-ase).

^b Enzyme Development Corporation (EDC); Amano Enzyme USA Co., Ltd.

^c Side activities as reported by supplier.

(loaf volume and score) was apparent with the addition of ≈ 200 – 300 SKB/100 g of flour (Fig. 1). Optimum loaf volume was maintained over a wider range of enzyme activity compared with bread score. At higher levels, bread score decreased more rapidly than loaf volume, with the former showing significantly lower scores compared with the control (0% enzyme addition) at the two highest activity levels. In addition to loaf volume, decreases in bread score at these higher activity levels could be attributed to a significant ($P < 0.05$) deterioration in both loaf appearance and crumb structure (data not shown). Crumb color did not appear to be affected by enzyme activity level (data not shown).

Both proteases attained optimum visual bread quality at ≈ 500 HUT/100 g of flour (Fig. 2). At this addition level, loaf volumes of bread prepared with both proteases (Pro1 and 2) and bread score with Pro2 were significantly higher than those of the control. Both of these parameters showed optimum values over a wide range of activity. At the highest levels (2,000 and 3,000 HUT/100 g of flour), scores were significantly lower than those of the control because of significant ($P < 0.05$) reductions in loaf

appearance and crumb structure values (data not shown). Loaf volume decreases at higher levels were less evident.

Although addition of both xylanases (Xyl1 and 2) appeared to increase loaf volume and bread score (Fig. 3), ANOVA (Table II) showed that Xyl1 had a significant effect on bread score, while Xyl2 had a significant effect on loaf volume. However, in the case of the Xyl2, significant differences were evident in score among the means using Duncan's multiple range test. The optimum level of addition for the xylanases required to attain optimum visual bread properties was difficult to discern, but appeared to be $\approx 1,500$ BXU/100 g of flour for one enzyme and ≈ 300 units/100 g of flour for the other enzyme. At the highest levels of addition, loaf volume did not show any tendency to decrease, while bread score showed significant decreases compared with the highest values. This deterioration in bread score could be attributed to significantly lower bread appearance and, to a lesser extent, reduced crumb structure scores (data not shown).

Optimum values for overall visual bread quality were attained at ≈ 20 ECU/100 g of flour for one cellulase enzyme and at ≈ 100

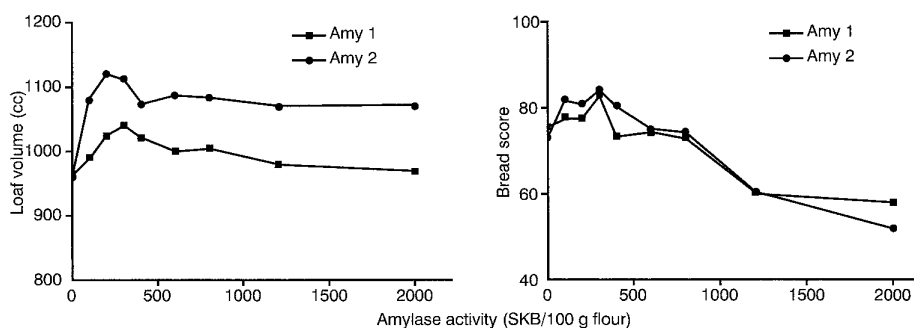


Fig. 1. Effects of increasing levels of commercial fungal α -amylases (Amy 1 and 2) on loaf volume and bread score using Canadian short process (CSP). α -Amylase activity in Sandstedt-Keen-Blish (SKB) units.

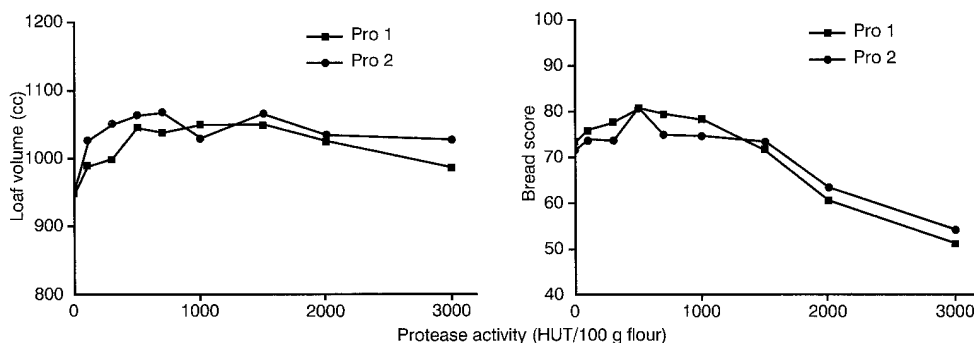


Fig. 2. Effects of increasing levels of commercial fungal proteases (Pro 1 and 2) on loaf volume and bread score using Canadian short process (CSP). Protease activity in hemoglobin units (HUT).

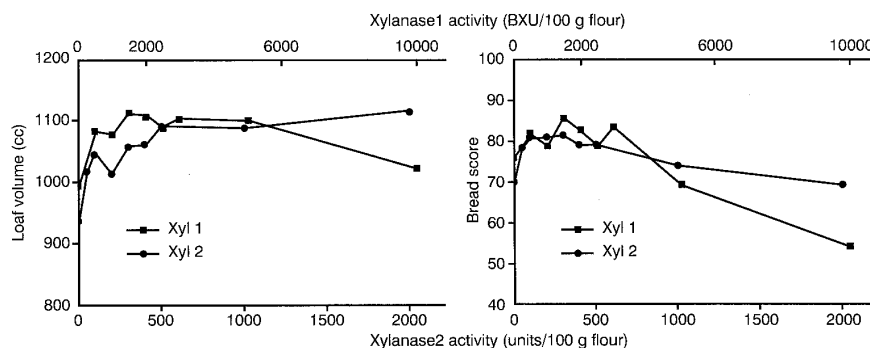


Fig. 3. Effects of increasing levels of commercial fungal xylanases (Xyl 1 and 2) on loaf volume and bread score using Canadian short process (CSP). Xylanase activity units on birch xylan substrate (BXU) or activity units on xylan substrate (u).

CMC-ase units/100 g of flour for the other (Fig. 4). Addition of both cellulases over a wide range of levels significantly increased bread loaf volume compared with that of the control. At the highest levels of addition, loaf volumes were not significantly different from the largest values attained. For bread score, values increased to a maximum then decreased at higher levels, although the effect was only significant with one of the enzymes. Loaf appearance, and to a lesser extent, crumb structure, were responsible for the decrease in bread score at high levels of activity (data not shown).

In almost all cases, optimum enzyme levels were associated with higher (superior) loaf appearance and crumb structure scores as compared with the corresponding control (data not shown). These higher values, in addition to increased loaf volume, contributed to higher bread scores. However, although this trend for loaf appearance and crumb structure was clear, none of the values obtained at optimum enzyme addition levels were significantly higher

than those of the control at the 5% level. Significant (negative) effects for these parameters were only evident at high levels of enzyme addition.

Effects of Enzymes on Crumb Firmness

All of the enzymes tested had significant effects on crumb firmness (Table II). Plots of the effects of level for all enzyme types on this parameter are shown in Fig. 5. Addition of both α -amylases at all levels significantly improved (reduced) crumb firmness when compared with the control. Optimum values were obtained over a wide range of activity. At the higher activity levels, a significant increase in crumb firmness compared with optimum enzyme addition was evident with Amy2, although values were still significantly lower than those of the control. Addition of protease also resulted in a decrease in crumb firmness. With Pro1, firmness values were significantly ($P < 0.05$) lower than the control at activity levels of 500–1,500 HUT/100 g of flour, while

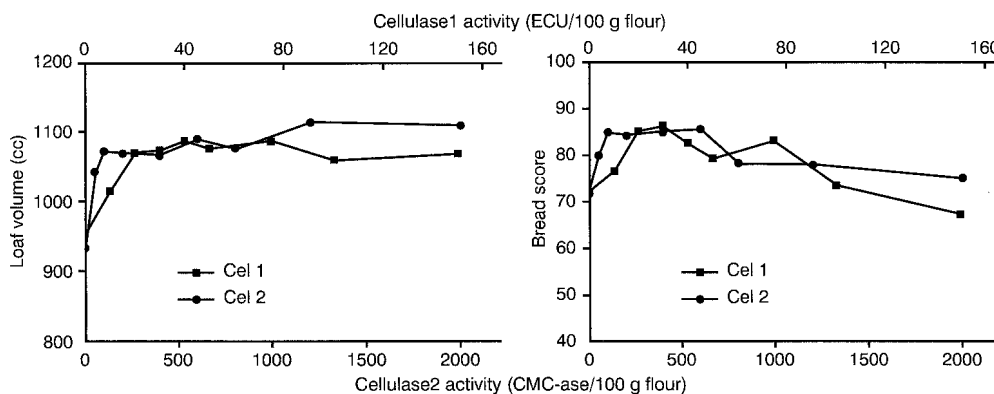


Fig. 4. Effects of increasing levels of commercial fungal cellulases (Cel 1 and 2) on loaf volume and bread score using Canadian short process (CSP). Cellulase units on hydroxyethyl cellulose (ECU) or carboxymethyl-cellulose hydrolyzing activity units (CMC-ase).

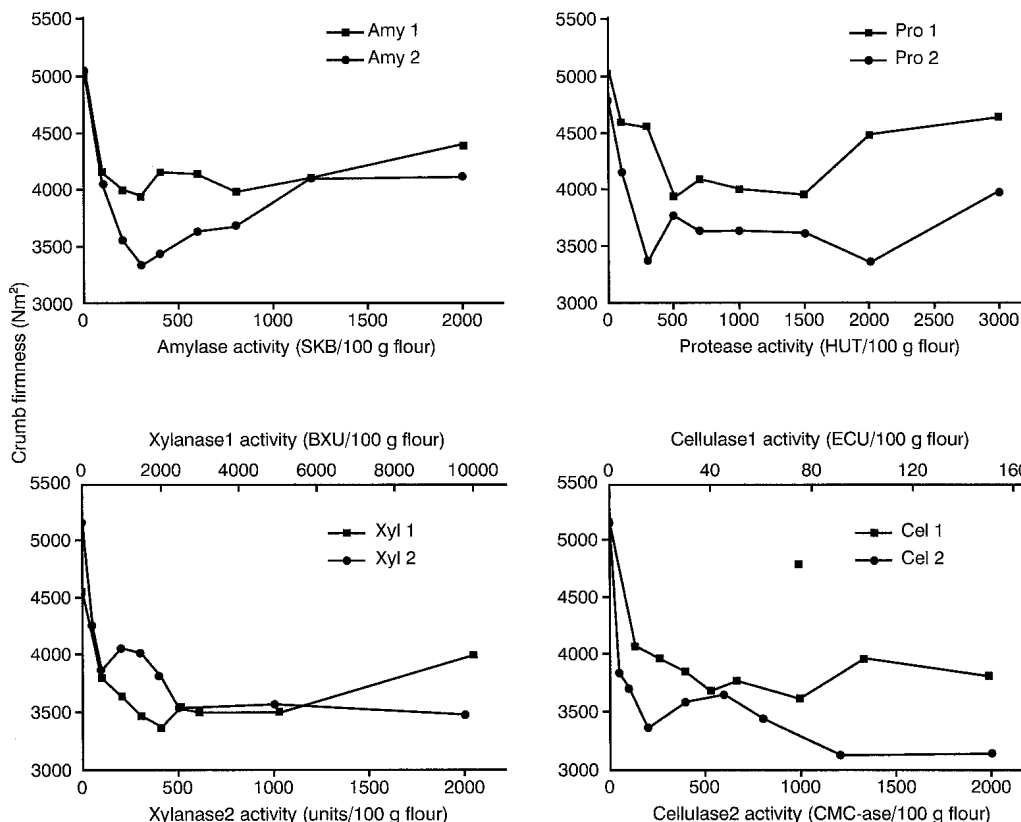


Fig. 5. Effects of increasing levels of commercial fungal α -amylases, proteases, xylanases, and cellulases on crumb firmness. See Table II for ANOVA and Duncan's critical range values ($P < 0.05$).

with Pro2, values were significantly lower than the control at all levels >100 HUT/100 g of flour. At the highest protease levels, there was a tendency to increased crumb firmness but values were not significantly different than the lowest (best) values.

Addition of both xylanases resulted in significant reductions in crumb firmness compared with the control. Optimum bread softness was evident over almost the entire range of addition. Crumb firmness values were also significantly reduced compared with the control at all levels of addition for both cellulase enzymes. A softer crumb was maintained over a wide range of activity, including the highest levels of addition.

In general, crumb softness (Fig. 5) and bread loaf volume (Figs. 1–4) showed similar positive trends to addition of all hydrolytic enzymes. Part of the increase in crumb softness, therefore, can be associated with these increases in loaf volume as well as crumb structure (Si 1997).

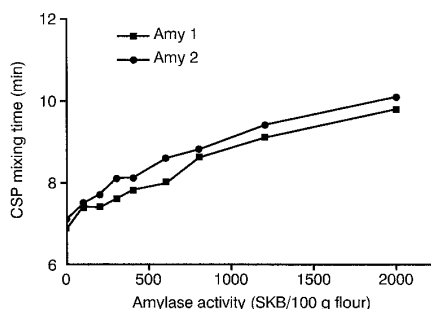


Fig. 6. Effects of increasing levels of α -amylases (Amy 1 and 2) on mixing time using Canadian short process (CSP). α -Amylase activity in Sandstedt-Keen-Blish (SKB) units.

Effects of Enzymes on CSP Mixing Properties

ANOVA showed that both fungal α -amylases had significant ($P < 0.0001$) effects on CSP mixing time (Table II). As shown in Fig. 6, increasing α -amylase activity resulted in increased mixing time. Responses were similar for both enzyme preparations. At the minimum level of addition required to attain optimum visual bread quality (≈ 300 SKB units for both enzymes), mixing times were significantly ($P < 0.05$) longer than the corresponding control. Further increases in α -amylase activity resulted in progressively longer mixing times. Mixing energy to peak also increased with increasing activity levels for both α -amylases, but only at the highest activity levels were they significantly different from the control (data not shown). In contrast, addition of proteases, xylanases, and cellulases over a wide range of activity had no significant ($P > 0.05$) effect on mixing time (Table II). Similar results ($P > 0.05$) were obtained with these enzymes for mixing energy (data not shown).

Effects of Enzymes on Sheeting Properties

Measurement of the work required to sheet dough provides a means of assessing the physical state of the dough during this processing stage (Kilborn and Preston 1982). The effects of increasing enzyme concentration on total sheeting work are shown in Fig. 7. Although a relatively high degree of variability in the sheeting work measurements were evident (high Duncan's critical range values), ANOVA results still clearly demonstrated significant ($P < 0.05$) effects of level for all of the enzymes tested (Table II). A strong trend to reduced sheeting work requirements was evident with increasing levels of each enzyme, indicating a dough softening effect. Concomitant with this decrease, curves showed reduced height and increased length that can be associated with a decrease in elasticity and an increase in extensibility (data not shown). At the minimum levels required to attain optimum visual bread quality, sheeting work requirements were similar for

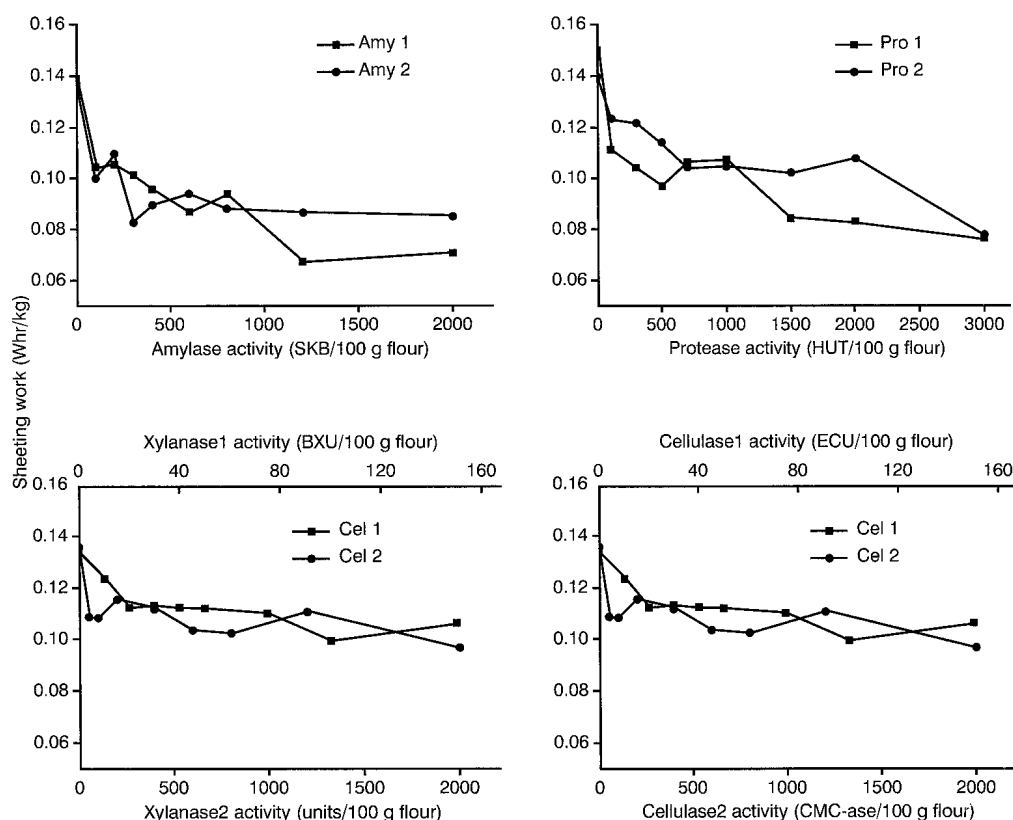


Fig. 7. Effects of increasing levels of commercial fungal α -amylases, proteases, xylanases, and cellulases on sheeting work. See Table II for ANOVA and Duncan's critical range values ($P < 0.05$).

most of the enzymes with a range of 0.097–0.117 Whr/kg. All of these values were within the average of the Duncan's critical range values obtained with the eight enzymes (0.024 Whr/kg), suggesting that there was no significant difference among sheeting energy requirements at the minimum enzyme level required to attain optimum bread properties. At the highest addition levels, the enzymes (Amy 1 and 2, Pro1 and 2, and Xyl1) that showed the lowest sheeting work values were clearly associated with the lowest bread scores.

DISCUSSION

Our results demonstrate that the addition of each of the four types of commercial hydrolytic enzymes (α -amylase, protease, xylanase, and cellulase) can lead to improved CSP bread quality. In general, these enzymes significantly improved loaf volume, bread score, and crumb firmness. Although no previous studies are available on the effects of these enzymes on CSP baking properties, studies with three of these enzyme types (α -amylase, protease, and xylanase) using processes involving primarily straight-dough have demonstrated their ability to increase loaf volume (Bayfield and Young 1964, Kulp, 1968, McDonald 1969, McCleary 1986, Cauvain and Chamberlain 1988, Bajwa 1990, Ranum and DeStefanis 1990, Rouau et al 1994, Martínez-Anaya and Jiménez 1997a, Monfort et al 1997, Si 1997). Information on the effect of these enzymes on bread score, which includes crust and crumb characteristics, is less definitive. Some studies have demonstrated improvements in crumb structure at optimum enzyme levels with hydrolytic enzymes (McDonald 1969, Rouau et al 1994, Si 1997), while other studies show no improvement (Bayfield and Young 1964, Martínez-Anaya and Jiménez 1997a). The improvement in CSP bread score with the enzymes at optimum levels compared with that of the controls was primarily associated with increased loaf volume. Although individual enzyme effects on loaf appearance and crumb structure were nonsignificant at optimum addition levels, there was a consistent trend to improvement in these parameters for most enzymes as compared with the control, which also contributed to superior bread scores. The significant reduction in crumb firmness with all enzymes tested is consistent with previous studies (Bajwa 1990, Ranum and DeStefanis 1990, Martínez-Anaya and Jiménez 1997a).

Few studies are available on the relative performance of different types of hydrolytic enzymes (Rouau et al 1994, Si 1997). Comparison of the bread property profiles shown in Figs. 1–5 suggests that at optimum levels, improvements in CSP loaf volume, bread score, and crumb firmness compared with those of the control are of similar magnitude for all enzyme types studied. All enzymes appeared to show good tolerance to addition at levels considerably higher than optimum. However, at very high levels ($\approx 10\times$ the initial optimum level), α -amylase and, in particular, protease showed large significant reductions in bread score that could be attributed to significant reductions in bread appearance and crumb structure. With cellulases and xylanases, this trend was much less evident, indicating greater dough tolerance to excess addition of these enzymes. Dough prepared with high levels of protease felt sticky and weak, which probably accounts for its poor performance. This weakness can be attributed to the hydrolysis of gluten proteins which are known to be the major determinant of dough strength (MacRichtie 1992). The tolerance of dough to addition of xylanases at levels considerably higher than optimum level is surprising. Previous work with commercial xylanases (Rouau et al 1994) reported slack and sticky dough at addition levels not much over twice the optimum level using a straight-dough French bread process. The improved tolerance with the CSP process is difficult to ascertain, although the lack of bulk fermentation, which would reduce reaction time between addition of the enzyme and its denaturation in the oven, may be an important factor. The use of a strong high-protein CWRS wheat flour in the present study may

have also influenced the ability of dough to withstand high levels of enzyme. These results suggest that both process and flour quality characteristics may strongly influence the impact of enzymes on processing and bread quality characteristics. It should be noted that in commercial bakeries, the range in tolerance to addition of all these enzymes would be expected to be narrower due to greater difficulties in the ability of commercial handling equipment to process softer dough and slice softer bread crumb as compared with laboratory baking equipment.

Improvements in bread properties obtained by the addition of hydrolytic enzymes have been associated with their impact on the physical properties of the dough during processing (Martínez-Anaya and Jiménez 1997b). However, with the exception of subjective dough feel, direct measurements of the physical properties of full formula dough during processing with enzymes are generally lacking. In this study, dough mixing and sheeting properties were measured to assess the effect of the enzymes on these properties and their possible relationship to bread quality characteristics. For CSP mixing requirements, α -amylase was the only hydrolytic enzyme to influence these parameters. The significant increase in mixing time and mixing energy requirements with this enzyme is probably related to dough slackening associated with rapid water release due to the synergistic action of α -amylase with natural β -amylase present in high levels in flour (Kruger 1972). The latter enzyme would rapidly reduce the size and water binding capacity of the initial α -amylase hydrolysis products (large dextrins) released from susceptible (damaged) starch. With the other enzymes, the initial hydrolysis products may be less prone to loss in water binding capacity during the relatively short mixing period due to lower levels of natural enzymes capable of rapidly reducing their size. Increases in mixing time with increasing dough water content were previously shown using a high-speed laboratory bread dough mixer (Larsen and Greenwood 1991) and a mixograph (Lang et al 1992). When water was added at several levels above and below the optimum absorption using the control flour with the GRL mixer, a significant ($P < 0.05$) positive relationship between CSP mixing requirements and absorption level was also evident (unpublished data).

The machining properties of dough during the makeup stage are critical in determining bread quality. One of the major functions of hydrolytic enzyme addition is to soften dough to improve machining properties and thus enhance bread quality (Martínez-Anaya and Jiménez 1997b). However, it has also been recognized that over addition can cause overly soft or sticky dough, resulting in machining problems at the sheeter and rounder that lead to a deterioration in bread quality (Si 1997). The measurement of sheeting properties with our equipment appears to be capable of differentiating these changes. Significant decreases in sheeting work requirements were evident with increasing levels of all enzymes, providing a direct measure of this softening effect. Optimum bread properties for all enzymes were attained within a relatively narrow range of sheeting work values which would presumably correspond to optimum dough handling properties. At very high levels of protease and α -amylase, the lower sheeting work requirements suggest overly soft dough that results in poor machining properties and inferior bread quality. These results suggest that the use of these measurements in commercial bakeries may prove beneficial in monitoring dough properties to optimize dough processing characteristics.

Both common and enzyme specific mechanisms have been put forward to explain the effects of hydrolytic enzymes on bread quality. Several researchers have suggested that the improvement in machining properties and bread quality can be at least partially attributed to the release of water that occurs when the appropriate natural substrates (starch, protein, pentosan, or cellulose) are sufficiently hydrolyzed to reduce their water binding capacity. The released water can reduce dough viscosity resulting in softer dough with better machining properties (Navickis et al 1982, Kulp 1993, Rouau et al 1994, Martínez-Anaya and Jiménez 1997b) and increased oven spring (Cauvain and Chamberlain 1988). Softer crumb would

result due to the increased bread loaf volume and improved crumb texture (Si 1997) associated with better oven spring and to more water being available to associate with the major components in the crumb influencing this factor, such as starch and gluten (He and Hoseneý 1990, Biliaderis et al 1995). At too high a level of enzyme addition, excess water release would produce dough that is overly soft and sticky (Rouau et al 1994) for proper handling. Other workers have suggested that enzyme-specific interaction effects of hydrolysis products with other dough and bread components may improve processing and bread quality characteristics, and in particular, crumb staling properties (Kulp 1968, D'Appolonia 1980, Martin and Hoseneý 1991, Bombara et al 1997, Biliaderis et al 1995). The similarity in response of bread characteristics to the addition of the four hydrolytic enzyme types at optimum level and the apparent close relationship between sheeting work requirements and optimum bread loaf volume and score found in the present study suggest that these enzymes exert their effect primarily through a common mechanism. Research by Larsen and Greenwood (1991) is consistent with this view. They showed that with a mechanically developed dough breadmaking system, good quality bread could be obtained at high water absorption levels as long as dough could be handled by sheeting and molding equipment. After sheeting and molding, additional release of water by hydrolytic enzymes during proofing may further enhance bread quality by additional promotion of oven spring and crumb softening.

Although some of the enzymes used had reported side activities (Table I), the similarity in effect of the two enzymes of each type tested argues that responses obtained in the present study can be directly attributed to their primary activity. Although other enzymes, particularly oxidases, in low levels can also influence results (D. Schofield, *personal communication*), recent studies in our laboratory with a range of purified oxidases did not show any major effect on bread quality characteristics using the CSP process and a similar flour (unpublished data).

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