Inactivation of Peroxidase as a Function of Corn Processing[1]

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ABSTRACT

Measurement of peroxidase activity is used as a method by which to quantitate enzyme destruction in processed corn. Dry-mill processing usually results in a small amount of enzyme destruction. Peroxidase activities in the dry-milled fractions vary, largely because of the anatomical origin of the fraction. The germ fraction is much more active than the fractions derived from endosperm or pericarp. Roll- and extrusion-cooking of grits often result in total inactivation of peroxidase activity. The degree of inactivation depends upon the amount of heat and moisture used in the process, and it can be correlated with either water-absorption properties of the product or consistency of a mixture of the product with water.

Peroxidase activity has been used in food quality control as a biochemical index of whether heating or blanching procedures are adequate (1,2,3). Similar studies have been made on sweet-corn peroxidase (4,5,6). The stability of peroxidase to heat is greater than that of most other enzymes, and peroxidase is also easy to assay. For these reasons it serves as an acceptable measure of inactivation for a broad range of enzymes.

In our study it was necessary to know if peroxidase inactivation could be measured in corn processed with semidry heat encountered in dry-milling and in roll-cooking and extrusion-cooking. Special attention was given to roll- and extrusion-cooked corn products specified as acceptable for use in "Blended Food Product, Formula No. 2," commonly called CSM (7). CSM is a food supplement for preschool children, consisting of a formulation of heat-processed corn meal, soybean meal, nonfat dry milk, vitamins, and minerals. Over 500 million lb. of this food product has been shipped to developing countries as of May 1968. The variable conditions under which CSM may be stored during and after shipment creates the special requirement for enzyme inactivation. Long periods of high humidity and heat could be disastrous in cereal products with large amounts of enzyme activity.

It is not known if peroxidase, per se, is partly responsible for the development of off-flavors during food storage, but a number of investigators have correlated the production of viny or strawlike flavors to peroxidase activity (1,2,8,9). Probably other enzymes are primarily responsible for loss of quality in foods inadequately processed, including corn products. Often lipid-active enzymes are implicated as the cause of off-flavors in cereals; among them are lipoxygenase (lipoxidase) in corn (10) and lipase in oat flakes (11).

MATERIALS AND METHODS

Corn Varieties

U.S. Grade No. 1 dent corn of a nonspecific variety was obtained from a local grain elevator in the fall of 1966 (presumably the 1965 crop).

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Bear X800 hybrid dent corn (1966 crop) was a gift from Bear Hybrid Corn Co., Inc. The hybrid was crib-dried and was not subjected to high temperatures before processing.

A high-lysine variety (1965 crop) was provided by Purdue University. Another high-lysine variety (1967 crop) came from D. E. Alexander, University of Illinois.

Sample Preparation

Dry-milled fractions were prepared from about 2 kg. corn in the following manner: The corn was first tempered by adding water to yield 21% moisture and blending for 3 hr. In the second temper, water was added to raise the moisture to 24% and blended in for 15 min. The tempered corn was then ground in a Mikro-Pulverizer operating at 1,200 r.p.m. and fitted with a two-layer hardware cloth screen, 1/2 in. by 1/2 in., with the openings of the top screen placed at a 45° angle to those on the bottom. This appeared to give results comparable to those obtained by Wichser (12) with an Entoleter for degermination in establishing a satisfactory experimental mill flow for dry-milling corn. The corn from the Mikro-Pulverizer was dried for 20 min. at 82°C. to proper moisture for milling, about 16%. Subsequent milling of this material was done with an Allis-Chalmers mill according to slightly modified Wichser flow. The particle sizes of the screened fractions were: degerminator fines, -50 mesh; grits, +24 mesh; meals, -24 +50 mesh; and flour, -50 mesh (U.S. Standard Sieve Series).

For yellow dent corn, roll- and extrusion-cooking studies were carried out with commercial grits (Illinois Cereal Mills, Paris, Ill.) containing 14% moisture, 8.1% protein, 0.37% ash, and 0.5% fat. Screen analysis was: 1% +14 mesh, 87% +20 mesh, and 1% -30 mesh (U.S. Standard Sieve Series).

For high-lysine corn, roll-cooking studies were carried out with a blend of corn flour and high-fat meal dry-milled from high-lysine corn grown in 1967 by the University of Illinois. Analyses of the blend were as follows: 12.7% moisture, 7.2% protein, 0.51% ash, 2.1% crude oil, 0.40% crude fiber.

A measure of the degree of cooking was determined by a ring consistency test (13) and by a water-absorption test (14) as modified for processed corn by Anderson et al. (15). Whenever the amount of sample was limited, the consistency was measured with a Bostwick Consistometer (16) instead of by the ring consistency test.

Peroxidase Assay

A modification of the method of Vetter et al. (17) was used to assay peroxidase activity. Essentially, 1-g. samples were soaked in 2 ml. citrate-phosphate buffer for 2 hr. to soften the material, and then they were thoroughly macerated with mortar, pestle, and sand, with a minimum of buffer used for the initial grinding. Because some of the corn fractions were extremely tough, reproducible results were ensured by grinding the sample completely into a paste. After the grinding, a total volume of 30 ml. buffer was added to the paste and mixed thoroughly. This homogenate was centrifuged at 8,000 × g for 15 min. and the supernatant was collected for assay. The enzyme reaction was started by mixing an equal volume of the supernatant with the reagent (1% *O*-phenylenediamine n 95% ethanol, 0.3% H_2O_2,

and citrate-phosphate buffer, 2:2:23 by volume) in a 1-cm. photometric cell. The temperature was held at 25° ± 0.5°C. by means of a constant-temperature cell jacket. The color development at 430 mμ was observed directly in a Cary 14 recording spectrophotometer with the chart speed serving as a measure of time. The reaction cell was balanced with a cell containing equal volumes of supernatant and buffer whenever cloudy supernatant solutions were encountered; however, a blank usually was not required, since the change in absorption with time was measured rather than absolute absorption values. Activity measurements commenced about 5 sec. after rapid mixing of the reagent and supernatant. The initial rate was determined as absorption units per min. and was corrected to be equivalent to a 1-g. sample (dry weight) extracted with 30 ml. buffer. Dry weights were determined by an AOAC method (18).

For microscopic examination, whole-corn parts and dry-mill fractions were stained with a more concentrated peroxidase reagent than that used for activity assays. The reagent (1% *O*-phenylenediamine in 95% ethanol, 0.3% H_2O_2, and citrate-phosphate buffer, 1:1:8 by volume) was mixed with the sample for 5 to 15 min., washed briefly with water, and dried.

RESULTS AND DISCUSSION

Assay of Peroxidase Activity

Since peroxidase is known to be relatively stable to heat, measurement of its activity was used as an indicator of general enzyme denaturation in corn and corn products. However, a few problems were encountered with these measurements. The use of guaiacol and H_2O_2 as a substrate proved unsatisfactory as an assay for activity in corn. Especially troublesome were the series-order or consecutive-reaction kinetics encountered when corn germ extracts were assayed with guaiacol. In some germ extracts no activity was apparent; however, considerable activity was detected when these same extracts were purified by $(NH_4)_2SO_4$ (4) or by acetone-ethanol precipitation (19). On the other hand, *O*-phenylenediamine and H_2O_2 as substrate proved to be satisfactory even for germ extracts. Initial rates were quantitative through 10^3-fold dilutions. Measurement of the initial rate during the first 10 sec. was especially important in some germ extracts because the rate deviated from the linear, resembling first-order kinetics, but in other extracts linear rates persisted for 1 to 2 min. Even though a linear rate lasting over 10 sec. was not necessary for measurement of the initial rate, the best accuracy could usually be achieved by diluting problem extracts two to four times before assaying.

It should be pointed out that only buffer-soluble peroxidase was measured. It is known that a significant portion of plant peroxidase is bound to the cell wall (20). In our study, different amounts of peroxidase activity were obtained from the same sample if the buffer or salt concentration was varied in the extraction procedure. The residue remaining after extraction also was active. Nevertheless, these observations have little bearing on the use of peroxidase as an index of enzyme inactivation. Rather, relative values with good reproducibility were desired.

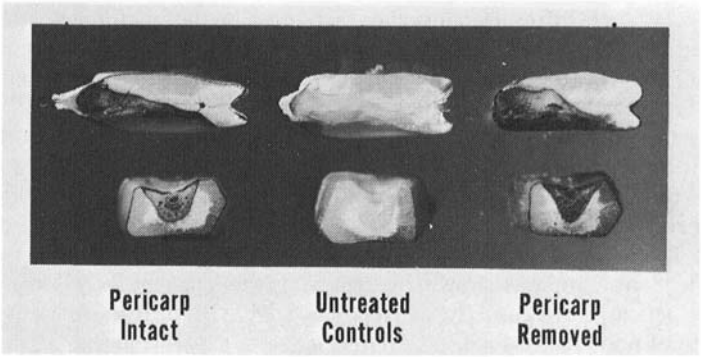

Fig. 1. In situ peroxidase activity of corn cross-sections.

Activity in Dry-Milled Fractions

Examination of cross-section pieces from whole corn stained with peroxidase reagent showed in a qualitative way that most of the peroxidase activity was concentrated in the aleurone layer, germ, and pericarp (Fig. 1). Actual analyses of activity in various anatomical parts demonstrated that endosperm and pericarp had slight activity, germ considerably more, and aleurone at least three times as much activity as germ. Owing to the difficulty of obtaining even small quantities of aleurone layer, a limited number of assays could be completed on aleurone-rich scrapings in order to determine that its activity was greater than germ tissue. Although Fig. 1 indicates that peroxidase activity is higher in the pericarp relative to endosperm tissue, assays of pericarp gave activities lower than for endosperm. Evidently, most of this activity was bound peroxidase not extractable by our method.

TABLE I. PEROXIDASE ACTIVITY[a] (A) AND OIL PERCENTAGE (B) OF DRY-MILLED CORN FRACTIONS

Fraction		Nonspecific Dent	Bear X800 Hybrid	High-Lysine[b] (1965 Crop) 1	2	High-Lysine (1967 Crop)
Germ	A	72.2	196	63.8	61.6	134
Germ	B	18	21	18	18	18
Grits	A	4.63	27.2	12.4	15.6	43.4
Grits	B	0.80	0.57	1.1	1.0	1.0
Meal	A	6.46	29.3	24.4	32.2	31.5
Meal	B	1.6	1.1	3.2	3.9	0.90
Flour	A	1.81	21.0	6.65	4.90	6.29
Flour	B	1.4	1.4	2.5	1.7	0.70
High-fat meal	A	13.9	48.3	48.5	47.0	31.9
High-fat meal	B	3.0	1.6	5.1	5.8	1.4
Degerminator	A	5.15	66.5	24.0	25.8	7.86
fines	B	.....	4.0	4.6	4.2	1.3
Feed	A	16.7	99.6	36.2	39.3	108
Feed	B	5.0	6.5	5.5	5.1	3.9

[a]Absorption units/min. X 10^2

[b]Activities and oil percentage values were determined for duplicate dry-mill runs of the same sample of high-lysine corn.

In view of such differing peroxidase activities in the anatomical parts, it was expected that peroxidase activity would vary greatly among dry-milled corn fractions (Table I). The germ fraction had the greatest activity and, in general, feed and high-fat meal had relatively high activity, probably as a result of inclusion of bits of germ or aleurone. Since the aleurone layer is the most active tissue, its presence is a significant contribution to activity assays, even though it amounts to only 2.2% of the total kernel (21). The germ, being about 12% of the kernel, also was assumed to be an important source of high activity in some of the dry-milled fractions. The contribution of germ to the high activity in the feed fraction of nonspecific dent corn was shown by removing the germ by hand so as to have essentially pericarp for assay. The activity was 2.28×10^{-2} or less than one-seventh the activity of nonselected feed.

Germ and aleurone tissue are high in oil content, whereas endosperm and pericarp are not; therefore, the percentage of oil in a sample can be considered an estimate of the amount of germ or aleurone in the sample. High activities resulting from inclusion of germ or aleurone in the dry-milled fractions can be demonstrated by correlating oil percentage with peroxidase activity. Figure 2 shows this correlation when values from dry-milled samples of the 1965 crop of high-lysine corn are used. Aleurone tissue, being more active than germ, may account for the lower germ activity values than might be expected in this correlation.

Other differences in values obtained among dry-milled fractions may also be explained by reasoning similar to that used previously. For example, there are large discrepancies among the values for flour, grits, and meal that need explaining, since these fractions are all primarily derived from the endosperm. Peroxidase activity in flour was low compared with that of grits or meal, although the percentage of oil in flour was at times comparatively higher. The values for flour may be low because this fraction is obtained from the floury endosperm of corn and is less likely to contain layers of the aleurone adhering to the flour particles. Microscopic examination of the flour treated with peroxidase reagent showed that very few particles were stained a decidedly darker color. On the other hand, treatment of grits or meal with the reagent revealed the presence of much aleurone tissue

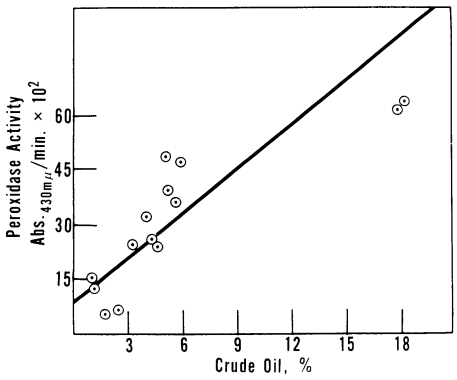

Fig. 2. Correlation of peroxidase activity with percentage of crude oil in dry-milled high-lysine corn fractions.

TABLE II. PEROXIDASE ACTIVITY[a] OF CORN GERM AND ENDOSPERM BEFORE AND AFTER DRY-MILLING

	Before Dry-Milling[b]			Dry-Milled		
Corn Variety	Germ	Aleurone Scrapings	Endosperm[c]	Germ Fraction	Hand-Selected Germ	Grits
Bear X800 hybrid	308	1,040	57.5	196	253	27.2
High-lysine						
1965 crop	177	...	67.5	62.7	...	14.0
1967 crop	169	...	56.5	134	145	37.0

[a]Absorption units per min. X 10^2.

[b]Germ and endosperm samples before dry-milling were obtained by hand-dissection of untreated corn.

[c]The endosperm samples included the aleurone layer.

appearing as a black layer on one side of the surface of a large number of particles. The color was especially dark around the severed edges of the aleurone layer.

The dry-milling process we employed included a drying step carried out at 82°C. for 20 min. on the streams after degermination. While there undoubtedly was a cooling effect from evaporation, this heat may have caused some peroxidase inactivation (Table II). Grits having low oil percentage values (Table I) were considered to be a representative sample of endosperm and likely to be mixed with a minimum of germ. However, grits are mostly derived from the horny endosperm and should have more aleurone and less floury endosperm than total endosperm. A comparison of the grits with the endosperm values given in the table shows that only about one-fifth to two-thirds of the initial activity remained after milling. Activities in the flour and meal were also lower than in hand-dissected endosperm. The apparent decrease in activities may not be wholy due to heat-denaturation of the enzyme; some loss in activity could have occurred through selective loss of some of the aleurone to other fractions during dry-milling. We have observed a loss in endosperm activity by as much as 82% after carefully scraping off all the aleurone layer. Fresh germ had about one-fifth more activity than hand-selected dry-milled germ. Hand-selection was necessary for comparison purposes because of the presence of bits of endosperm and pericarp. In general, insignificant amounts of activity were lost from the germ during dry-milling.

Tables I and II also suggest that differences in peroxidase activity occurred among the corn genotypes. These differences may be caused in part by the genetic make-up of the corn, but the history of the samples also must be considered.

Activity in Roll-Cooked and Extrusion-Cooked Corn

The roll- and extrusion-cooking methods used for processing grits, meal, or flour result in products with varying degrees of gelatinization, depending on the heat and moisture conditions selected. To meet the specifications for the CSM food supplement, the consistency value of partially gelatinized products must fall in the

TABLE III. EFFECT OF PROCESSING CONDITIONS ON PEROXIDASE ACTIVITY OF COOKED GRITS[a]

Peroxidase Activity[b]	Ring Consistency	Water-Absorption Index	Conditions: Moisture	Conditions: Temperature	Cooking Method
	in.	g. gel/g. dry sample	%	°C.	
2.01	...	2.4	...	...	None[c]
0.71	10+	2.7	15	124	Roll
0.13	10+	3.2	15	149	Roll
0.08	10+	3.4	20	141	Roll
0	10+	3.3	20	110	Extrusion
0	8.5	3.7	20	116	Extrusion
0	7.4	4.5	25	107	Extrusion
0	5.8	5.0	25	110	Extrusion
0	4.8	5.3	25	116	Extrusion
0	4.1	5.9	25	121	Extrusion
0	3	7.5	35	149	Roll

[a]Processed samples were commercial grits from Illinois Cereal Mills, Paris, Ill.

[b]Absorption units per min. X 10^2.

[c]Unprocessed grits.

range of 4.5 to 7.5 in. when the test is carried out by the specified procedure (13). For assays of peroxidase activity, samples having consistencies ranging from 3 to 10+ in. were selected, as can be seen in Table III. An additional measure of the degree of cooking, the water-absorption index (15), was also determined. In Fig. 3, peroxidase activity is plotted against water-absorption index. It was evident that certain processing conditions denatured the enzyme sufficiently so that measurement of activity was not possible. Although these samples were apparently inactive, they actually may contain residual amounts of activity below the limits of detection. The processed corn meal specified as acceptable for use in CSM was one of the inactive products. While milder treatment did not eliminate activity, the least strenuous conditions preserved only 35% of the original activity in the grits.

To observe the progress of inactivation better, high-lysine corn flour, which had relatively high peroxidase activity, was roll-cooked to give samples with varying degrees of consistency. When cooked dent corn was tested for acceptable

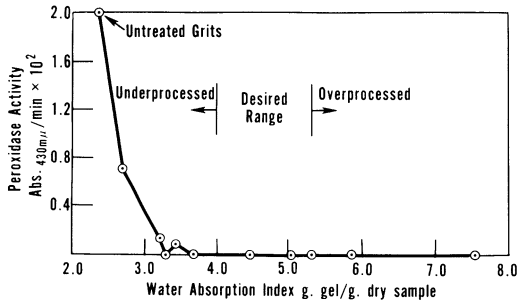

Fig. 3. Peroxidase activity of corn grits as affected by the degree of processing.

TABLE IV. EFFECT OF CONDITIONS OF PROCESSING ON PEROXIDASE ACTIVITY OF ROLL-COOKED HIGH-LYSINE CORN FLOUR[a]

Peroxidase Activity[b]	Bostwick Value cm.	Water-Absorption Index g. gel/g. dry sample	Conditions Moisture %	Conditions Temperature °C.
11.2	24+	2.6	unprocessed	unprocessed
3.82	24+	2.4	20	124
0.71	24+	2.9	20	141
0.53	24+	3.4	20	149
0.26	11.5	3.9	25	124
0.20	3.5	4.6	25	141
0.17	0	5.1	25	149
0.06	0	5.2	29	124
0	0	5.6	29	141
0	0	6.0	29	149
0	0	7.2	70	149

[a]See text.

[b]Absorption units per min. X 10^2.

consistency, water-absorption indices from 4.0 to 5.3 (15) and Bostwick consistencies from 4 to 14 cm. (16) were about equivalent to the range of ring consistencies specified for CSM. The present study showed that these correlations may not be valid for cooked high-lysine corn. Some evidence for a difference can be noted in Table IV where samples acceptable for CSM by using the water-absorption index values are not acceptable by the Bostwick Consistometer measurements. Regardless of which consistency measurement is selected, it can be seen that slight peroxidase activity persists in all samples within the range of CSM acceptability. Nevertheless, only about 2% of the activity remains in most of these samples.

The dry-milling process used in this study was not effective in peroxidase inactivation. Extruder- or roll-cooking was effective. Properly treated corn used in CSM is not likely to cause serious problems originating from enzymatic activity, assuming that peroxidase is the ultimate indicator. Some of the cooked high-lysine corn flour had slight residual activity.

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