

Effects of Selected Commercial Enzymes on Cookie Spread and Cookie Dough Consistency

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ABSTRACT

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Seventeen commercial enzyme preparations having cellulase, protease, β -glucanase, β -glucosidase, or cellobiase activities were added to cookie doughs made with chlorine-treated soft red winter wheat flour. Cookie diameters and Instron universal testing machine physical-texture measurements of the doughs were made immediately after mixing and after holding for 2 hr at 32° C. Several cellulase preparations from *Trichoderma reesei* were the most effective at maintaining stability of dough consistency

and cookie size when doughs were held for 2 hr before processing. When untreated doughs were held 2 hr they became more stiff and baked smaller cookies. The protease papain was the most effective at increasing cookie spread and top grain. At the concentrations studied, papain produced sugar-snap cookies from hard red winter and hard spring wheat flours that were as large as control cookies made from soft red winter wheat flour.

The sugar-snap cookie baking test is one method used to evaluate wheat flour for soft wheat baking quality. Cultivars that produce larger cookies are considered to have better quality. The wheat classes usually distinguish themselves on the basis of cookie spread. Soft wheats have the largest cookie spread, hard winter wheats produce smaller cookies, and hard spring wheats usually produce still smaller cookies.

Several recent studies have investigated the mechanism by which the wheat classes differ in cookie baking quality. Building on the earlier work of Yamazaki (1955, 1959), Abboud et al (1985) observed that cookie diameter is determined by the rate at which dough spreads during heating and by the rate at which dough stops spreading (sets up). Soft wheat doughs spread faster because they become less viscous during baking than do hard wheat doughs, and soft wheat doughs set later in the baking cycle than hard wheat doughs do. Doescher et al (1987a) investigated the relative

differences in dough setting and observed that the glass transition temperature of hard wheat flour gluten is lower than that of soft wheat gluten. They theorized that, when the glass transition temperature is reached, gluten swells and forms a continuous phase network that decreases water mobility and rapidly increases dough viscosity, thus counteracting the influence of gravity and stopping the spread of the dough. That process occurs at a lower temperature in doughs made from hard wheat flours.

Slade et al (1989) suggest that cookie volume rather than cookie diameter or height/diameter ratio is a better descriptor of cookie flour quality. They also theorize that the glass transition temperature of wheat gluten is not involved in the sugar-snap baking mechanism, at least to the extent that it makes cookies set during baking. They theorize that cookie dough does not actually "set"; doughs from flours having poor cookie baking quality (hard wheats) exhibit elastic expansion followed by elastic shrinkage during baking, and doughs from good cookie baking flours (soft wheats) exhibit viscous expansion and creep followed by structural collapse. They suggest that hard wheat flour produces a dough having a three-dimensional, self-supporting, elastic, composite film-network that first expands upon heating and then shrinks above glass transition temperature. Soft wheat doughs are rubbery and expand by a two-dimensional film formation with no significant functional network formation and then collapse above glass transition temperature.

Another predictive flour quality aspect of cookie test baking is the cracking that occurs on the top surface of the cookie. This is

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referred to as cookie "top grain." Doescher and Hoseney (1985) determined that good top grain (many surface cracks) results from the recrystallization of sucrose at the cookie surface during baking. Cookie top grain is correlated with cookie diameter, and wheats that produce relatively small cookies also have fewer cracks, resulting in a pattern of larger "islands" on the cookie surface. Hard wheat flours usually have few, if any, cracks.

Chlorine treatment of flour reduces its baking quality as measured by the sugar-snap cookie baking test. With the recent introduction and success of dual-textured cookies (in which cookie spread must be controlled and cookie texture is a paramount concern), much more chlorine-treated flour is used. However, chlorinated flour produces problems for the baker. Chlorine-treated flour bakes smaller cookies and has a much reduced dough machining tolerance if it contains much damaged starch or received much holding time before processing (Gaines et al 1988). It is possible that added enzymes can be used to reduce those problems.

The use of protease in bread and cracker production has a long history. Bread bakers use proteases to reduce mixing time and create "mellow" gluten (Pratt 1971) and to reduce dough buckiness (consistency) (Bloksma 1971). Cracker manufacturers, who often require even greater control over dough consistency, routinely add standardized protease tablets from *Aspergillus oryzae* to cracker sponges to increase dough extensibility, thus ensuring proper machinability (Matz and Matz 1978). Also, proper supplementation of bread and cracker sponges with (usually fungal) protease improves the texture of those products, making them more soft and tender (Pylar 1973).

The hydrophilic tailings flour fraction is usually greater in hard wheats than in soft wheats. This is considered one of the main quality differences between the two wheat classes (Yamazaki 1955). Also, hard wheat flours usually have greater gluten strength than do soft wheat flours. To our knowledge, the application of proteases, cellulases, pentosanases, hemicellulases, β -glucanases, or β -glucosidases to sugar-snap cookie doughs has received no attention in the literature.

West (1988) reviewed the problems of choosing one of the several available commercial enzymes for a specific food system application. Some difficulties are 1) wide ranges in enzyme activities that are often assayed by different methods, 2) batch-to-batch variability, 3) contaminating enzymes having different activities, 4) different pH and temperature profiles depending upon the source, 5) various required activators and inhibitors, and 6) compatibility of combining two or more enzymes.

The following study partially overcomes some of those

difficulties by observing dynamic (time related) treatment effects in order to better compare the enzyme in a standardized dough system rather than to choose an optimum enzyme for a laboratory-type cookie dough. For purposes of comparison, it is assumed that the activity described by the commercial enzyme suppliers as the predominant activity is actually the activity observed when the enzymes are added to sugar-snap cookie doughs. However, it is possible that, due to differences in pH and temperature optima, activators, and inhibitors of the several (usually related) enzymes in the commercial preparations, the contaminating enzymes are causing the observed treatment effects. It is not our purpose to assay their degree of purity but to observe any beneficial application of several types and sources of commercial enzymes (which always have various levels of purity, usually stated by the distributor) on the dough handling characteristics and baking mechanism of the sugar-snap cookie formula.

The objective of this study was to apply a wide range of commercial grade and more purified examples of two general categories of enzymes (cellulases and proteases) to sugar-snap cookie doughs made from poor quality and chlorine-treated flours to determine if cookie quality can be improved using one or more enzymes. Enzymes originated from fungal, bacterial, plant, or animal sources and were chosen from several suppliers. Exo- and endoactivities were variously represented, as were various levels of enzyme purity. Reed (1966) suggests that such a practical approach is necessary because enzyme specificity is usually unknown in new applications.

MATERIALS AND METHODS

Flours and Baking

Four commercially milled flours were used to prepare sugar-snap cookie doughs and cookies according to AACC method 10-52 (AACC 1984). The flours were 1) a soft wheat flour (8.5% protein, 0.39% ash); 2) a chlorinated (pH 4.8) soft wheat 40% patent flour (7.9% protein, 0.38% ash); 3) a hard red winter wheat (9.5% protein, 0.38% ash); and 4) a hard red spring wheat flour (9.8% protein, 0.39% ash). Baking laboratory ambient conditions were controlled at 21–22°C and 37–46% relative humidity. Some doughs were sealed in plastic bags and held for 2 hr in a fermentation cabinet at 32°C before processing

Enzymes

Seventeen commercial enzymes (six cellulases, a cellobiase, six proteases, two β -glucanases, a β -glucosidase, and a proprietary mixture) were added to the cookie doughs made with the

TABLE I
Enzyme Descriptions and Quantities Added to Sugar-Snap Cookie Doughs

Manufacturer	Manufacturer's Description	Enzyme Type	Enzyme Source	Quantity Added to Doughs on 40 g Flour Basis				
				Activity (Manufacturer's Units/mg)	Preparation Density (g/ml)	Quantity Added	Manufacturer's Units Added	
Genencor	GC123	Cellulase	<i>Trichoderma reesei</i>	0.09	1.1	1.0 ml	100	
Novo	Celluclast	Cellulase	<i>T. reesei</i>	1.5	1.2	1.0 ml	1,800	
Serva	Onozuka R-10	Cellulase	<i>T. reesei</i>	1.75	...	52 mg	100	
Serva	TC	Cellulase	<i>T. reesei</i>	0.5	...	200 mg	100	
Sigma	C-0901	Cellulase	<i>Penicillium funiculosum</i>	6.7	...	15 mg	100	
US Biochem. Corp.	13285	Cellulase	<i>Aspergillus niger</i>	4.04	...	25 mg	100	
Novo	Novozyme 188	Cellobiase	<i>A. niger</i>	0.25	1.2	0.5 ml	300	
Genencor	P41	Protease	<i>A. oryzae</i>	17.0	...	16 mg	272	
Genencor	P53	Protease	<i>Bacillus subtilis</i>	15.0	...	16 mg	240	
Sigma	P-4032	Protease	<i>A. oryzae</i>	3.5	...	14 mg	50	
Sigma	P-4880	Protease	Papaya	0.3	...	167 mg	50	
Sigma	P-4630	Protease	Bovine pancrease	8.0	...	6 mg	50	
Sigma	P-5380	Protease	<i>B. subtilis</i>	11.6	...	4 mg	50	
Novo	Finizyme	β -Glucanase	<i>A. niger</i>	0.2	1.3	1 ml	260	
Novo	Cereflo	β -Glucanase	<i>B. subtilis</i>	0.2	1.2	1 ml	240	
Sigma	G-0395	β -Glucosidase	Almonds	4.2	...	24 mg	100	
Novo	Ceremix	α -Amylase	} <i>B. subtilis</i>	0.04	} 1.25	0.5 ml	{ 25	
		β -Glucanase		0.15				} 0.1
		Protease		0.00016				

chlorinated soft wheat flour. Descriptions and quantities added are shown in Table I. Also, four concentrations each of three enzymes (Novo Ceremix β -glucanase and protease mixture, Sigma Protease P-4880, and Serva TC cellulase) were added to doughs prepared with the nonchlorinated soft wheat flour, the hard red winter wheat flour, and the hard red spring wheat flour.

Dough and Flour Physical/Texture Measurements

Resistance to compression (consistency) of cookie doughs was measured with an Instron model 1000 universal testing machine (UTM). A 5-kg transducer was used with the 3.5-cm diameter plunger attached. Crosshead speed was 50 mm/min. Doughs were compressed to 3 mm. Alkaline water retention capacity of the chlorinated flour with enzymes added (at the same concentration based on flour weight as was added to the cookie doughs) was determined by AACC method 56-20 (AACC 1984).

Analysis

Data were replicated using different doughs and analyzed by analysis of variance. Least significant difference values were calculated from within mean square error terms for duplicate observations. The amount of change in dough consistency or cookie diameter was that value at 2-hr holding time subtracted from the comparable value at zero holding time.

RESULTS AND DISCUSSION

The enzymes described in Table I were added to cookie doughs made from chlorinated flour in an effort to reverse the increased dough stiffness and reduction in cookie spread caused by chlorine treatment and accentuated by holding doughs for 2 hr at 32°C (Gaines et al 1988). Doughs were then evaluated for resistance to UTM compression as an indication of dough consistency and for sugar-snap cookie diameter as an indication of soft wheat baking quality.

The commercial-grade enzymes used in this study contain a variety of specific activities, and the various substrates of those enzymes' activities in wheat flour are not well defined. The optimum pH and ionic strength for the various enzymes are likely different. Also, enzyme suppliers use different assays to define a

specific enzyme activity. Therefore, quantitative comparisons among the enzymes are not possible. However, a similar number of each supplier's units was chosen (100 units of cellulase, 50 units of protease, 240–250 units of β -glucanase, and 1 ml of the liquid enzymes). At those concentrations the Novo Novozyme 188 cellobiase and Ceremix β -glucanase/protease caused excessive browning, and the amounts of those enzymes were reduced by half. Genencor P41 and P53 protease were not effective at those concentrations and additional units were added. That suggests that any enzyme may be functional if enough is added, although the ratio of exo- and endoactivities may be optimized in specific preparations. Liquid enzyme preparations were substituted for added dough water based on density. The units of liquid enzymes added are described in Table I.

Cookie Size and Dough Consistency Measured Immediately After Mixing

Compared to the control dough, when treated doughs were evaluated immediately after mixing, only two enzymes (Sigma P-4880 protease and Novo Finizyme β -glucanase) increased dough consistency (resistance to UTM compressions) significantly (Table II). One enzyme (Novo Novozyme 188 cellobiase) significantly decreased dough consistency. Most enzymes caused dough formation to occur sooner during mixing. Compared to the control cookie, 11 enzymes caused cookies from doughs baked with no holding time to become larger. Two of the cellulases from *Trichoderma reesei* (Genencor GC123 and Novo Celluclast) decreased cookie spread. Figure 1 shows representative cookies made from the chlorinated flour and the Serva TC cellulase and the Sigma P-4880 protease.

Cookie Size and Dough Consistency Measured 2 hr After Mixing

decreased dough consistency when doughs were held for 2 hr at 32°C (Table II). The *T. reesei* cellulases from Genencor and Serva, respectively, produced the greatest reductions. Compared to the control cookie, only two cellulases (Novo Celluclast and Sigma C-0901) decreased cookie spread, whereas 10 enzymes increased cookie diameter. The most effective was Sigma P-4880 protease from papaya. Novo Ceremix β -glucanase/protease, Sigma P-5380

TABLE II
Effect of Enzymes on Resistance to Compression of Sugar-Snap Cookie Dough, Cookie Diameter, and Alkaline Water Retention Capacity Using a Chlorinated Flour

Enzyme	No Hold Time				2-hr Hold Time				Change from 0 to 2 hr Hold Time				Alkaline Water Retention Capacity	
	Resistance to Compression		Cookie Diameter		Resistance to Compression		Cookie Diameter		Resistance to Compression		Cookie Diameter		Alkaline Water Retention Capacity (%)	Rank
	(kg)	Rank	(cm)	Rank	(kg)	Rank	(cm)	Rank	(kg)	Rank	(cm)	Rank		
Control	5.8	10	16.4	15	12.5	13	15.5	13	6.8	15	-0.8	9	60.7	13
Genencor GC123	5.7	9	16.1 ^a	18	8.7 ^a	1	15.7 ^b	9	3.0 ^a	1	-0.4 ^a	5	50.9 ^a	1
Novo Celluclast	5.7	7	16.2 ^a	17	12.2	12	15.1 ^a	18	6.5	13	-1.1 ^b	18	51.0 ^a	2
Serva Onozuka R-10	5.7	6	16.6 ^b	6	9.7 ^a	3	16.3 ^b	5	4.0 ^a	3	-0.3 ^a	3	53.8 ^a	6
Serva TC	5.6	5	16.7 ^b	5	8.9 ^a	2	16.5 ^b	4	3.3 ^a	2	-0.2 ^a	1	51.4 ^a	3
Sigma C-0901	5.4	2	16.4	14	10.3 ^a	4	15.3 ^a	17	4.9 ^a	5	-1.0 ^b	15	60.9	14
USBC 13285	6.0	14	16.5 ^b	9	12.9	16	15.6	12	6.9	16	-1.0	14	61.5 ^b	16
Novo Novozyme 188	5.2 ^a	1	16.5 ^b	8	11.1 ^a	7	15.7 ^b	10	6.0	8	-0.9	10	61.0	15
Genencor P41	6.3	16	16.6 ^b	7	12.8	14	15.5	15	6.5	12	-1.1 ^b	17	61.5 ^b	17
Genencor P53	5.8	12	16.4	13	12.8	15	15.5	14	7.0	17	-0.9	11	55.6 ^a	8
Sigma P-4032	5.6	4	16.9 ^b	3	10.6 ^a	6	16.2 ^b	6	5.1 ^a	6	-0.7	8	55.5 ^a	7
Sigma P-4880	6.3 ^b	17	17.9 ^b	1	11.6	8	17.3 ^b	1	5.2 ^a	7	-0.6 ^a	6	53.1 ^a	5
Sigma P-4630	5.6	3	16.4	12	12.1	11	15.8 ^b	8	6.6	14	-0.6 ^a	7	57.6 ^a	11
Sigma P-5380	6.0	13	17.7 ^b	2	10.5 ^a	5	16.6 ^b	3	4.5 ^a	4	-1.1 ^b	16	56.8 ^a	10
Novo Finizyme	6.8 ^b	18	16.5 ^b	11	13.2	17	16.1 ^b	7	6.4	11	-0.4 ^a	4	59.9 ^a	12
Novo Cereflo	6.1	15	16.3	16	12.1	9	15.4	16	6.0	9	-1.0	13	55.8 ^a	9
Sigma G-0395	5.8	11	16.5 ^b	10	12.1	10	15.6	11	6.3	10	-0.9	12	61.7 ^b	18
Novo Ceremix	5.7	8	16.9 ^b	4	13.5	18	16.6 ^b	2	7.9 ^b	18	-0.2 ^a	2	51.6 ^a	4
LSD ($P = 0.05$)	0.51		0.11		1.02		0.14		1.11		0.19		0.71	

^aIndicates values that are significantly smaller ($P = 0.5$ level of probability) than the control dough.

^bIndicates values that are significantly larger ($P = 0.5$ level of probability) than the control dough.

protease from *Bacillus subtilis*, and Serva TC cellulase from *Penicillium funiculosum* also were relatively effective. The dramatic effects of Serva TC cellulase and Sigma P-4880 protease on cookie size and top grain of holding doughs for 2 hr are evident in Figure 1.

Stability of Cookie Size and Dough Consistency Over 2-hr Holding Time

Stability of dough consistency and cookie diameter was that value at 2 hr subtracted from the comparable 0-hr value. Ideally, a commercially stable dough is one that can be held after mixing for a longer time before baking without the holding time generating changes in machining and baking properties, especially if the dough is made with chlorinated flour.

Stability over time of dough consistency was best in doughs with added cellulase from *T. reesei* (Genecor GC123, Serva TC, and Serva Onozuka R-10). The Novo Ceremix enzyme mixture caused significantly less stable dough consistency. However, it and the two Serva cellulases created the most stable cookie diameters. The best stability for both dough consistency and cookie spread resulted from doughs treated with Serva TC and Serva Onozuka R-10 cellulases and from the Genecor GC123 cellulase.

Although the alkaline water retention capacity (AWRC) testing method contains excess water (especially compared to the relatively limited water system of the sugar-snap cookie doughs) the ranking of the effects of the enzymes on the AWRC of chlorinated flour was somewhat similar to the rankings of the enzymes' effect on dough and cookie size stability (Table II). Two exceptions were the Novo Celluclast, which produced a low AWRC but did not produce stable dough consistency or cookie diameter, and the Novo Ceremix, which produced good cookie diameter stability but caused a relatively large increase in dough consistency.

Cookie Spread and Top Grain Improvement from a Hard Red Winter and a Hard Red Spring Wheat Flour

Four concentrations each of the Novo Ceremix enzyme mixture, Sigma P-4880 protease from papaya, and Serva TC cellulase were added to sugar-snap cookie doughs made from a soft red winter wheat, a hard red winter wheat, and a hard red spring wheat. Cookie doughs received no holding time between mixing and baking.

Each enzyme significantly improved the cookie spread of the three wheats, but papaya protease was most effective (Fig. 2). At the higher concentrations, the papaya protease produced cookie

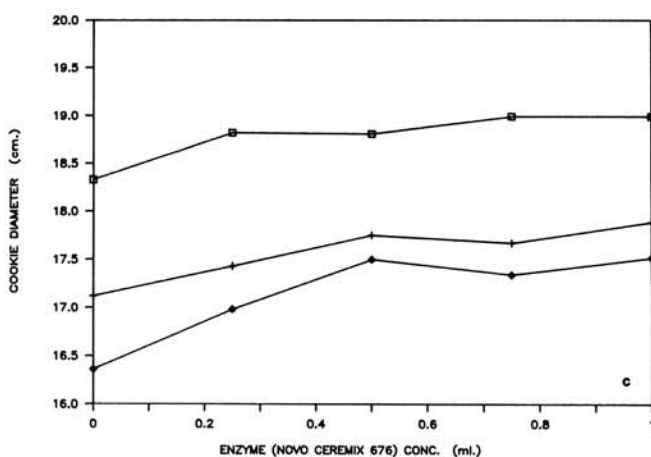
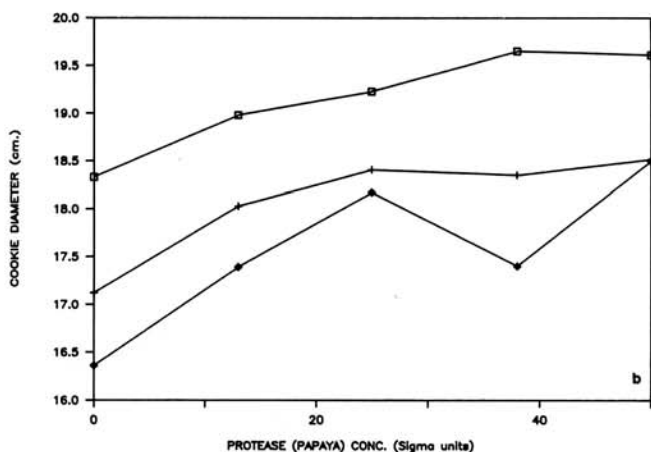
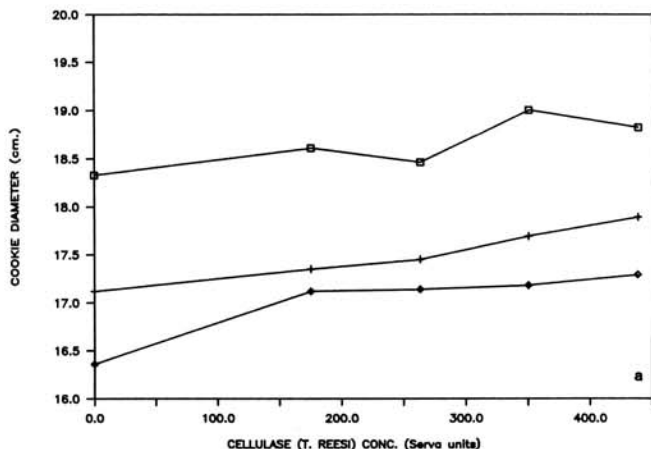


Fig. 2. Effect of four concentrations of an added Serva TC cellulase (top), Sigma p-4880 protease (middle), and Novo Ceremix enzyme mixture (bottom) on sugar-snap cookie diameter (LSD = 0.16). Square, plus, and diamond represent the soft wheat, hard winter, and hard spring flours, respectively.

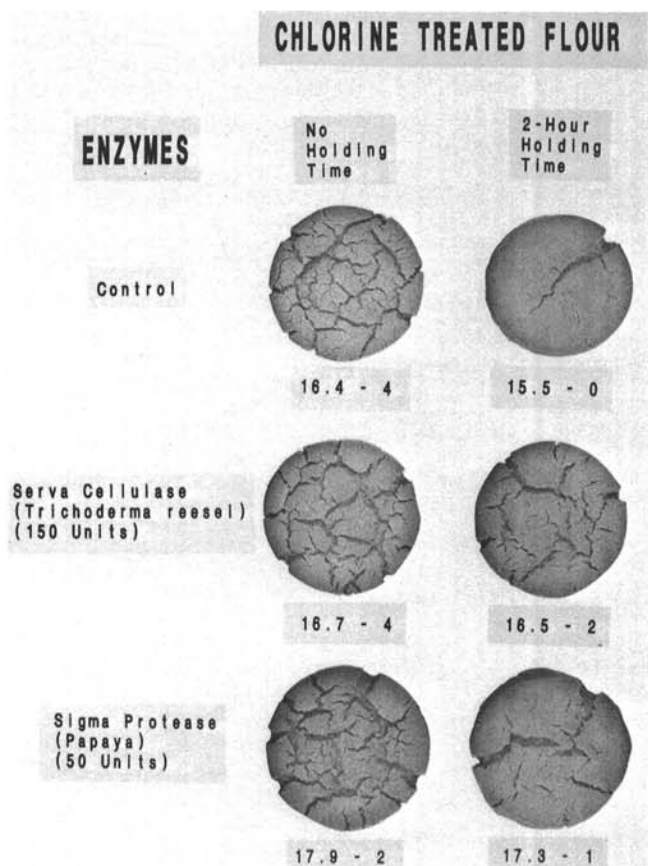


Fig. 1. Sugar-snap cookies (containing added Serva TC cellulase and Sigma P-4880 protease) made with a chlorine-treated flour which was held for zero and 2 hr before baking. First number under cookie is the two-cookie diameter. Second number is the top-grain score (0-9).

diameters from the hard winter and hard spring wheat flours that were as large as that of the soft wheat control. The papaya protease also improved the top grain of those cookies (Fig. 3). The top grain of the hard winter flour equalled that of the soft wheat flour and the top grain of the hard spring flour improved with increased concentrations. The Novo Ceremix did not improve cookie top grain, although it did increase cookie spread. At the higher concentrations, Serva TC cellulase also improved the top grain of the hard winter and spring wheat flours. Figure 4 is representative of the effects of the Serva TC cellulase, Novo Ceremix β -glucanase/protease, and Sigma P-4880 protease on the cookie

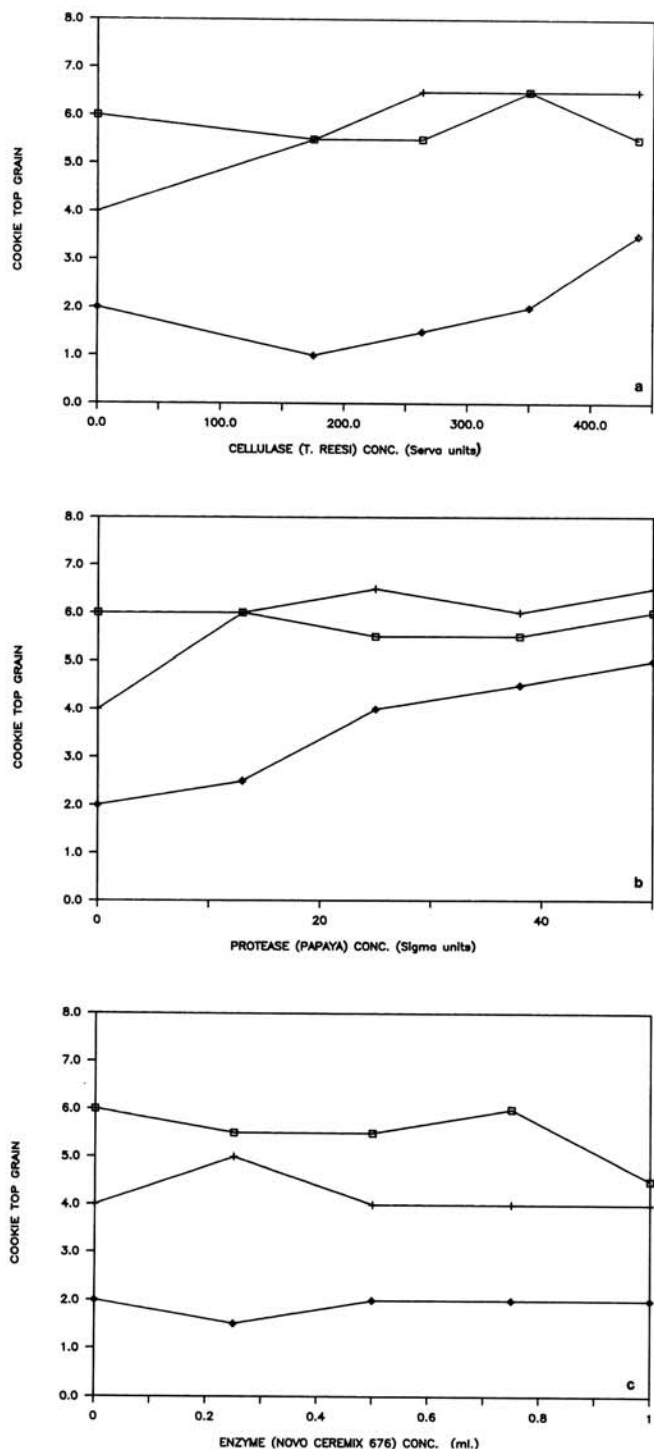


Fig. 3. Effect of four concentrations of an added Serva TC cellulase (top), Sigma P-480 protease (middle), and Novo Ceremix enzyme mixture (bottom) on sugar-snap top grain score (LSD = 1.4). Square, plus, and diamond represent the soft wheat, hard winter, and hard spring flours, respectively.

spread and top grain of the soft red winter, hard red winter, and hard red spring flours.

Sugar-snap cookie top grain is a heritable flour quality trait that is normally only partially correlated with cookie diameter. Usually, hard wheats do not produce cookie diameters much larger than their controls in this study. Hard winter wheat, and especially hard spring wheat, flours have cookie top grain values below five on a scale from zero to nine.

As theorized below, the enzymes may have reversed a genetically controlled negative influence on the cookie baking mechanism (cookie spread) of the two hard wheat flours. Because the substrates of the cellulase, the protease, and the mixture of α -amylase, β -glucanase, and protease are so different and the enzymes do not produce common enzymatic by-products, it is possible that the improvement in baking resulted from common macrophysical effects of the various enzymatic activities.

Concerning the various theories of cookie baking mechanisms stated in the introduction, those common macrophysical results may be an increased freedom of water mobility, causing a reduction of dough viscosity during baking. Yamazaki (1959) showed that cookie spread is a function of dough viscosity and that poorer quality flours and flours from the hard wheat class produce higher dough viscosities during baking, thus restricting cookie spread.

The papaya protease, which has extensive exo- and endoproteolytic activities, produces substantial degradation of gluten proteins (Reed 1966). Concerning the several theories on the influence of protein on cookie spread, the papaya protease may have decreased the glass transition temperature of the flours or decreased the rate at which the gluten expanded. It may have also lowered the dough viscosity and raised the rate at which the doughs spread. It may also have raised the temperature and time at which

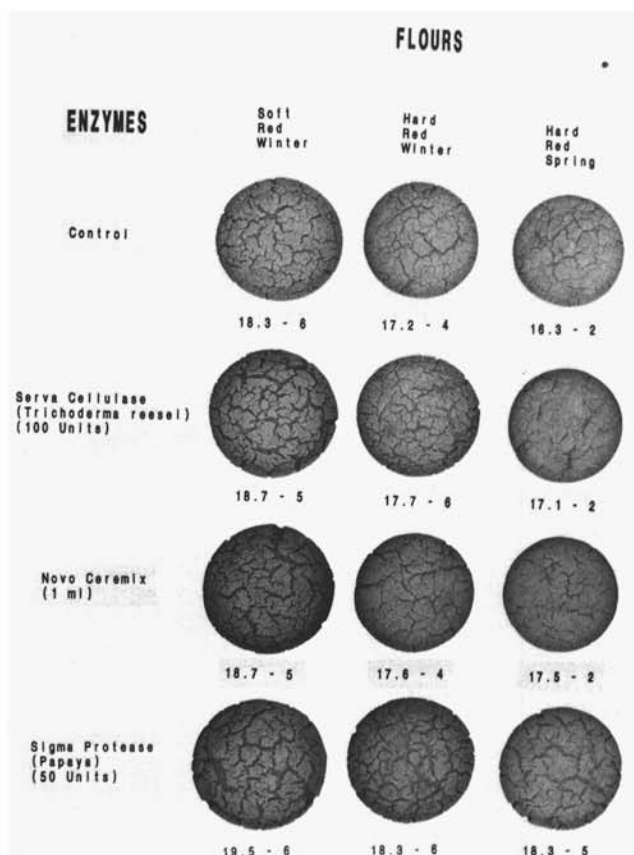


Fig. 4. Sugar-snap cookies (containing added Serva TC cellulase, Novo Ceremix enzyme mixture, and Sigma P-4880 protease) made with soft red winter, hard red winter, and hard red spring wheat flours. First number under cookie is the two-cookie diameter. Second number is the top-grain score (0-9).

the baking doughs set, or partially disrupted the two- or three-dimensional gluten network.

Because commercial cellulase preparations have various degrees of related hemicellulase, pectinase, gluconase, etc., activities, it is possible that the lack of change over time in cookie spread and dough consistency observed resulted from hydrolysis by those preparations of the various components of the tailings fraction of the flour, increasing water mobility. The flour tailings fraction has been shown to be detrimental to hard wheat flour bread loaf volume (Kulp and Bechtel 1963a, b) and to soft wheat flour cookie diameter (Yamazaki 1955).

Cookie spread is known to be increased by 1) better cultivar selection (Finney et al 1987), 2) lower flour protein content (Yamazaki 1954), 3) reduction of formula sugar particle size, (Kissell et al 1973), 4) surfactants (Tsen et al 1975) 5) sodium metabisulfite (Wade 1972), 6) greater sugar/shortening ratio (Finney et al 1950), 7) increased flour moisture content, (Gaines et al 1988) 8) reduction of damaged starch content (Gaines et al 1988), 9) using all granular sucrose in the formula (Doescher et al 1987b), 10) increased leavening (Finney et al 1950), and 11) lecithin (Kissell and Yamazaki 1975). Protease and cellulase enzymes should also be added to the list.

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