



Factors Affecting Starch Analysis of Feeds¹

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Most starch analyses are enzymatic, relying on the specificity of the enzymes to distinguish starch from other glucose-containing carbohydrates. The steps in a starch assay are generally gelatinization, hydrolysis, and measurement of end products.

Critical elements in an accurate starch analysis are:

1. Complete gelatinization of starch.
2. Specificity and purity of enzymes.
3. Complete hydrolysis of starch to glucose.
4. Measurement of glucose produced from hydrolyzed starch.
5. Minimization of interference.

Gelatinization is the breaking of hydrogen bonds among and within starch molecules that opens the granules to hydration and enzymatic hydrolysis. Before gelatinization, starch, especially unprocessed starch, is partially crystalline. The linear portions of starch molecules are partially aligned and hydrogen bonded to each other in such a fashion that they exclude water and resist enzymatic activity. That crystalline structure must be disrupted for complete enzymatic hydrolysis of the starch to take place in a reasonable amount of time. Gelatinization is typically accomplished with heating (90 – 100°C) in water,

or, alternatively, with use of a base (e.g., potassium hydroxide) followed by neutralization. Incomplete gelatinization can lead to incomplete hydrolysis of starch to glucose.

The enzymes must release glucose by specifically and completely hydrolyzing only the bonds between glucose molecules in starch. Since there are many glucose-containing carbohydrates in plants, specific hydrolysis of the alpha-(1 --> 4) (linear chain) and alpha-(1 --> 6) (branch) linkages in starch is required to make a method specific for starch (Figure 1). Heat-stable alpha - amylase, which can be added during the gelatinization step, and amyloglucosidase, which hydrolyzes starch to glucose, are commonly used. Care must be taken to ensure that a given enzyme is incubated at the correct pH and temperature to optimize its effectiveness. Since starch is estimated as the glucose released by enzymatic hydrolysis, the hydrolysis must be complete, or starch content will be underestimated. Presence of other enzymes such as invertase (digests sucrose), or cellulase (digests cellulose) that release glucose through hydrolysis will inflate the calculated starch value.

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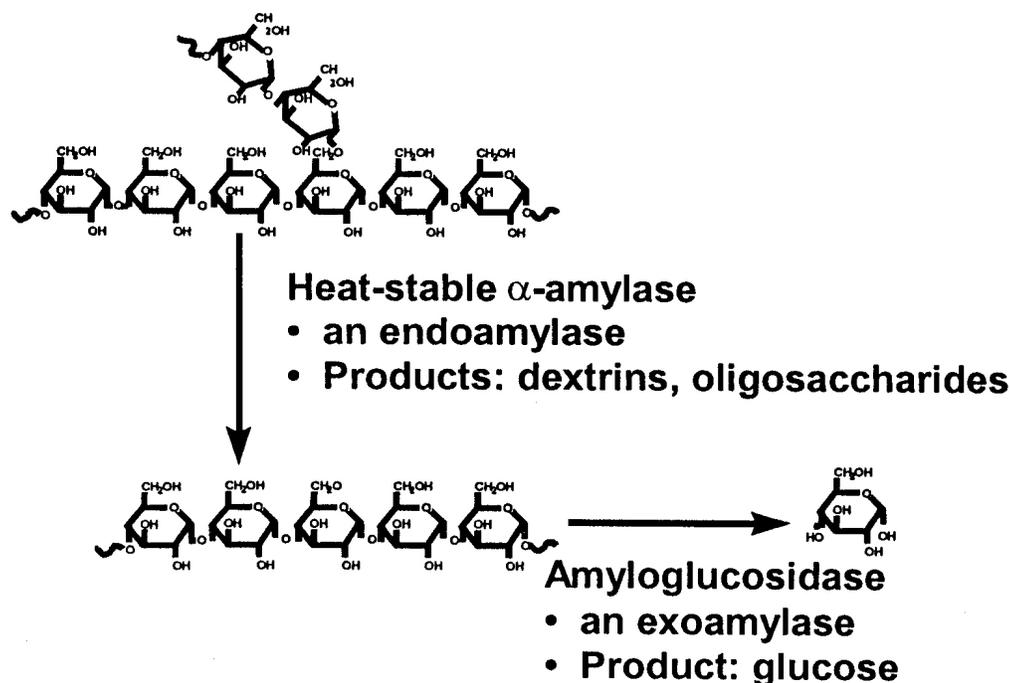


Figure 1. Enzymic action on starch

Measurement of the glucose from starch hydrolysis is the final step for calculation of a starch value. Starch is computed as glucose times 0.9, because the total weight of glucose released is higher than the weight of the starch. One molecule of water (M.W. = 18) is required for every covalent bond hydrolyzed, which adds the weight of water used for hydrolysis to the weight of the starch in the sample. Commonly, glucose is measured with a glucose-specific assay such as the glucose oxidase-peroxidase assay (Karkalas, 1985). Although either purified starch carried through the gelatinization and hydrolysis procedures or glucose may be used as standards for the end product assay, glucose is preferred. Use of starch as the standard relies upon its complete hydrolysis to glucose, and presumes similar recoveries for starch from all sources. Use of glucose as a standard removes the question of percent recovery. Including starch as a reference sample in starch analyses allows assessment of recovery and enzyme efficacy.

An alternative method for measuring glucose resulting from starch hydrolysis is the reducing sugar assay. This second approach carries a greater risk of including monosaccharides not derived from starch. Such sugars may be present in the sample as mono-

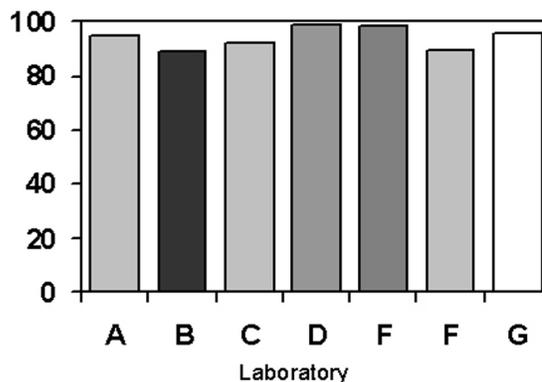


Figure 2. Starch assay of corn starch

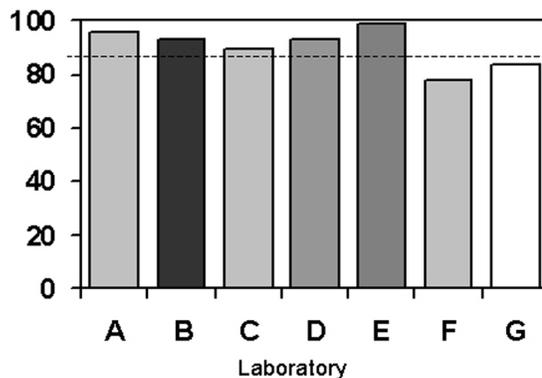


Figure 3. Starch assay of corn starch+glucose

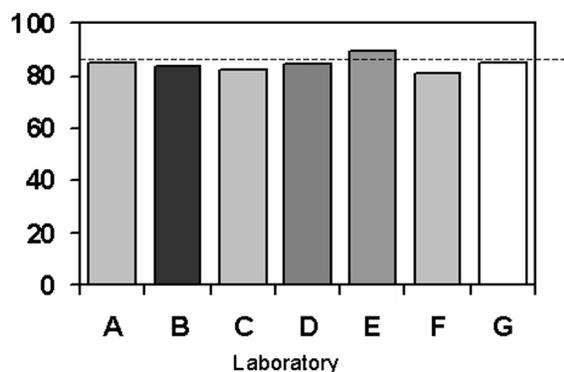


Figure 4. Starch assay of corn starch+fructose

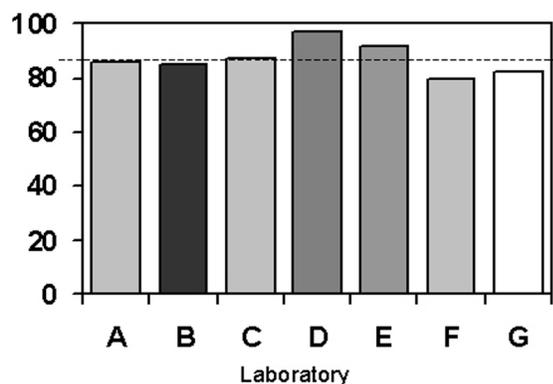


Figure 5. Starch assay of corn starch+cellulose

or disaccharides, or released by hydrolysis of non-starch carbohydrates. Particularly when the enzyme preparations used contain invertase, fructanase, or other contaminating enzymes, simultaneous measurement of hydrolysis products from sucrose, fructans, and other carbohydrates can significantly inflate starch estimates.

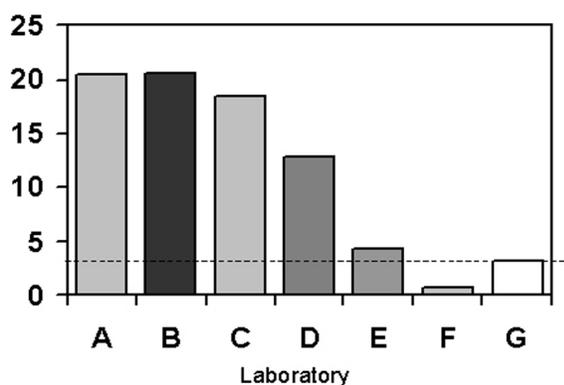


Figure 6. Starch assay of confectioner's sugar

Interfering substances include any substance that increases or decreases the starch estimate. The method of glucose measurement used, either for

glucose or reducing sugars, determines what substances are measured. Some commercial amyloglucosidase preparations contain glucose and should not be used for starch analysis. Non-carbohydrate substances that absorb at the appropriate wavelength in colorimetric analyses can unduly alter starch values. Interference from low molecular weight carbohydrates can be eliminated by pre-extracting them with 80% ethanol:water (v:v) before starch analysis. Alternatively, free glucose times 0.9 (to put it on a starch basis) can be measured and subtracted from the analyzed starch value if there is certainty that the enzymes release glucose only on starch. The effect of glucose, sucrose, and oligosaccharides on starch analysis values can be seen in figures 3, 6, 8, 9, and 11 (Hall et al., 2000). In this comparison of laboratories, starch values from samples such as confectioners sugar, starch+glucose, soybean meal, and citrus pulp were inflated by the inclusion of the nonstarch carbohydrates in the starch value. Samples from the UF lab were extracted with 90% EtOH prior to starch analysis. Another method of accounting for free glucose or reducing sugars, is to measure a sample blank untreated with enzymes. This approach requires that the enzymes used be of sufficient purity so that they do not hydrolyze non-starch carbohydrates to any appreciable extent and thereby add to the free monosaccharide pool. The extent to which one should be concerned about interfering substances will depend upon the type of sample. Mature grain samples and silage samples will likely have little sugar remaining to interfere with starch analysis. Interfering carbohydrates may be an issue in by-product feeds such as bakery waste, almond hulls, and citrus pulp.

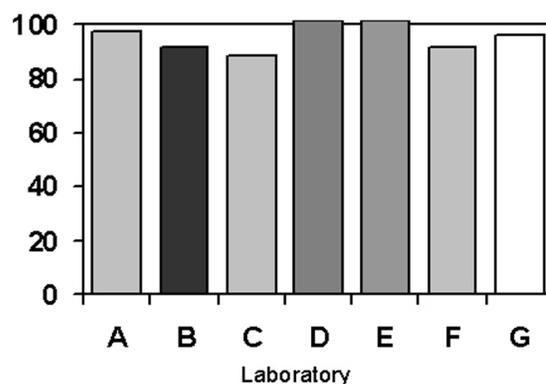


Figure 7. Starch assay of potato starch

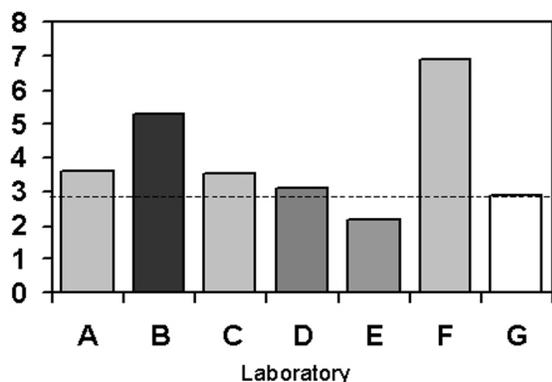


Figure 8. Starch assay of 48% soybean meal

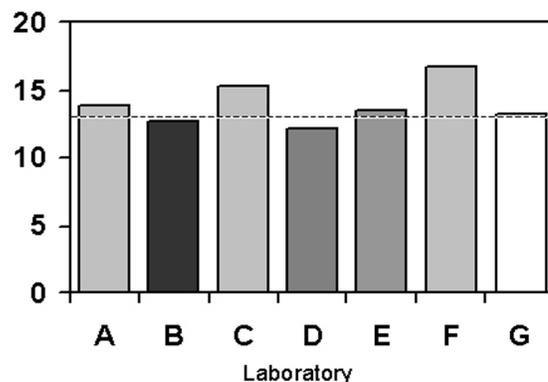


Figure 11. Starch assay of a total mixed ratio containing citrus pulp

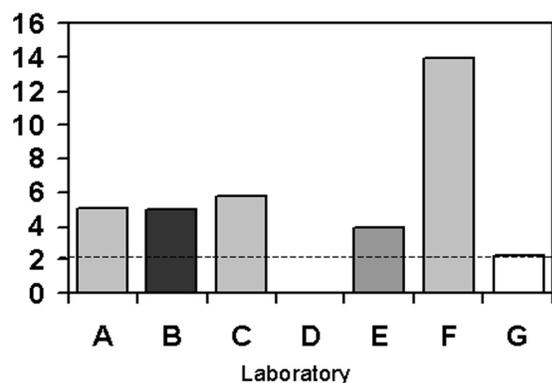


Figure 9. Starch assay of citrus pulp

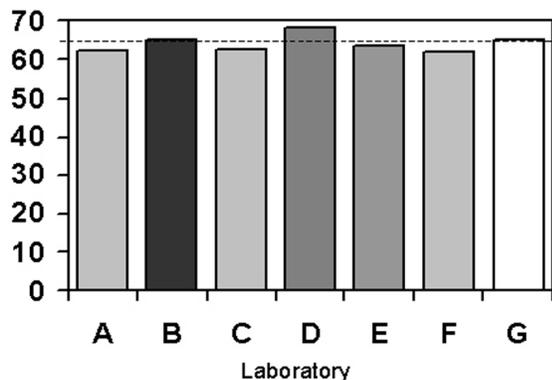


Figure 10. Starch assay of hominy feed

References

Hall, M. B. 1998. Analyzing nonstructural carbohydrates: methods and relevance. Proc. National Forage Testing Association Workshop, Midwest Section AOAC International Meeting, June 8 – 10, 1998, Madison, WI.

Hall, M. B., J.P. Jennings, B.A. Lewis, and J. B. Robertson. 2000. Evaluation of starch analysis methods for feed samples. *Journal of the Science of Food and Agriculture*. 81:17-21.

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