Simpler Method to Compare Starch Hydrolysis Rate and In Vitro Expected Glycemic Index of Flours

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ABSTRACT

In vitro expected glycemic index (eGI) is a reliable tool to predict postprandial blood glucose concentrations. Evaluating foods for eGI is important particularly for diabetes patients who must manage their health condition by consuming products with more slowly digestible carbohydrates. In this study, flour samples were digested with continuous agitation for 3 hours with the help of alpha-amylase enzyme. Digestion was monitored by measuring brix every 20 minutes using a refractometer. Brix was plotted to produce a hydrolysis curve for each flour sample. Measuring brix eliminated the need for glucose oxidase/peroxidase (GOPOD) reagent and spectrophotometer steps required by current methods used to determine eGI. Results indicate significant differences in hydrolysis index (HI) and eGI among flour samples ranging from 44.9 (pinto bean flour) to 87.6 (green split peas). With the exception of split green peas which had a much higher eGI than previously reported, all other values were in close proximity to values obtained using GOPOD.

METHOD

Buffer Preparation

Maleate Buffer (pH 6, 100 mM) was prepared following method described in Megazyme Resistant Starch Kit (Megazyme, UK).

Digestion

Twenty grams of flour sample was collected in a 400 ml beaker and 250 ml of maleate buffer added. The mixture was homogenized for 30 s at 10,000 rpm using a Polytron 2500E homogenizer to remove clumps (Kinematica Inc, NY). Beakers were then placed in a water bath at 60 ºC and mixed continuously with a 20 rpm rotating blade. Exactly 500 µl of Amylex 3T enzyme (DuPont Nutrition and Health, New Century, KS) was added when the internal temperature in the beakers reached 60 ºC. A refractometer was used to measure brix at 20 minutes intervals over 3 hours.

Determination of eGI

The eGI was determined by first calculating the hydrolysis index (HI) and then applying the eGI equation below (Grandfeldt, et al. 1992).

$$HI = \frac{Area\ Under\ Curve\ Representing\ Sample}{Area\ Under\ Curve\ Representing\ White\ Bread}\ x\ 100$$

$$eGI = 8.198 + 0.862(HI)$$

Statistical Analysis

SPSS Software (IBM SPSS Statistics for Windows, Version 24.0) was used to conduct analysis of variance (ANOVA) followed by Tukey's multiple comparison test to determine differences in eGI. Differences were considered to be significant at $p < .05$.

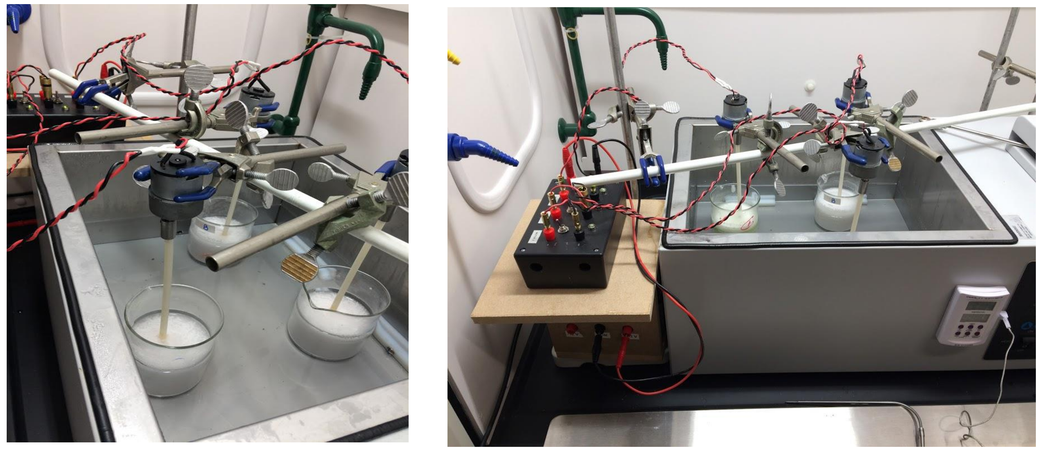

Fig 1. Water bath with rotating agitators used for starch digestion

RESULTS

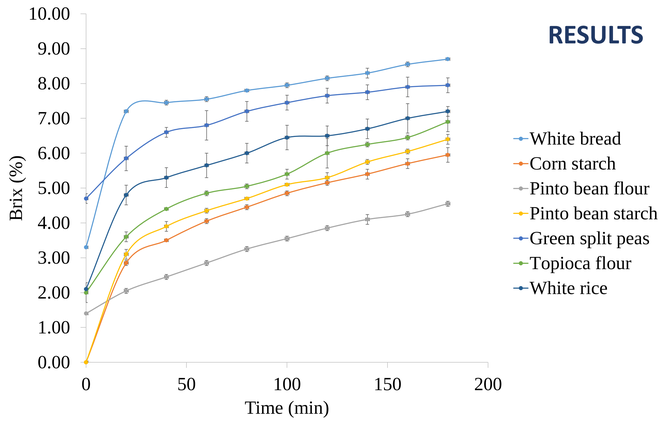

Fig. 2 Hydrolysis Curves

Fig 3. Refractometer

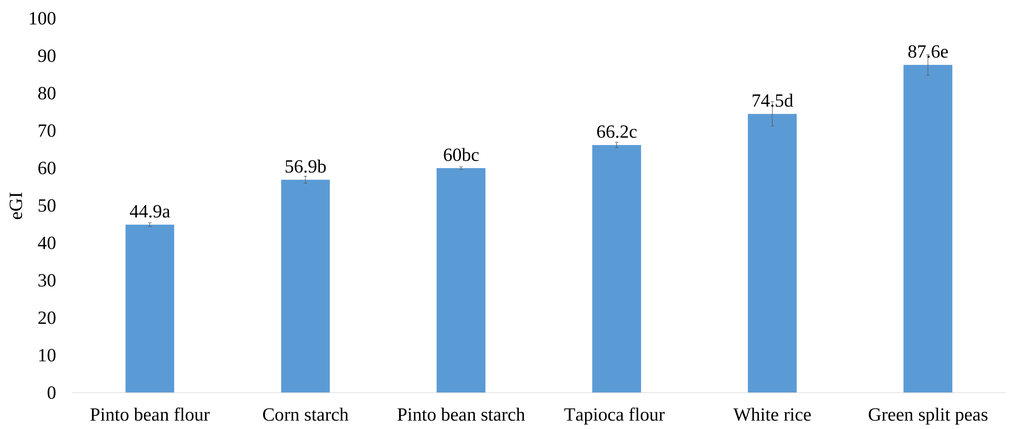

Fig 4. Expected glycemic index (eGI) of flour samples. Different letters indicate significant differences (p<.05)

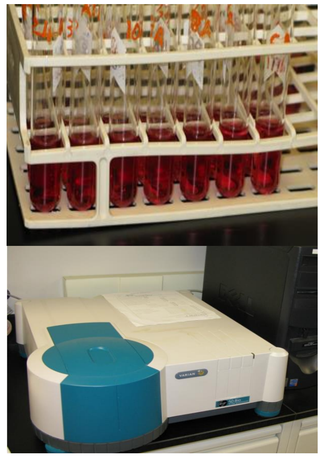

Fig 5. Samples reacted with GOPOD (top) in preparation for spectrophotometer (bottom) measurement. This new method eliminates these time-consuming and costly steps.

DISUSSION

Alpha amylase enzyme digestion of starch produced starch degradation resulting in detectable changes in brix. This allowed preparation of hydrolysis curves (Fig 2) which were used to calculate eGI. Calculated eGI following method by Granfeldt et al. (1992) indicate significant differences among flour samples; with pinto bean flour having the lowest eGI (44.9) and green split pea the highest (87.6). The eGI of flour samples were similar, although generally higher than those that have been previously reported in literature. The exception was green split peas which had a much higher eGI value than reported. Disparities could be due to differences in cultivars used, enzyme activity, flour particle size, and digestion temperature. Nevertheless, the method proved to be a simpler, cheaper and effective method to determine and compare hydrolysis rates of flours.

CONCLUSIONS

- Simple brix determination using a refractometer is an effective method to monitor changes in starch digestion and determining HI and eGI
- This method eliminates the need for expensive steps in current eGI determination protocols including reaction with GOPOD and spectrophotometer measurements. Hence it saves time and money

REFERENCES

1. AACC (2000). Approved methods of the AACC (10th ed.), St. Paul, MN.
2. Granfeldt, Y., Björck, I., Drews, A., & Tovar, J. (1992). An in vitro procedure based on chewing to predict metabolic responses to starch in cereal and legume products. European Journal of Clinical Nutrition, 46, 649–660.