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Air Classification of Corn Grits. I. Softening Grits with Enzymes and Chemicals

J. SPANHEIMER, J. E. FREEMAN, R. E. HEADY, and V. E. HEADLEY, Biochemical Research Department, CPC International, Argo, Illinois 60501

ABSTRACT

Capacity of five chemicals and eight different commercial protease preparations to soften corn grits at 30% moisture was determined. Moist grits with added chemical or enzyme were held 24 hr. at 120°F., then dried overnight at 50°C. Treated samples were slurry-milled and wet-screened on nylon bolting cloth. The proportion of insoluble grit dry-substance passing a 36 Nitex cloth was the primary criterion of treatment effectiveness. Effects of moisture level and time and temperature of incubation were determined for selected enzymes. The only chemical investigated that softened grits significantly was sulfur dioxide. All proteases solubilized a portion of the endosperm protein and weakened grit structure. Osborne fractionation indicated that the matrix protein glutelin was the primary substrate for protease enzymes, whereas zein was not measurably affected.

Corn flour and grits have been fractionated by fine grinding and air classification (1,2), but variation in composition of fractions has not been pronounced, particularly with grits. Limited studies of ways to improve air classification have provided discouraging results.

A recently described experimental procedure was reported to have some benefit in preliminary tests (3). Endosperm material was ground to pass 60 mesh (U.S.), hydrated with an isotonic buffer at pH 7.2 by 24-hr. stirring at 4°C. in a large excess of buffer solution (1 g. grit per 10 ml. buffer), and freeze-dried. By starting with a very low protein (5.5%) flour from dent corn, a yield of more than 50% of a fraction containing 2.7% protein was obtained by repeated grinding and air classification of selected fractions. Percentage protein shift, as computed by Gracza's protein-shift-index formula (4), was approximately twice that achieved with untreated flour similarly processed. Even with the buffer treatment and with extensive grinding and air classification, however, the percentage protein shift with the corn flour was no greater than that attainable with soft wheat flours without grinding and with no pretreatment (4).

A more practical pretreatment for air classification of grits or flour recently was patented (5). Moisture content is adjusted to 15 to 28% with a dilute (0.1 to 1.0%) sulfur dioxide (SO_2) solution, and the moistened endosperm material is held 4 to 18 hr. at room temperature. Preferably, grits should be ground to a very small particle size (50% smaller than 18 μ) prior to treatment. Air-classification data for corn-derived products were not given, but the improvement in protein shift achieved with sorghum flour was comparable to that reported for buffer-treated corn flour (3).

Treatment of a low-protein wheat-flour fraction for 18 hr. at 140°F. with a proteolytic enzyme added at a level of 1% of the flour and dispersed in enough 0.2M phosphate buffer at pH 7 to raise flour moisture content to 18%, failed to improve air-classification results (6). This approach, however, appeared to us to merit further consideration. Advantages to be gained from proteolytic action might be especially great with corn grits, since protein is principally responsible for their extreme hardness and resistance to grinding.

MATERIALS AND METHODS

Corn Grits Used

Dry-milled brewer's grits from commercially processed yellow dent corn were used in this study. U.S. standard sieve approximate size-weight relationships for the grits were 32% larger than 10 mesh (2,000 μ), 66% smaller than 10 mesh but larger than 16 mesh (1,168 μ), and 2% smaller than 16 mesh. The grits contained 8.5% moisture and analyzed 9.5% protein, dry basis (d.b.).

Enzymes and Chemicals Evaluated

Major emphasis was given to the evaluation of commercially available protease preparations. Some known properties of enzymes included in this study are given in Table I. For comparison, grits also were treated with SO_2 according to Weiss (5). Because of their known or presumed ability to react with protein, hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), sodium sulfite (Na_2SO_3)-NaOH in combination, and hydrochloric acid (HCl) also were included for limited evaluation. Another reason for including HCl and NaOH was to provide data needed to separate enzymatic and pH influences when evaluating pepsin (pH adjusted with HCl) and an alkaline bacterial protease (pH adjusted with NaOH).

Techniques Used to Evaluate Treatment Effectiveness

A procedure was devised for quickly measuring the enzymatic or chemical effects on a small quantity of grits. One-hundred-gram samples were placed in 8-oz. bottles. Enzymes or chemicals were dissolved in water and the solution was transferred to the bottle containing the grits. Except when variable moisture levels were studied, the amount of water added was calculated to give a moisture content of 30%.

Bottles were capped immediately after addition of the reagent solution to the grits and transferred to a pair of revolving rubber rolls to assure uniform distribution of moisture and reagent. When unabsorbed water was no longer apparent, samples were placed in a constant-temperature environment (air-oven) and held for 20 hr. Except when temperature effects were being studied, the

TABLE I. SOURCE AND SOME PROPERTIES OF ENZYMES USED TO TREAT CORN GRITS

General Source	Specific Source	pH Range[a]	Temperature Range,[a] °C.
Bacterial	Bacillus	5–10	50
Animal	Hog stomach mucosa (pepsin)	1.5–3.0	30–50
Animal	Sheep pancreas	6.0–8.5	30–55
Fungal	Aspergillus oryzae	4.0–7.5	30–50
Actinomycetes	Streptomyces griseus	7.0	50
Plant	Pineapple plant (bromelain)	5.5–7.5	30–50
Plant	Latex of fig tree (ficin)	5.5–7.5	30–50
Plant	Fruit of Carica papaya (papain)	6.0–8.0	30–75

[a]These are generally recommended ranges provided by the manufacturer from which each enzyme was obtained. Optimum conditions vary for different substrates; the best temperature and pH range for corn-grit substrate is not known.

temperature was 50°C. At the end of the 20-hr. incubation period, samples were removed from the containers and dried overnight at 50°C. This was done so that all samples, regardless of incubation-moisture levels, could be processed at a common equilibrium-moisture level, thereby permitting direct comparison of all results. Susceptibility of grits to particle-size reduction and amount of soluble protein extracted from grits during milling were primary criteria of treatment effectiveness.

Relative softness of samples was determined by the prime-starch milling procedure used to evaluate millability of steeped grain (7). The entire sample was milled in 250 ml. of water in a Waring Blendor with dulled blades, operated for 1 min. at 85 v. After milling, samples were screened and washed successively over 53 Nitex and 36 Nitex bolting cloths on a reciprocal shaker.

Material passing the 36 Nitex cloth was filtered on a Buchner funnel with Whatman No. 1 paper. The filter cakes consisted of insoluble particles in the general size range of starch granules. Microscopic examination revealed a mixture of free starch granules, free protein particles, and associated starch granules and protein particles.

The filtrate contained the soluble carbohydrate and soluble protein. Any increase in nonprotein soluble dry substance resulting from enzyme treatment was assumed to result from starch hydrolysis.

Protein Solubility Classification

Cereal proteins commonly are classified on the basis of solubility in a series of

four solvents, first employed by Osborne (8). Successive extraction with water, saline, alcohol, and alkaline solutions removes albumins, globulins, prolamines and glutelins. To determine enzyme specificity for type of endosperm protein, we did Osborne solubility classifications of protein in grits both before and after treatment with proteases.

Samples were defatted prior to protein solubility studies. Two 7.5-g. aliquots of each grit sample were defatted separately, combined, and blended. Defatting was accomplished by placing sample aliquots in 20 ml. of hexane and milling 5 min. in a Spex Mixer/Mill, Model 8000 (ball mill). The milled product was filtered on Whatman No. 1 paper and washed with hexane. The washed filter cake was returned to the Spex Mixer/Mill and the entire procedure repeated twice (total of three milling-extractions).

The procedure of Schneider et al. (9) was used to fractionate protein in defatted samples. The only deviation was sample size. Schneider and associates used 4-g. samples of endosperm material; we used 5-g. samples.

The volume of solubles from each extraction was measured and protein estimated by the Kjeldahl procedure, assuming all nitrogen to be in protein and using the conversion factor of 6.25. Protein contents of initial defatted grit samples and the residue after alkali extraction also were determined by Kjeldahl.

RESULTS AND DISCUSSION

Effects of Different Enzymes under Standard Conditions

Each of the enzymes listed in Table I was applied to grits at a level of 0.5, 1, and 2 mg. protease preparation per g. grits, d.b. Bromelain was applied also, at a level of 10 mg. per g. in one case. Control samples of grits were tempered without the addition of enzyme, dried, and processed in parallel with enzyme-treated grits.

Since enzymes were not applied on a standardized-activity basis, a direct comparison of prime-starch yields or percentage of protein solubilized with a given level of the different enzymes is somewhat meaningless. By plotting prime-starch yields against soluble protein, however, the confounding effects of nonuniformity of enzyme concentration were eliminated and relative effects of the different enzymes were accurately compared. Relationships between prime-starch yields and percentage of total endosperm protein solubilized with each enzyme are shown in Fig. 1. Results obtained with control samples processed in comparison with each enzyme are not shown in the figure, but prime-starch yields without enzyme treatment ranged from about 18 to approximately 22%, with an average of about 2% of the nitrogen being soluble in water. All enzyme treatments resulted in increased prime-starch yields and increased protein solubilization. Prime-starch yield was directly related to percentage of endosperm protein solubilized with each enzyme. Furthermore, the relationship between prime-starch yield and protein solubilized differed only slightly for most of the enzymes, indicating a similar effect on the protein. The higher yield of soluble protein at any prime-starch yield achieved with pepsin apparently was a function of pH. About 5% of the protein was solubilized by HCl alone at the pH used for pepsin with no increase in prime-starch yield (see Table VI).

Three of the enzymes (alkaline bacterial protease, sheep pancreas protease, and fungal protease) apparently had starch-hydrolyzing capabilities. These were the

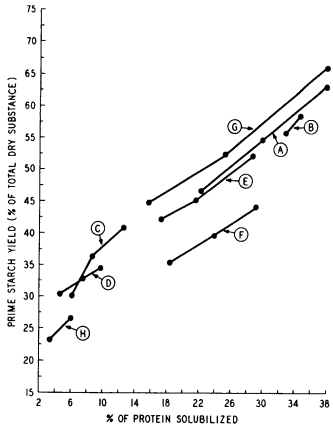

Fig. 1. Relationship between prime-starch yield and percentage of corn endosperm protein solubilized by protease enzymes applied to grits at levels of 0.5, 1 and 2 mg. protease preparation per g. grits, d.b. A, Bromelain; B, ficin; C, fungal protease; D, maxatase (pH 9.0); E, papain; F, pepsin (pH 2.5); G, pronase; and H, sheep pancreas protease.

only enzymes for which nonprotein solubles increased. In each case, an estimated 4 to 5% of the granular starch was solubilized at maximum enzyme dosage. Of all the enzymes investigated, these three produced the smallest amount of water-soluble protein and gave lowest prime-starch yields at any given addition level (weight basis). Purity was not determined for any of the enzymes, but the measurable starch-hydrolyzing ability and relatively low protein-hydrolyzing ability of these enzymes may indicate low purity and not low intrinsic proteolytic activity. Partial starch hydrolysis did not seem to be of any particular benefit in promoting particle-size reduction, as the relationship between soluble protein and prime-starch yield was independent of starch hydrolysis. Since starch hydrolysis had no beneficial effect and would reduce both quantity and quality of starch potentially recoverable, enzymes with this property should be avoided in treatment of grits for starch recovery.

Weakening of the protein matrix appears to be the essential requirement for facilitating grit disintegration and starch release. Zein, the prolamine of corn, is the predominant protein type in corn endosperm. Importance of zein in determining structural strength of the endosperm may be minimal, however, since it exists primarily as small granules embedded in the protein matrix (10). Glutelin, the second most abundant type of protein in corn endosperm, is probably the principal constituent of the matrix in which starch granules are embedded and almost certainly would have to be affected by proteases to achieve the observed beneficial effect.

Proteins Solubilized by Enzymes

The prime-starch milling test showed that almost 40% of the total protein was water-soluble after some enzyme treatments, as compared to only about 2% water-soluble protein from untreated grits. To determine if any particular class of

TABLE II. SOLUBILITY OF CORN ENDOSPERM PROTEIN BEFORE AND AFTER TREATMENT WITH FUNGAL PROTEASE OR BROMELAIN

Fraction	Untreated Grits (Control) % of Total[a]	Fungal Protease-Treated Grits % of Total[a]	Bromelain-Treated Grits % of Total[a]
Water-soluble (albumins)	3.8	8.7	34.1
Salt-soluble (globulins)	3.3	3.7	4.8
Alcohol-soluble (prolamines)	43.4	43.9	41.1
Alkali-soluble (glutelin)	29.1	32.2	16.2
Insoluble	20.3	11.5	3.8

[a]Total based on sum of fractions (sum of fractions was equivalent to 91.6 to 98.6% of total protein in grits before treatment).

proteins was preferentially hydrolyzed by the protease enzymes, two different enzymes, bromelain and fungal protease, were evaluated. Each was applied to grits at a level of 2 mg. enzyme per g. grits. The two samples with enzyme and a control sample (no enzyme) were held 20 hr. at 50°C. and 30% moisture. All three samples were dried overnight in a forced-air oven at 50°C.

Proportions of the various classes of protein in untreated grits (Table II) were very similar to the average values reported by Schneider et al. (9) for corn endosperm from a number of sources. The two protease enzymes had little if any measurable effect on the alcohol-soluble protein, but had a pronounced effect on the alkali-soluble protein and insoluble protein after alkali extraction. The proportion of the total protein soluble in alkali was increased by treatment with fungal protease, but decreased with bromelain. Protein hydrolysis was more extensive with bromelain than with fungal protease, and much of both the alkali-soluble and insoluble protein was converted to water-soluble forms.

With the limited data we have, a hydrolysis sequence cannot be established. A more detailed study involving various enzyme concentrations and reaction times, however, could help to clarify the relationship between the various solubility classes of corn endosperm protein.

Effects of Moisture Content on Enzyme Activity

Moisture content of grits was varied at 5% increments from 15 to 30% with fungal protease and from 30 to 40% with bromelain. At each moisture level, enzyme concentrations of 0, 0.5, 1, and 2 mg. enzyme per g. grits were used. Samples were incubated 20 hr. at 50°C. In addition, each enzyme was applied at

the highest level (2 mg. per g. grits) in an excess of water (500 ml. per 200-g. grits) and held for 20 hr. at 50°C. Results are tabulated in Tables III and IV.

No measurable effect of fungal protease was detected at the lowest moisture level (15%), even though various types of enzymatic reactions have been observed at moisture levels considerably lower than this (11). If the enzyme and substrate had been held in contact for a longer period of time, some effect might have been observed, since most of the reported enzymatic reactions at very low moisture levels were measured after several days.

Failure to see any difference between the control and enzyme-treated grits at 15% moisture is evidence that all observed activity at higher incubation-moisture levels occurred during the extended incubation periods and not during the short time that the grits were in slurry for milling and sizing.

A small effect was observed with fungal protease at 20% moisture, a level just above that at which no benefit was obtained from protease treatment of wheat flour (6). Enzyme action increased rapidly as moisture was raised to 30%. Providing an excess of moisture did not improve prime-starch yields, but did increase protein

TABLE III. EFFECTIVENESS OF FUNGAL PROTEASE (A. ORYZAE) IN SOLUBILIZING PROTEIN IN CORN GRITS AND PROMOTING INCREASED PRIME-STARCH YIELDS AT VARIOUS MOISTURE LEVELS[a]

Moisture %	Enzyme Dosage mg./g. grits	% of Total Protein Solubilized	% of Total Grit Dry Substance Recovered in Prime Starch
15	0	1.4	16.9
	0.5	1.9	16.6
	1	1.8	16.2
	2	2.3	16.7
20	0	2.0	16.3
	0.5	2.3	20.0
	1	2.5	19.4
	2	4.2	22.0
25	0	1.8	15.9
	0.5	5.7	25.2
	1	7.5	29.7
	2	10.4	34.2
30	0	2.0	22.3
	0.5	6.3	30.2
	1	8.9	36.4
	2	11.9	40.9
Excess[b]	0A[c]	5.4	31.4
	0B[c]	4.8	22.4
	2A[c]	18.4	39.7
	2B[c]	18.9	37.1

[a] Values for 30% moisture are averages from duplicate tests; values for other moisture levels are from unreplicated tests.

[b] 200 g. grits per 500 ml. water.

[c] A indicates samples milled without drying; B, samples dried for 16 hr. at 50°C. prior to milling.

TABLE IV. EFFECTIVENESS OF BROMELAIN IN SOLUBILIZING PROTEIN IN CORN GRITS AND PROMOTING INCREASED PRIME-STARCH YIELDS AT VARIOUS MOISTURE LEVELS[a]

Moisture %	Enzyme Dosage mg./g. grits	% of Total Protein Solubilized	% of Total Grit Dry Substance Recovered in Prime Starch
30	0	2.0	20.9
	0.5	21.7	49.2
	1	25.6	51.0
	2	30.1	55.3
	10	38.3	63.1
35	0	2.5	21.9
	0.5	...	57.4
	1	32.1	60.7
	2	38.3	65.7
40	0	2.6	23.3
	0.5	30.1	56.5
	1	33.7	62.1
	2	40.6	64.6
Excess[b]	0A[c]	5.4	31.4
	0B[c]	4.8	22.4
	2A[c]	30.8	52.1
	2B[c]	30.2	54.6

[a] Values for 30% moisture are averages from duplicate tests; values for other moisture levels are from unreplicated tests.

[b] 200 g. grits per 500 ml. water.

[c] A indicates sample milled without drying; B, sample dried for 16 hr. at 50°C. prior to milling.

solubilization. Also, the quantity of water-soluble protein extracted from the control was two to three times greater at the higher moisture levels than at lower moisture levels, and about 50% greater than that obtained by exhaustive extraction of unsteeped grits (Table II). Apparently, a small amount of protein that initially was insoluble in water became soluble during the 20-hr. steeping. This very likely is a result of indigenous protease enzymes. The similar quantities of soluble protein recovered from samples milled before and after drying overnight at 50°C. indicate that drying under these relatively mild conditions did not irreversibly insolubilize any of the protein solubilized by the enzyme treatment.

Bromelain was more effective at 35% moisture than at 30%. In the presence of excess moisture, less protein was solubilized than when grit moisture was 35%. This apparently was a function of effective enzyme concentration. With the temper treatment, all the enzyme was on or inside the grit and in close proximity to the protein substrate. With the large excess of moisture, some of the enzyme may never have come into contact with the protein. Relative stability of the enzyme in the two systems also may be a factor.

Effect of Incubation Time on Enzyme Activity

Incubation was varied from 4 to 64 hr. with bromelain at 30% moisture. Enzyme action, as measured by both soluble protein and prime-starch yield, was

greatest with longest holding time (Table V). Samples held for the maximum time, 64 hr., were beginning to show signs of incipient spoilage, so a longer holding time was not feasible.

Effects of Selected Chemicals and Some Interactions with Enzymes

A 30% solution of H_2O_2 added at a 30% moisture-equivalent level increased soluble protein to approximately fourfold that of the untreated grits and caused grits to become "sticky", but did not improve prime-starch yield (Table VI). Both NaOH at pH 9 and the combination of Na_2SO_3 (added at a level equivalent to 1% of the protein in the grits) and NaOH (added at a level required to adjust grit pH to 9.0) gave slight, but practically unimportant, increases in both soluble protein and prime-starch yield. (NaOH also was used to adjust the pH of grits for testing an alkaline bacterial protease.) HCl at pH 2.5 increased soluble protein, but did not improve prime-starch yields. (This chemical was used to adjust grit pH for evaluating pepsin.)

The unique ability of SO_2 to soften and partially solubilize corn endosperm protein was apparent even at the very low moisture level utilized, confirming the results of Weiss (5). The SO_2 was more effective at pH 4 than at pH 2, as judged by either protein solubilization or prime-starch yield. Increasing moisture level to 35% and incubation time to 48 hr. resulted in a slight increase in protein solubilization and quite significant increases in prime-starch yield. Under best conditions (pH 4, 48-hr. incubation, 35% moisture), prime-starch yield was approximately twice that obtained with untreated grits.

TABLE V. RELATIONSHIP BETWEEN INCUBATION PERIOD AND ACTIVITY OF BROMELAIN ON MOIST CORN GRITS AS MEASURED BY PROTEIN SOLUBILITY AND PRIME-STARCH YIELD

Incubation Period hr.	Enzyme Dosage mg./g. Grits	% of Total Protein Solubilized	% of Total Grit Dry Substance Recovered in Prime Starch
4	0	2.5	18.5
	0.5	15.9	41.9
	1	18.6	44.6
	2	22.4	49.0
8	0	2.7	18.1
	0.5	15.5	45.8
	1	18.8	50.1
	2	21.9	53.0
16	0	2.6	17.0
	0.5	19.9	49.4
	1	24.6	50.9
	2	31.2	55.4
32	0	2.1	16.8
	0.5	23.6	52.2
	1	28.1	54.5
	2	29.8	61.1
64	0	3.0	20.1
	0.5	30.8	57.5
	1	36.5	60.8
	2	40.1	67.2

TABLE VI. CAPACITY OF SELECTED CHEMICALS TO SOLUBILIZE PROTEIN AND PROMOTE INCREASED PRIME-STARCH YIELDS WITH CORN GRITS AT LOW MOISTURE LEVELS[a]

Chemical Additive	pH	Protein Solubilized, % of Total	% of Total Grit Dry Substance Recovered in Prime Starch
	20-hr. incubation at 120°F. and 30% moisture		
None	ca. 5.5	2.0 ±0.4[b]	19.5 ±5.1[b]
H_2O_2[c]	?	8.4	23.3
NaOH	9.0	2.5	25.4
Na_2SO_3-NaOH[d]	?	3.2	26.8
HCl	2.5	7.9	19.2
SO_2[e]	2.3	6.6	31.6
	4.0	11.1	34.6
	48-hr. incubation at 120°F. and 35% moisture		
SO_2	2.3	7.5	38.2
	4.0	12.5	40.9

[a]Values for HCl and SO_2 are averages from duplicate tests; all other values are from unreplicated tests.

[b]Mean values and standard deviations for control samples were calculated from eight separate tests.

[c]A 30% H_2O_2 solution was substituted for water on an equal-volume basis for adjusting grits to a 30% moisture-equivalent level.

[d]Na_2SO_3 was added at a level of 1% of the protein content of grits; NaOH was added at a level required to adjust grit pH to 9.0.

[e]Approximately 0.6 g. SO_2 per 100 g. grits was required to reduce pH to 2.3, and approximately 0.1 g. SO_2 per 100 g. grits was required to reduce pH to 4.0.

TABLE VII. EFFECTS OF SO_2 AND BROMELAIN, ALONE AND IN COMBINATION, ON PRIME-STARCH YIELD AND PROTEIN SOLUBILIZED WITH CORN GRITS AT LOW MOISTURE LEVELS

	Bromelain			SO_2	Bromelain + SO_2		
	0.5[a]	1.0	2.0	pH 4	0.5	1.0	2.0
Protein solubilized, % of total	22.2	24.8	30.0	10.1	21.9	25.8	30.0
Prime starch yield, % of total grit dry substance	46.7	49.4	54.8	39.6	55.9	59.3	60.8

[a]Enzyme dosage expressed as mg. enzyme per g. grits, d.b.

Effects of SO_2 on specific classes of protein were not investigated in this study. Results of an earlier study, however, indicated that glutelin was solubilized by SO_2 used in steeping whole corn for wet-milling, whereas zein apparently was not affected (12).

A combination of bromelain and SO_2 (pH 4) resulted in greater prime-starch yields than either treatment alone without any increased protein solubilization (Table VII). Since SO_2 altered pH, it is not possible to say if the greater effect achieved by the combined treatment was owing to enhanced activity of the enzyme at the lower pH or to the additive effects of the separate actions of chemical and

enzyme. The latter alternative, however, appears most likely. Protein which might have been soluble at the natural pH of the grain possibly was insoluble at pH 4.0, thus accounting for the increased prime-starch yield achieved without a concomitant increase in soluble protein when SO_2 was included.

CONCLUSIONS

All protease enzymes tested solubilized endosperm protein and softened the grits. Each enzyme appeared to have approximately the same type of effect, as judged by the ratio of water-soluble protein to prime-starch yield. This was further substantiated with bromelain and fungal protease by showing that each enzyme affected the alkali-soluble protein (glutelin) while not measurably altering solubility of the alcohol-soluble protein (zein). Therefore, the eventual selection of an enzyme for commercial usage could be made on a cost/performance basis.

The slurry milling test used to evaluate the various grit treatments studied provides an index of relative softness. The real value of such treatments, however, can be measured only by actual dry-grinding and air-classification tests.

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