EFFECT OF VARIETY AND DRYING TEMPERATURE ON PROTEOLYTIC ENZYME ACTIVITY OF YELLOW DENT CORN

by

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INTRODUCTION

Yellow dent corn or maize (*Zea mays, L.*) ranks as one of the four principal cereal crops of the world. It is grown by many countries in the world, but more than half of the total world corn crop is grown in North America. The largest single area of production is the U.S. Corn Belt, which is made up of 12 states (Iowa, Illinois, Nebraska, Minnesota, Indiana, Ohio, Wisconsin, Michigan, Missouri, South Dakota, Kentucky, and Kansas). The Corn Belt produces 40% of the world's total production and is followed by China (14%), the Balkan area (8%), Western Europe (5%), Brazil (5%), and Mexico, the U.S.S.R., Argentina and South Africa (2% each) (Benson and Pearce, 1987).

Corn, today, is the most important crop in the U.S. agriculture. In fact, corn has had a dominant influence on American economics, politics, history, literature, even art. Since corn is plentiful, relatively cheap, has a high starch content, and an adequate protein content, it is used primarily for animal feed. It can also be processed into food and industrial products through the process of dry milling and wet milling.

Starch is the major product of the corn wet-milling industry. More than 90% of the starch used in the United States comes from corn. Food products, such as fructose, corn oil, modified starches, and dextrose, and nonfood products, such as ethanol, corn gluten feed, corn gluten meal, and industrial starches, are also derived from the wet-milling process.

The wet-milling process involves an initial sulfurous acid steep under controlled conditions to soften the kernels and make separation of starch and protein possible by

disrupting the endosperm protein matrix. The corn is then milled and its components are separated by various processes, such as washing, screening, and centrifuging. The corn wet-milling process can be affected by numerous factors, such as drying temperature, amount of sulfur dioxide in steeping, and corn genotype.

The effects of variety and drying temperatures on the wet-milling characteristics of corn have been reported by several researchers. Increasing the drying air temperature results in a significant decrease in percent starch recovery among the varieties. Watson and Hirata (1962) found that corn dried at 82°C or higher showed evidence of reduced millability. Weller (1987) showed that starch recovery decreased from 99.0 to 92.4% as drying air temperature was increased from 22 to 93°C. Vojnovich et al. (1975) found that high-temperature drying reduced starch yield from 86.7% at 49°C to 50.5% when dried at 149°C. They also found that starch viscosity and oil recovery decreased at the higher drying temperatures. Lasseran (1973) found that drying at 140°C reduced the amount of ethanol-soluble proteins as well as starch yield.

The explanation generally given for the decreased starch yield resulting from high-temperature drying is that there is some denaturation of the corn proteins at high temperatures (Watson, 1984) or there is a formation of intermolecular disulfide bonds during heating (Wall et al., 1975). An alternative explanation is that indigenous protease activity is decreased due to thermal inactivation of the proteases. Solubilization of the proteins during steeping cannot be achieved by disulfide bond cleavage alone and requires some breakdown of the matrix proteins by proteases. The source of proteases needed in steeping has been thought to be the lactic acid bacteria

which grow during the early stages of steeping (Watson et al., 1955). However, Wahl (1971) reported that natural enzymes in the corn were the source of the proteolytic activity, and that the proteases were activated by sulfur dioxide. This promotion effect has been shown to cause the loss of strength by wheat dough after the addition of oxidizing agents (Elion, 1943; Olcott et al., 1943).

The purpose of this project was to determine if there was a correlation between the indigenous protease activity of corn dried at different temperatures and the resultant starch yield. Varietal differences were also explored to ascertain if there were differences in the level of indigenous proteases between varieties.

Objectives of the Study

1. To investigate the relationship between drying temperature, corn variety, and the indigenous proteolytic enzyme activity in corn.
2. To determine if lower indigenous proteolytic activity results in lower starch yield.

LITERATURE REVIEW

Uses of Corn

Corn, known botanically as *Zea mays Linnaeus*, is one of the world's most versatile seed crops. It is grown in warm temperature regions, as well as in humid sub-tropical and tropical zones. The world corn crop has international importance and is grown on more than 130 million hectares each year, with a production of about 430 million metric tons. The United States produces about 46% of the world crop (USDA, 1983).

Corn has hundreds of uses. It is used primarily as food for humans in some areas of the world, in contrast to the United States, where about 85% of the crop is used as animal feed. Corn's large grain size, good yield, ease of cultivation, versatile food uses, and storage character are responsible for its acceptance as a food staple.

Corn production holds a special position in the agriculture of the United States historically, agronomically, and economically. It is grown, to a certain extent, in all of the states, but plays an important role especially in the midwestern states. It provides more animal feed and yields more industrial products than any other grain. The industrial use of corn in the United States may be divided into four categories: mixed feed manufacture, dry milling, wet milling, and distillation and fermentation.

The principal food products of the dry-milling industry are corn meal, corn flour, grits, oil, and breakfast cereals. The wet millers also utilize large quantities of corn. They manufacturer starch, feed, syrup, oil, and dextrines from corn. The

distilling and fermentation industries in the United States rank fourth in the industrial use of the corn crop. Ethyl alcohol, butyl alcohol, propyl alcohol, acetaldehyde, acetic acid, acetone, lactic acid, citric acid, glycerol, and whiskey are the major products of these industries.

Structure of Corn

Botanically, the corn kernel is a caryopsis, which is a dry, one-seeded berry with a single seed enclosed in a dry outer covering fused to the seed coat. Shape, size, structure, and composition of the kernel are determined by its genetic background (Inglett, 1970).

The corn kernel, enclosed within the pericarp (hull or bran), consists of the embryo (germ), endosperm, and tip cap. An average yellow dent corn kernel is comprised of 82% endosperm, 11.6% embryo, 5.5% pericarp, and 0.8% tip cap (Earle et al., 1946).

Tip cap, the smallest fragment of the kernel, is the tissue that connects the kernel to the cob. It consists mostly of starch and protein.

The pericarp of a corn kernel is composed of an outer layer of dead, thickwalled cells forming a tough, dense tissue. Beneath this is a spongy layer of cells that is continuous with the spongy cells of the tip cap. A thin, suberized membrane, known as the seed coat or testa, comes next. The aleurone cell layer, which constitutes about 3% of the kernel weight, lies between the testa and endosperm. Morphologically, it is part of the endosperm.

The endosperm, beneath the pericarp, is composed of floury and horny regions. The floury endosperm region is characterized by large cells, large round starch granules, and a thin protein matrix, which ruptures during drying. The protein matrix in the horny endosperm is thicker and does not rupture upon drying. The horny region has a 1.5 to 2.0% higher protein content than the floury region.

The scutellum and embryonic axis are the two major parts of the embryo (germ) of the kernel. The scutellum makes up about 90% of the germ and stores nutrients mobilized during germination. The embryo contains high oil content and is difficult to remove during dry or wet milling.

Composition of Corn

The average composition of the corn kernel is: 13.5% water, 10.0% protein, 4.0% oil, 61.0% starch, 1.4% sugars, 6.0% pentosans, 2.3% crude fiber, 1.4% ash, and 0.4% other substances (Leonard and Martin, 1963). The distribution of the component in various parts of the yellow dent corn kernel is given in Table 1. Starch is the major component of corn, although other carbohydrates are present in small amounts. The starch granule contains two kinds of molecules: amylose (27%) and amylopectin (73%). Both molecules are high molecular weight polymers made up of units of D-glucose. Amylopectin is a branched molecule and contains 40,000 or more glucose units. Amylose, a linear molecule, has around 1000 glucose units. The starch granules make up the major portion of the corn endosperm. The central portion contains the largest granules (10-30 μm), and the portion closer to the pericarp contains smaller granules (1-10μm).

TABLE 1. Chemical composition of the component parts of the yellow dent corn kernel[a].

Chemical Component	Portion of kernel (%)[b]			
	Endosperm	Embryo	Pericarp	Tip cap
Protein	73.1	23.9	2.2	0.8
Oil	15.0	83.2	1.2	0.6
Sugar	28.2	70.0	1.1	0.7
Starch	98.0	1.3	0.6	0.1
Ash	18.2	78.5	2.5	0.8

[a] From Leonard and Martin (1963).
[b] On a moisture-free basis.

Corn seems to be variable in protein content due to a complex interaction between genetic, environmental, and physiological factors (Leonard and Martin, 1963). Prolamin (zein) and glutelin are the principal proteins of the corn endosperm. These two fractions compose approximately 89% of the total nitrogen content in normal corn (Russell and Tsao, 1985). Zein occurs in corn as cytoplasmic deposits called protein bodies, ranging in size from 1 to 3 μm diameter, and is generally low in the essential amino acids, lysine and tryptophan. It is soluble in relatively strong alcohol or dilute aqueous alkaline solution, but insoluble in water or in solutions of neutral inorganic salts. On the other hand, glutelin is insoluble in water, saline solutions, or alcohol, but readily soluble in dilute sodium or potassium hydroxide solutions.

The lipids of corn influence the flavor and storage characteristics of the grain and the fractions. They are soluble in ether or chloroform. The major substances are fats, waxes, phosphatides, steroids, and carotenoids. Almost 85% of corn lipids occur in the germ, which is the commercial source of corn oil (Inglett, 1970).

Proteolytic Enzymes

Proteases (proteolytic enzymes) are enzymes which are capable of hydrolyzing peptide bonds of proteins and peptides. They consist of two different groups: proteinases (endopeptidases) and the peptidases (exopeptidases) (Myrback, 1951). The exopeptidases act only on peptide bonds adjacent to a free amino or carboxyl group; i.e., on compounds which possess one or more of the free terminal polar groups, such as the alpha amino or alpha carboxyl groups, hence they cannot act on proteins. In contrast, the endopeptidases can hydrolyze compounds which do not possess free terminal amino or carboxyl groups, or which contain polar groups other than alpha in relation to the sensitive peptide bonds. These enzymes act on the interior peptide bonds of proteins; they can, however, also split peptide bonds in suitable simple peptides and their derivatives.

The importance of proteolytic enzymes in corn processing is their effect during wet milling. Proteolytic enzymes, with amylolytic enzymes, can affect the breakdown of the corn starch in the first phase of steeping during the corn wet milling (Wahl, 1971). It is assumed that, during steeping, proteolytic enzymes may be activated by sulfur dioxide present and hydrolyze the disulfide bonds of the protein matrix surrounding starch granules, so amylolytic enzymes can break starch down easily.

Spanheimer et al. (1972) studied the effects of enzymes on corn grits and reported that all enzyme treatments resulted in increased starch yield and increased protein solubilization. Each enzyme tested affected the alkali-soluble protein (glutelin), while not measurably altering the solubility of the alcohol-soluble protein (zein) of corn.

Corn Wet Milling

Approximately 12% of the corn produced in the United States is used in the wet-milling industry to produce starch, gluten, oil, and fiber. The physical condition of the grain used for wet milling is important. The ground should be sound and it should have a moisture content of 16% or less. Corn damaged by heating, insects, or mold is undesirable for wet milling because processing becomes more difficult and starch and oil yields are reduced (Inglett, 1970).

The recovery of starch and other products by corn wet milling is a straight-forward process which starts with the cleaning of the grain. The grain is cleaned by screening and aspirating, which remove undesirable materials such as cob, chaff, sand, stones, insects, dust, and other foreign material. Cleaning the corn is an important first step because the presence of undesirable materials can alter the flow of steepwater through the grain mass, resulting in non-uniform steeping.

The next step in the corn wet-milling process is to soften the corn through the steeping process, which produces optimum milling conditions and separation of corn components. Steeping is more than simple water soaking of corn. It involves maintaining the correct balance of water flow, temperature, sulfur dioxide

concentration, and pH. Steeping is done in a series of stainless steel tanks in which the lactic acid fermentation is controlled by using a countercurrent flow of steeping water and the addition of sulfurous acid. Corn is normally steeped 20-30 hours at a temperature of 48-52°C. According to Watson (1984), at the end of the steeping process, the corn kernels absorb water to about 45% (wet basis), release about 6.0-6.5% of its dry substance as solubles into the steepwater, absorb about 0.2-0.4 g of sulfur dioxide per kg, and become sufficiently soft for the milling of the kernel.

Next, the corn is run through an abrasive milling operation. The milling breaks the kernel apart to allow for appropriate separation of its components. Subsequent processing results in the separation of starch from gluten, germ, and hulls.

Protein-Starch Separation

After steeping and the separation of germ and fiber, the mixture of starch and protein, known as "mill starch," is ready for separation. The mill starch, which carries 5-8% insoluble protein content, is first concentrated and then the protein or gluten is separated from the starch in high speed centrifuges. The separation is possible due to the difference in specific gravity; the relatively heavier starch is separated from the lighter gluten by centrifugal force.

The centrifuging of starch is made in two stages. In the first stage, good quality gluten which contains 60-70% protein and 1.5-2.0% solid, is separated. In the second stage, the hydroclones are used to remove the remaining soluble and insoluble protein from starch. After the second separation, the starch contains an insoluble protein level of approximately 0.33% (dry basis).

Role of Sulfur Dioxide and Lactic Acid in Steeping

During the wet-milling process, the corn is steeped for 24-48 hours in 55°C steep water containing 0.2% sulfur dioxide. The primary action of sulfur dioxide appears to be its reaction with disulfide bonds of the protein encapsuling the starch granules. This leads to degradation of cell walls and protein fibers throughout the corn kernel. Sulfur dioxide also controls fungal growth, while allowing some lactic acid bacteria to grow. Wall and Paulis (1978) also suggested that sulfur dioxide activates indigenous proteolytic enzymes which help break down and solubilize the protein. Cox et al. (1944) demonstrated that during steeping with sulfur dioxide over a 24-hour period at 50°C, the protein matrix gradually swells, becomes globular, and finally disperses. The degree of globularity is directly related to the ease of starch recovery on grinding. Wahl (1969) showed that, during steeping, the reaction of bisulfite ion with endosperm protein is completed in whole kernels in 6-10 hours. Both sulfite and disulfite ions are capable of reducing disulfide bonds, but pH conditions inside the kernel determine which ion predominates. A kernel pH of 3.6-4.0 would favor disulfide ion as the reactant, but pH levels are normally closer to 4.0-4.5 in most commercial steeping.

The influence of lactic acid on the steeping process and on milling results is very important. Wahl (1970) has shown that lactic acid increases kernel softening and participates in protein breakdown. Lactic acid is produced mostly by lactic acid bacteria that are found naturally on the kernel. During the steeping process, the formation of lactic acid takes place in 8-20 hours. The lactic acid enters the interior of the kernel and causes a decrease in pH. This decrease in pH helps in the softening of

the corn kernel (Wahl, 1970). Roushdi et al. (1981) showed that the increasing levels of sulfur dioxide can adversely affect lactic acid fermentation, and this may disturb the course of steeping.

Effect of High Drying Temperatures on Starch Yield

Application of different drying temperatures after harvest is one of the critical quality factors that affect the efficient production of starch by wet milling. The amount of starch recoverable from a wet-milling process is inversely related to the severity of the drying treatment of incoming corn (Weller, 1987).

Le Bras (1982) reported that the starch recovery rate decreased and the percentage of proteins in starch increased with increasing temperatures from 80 to 150°C. Higher drying temperatures can lower the quality of corn and reduce the amount of starch recovered by the wet-milling process.

Weller (1987) treated four varieties of yellow dent corn with four different drying temperatures (22, 49, 71, and 93°C) and wet milled them. He found that increasing drying air temperatures resulted in a significant linear decrease in percent starch recovery among the varieties. Starch recovery decreased from 99.0 to 92.4% as drying air temperatures increased from 22 to 93°C.

MATERIALS AND METHODS

Materials

Four genotypes of yellow dent corn (FR27xTRMo17, B73xLH38, LH51xLH119, and FR27xVa22) were harvested at a moisture content of 30% on wet basis (wb) and dried to 14% (wb) at four drying temperatures (22, 49, 71 and 93°C) by Dr. Curtis Weller of the University of Illinois at Urbana-Champaign. The B73xLH38 cross was a soft genotype, FR27xTRMo17 was a medium-soft genotype, LH51xLH119 was a medium-hard genotype, and FR27xVa22 was a hard genotype. Dr. Weller then ran these dried samples through a wet-milling process and determined the final starch yields for each sample, as reported in his study (Weller, 1987). A total of 64 corn samples (4-variety; 4-temperature treatment; and 4-replicates) was obtained from Dr. Weller. However, two of the four replicates run by Weller were chosen according to their starch contents and were used for this study.

Measurement of Proteolytic Activity

Enzymatic activity was determined on ground samples using a modification of the fluorometric assay reported by Mathewson et al. (1988). The assay is based on the measurement of the fluorescent adduct formed between the alpha-amino group, resulting from proteolysis and o-phthalaldehyde (OPA) in the presence of ethanethiol. This adduct was measured fluorometrically at its emission wavelength of 450 nm.

The corn samples were ground twice through a 1.0-mm screen by a modified Udy/Weber mill (hammer mill) and the ground samples were stored at 4°C in sealed containers for two weeks to equilibrate the moisture. Approximately 3 g of the ground corn sample corresponding to 0.27 g protein was then extracted, with 6.0 ml buffer (0.025 M MES [2(N-morpholino) ethanesulfonic acid], 0.001 M EDTA [ethylenediamine-tetraacetic acid], 0.002 M BME [beta-mercaptoethanol], pH 6.0) at 4°C. All solutions used in the assay were as reported by Mathewson et al. (1988). The sample solution was stirred for two hours and then centrifuged at 20,000 rpm (about 48,246 x *g*) for 30 minutes at 4°C. The supernatant was collected and filtered through a Whatman #2 filter, and then assayed.

For determination of protein content, the corn extract (40μl) was diluted to 2.0 ml with distilled water, mixed, and transferred to a 1.0 cm square spectrophotometer cuvette. Its absorbance was measured with a Pye-Unicam SP1750 UV-VIS Spectrophotometer at 280 nm. Each sample determination was replicated twice and each replicate was duplicated.

For determination of proteolytic activity, a control was prepared using 200 μl of substrate (Cytochrome-C) solution (1 mg/ml) equilibrated in a water bath at 40°C. After corn extract (30 μl) was added to the substrate and mixed, 10 μl of the sample was immediately removed. This 10 μl was added to a 75 x 10 mm round fluorometer tube containing 200 μl of saturated borate buffer (pH 9.4) with 1% SDS (sodium dodecyl sulfate) to terminate the proteolysis. Twenty-five μl of OPA reagent solution was pipeted into the tube, mixed, and the extract was left at room temperature for 15 minutes. One ml of methanol was then added to the extract and mixed. Fluorometric

activity was measured using a Farrand Optical Model A4 fluorometer set on the second smallest aperture (#2). An interference filter having a band pass of less than 10 nm was used for emission at 450 nm. This measurement procedure was repeated for each sample at time intervals of 8, 16, and 24 hours. Specific activities were then calculated using the following formula:

$$\text{Specific Activity} = \frac{\text{Vol (Rx Sol)} \times \Delta\text{RF}}{\text{Abs} \times \text{Vol (Sample)} \times \text{Rx Time} \times \text{Vol (Rx Sample)}}$$

where:
- Vol (Rx Sol) = Volume of reaction solution: 1.235 ml
- ΔRF = Change in relative fluorescence (RF) = RF (final) - RF (initial)
- Abs = Spectrophotometric absorbance at 280 nm of the extract
- Vol (Sample) = Sample volume: 0.05 ml
- Rx Time = Reaction time: 8, 16, or 24 Hours
- Vol (Rx Sample) = Volume of reaction sample: 0.01 ml

Proximate Analysis

Moisture content was determined by drying 2 g ground corn samples at 103°C for one hour in a Blue M air oven. Protein content was determined by the AACC Improved Kjeldahl Method 46-10 and expressed on a 14% moisture basis. Starch yields of the corn samples were as reported by Weller (1987), who determined the starch contents using a Yellow Springs Instruments Glucose Analyzer.

Statistical Evaluation

Statistical analyses were used to evaluate the effect of variety and drying temperature on yellow dent corn starch yield and proteolytic enzyme activity. Analysis of variance (ANOVA) procedure was used for comparisons. To determine significant differences in means, Duncan's Multiple-Range Test was applied. All statistical statements for significance were made at the 5% probability level.

RESULTS AND DISCUSSION

Increasing drying temperatures of all four genotypes of yellow dent corn resulted in a decrease in proteolytic enzyme activity. Specific activities decreased with increased drying temperatures at the 8-, 16-, and 24-hour reaction times (Figures 1-3). The linear regression equations and their coefficient (r) values of Figures 1-3 are given in Table A in the appendix. The means and standard deviations for Figures 1-3 are also given in Table 2. The variability in the measured activity decreased with longer reaction times. At all three reaction times, activity dropped over 50% as temperature increased. At a reaction time interval of 24 hours, the mean specific activity of four genotypes decreased from 6.9 to 3.2 units/min/ml/mg as the drying temperature increased from 22 to 93°C (Figure 3). Table 3 shows the linear correlation coefficients between starch yield and drying temperature. It also shows coefficients between protease activity vs. drying temperature and protease activity vs. starch yield. For three of the varieties (varieties 2, 3, and 4), the correlation coefficient relating specific activity to drying temperature was highest when the specific activity was measured at 24 hours. Unlike these three varieties, variety 1 had its highest correlation value when the specific activity was measured at the 16-hour reaction time. At all three reaction times, variety 1 showed, in general, lower correlation values than did the other three varieties.

Starch yield correlated negatively to the drying temperature (Table 4), as previously reported by Weller (1987). Correlation coefficients relating starch yield to

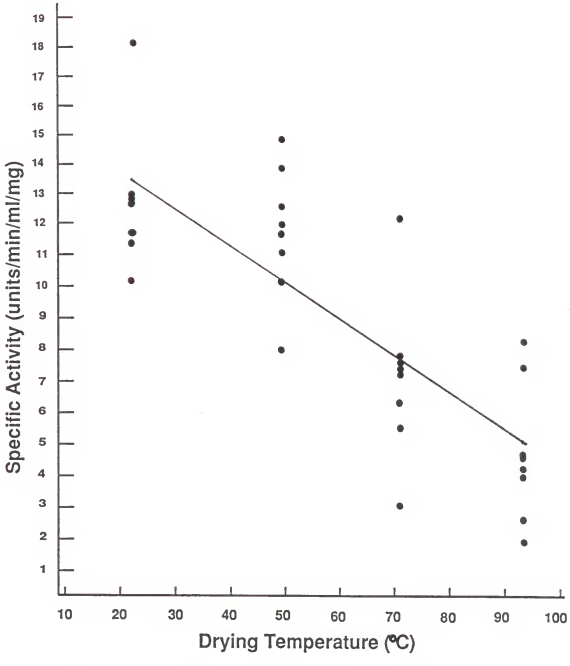

Fig. 1. Effect of drying temperature on specific activity of proteolytic enzymes measured at the 8-hour reaction time.

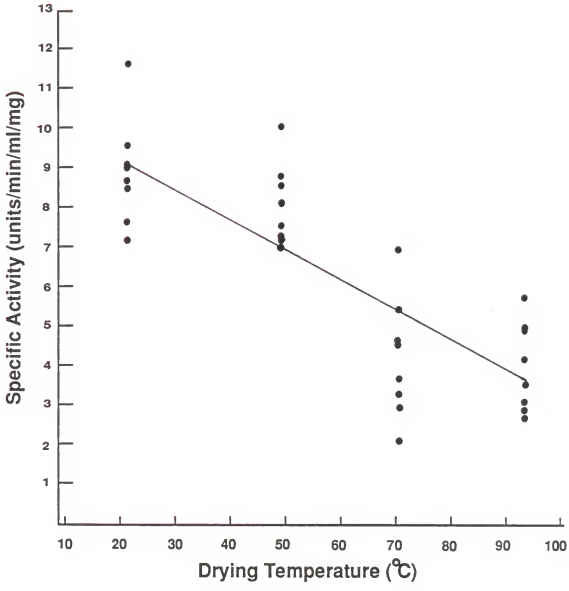

Fig. 2. Effect of drying temperature on specific activity of proteolytic enzymes measured at the 16-hour reaction time.

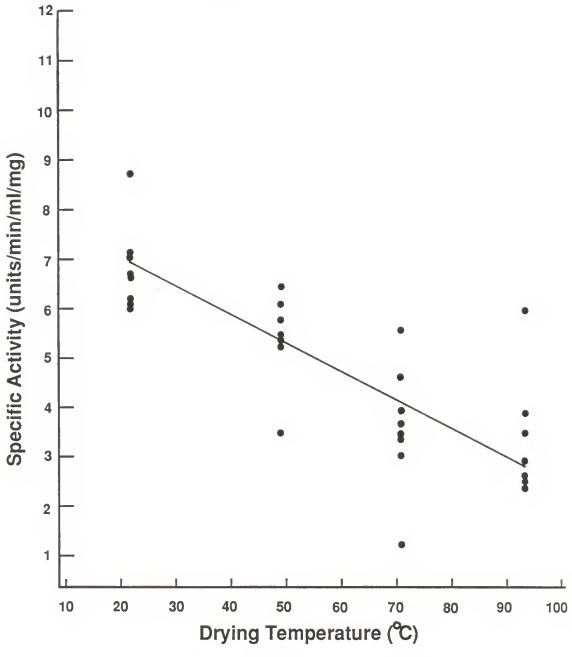

Fig. 3. Effect of drying temperature on specific activity of proteolytic enzymes measured at the 24-hour reaction time.

TABLE 2. Means and standard deviations for data in Figures 1, 2, and 3.

Figure	Temperature (°C)	Mean specific activity	Standard deviation
1	22	12.61	2.25
	49	11.74	1.98
	71	7.13	2.37
	93	4.69	1.98
2	22	8.84	1.23
	49	8.02	0.93
	71	4.18	1.39
	93	3.98	1.01
3	22	6.91	1.08
	49	5.49	0.84
	71	3.60	1.12
	93	2.90	0.54

TABLE 3. Pearson correlation coefficients between protease specific activity and drying temperature or starch yield.

Corn variety	Protease reaction time (hrs)	r value	
		Drying temp vs. proteolytic activity	Starch yield vs. proteolytic activity
1	8	-0.681	0.705
(FR27xTRMo17)	16	-0.736	0.812
	24	-0.673	0.659
2	8	-0.937	0.861
(B73xLH38)	16	-0.920	0.872
	24	-0.961	0.870
3	8	-0.810	0.843
(LH51xLH119)	16	-0.771	0.599
	24	-0.911	0.912
4	8	-0.894	0.907
(FR27xVa22)	16	-0.933	0.895
	24	-0.957	0.895

TABLE 4. Pearson correlation coefficients between starch yield and drying temperature.

	Corn variety	Starch yield vs. drying temperature
1	(FR27xTRMo17)	-0.883
2	(B73xLH38)	-0.872
3	(LH51xLH119)	-0.902
4	(FR27xVa22)	-0.939

drying temperature ranged from -0.872 to -0.939 for the four varieties (Table 4). The raw experimental data are given in Table B in the appendix. It should be noted that the data on starch yield was taken from Weller (1987).

Figures 4-7 show the relationship between drying temperature and the specific activity measured at the 24-hour reaction time for each variety. The linear regression equations and their coefficient (r) values of Figures 4-7 are given in Table A in the appendix. The means and standard deviations for Figures 4-7 are also given in Tables 5-8. Figure 4 shows that the replicate points for variety 1 showed larger variability in protease specific activity at each drying temperature as compared to the other three varieties, as shown in Figures 5-7. The cause of the larger variability in activity of variety 1 can only be speculated and might related to some extent to differences in the experimental drying conditions. This explanation is only partially satisfactory, as more variability existed in the duplicated activity measurements for each replicate of

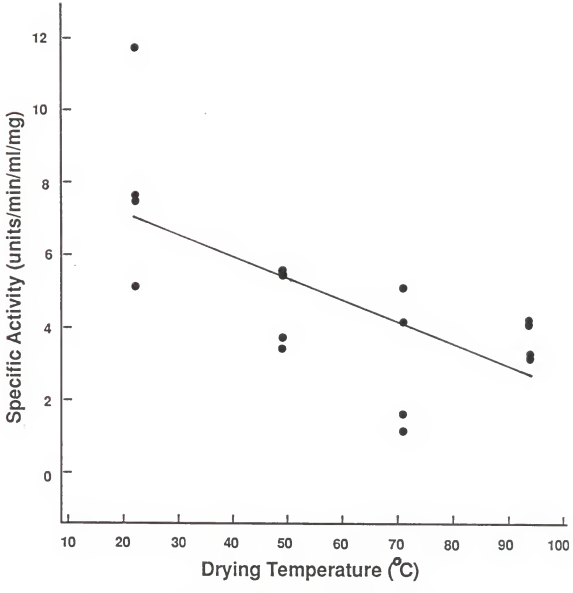

Fig. 4. Effect of drying temperature on specific activity of proteolytic enzymes measured at the 24-hour reaction time for Variety 1 (FR27xTRMo17).

TABLE 5. Means and standard deviations for data in Figure 4.

Temperature (°C)	Sample[a]	Rep #	Specific activity	Mean (rep)	Mean (sample)	Standard deviation
22	1	1	7.54	9.60		
		2	11.66		7.87	2.41
	4	1	7.31	6.14		
		2	4.96			
49	5	1	5.42	5.37		
		2	5.32		4.41	0.96
	6	1	3.32	3.45		
		2	3.58			
71	9	1	4.89	4.58		
		2	4.27		2.94	1.66
	12	1	1.14	1.29		
		2	1.44			
93	14	1	3.12	3.15		
		2	3.18		3.57	0.42
	16	1	3.91	3.99		
		2	4.07			

[a] See Variety 1 in Table B in the appendix.

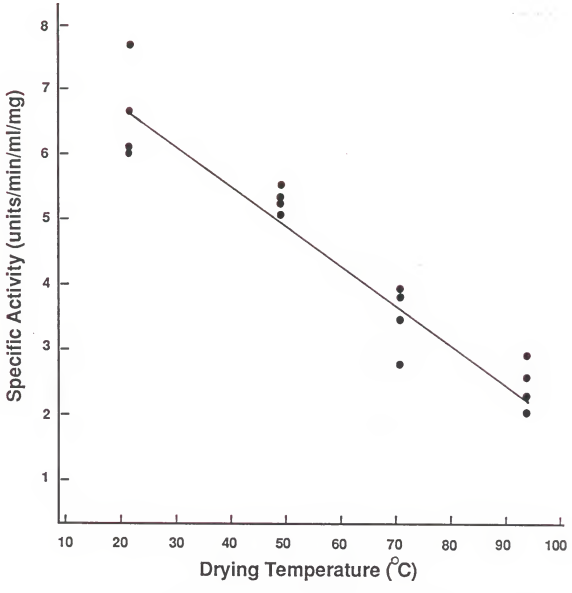

Fig. 5. Effect of drying temperature on specific activity of proteolytic enzymes measured at the 24-hour reaction time for Variety 2 (B73xLH38).

TABLE 6. Means and standard deviations for data in Figure 5.

Temperature (°C)	Sample[a]	Rep #	Specific activity	Mean (rep)	Mean (sample)	Standard deviation
22	2	1	7.53	7.05		
		2	6.56		6.53	0.62
	3	1	5.96	6.02		
		2	6.08			
49	6	1	5.24	5.16		
		2	5.08		5.24	0.13
	7	1	5.43	5.32		
		2	5.20			
71	9	1	2.60	2.96		
		2	3.32		3.32	0.44
	10	1	3.68	3.69		
		2	3.69			
93	15	1	2.50	2.28		
		2	2.05		2.39	0.28
	16	1	2.20	2.49		
		2	2.79			

[a] See Variety 2 in Table B in the appendix.

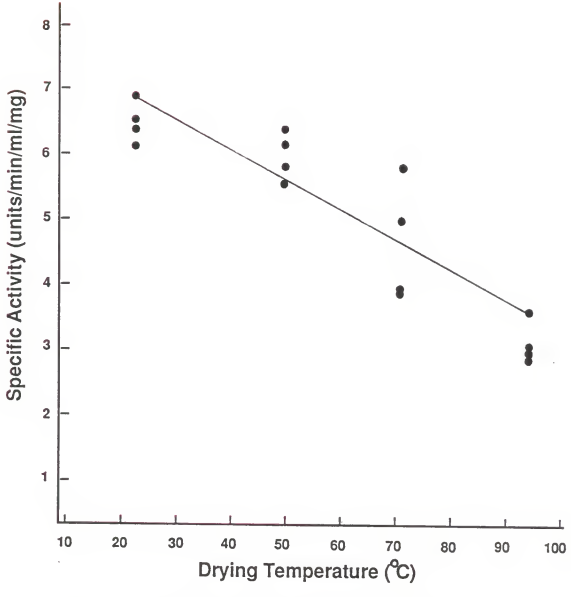

Fig. 6. Effect of drying temperature on specific activity of proteolytic enzymes measured at the 24-hour reaction time for Variety 3 (LH51xLH119).

TABLE 7. Means and standard deviations for data in Figure 6.

Temperature (°C)	Sample[a]	Rep #	Specific activity	Mean (rep)	Mean (sample)	Standard deviation
22	1	1	6.84	6.67		
		2	6.50		6.46	0.27
	4	1	6.39	6.24		
		2	6.09			
49	5	1	6.37	6.25		
		2	6.13		5.97	0.30
	6	1	5.59	5.68		
		2	5.77			
71	10	1	5.73	5.35		
		2	4.97		4.63	0.77
	11	1	3.88	3.90		
		2	3.92			
93	15	1	3.09	3.32		
		2	3.55		3.13	0.25
	16	1	2.96	2.94		
		2	2.91			

[a] See Variety 3 in Table B in the appendix.

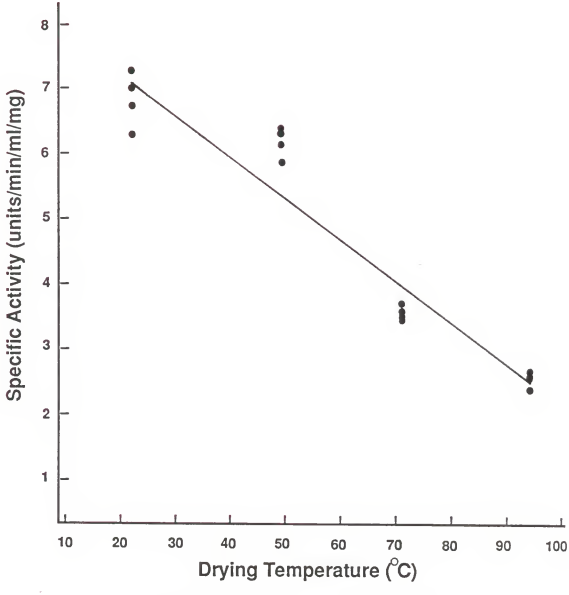

Fig. 7. Effect of drying temperature on specific activity of proteolytic enzymes measured at the 24-hour reaction time for Variety 4 (FR27xVa22).

TABLE 8. Means and standard deviations for data in Figure 7.

Temperature (°C)	Sample[a]	Rep #	Specific activity	Mean (rep)	Mean (sample)	Standard deviation
22	1	1	7.37	7.06		
		2	6.74		6.83	0.39
	3	1	6.28	6.60		
		2	6.91			
49	5	1	6.19	5.97		
		2	5.75		6.12	0.21
	7	1	6.23	6.26		
		2	6.29			
71	11	1	3.41	3.40		
		2	3.39		3.46	0.08
	12	1	3.46	3.53		
		2	3.59			
93	14	1	2.52	2.52		
		2	2.52		2.49	0.07
	16	1	2.38	2.47		
		2	2.55			

[a] See Variety 4 in Table B in the appendix.

samples dried at 22°C than the variability between replicates.

Starch yield correlated positively with the specific activity measured at each reaction time. In general, the correlation values were satisfactorily high with the 24-hour reaction time for three varieties, even though the correlation value was highest for variety 1 at the 16-hour reaction time (Table 3). The 24-hour reaction time was satisfactory for the measurement of protease activity in relation to both drying temperatures and starch yields.

The ANOVA for all samples showed that there was significant effect of drying temperature on protease specific activity, but the varietal effect was not significant. However, the interaction effect of drying temperature and variety was significant on specific activity.

Even though there were no significant differences in mean specific activities between 71 and 93°C drying temperatures, the mean specific activities of all four corn samples dried at the lowest temperature differed significantly from those of samples dried at the highest temperature. The protease activities of varieties 2, 3 and 4, dried at 49°C, differed significantly from those of samples dried at 93°C.

The ANOVA for each variety showed significant drying temperature effect on the mean specific activity and the results of Duncan's multiple range test are also given in Table 9.

Figure 8 shows the relationship between the starch yield and specific activity of proteolytic enzymes measured at the 24-hour reaction time for all four varieties. The strong positive correlation observed between the starch yield and protease activity (Figure 8) is consistent with the postulate that high temperature drying decreases

TABLE 9. Results of Duncan's multiple range test for protease specific activity measured at the 24-hour reaction time.

Variety	Drying temp. (°C)	Mean starch* yield (%)	Mean specific activity (units/min/ml/mg)	Grouping** between all samples	Grouping** within genotypes
1	22	96.91	7.867	A	a
(FR27xTRMo17)	49	96.09	4.410	EFG	b
	71	92.19	2.935	GH	c
	93	92.90	3.570	FGH	d
2	22	98.81	6.532	ABC	a
(B73xLH38)	49	100.58	5.237	CDE	b
	71	93.51	3.322	FGH	c
	93	88.11	2.385	H	d
3	22	101.55	6.455	ABC	a
(LH51xLH119)	49	100.26	5.965	BCD	b
	71	98.00	4.625	DEF	c
	93	90.05	3.127	FGH	d
4	22	100.67	6.825	AB	a
(FR27xVa22)	49	98.53	6.115	BCD	b
	71	96.89	3.462	FGH	c
	93	90.79	2.492	H	d

* Starch yield data was obtained from Weller (1987).
** Means with same letter are not significantly ($P > 0.05$) different.

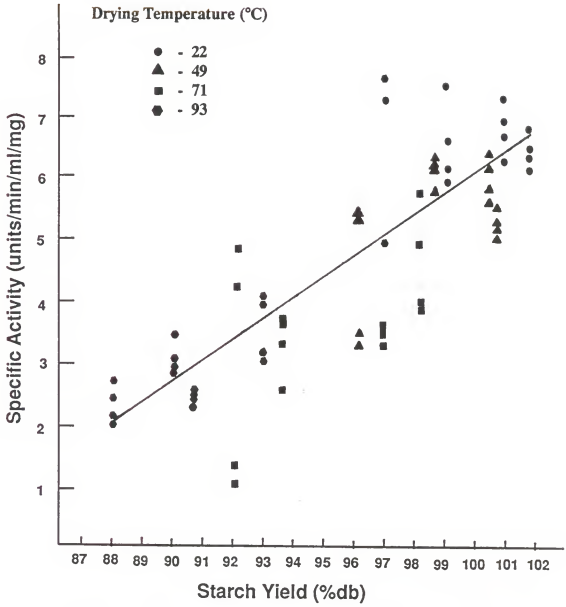

Fig. 8. Relationship of the starch yield and specific activity of proteolytic enzymes measured at the 24-hour reaction time. The numbers denote the drying temperatures of four genotypes of yellow dent corn.

TABLE 10. Means and standard deviations for data in Figure 8.

Starch yield (%)[a]	Temperature (°C)	Mean specific activity	Standard deviation
88.11	93	2.89	0.28
90.05	93	3.13	0.25
90.79	93	2.49	0.07
92.19	71	2.94	1.66
92.90	93	3.57	0.42
93.51	71	3.32	0.44
96.09	49	4.41	0.96
96.89	71	3.46	0.08
96.91	22	7.87	2.41
98.00	71	4.63	0.77
98.53	49	6.12	0.21
98.81	22	6.53	0.62
100.26	49	5.97	0.30
100.58	49	5.24	0.13
100.67	22	6.83	0.39
101.55	22	6.46	0.27

[a] Starch yield data was obtained from Weller (1987).

starch yield by inactivating indigenous proteases. The linear regression equation and its coefficient (r) value of Figure 8 are given in Table A in the appendix. The means and standard deviations for Figure 8 are also given in Table 10. The proteolytic activity observed during wet milling would have resulted from indigenous proteases, although Watson et al. (1955) speculated that the lactic acid bacteria were the source of proteolytic activity during corn wet milling. The bacteria and the proteolytic enzymes are too large to diffuse into the kernel to a level where they could be useful. The diffusion coefficient for urease and lipoxidase in aqueous solutions is on the order of $4x10^{-5}$ cm/s at 25°C (Geankoplis, 1983). This is approximately the same order of magnitude as the diffusion of steep water into corn kernels (Fan et al., 1965). Thus, the semi-permeable membranes on the corn kernel may act to exclude the larger molecular weight enzymes from penetrating into the kernel. Spanheimer et al. (1972) showed that corn grits could be treated with enzymes to enhance starch yield. In whole kernels, the same effect has not been observed, most likely due to the exclusion of the added enzymes from the endosperm by the seed coat and pericarp.

Thermal inactivation of the indigenous proteases does not appear to be a function of temperature alone. Most corn drying models predict that the kernel temperature will be fairly uniform during drying. Litchfield and Okos (1982) predicted that within 90 minutes the kernel temperature would be a uniform 92°C in 97°C drying air. It is likely that the inactivation could require either the absence or the presence of moisture. Both conditions exist at some part of the kernel during drying. It is also possible that the inactivation requires an intermediate reaction to occur which is site specific within the kernel. More data will be needed to resolve this issue.

At the current level of accuracy of the analytical procedure, use of the protease activity measurement to predict starch yield does not seem plausible due to large variabilities in both protease activity and starch yields among different varieties. The spread of starch yield values at any given specific activity is too wide to be commercially usable for prediction (Figure 8). For example, at a specific activity of 4.00, the starch yield range would be from 92 to 98%. However, the procedure could be used to screen out corn cuts with lower activity levels. If a cut-off level of 3.5 is used, 6 out of 8 of the 93°C-dried samples could be segregated out. One 49°C-dried sample and three 71°C-dried samples out of eight could have been separated out as well.

CONCLUSIONS

1. Protease specific activity decreased linearly at each reaction time interval tested as drying temperature increased. The largest decrease in specific activity was seen at the 24-hour time interval for all varieties except variety #1.
2. No significant differences in proteolytic activities were shown between corn varieties.
3. There was a significant linear relationship between starch yield and specific activity. Proteolytic specific activity increased as starch yield increased for all varieties. This relationship indicated that proteolytic specific activity decreased as drying temperature increased, and this effect resulted in a decrease in starch yield.

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APPENDIX

TABLE A

LINEAR REGRESSION EQUATIONS
AND THEIR COEFFICIENT VALUES

Figure No.	Equation (Y = mX + b)		Linear Coefficient Value [a] (r)
	m	b	
1	-0.065	17.987	-0.801
2	-0.042	12.051	-0.826
3	-0.032	9.165	-0.854
4	-0.063	8.386	-0.673
5	-0.061	7.950	-0.961
6	-0.047	7.836	-0.911
7	-0.066	8.599	-0.957
8	0.340	-27.900	0.736

[a] Significant at 5% probability level.

SAMPLE	VARIETY	DT	STARCH*	PROT	MOIST	WTCP	ABS	RFI	RF8	RF16	RF24	ΔRF8	ΔRF16	ΔRF24	SP8	SP16	SP24
1	1	22	96.91	8.53	11.24	3.066	13.20	0.792	1.050	1.090	1.140	0.258	0.298	0.348	16.81	9.53	7.54
1	1	22	96.91	8.53	11.24	3.066	8.65	0.729	0.929	1.010	1.090	0.200	0.281	0.361	19.40	13.41	11.66
4	1	22	96.91	8.50	10.92	3.066	13.80	0.792	1.026	1.120	1.146	0.233	0.326	0.353	14.51	10.03	7.31
4	1	22	96.91	8.50	10.92	3.066	13.16	0.776	0.949	1.030	1.090	0.171	0.252	0.312	11.15	8.09	4.96
5	1	49	96.09	8.45	10.46	3.066	12.03	0.852	1.090	1.140	1.080	0.238	0.288	0.228	16.96	10.06	5.42
5	1	49	96.09	8.45	10.45	3.066	11.88	0.819	0.968	1.100	1.040	0.149	0.281	0.221	10.76	9.94	5.32
6	1	49	96.09	8.56	10.50	3.034	13.50	0.841	1.055	1.070	0.998	0.214	0.229	0.157	13.59	7.13	3.32
6	1	49	96.09	8.56	10.50	3.034	13.40	0.841	0.976	1.090	1.010	0.136	0.249	0.169	8.56	7.76	3.58
9	1	71	92.19	8.64	10.38	3.000	10.23	0.761	0.902	0.918	0.926	0.151	0.167	0.175	12.66	6.99	4.89
9	1	71	92.19	8.64	10.38	3.000	10.30	0.762	0.900	0.924	0.916	0.138	0.162	0.154	11.49	6.74	4.27
12	1	71	92.19	8.37	10.63	3.103	10.03	0.779	0.812	0.819	0.819	0.033	0.040	0.040	2.82	1.71	1.14
12	1	71	92.19	8.37	10.63	3.103	9.93	0.761	0.816	0.839	0.831	0.037	0.058	0.050	3.19	2.50	1.44
14	1	93	92.90	8.34	10.31	3.110	10.15	0.761	0.831	0.862	0.862	0.080	0.111	0.111	6.76	4.49	3.12
14	1	93	92.90	8.34	10.31	3.110	9.52	0.695	0.792	0.802	0.791	0.107	0.117	0.106	9.64	5.27	3.18
16	1	93	92.90	8.50	10.04	3.034	9.35	0.712	0.800	0.827	0.839	0.088	0.115	0.127	8.07	5.27	3.91
16	1	93	92.90	8.50	10.04	3.034	9.28	0.701	0.772	0.832	0.833	0.071	0.131	0.132	6.56	6.05	4.07
2	2	22	98.81	9.53	11.60	2.756	12.23	0.477	0.600	0.772	0.799	0.183	0.296	0.323	12.83	10.34	7.53
2	2	22	98.81	9.53	11.60	2.756	12.08	0.459	0.600	0.697	0.736	0.141	0.238	0.277	10.01	8.45	6.56
3	2	22	98.81	9.13	10.53	3.103	13.95	0.510	0.711	0.750	0.801	0.201	0.240	0.291	12.36	7.38	5.96
3	2	22	98.81	9.13	10.53	3.103	13.60	0.503	0.710	0.749	0.792	0.207	0.246	0.289	13.05	7.76	6.08
6	2	49	100.58	8.89	11.05	2.935	12.92	0.602	0.733	0.851	0.839	0.131	0.249	0.237	8.70	7.35	5.24
6	2	49	100.58	8.89	11.05	2.935	12.15	0.595	0.698	0.825	0.811	0.103	0.230	0.216	7.27	7.22	5.08
7	2	49	100.58	8.88	10.87	2.935	13.22	0.561	0.711	0.809	0.802	0.150	0.250	0.251	10.38	7.44	5.43
7	2	49	100.58	8.88	10.87	2.935	13.47	0.553	0.710	0.801	0.798	0.157	0.248	0.245	9.99	7.02	5.20
9	2	71	93.51	9.12	10.40	2.842	10.08	0.552	0.610	0.642	0.649	0.058	0.090	0.097	4.66	3.21	2.60

TABLE B : Quality Factor and Specific Activity Data

SAMPLE	VARIETY	DT	STARCH*	PROT	MOIST	WTCP	ABS	RFI	RF8	RF16	RF24	ΔRF8	ΔRF16	ΔRF24	SP8	SP16	SP24
9	2	71	93.51	9.12	10.40	2.842	11.62	0.532	0.622	0.660	0.666	0.090	0.128	0.134	6.70	4.23	3.32
10	2	71	93.51	9.21	10.35	2.813	11.97	0.666	0.692	0.711	0.720	0.126	0.146	0.154	9.03	5.19	3.68
10	2	71	93.51	9.21	10.35	2.813	11.40	0.552	0.638	0.698	0.699	0.086	0.146	0.147	9.47	5.49	3.69
15	2	93	88.11	9.14	10.59	2.842	10.30	0.539	0.564	0.618	0.629	0.025	0.079	0.090	2.08	3.29	2.50
15	2	93	88.11	9.14	10.59	2.842	9.93	0.549	0.572	0.602	0.620	0.023	0.053	0.071	1.99	2.29	2.05
16	2	93	88.11	9.32	10.54	2.783	10.00	0.536	0.565	0.607	0.615	0.027	0.069	0.077	2.31	2.96	2.20
16	2	93	88.11	9.32	10.54	2.783	10.32	0.545	0.562	0.642	0.646	0.037	0.097	0.101	3.07	4.03	2.79
1	3	22	101.55	8.26	11.47	3.176	13.70	0.471	0.692	0.769	0.799	0.231	0.298	0.328	13.28	9.33	6.84
1	3	22	101.55	8.26	11.47	3.176	12.66	0.476	0.660	0.710	0.761	0.186	0.236	0.286	12.11	8.01	6.50
4	3	22	101.55	8.11	10.87	3.214	14.72	0.470	0.652	0.715	0.796	0.182	0.243	0.329	10.18	7.08	6.39
4	3	22	101.55	8.11	10.87	3.214	14.66	0.481	0.660	0.722	0.793	0.179	0.241	0.312	10.06	7.05	6.09
5	3	49	100.26	8.26	11.50	3.176	13.87	0.486	0.707	0.748	0.772	0.222	0.263	0.287	14.79	8.76	6.37
5	3	49	100.26	8.26	11.50	3.176	13.60	0.493	0.710	0.726	0.761	0.217	0.259	0.268	14.69	8.06	6.13
6	3	49	100.26	8.11	10.96	3.214	13.63	0.481	0.665	0.679	0.732	0.184	0.198	0.251	12.30	6.62	5.59
6	3	49	100.26	8.11	10.96	3.214	15.02	0.499	0.675	0.724	0.762	0.176	0.225	0.263	11.59	7.41	5.77
10	3	71	98.00	8.10	10.83	3.214	11.18	0.547	0.640	0.690	0.771	0.093	0.143	0.224	7.13	4.17	5.73
10	3	71	98.00	8.10	10.83	3.214	10.75	0.514	0.607	0.637	0.701	0.093	0.123	0.187	7.42	3.74	4.97
11	3	71	98.00	8.14	10.26	3.176	10.97	0.549	0.631	0.648	0.698	0.082	0.099	0.149	6.41	2.95	3.88
11	3	71	98.00	8.14	10.26	3.176	10.86	0.528	0.604	0.626	0.677	0.076	0.100	0.149	6.16	3.01	3.92
15	3	93	90.05	7.99	10.65	3.253	9.98	0.464	0.610	0.669	0.672	0.046	0.105	0.108	3.95	4.51	3.09
15	3	93	90.05	7.99	10.65	3.253	10.05	0.474	0.617	0.595	0.599	0.043	0.121	0.125	3.67	5.16	3.55
16	3	93	90.05	8.10	10.82	3.214	10.33	0.509	0.549	0.611	0.616	0.040	0.102	0.107	3.32	4.23	2.96
16	3	93	90.05	8.10	10.82	3.214	10.13	0.498	0.556	0.598	0.601	0.058	0.100	0.103	4.91	4.23	2.91
1	4	22	100.67	9.15	10.71	2.884	12.38	0.532	0.705	0.801	0.851	0.173	0.269	0.319	11.98	9.32	7.37
1	4	22	100.67	9.15	10.71	2.884	11.83	0.528	0.684	0.769	0.807	0.156	0.241	0.279	11.31	8.73	6.74

TABLE B : (continued)

SAMPLE	VARIETY	DT	STARCH*	PROT	MOIST	WTCP	ABS	RFI	RF8	RF16	RF24	ΔRF8	ΔRF16	ΔRF24	SP8	SP16	SP24
3	4	22	100.67	9.01	11.22	2.903	12.20	0.530	0.686	0.753	0.796	0.156	0.223	0.266	10.97	7.84	6.28
3	4	22	100.67	9.01	11.22	2.903	11.75	0.521	0.681	0.769	0.805	0.160	0.248	0.284	11.08	9.05	6.91
5	4	49	98.53	8.96	10.75	2.903	11.92	0.589	0.755	0.822	0.847	0.166	0.233	0.258	11.94	8.38	6.19
5	4	49	98.53	8.96	10.75	2.903	11.38	0.579	0.729	0.788	0.808	0.150	0.209	0.229	11.30	7.88	5.75
7	4	49	98.53	8.86	10.72	2.935	11.20	0.572	0.732	0.799	0.816	0.160	0.227	0.244	12.25	8.69	6.23
7	4	49	98.53	8.86	10.72	2.935	11.46	0.663	0.761	0.813	0.835	0.168	0.230	0.252	12.58	8.61	6.29
11	4	71	96.89	8.89	10.99	2.935	9.88	0.607	0.702	0.712	0.725	0.090	0.105	0.118	8.26	4.56	3.41
11	4	71	96.89	8.89	10.99	2.935	10.53	0.611	0.695	0.723	0.736	0.084	0.112	0.125	6.84	4.56	3.39
12	4	71	96.89	8.93	10.44	2.903	9.25	0.602	0.682	0.707	0.716	0.080	0.105	0.114	7.42	4.77	3.46
12	4	71	96.89	8.93	10.44	2.903	9.03	0.606	0.689	0.704	0.729	0.083	0.098	0.123	7.30	4.27	3.59
14	4	93	90.79	8.75	10.51	2.967	8.58	0.562	0.604	0.618	0.629	0.052	0.066	0.077	5.20	3.23	2.52
14	4	93	90.79	8.75	10.51	2.967	9.26	0.566	0.631	0.644	0.669	0.045	0.058	0.083	4.17	2.63	2.52
16	4	93	90.79	8.96	10.68	2.903	9.90	0.594	0.641	0.661	0.678	0.047	0.067	0.084	4.07	2.84	2.38
16	4	93	90.79	8.96	10.68	2.903	9.68	0.599	0.666	0.673	0.687	0.069	0.074	0.088	6.23	3.21	2.55

SAMPLE - Sample Number
VARIETY - Variety Number
DT - Drying Air Temperature, °C
STARCH - Starch Yield, %db.
PROT - Protein Content, %db.
MOIST - Moisture Content, %wb.
WTCP - Test Weight at Constant Protein, g.
ABS - Absorbance at 280 nm

RFI - Initial Relative Fluorescence at 450 nm
RF8 - Relative Fluorescence at 8 Hours
RF16 - Relative Fluorescence at 16 Hours
RF24 - Relative Fluorescence at 24 Hours
ΔRF8 - Change on Relative Fluorescence at 8 Hours
ΔRF16 - Change on Relative Fluorescence at 16 Hours
ΔRF24 - Change on Relative Fluorescence at 24 Hours
SP8 - Specific Activity at 8 Hours
SP16 - Specific Activity at 16 Hours
SP24 - Specific Activity at 24 Hours

Variety 1 = FR27xTRMo17 - Medium Soft
Variety 2 = B73 x LH38 - Soft
Variety 3 = LH51xLH119 - Medium Hard
Variety 4 = FR27 x Va22 - Hard

TABLE B : (continued)

* - Averaged Starch Yield Values Taken From Weller (1987)

EFFECT OF VARIETY AND DRYING TEMPERATURE ON PROTEOLYTIC ENZYME ACTIVITY OF YELLOW DENT CORN

by

MEHMET R. KERPISCI

B.S., Hacettepe University, Turkey, 1984

AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

Corn is one of the world's four major cereal crops. About 46% of the world crop is produced in the United States. In commercial preparation of corn starch by the process of wet milling, even a small increase in starch yield is of major economical importance. The objectives of this study were to investigate the effect of drying temperatures on corn proteolytic activities and their relationship to starch yield.

Proteolytic activity was measured in four commercial corn varieties, each dried at 22, 49, 71, and 93°C, using a procedure based on measurement of the fluorescent adduct formed between the alpha-amino group resulting from proteolysis and o-phthaldehyde in the presence of ethanethiol. Proteolytic activity decreased significantly with an increasing drying temperature: the overall mean proteolytic specific activity value of the four varieties decreased from 6.9 to 3.2 units/min/ml/mg as drying temperature increased from 22 to 93°C at the 24-hour reaction time.

There was a significant positive linear relationship between starch yield and proteolytic specific activity. This relationship indicates that proteolytic specific activity decreased as drying temperature increased and this effect resulted in a decrease in starch yield.