REFRACTIVE INDEX: A RAPID METHOD FOR DETERMINATION OF STARCH AVAILABILITY IN GRAINS

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Summary

Steam-flaked corn samples were used in a series of experiments to determine if refractive index could be used as a rapid, inexpensive method to predict starch availability. Results were best when samples were incubated for 15 min with 500 to 600 active units of enzyme/gm of grain prior to measuring on a hand-held refractometer. Correlations to starch availability determined from gas production by a commercial lab were R=.64 for whole flakes and R=.79 when samples were ground. Samples of corn flaked to different densities produced estimates of solubility similar to an in situ dry matter disappearance assay (R^2 = .84, P < .01). Furthermore, refractive index yielded estimates of starch solubility that were well related to a commercial lab's measures of glucose release for samples of flaked corn that were stored for 0 to 48 hours subsequent to flaking.

(Key Words: Starch Availability, Steam-Flaked Corn, Refractive Index.)

Introduction

When grain is steam flaked, the starch matrix is disrupted and ultimately gelatinized, increasing its susceptibility to degradation by digestive enzymes and improving energy value. Current starch availability methods take too much time to be useful for making mill adjustments. Our objective was to develop a rapid, reliable, inexpensive procedure that could be used by mill operators to assess quality of flaked grains. If successful, the method could be used to determine the effects of milling and grain storage procedures on gelatinization and (or) extent of retrogradation of grains.

Experimental Procedures

Experiment 1 – Effect of Amyloglucosidase Concentration

A dose titration was performed to determine optimum concentration of amyloglucosidase required for measuring starch availability. Steam-flaked corn (26 lb/bu) was prepared using an 18-in × 24-in Ferrel-Ross flaker equipped with a 96-cubic ft steam cabinet. Grain was conditioned for approximately 45 min before being flaked. Samples were collected, packed in dry ice, transferred to the laboratory, and frozen. Grain was ground to pass through a 1-mm screen. Samples then were divided into 10-gm subsamples that were placed into Erlenmeyer flasks. Buffered-enzyme solutions (40 ml) containing amyloglucosidase (A3407, Sigma Chemical Company; 6,100 enzyme units per ml) were added at levels of 30, 60, 153, 305, or 610 active enzyme units/gm of grain. Samples were incubated for 15 min in a 130° F water bath and filtered through Whatman 541 filter paper. Several drops of the filtrate were placed on the prism of a hand-held refractometer, and the percentage of solubles in each sample was read from a Brix scale.

Experiment 2 – Effects of Enzyme Concentrations and Incubation Time

Samples from Exp.1 were used to further evaluate different enzyme concentrations and incubation times. Ground subsamples were incubated at 130°F for 10, 15, 20, or 30 min in 40 ml of buffered amyloglucosidase enzyme solution containing 153, 305, 610, or 1220 active enzyme units/gm of grain. Per

cent solubles was determined for each filtrate with a hand-held refractometer.

Experiment 3 – Refractive Index vs. Other Methods of Estimating Starch Availability

A series of grain samples was submitted to a commercial laboratory to obtain measures of in vitro gas production, total starch, and starch availability (by glucose release). Correlations were used to compare these procedures to the enzyme/refractive index method for both ground and whole-flaked grain samples.

Experiment 4 – Refractive Index vs. *in Situ* Ruminal Disappearance of Grains

Corn was conditioned for approximately 45 min and then flaked to densities of 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, and 31 lb bu. After collection, samples were frozen immediately in liquid nitrogen, transferred to the laboratory, and incubated with buffered enzyme (610 active units/gm of grain) for 15 minutes at 130°F. Ruminal dry matter disappearance was measured by placing subsamples of each flake density in Dacron bags and suspending them in the rumen for 8 hrs. Samples were removed from the rumen, rinsed, dried, and weighed to determine in situ dry matter disappearance.

Experiment 5 – Solubility by Refractive Index vs. Starch Availability by Glucose Release

Approximately 6 tons of corn were steam conditioned for 45 min, flaked to a density of 26 lb/bu, and conveyed onto a concrete slab for storage for a period up to 48 hrs. Samples of grain were placed in permeable nylon bags and positioned in the flake pile. The interior of the pile maintained a temperature of 120°F or greater throughout the 48-hr storage period. Sample bags were removed from the core of the pile at 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, and 48 hrs after flaking and immediately placed into a freezer. Unground flake samples were incubated in enzyme solution as in Experiment 4. Percent available starch also was measured by a glucose release method.

Results and Discussion

Experiments 1 and 2 indicated that optimal results were achieved when enzyme was added at approximately 600 units per gm of grain (Figure 1). An incubation time of 15 min was adequate to produce concentrations of solubles that could be measured easily on a low-range refractometer (Figure 2). Correlations among various methods of estimating starch availability are shown in Table 1. Correlations between gas production and glucose release were very high. However, comparison of the refractive index method to the gas production technique gave a lower correlation of R=.64. Proteins and other solubles likely are measured in the refractive index procedure, whereas other procedures are more specific to carbohydrates. Grinding the flakes through a 1-mm screen improved the correlation with gas production. Flake density was correlated negatively to starch availability. In Exp. 4, the refractive index method yielded results similar to an in situ procedure for corn of different flake densities (Figure 3). The proportion of grain that was digested in situ decreased with increasing flake density. Exp. 5 examined length of storage vs. starch availability. The refractive index method followed a trend similar to the gas production procedure in determining starch availability (Figure 4). Available starch decreased in the early hours of storage and was lowest at 12-30 hours. From 30 to 48 hours, starch availability increased as measured by both procedures, but to a greater degree for the gas production procedure.

Applications

Estimating starch availability in grains by enzymatic starch hydrolyses/refractive index is inexpensive and rapid. We suggest that grain samples be incubated for 15 min using 500 to 600 enzyme units/gm of grain. After filtration, the resulting solubles can be measured easily using a simple hand-held refractometer under direct lighting. Starch availability can be assessed without further processing of flaked grain and requires as little as 15 to 20 min.

Table 1. Coefficients of Correlation (R) for Availability of Starch Measured by Various Methods[1] (Exp. 3)

Item	Gas Production	Glucose Release	Refractive Index, w[1]	Refractive Index, g[2]	Flake Density
Gas production	1.0	.97	.64	.79	-.64
Glucose release		1.0	.59	.80	-.65
Refractive index, w[2]			1.0	.75	-.72
Refractive index, g[2]				1.0	-.76
Flake density					1.0

[1]P<.01 for all correlations.
[2]Measured using whole flakes.
[3]Measured after grinding flakes through a 1-mm screen.

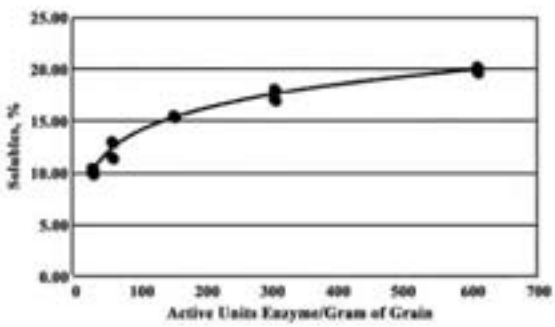

Figure 1. Effect of Enzyme Concentration on Solubles in Steam-Flaked Corn Incubated for 15 Min, as Measured by Refractive Index (Exp. 1).

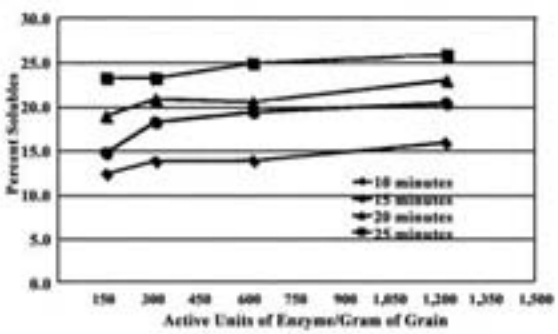

Figure 2. Effects of Incubation Time and Enzyme Concentration on Steam-Flaked Corn Solubility as Measured by Refractive Index (Exp. 2).

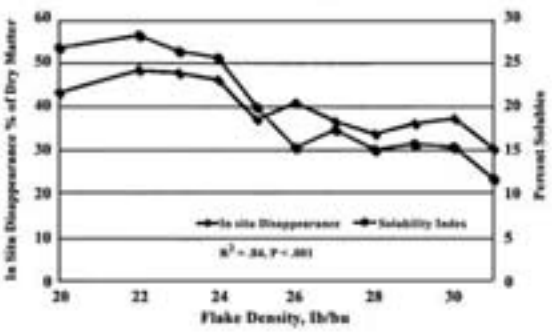

Figure 3. Flake Density Effects on Percent in Situ Dry Matter Disappearance and Percent Solubles Measured by Refractive Index (Exp. 4).

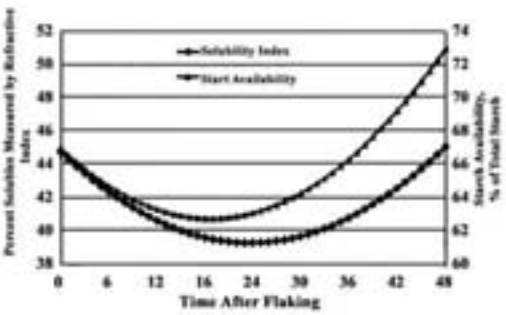

Figure 4. Comparison of Starch Availability Measured by Glucose Release and Solubility as Measured by Refractive Index for Steam-Flaked Corn Piled and Stored for Various Lengths of Time (Exp. 5).