THE DEVELOPMENT OF A RAPID ASSESSMENT METHOD FOR THE PREDICTION OF THE GLYCEMIC INDEX

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Abstract

The glycemic index (GI) is a measurement used to classify foods according to their potential for raising blood glucose levels. The GI of a foodstuff is generally measured by determining the increment in blood glucose concentration after the consumption of a test meal over a set period of time and comparing it with an isoglucosidic control meal (normally white bread or glucose) and expressed as a percentage within a group of individuals (*in vitro*). Rapid analysis methods (*in vivo*) have been evaluated worldwide, and in many cases these values have correlated with the GI values determined by *in vitro* methods. The critic against rapid analysis methods are that the methods do not provide a numerical GI values, although proposed labelling legislation in South Africa recommends that suppliers should only indicate if the product has a high, intermediate or low GI. The purpose of this study was to investigate existing rapid assessment methods for the prediction of GI, and develop such a method for South Africa to be used by food producers in line with the newly proposed national labelling requirements. The preliminary

studies on the developed rapid assessment method indicated good repeatability (CV 0.78%), reproducibility and precision (CV 3.5%), and can accurately predict a foods GI category, being high, intermediate or low GI.

INTRODUCTION

The glycemic index (GI) is a measurement used to classify foods according to their potential for raising blood glucose levels (Whitney & Rolfes, 2002). The GI of a foodstuff is generally measured by determining the increment in blood glucose concentration after the consumption of a test meal over a set period of time and comparing it with an isoglucosidic control meal (normally white bread or glucose) and expressed as a percentage (Goni *et al.,* 1997.)

High glycemic foods are generally percieved as culprits in weight gain and obesity (Ludwig *et al.*, 1999; Ludwig, 2000; Liu *et al,* 2000), and high consumption has been linked to an increased risk of developing type 2 diabetes (Foster-Powell *et al.,* 2002; Rondini & Bernink, 2007), certain cardiovascular diseases and cancer (Liu *et al.,* 2000; Foster-Powell *et al*, 2002). In 2003, 56.2% of the adult population in South Africa was recorded as either overweight or obese (as determined by BMI), with the highest prevalence in the female population (23.3% obesity) (Demographic and Health Survey, 2003). A committee brought together in 1997 by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), endorsed the use of the GI method for classifying carbohydrate rich foods and recommended that GI values along with other food composition data be used in the guiding of healthy food choices (Foster-

Powell *et al.,* 2002). Low GI foods, such as low fat animal products and legumes, have been recommended as part of many weight loss strategies aimed at improving health.

The FAO consultation report on dietary carbohydrates in human nutrition (1998) concluded that glycemic response data should be supplemented with values for local foods and meals, as food variety and cooking could have significant effects on glycemic response. Thus the need for a great variety and amount of GI indications are needed in countries such as South Africa with its diverse cultures and unique traditional foods.

A South African task force, assembled in 2002 by the Directorate of Food Control, has been put into place to standardise the methodology used in South Africa, aimed at paving the way for GI labelling and consumer education. Aspects considered included the methodology used in determining the GI of a food, how to express GI on food labels as well as how to handle the variations in GI of the same food, or variations between individuals consuming the same food (Pieters & Jerling, 2005).

The South African draft labelling regulations (Amendment Foodstuffs, Cosmetics and Disinfectants Act, 54/1972) include recommendations for making GI claims on the label of foods, but to date this legislation has not yet been approved, mostly due to opposition from the food industry. They argue that South Africa as a developing country with limited resources to enforce and monitor labelling, and that no regulation should be stricter than Codex regulations, due to resulting barriers to trade. The proposed legislation states that the GI of a food will be presented as a category claim. The proposed GI label should not indicate a specific numerical

value, but indicate either low (<55), intermediate (55-70) or high GI (>70). GI indication in South Africa will be allowed as either a logo, or within the nutritional information table on the package (Department of Health, 2007).

Currently in South Africa, as in most countries around the world, only *in vivo* methods are used to determine the GI of a food. This method entails that a specific food is ingested by human subjects and the glycemic response of the food is presented as a percentage of a reference food, namely white bread. A specific GI value is then given to the food (Goni *et al.,* 1997). The current *in vivo* methods, which are expensive and time consuming, might not be feasible in delivering on such a task at a country level as required for labeling.

Rapid analysis methods have been developed and evaluated worldwide (Goni *et al.,* 1997; Englyst *et al.,* 2003). The values correlated well with GI values determined by the previously mentioned *in vivo* methods. The critic against rapid analysis methods (*in vitro)* are that the methods do not provide a numerical GI values. Rapid analysis methods only produce an indication of the specific food's glycemic response as high, intermediate or low. With the proposed South African draft regulations (Department of Health. 2007), published for comment in 2008, including the indication of the GI of carbohydrate rich foods should be declared as high, intermediate or low GI on food labels. Thus the need for an i*n vitro* method that comply to this regulation is validated.

The purpose of this study was to investigate an *in vitro* GI method, and once developed, determine its reliability to produce scientifically sound results for describing a foods GI classification as either low, intermediate or high in line with the proposed regulation.

MATERIALS AND METHODS

Hydrolysis Index methodology

The *in vitro* method for evaluating starch digestibility has previously been developed by the Campden and Chorleywood Food Research Association (CCFRA). It involves mechanical disruption and multienzymic digestion based on proteolysis, followed by incubation with pancreatic a-amylase. This method allows the calculation of a hydrolysis index (HI), which is a prediction of the food's GI. The rapid assessment method was adapted in consultation with Dr AJ Alldrick (Campden & Chorleywood Food Research Association, U.K.), Prof AWH Neitz (Biochemistry, University of Pretoria) and the Agricultural Research Council (ARC) analytical laboratory (Irene).

A sample of each food as eaten, containing 2 g of carbohydrate was sliced and grinded in a flask with 20 ml of a 0.1 M potassium phosphate buffer solution buffer solution (pH 6.9) kept at 37 °C was added. After grinding the samples were homogenized with a Altra Turrax at a constant speed, and rinsed with an additional 20ml buffer solution. The pH of the samples was decreased to pH 2.5 with Ortho phosphoric acid, after which 1 ml pepsin enzyme (Sigma-Aldrich) was added. The samples were placed in a 37 °C stirring waterbath for 1 hour to simulate the time that

food would be churned in the human stomach. Each sample was then buffered back to pH 6.8 with KOH, and 2ml alpha Amylase enzyme (Sigma-Aldrich) was added. The whole contents of the flask was then transferred into a dialysis tube. The tube was closed and placed in flasks containing 500 ml buffer solution. The flasks were placed in the stirring waterbath and 40 ml of the buffer solution was extracted every 30 minutes in order to determine the rate of hydrolysis of carbohydrate from the dialysis tube into the buffer solution. The volume of the buffer solution was allowed to decrease during the analysis. In Figure 1 the methodology is summarized.

Reduced sugars were determined using Infra-red (Milkoscan). The Milkoscan works on the principle of an Infrared Spectrophotometer. All the experimental work was performed at the accredited laboratory at the ARC-Irene Analytical Services, Agricultural Research Council: Animal Production Institute.

The values were plotted on a graph and the area under the concentration-over-time curve (AUC) was determined. The Hydrolysis Index (HI) values were calculated as the relation between the AUC of the specific food compared to the AUC of maltose as the reference food. The following equation was followed:

AUC food tested ÷ AUC reference food = Hydrolysis Index of food

In order to determine the relationship between the derived HI values and GI values, the HI values were correlated to known GI values tabulated in the South African Glycemic Index and Glycemic Load Guide (Steenkamp & Delport, 2005), published by the Glycemic Index Foundation of South Africa determined by means of *in vivo* analysis.

Samples and repetitions

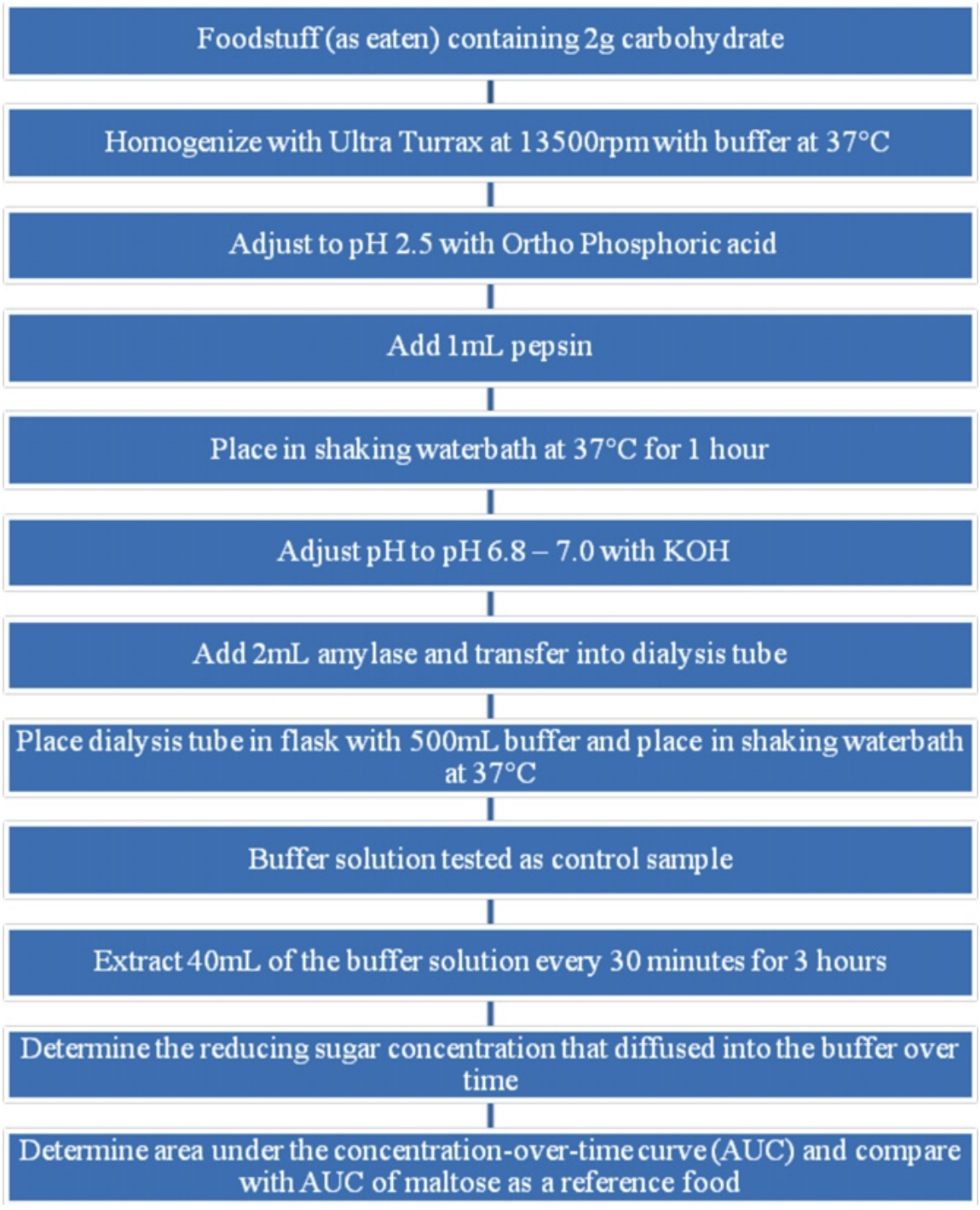


Fig. 1. The method used to determine the hydrolysis index (HI) of foodstuffs.

Based on published GI values, food samples were selected as to represent all three GI ranges, namely high, intermediate and low GI. Maltose and white bread (from the same loaf of bread from a specific retailer) were both used as control samples during the trial. The analysis took place over a three day period. On day one, white bread was analyzed seven times to enable determining the repeatability of the method.

On day two, two potato cultivars, namely Mondial and Darius, were selected along with baby potatoes from the Mondial cultivar. Potatoes are considered high GI, irrespective of cultivar. Baby potatoes are known to have an intermediate GI, irrespective of cultivar. Maltose and bread were analysed as control samples.

On day three canned butterbeans, pumpkin leaves (*Curcurbita*) and Taro (African potato, *Colocasia esculenta*) were analysed along with maltose and white bread as control samples.

Samples were procured form various places. Baby potatoes, canned butterbeans and white bread were purchased from retail shelves. Mondial and Darius potatoes were supplied from distributors. Pumpkin leaves (*Curcurbita*) and Taro (African potato, *Colocasia esculenta*) were harvested fresh at the Agricultural Research Council, Roodeplaat, South Africa.

Two composite samples of each food product was analysed. A pilot study was conducted prior to analyses to familiarize the researcher and assistants to the process.

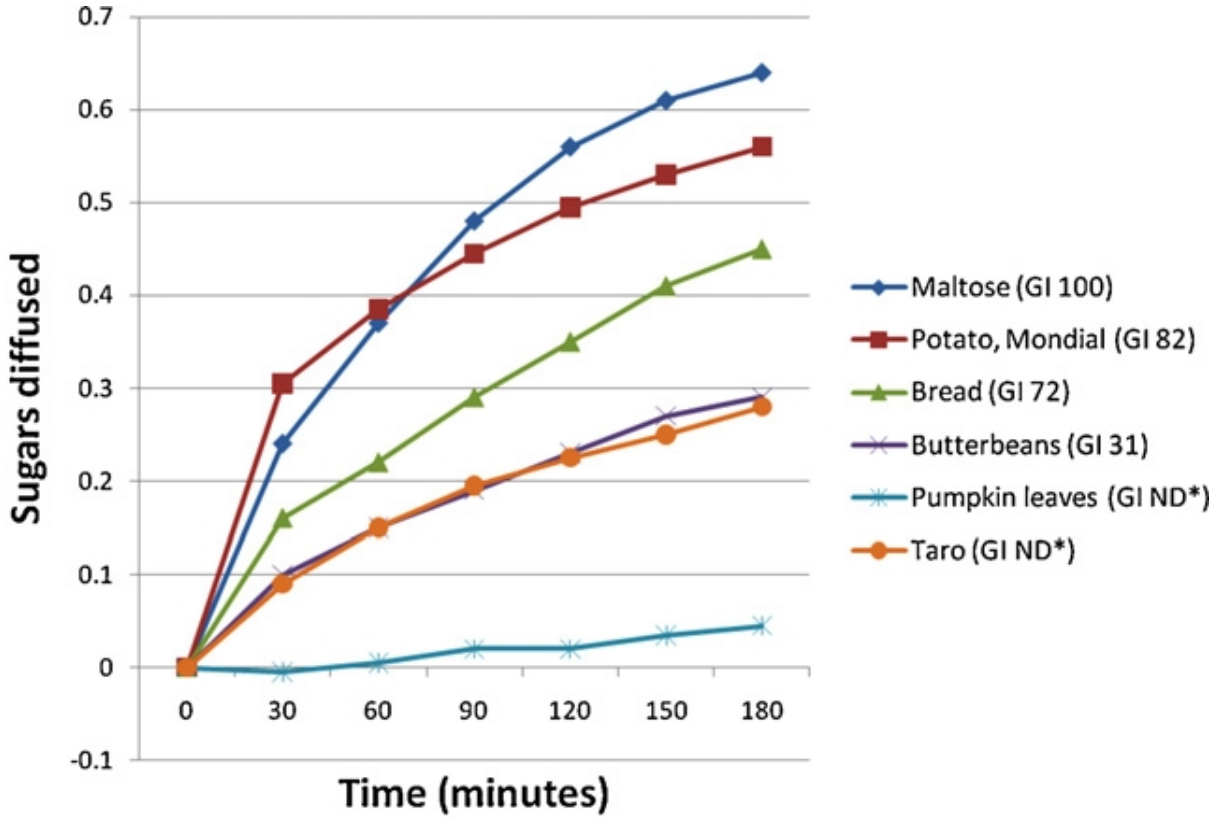
Statistical analysis

The data obtained from each experimental procedure was entered into a spreadsheet on Microsoft Excel (2000). Data was analysed by the using the statistical computer program GenStat for Windows (2003). Mean values, standard deviations and coefficient of variations were determined to present the repeatability and reproducibility of the method.

RESULTS AND DISCUSSION

The mean area under the concentration-over-time curve (AUC) for each food product tested was determined (Figure 2) and the Hydrolysis Index (HI) values were calculated as the relation between the AUC of the specific food compared to the AUC of maltose as the reference food following the equation AUC / AUC reference food = Hydolysis Index value. In Tables 1 to 3 the AUC and HI values determined over the three day trial period are presented. As comparison, GI values obtained from the South African Glycemic Index and Load Guide (Steenkamp & Delport, 2005) are included.

In Table 1 the HI of the seven white bread samples, along with one maltose sample, is reported. The maltose value was adjusted mathematically to represent an HI value of 100, and the calculation was applied to all the other samples in the same trial. This action was repeated for every trial. The mean HI value for white bread was 67.00 (SD 3.45), compared to the GI value of 72.



*Not determined

GI derived from the South African Glycemic Index and Glycemic Load Guide (Steenkamp & Delport, 2005)

Fig. 2. The hydrolysis of carbohydrate from treated food samples through the dialysis tube into surrounding fluid over time. *Not determined GI derived from the South African Glycemic Index and Glycemic Load Guide (Steenkamp and Delport, 2005).

While the determined HI value of white bread during trial 2 (HI 72.52) corresponded well to the reported GI of white bread (GI 72), when comparing the determined HI values of two different South African potato cultivars, greater variation was observed from the reported GI values (Table 2). All potatoes are reported to have a GI of 82 according to the South Africa Glycemic

Table 1
Area under the concentration-over-time curve (AUC), hydrolysis index (HI) and glycemic index (GI) values for maltose and white bread tested during trial one.

	Area under curve (AUC)	Hydrolysis index (HI)[a]	glycemic index (GI)[b]
Maltose	77.4	100	100
Bread mean	51.9	67.0	72
SD	2.68	3.46	–
95% CI (lower)	–	63.8	–
95% CI (higher)	–	70.2	–
Variance	0.008	11.9	–
Sample 1	56.0	72.3	–
Sample 2	51.6	66.7	–
Sample 3	49.7	64.1	–
Sample 4	49.8	64.3	–
Sample 5	50.6	65.3	–
Sample 6	50.1	64.7	–
Sample 7	55.4	71.5	–

[a] Determined: AUC of sample/AUC of Maltose X 100.
[b] Obtained from The South African Glycemic Index and Load Guide (Steenkamp and Delport, 2005).

Table 2
Area under the concentration-over-time curve (AUC), hydrolysis index (HI) and glycemic index (GI) values for maltose, white bread, Mondial, Darius and Mondial baby potatoes tested during trial two.

	Area under curve (AUC)	Hydrolysis index (HI)[a]	Glycemic index (GI)[b]
Maltose	3.33	100	100
White bread	2.42	72.5	72
Mondial mean	2.44	73.3	83
SD	0.37	11.0	
Mondial 1	2.18	65.5	–
Mondial 2	2.7	81.1	–
Darius average	2.21	66.4	83
SD	0.007	0.21	
Darius 1	2.21	66.2	–
Darius 2	2.22	66.5	–
Baby potatoes average	2.72	81.7	62
SD	0.05	1.49	
Baby potatoes 1	2.76	82.7	–
Baby potatoes 2	2.69	80.6	–

[a] Determined: AUC of sample/AUC of Maltose X 100.
[b] Obtained from The South African Glycemic Index and Load Guide (Steenkamp and Delport, 2005).

Table 3
Area under the concentration-over-time curve (AUC), hydrolysis index (HI) and glycemic index (GI) values for maltose, white bread, canned butterbeans, pumpkin leaves (*Curcurbita*) and Taro (African potato, *Colocasia esculenta*) tested during trial three.

	Area under curve (AUC)	Hydrolysis index (HI)[a]	Glycemic index (GI)[b]
Maltose	2.55	100	100
White bread	1.61	63.1	72
Canned butterbeans mean	1.09	42.7	31
SD	0.004	0.14	–
Sample 1	1.09	42.6	–
Sample 2	1.09	42.8	–
Pumpkin leaves mean	0.10	3.82	ND[c]
SD	0.13	5.13	–
Sample 1	0.19	7.45	–
Sample 2	0.01	0.20	–
Taro mean	1.05	41.2	ND[c]
SD	0.11	4.16	–
Taro 1	0.98	38.2	–
Taro 2	1.13	44.1	–

[a] Determined: AUC of sample/AUC of Maltose X 100.
[b] Obtained from The South African Glycemic Index and Load Guide (Steenkamp and Delport, 2005).
[c] ND, no GI has yet been determined in South Africa.

Table 4
Correlating calculated HI values with previously reported GI values.

	Hydrolysis index (HI)			Glycemic index (GI)[b]
	HI[a]	SD	Variance	
Maltose	100	–	–	100
Bread	70.0	3.45	11.9	72
Darius	66.4	0.21	0.05	83
Mondial	73.3	11.0	122	83
Baby potatoes	81.7	1.49	2.21	62
Butterbeans	42.7	0.14	0.02	31
Pumpkin leaves	3.82	5.13	26.3	ND[c]
Taro	41.2	4.16	17.3	ND[c]

[a] Determined: AUC of sample/AUC of Maltose X 100.
[b] Obtained from The South African Glycemic Index and Glycemic Load Guide (Steenkamp and Delport, 2005).
[c] ND, no GI has yet been determined in South Africa.

Index and Load Guide. The determined mean HI of the Mondial potatoes was 66.37 (SD 0.21), while Darius potatoes had a mean HI of 73.27 (SD 11.04). Both these values fall within the intermediate GI range of between 55 and 70. Baby potatoes are reported to have an intermediate GI value of 62 within the South African Glycemic Index and Load guide. The determined mean HI value of the Mondial baby potatoes were 81.68 (SD 1.49) which rather indicates it to be a high GI food than an intermediate GI food.

In various scientific articles published in previous years the GI of different potato cultivars in different countries, prepared by different methods, varied between intermediate GI values of 59 to 70 (Jenkins, Wolever, Taylor, Barker, Fielden, Baldwin, Bowling, Newman, Jenkins & Goff, 1981) and high GI values of 87 to 100 (Soh & Brand-Miller, 1999). In the International Table of Glycemic Index and Glycemic Load values (Steenkamp & Delport, 2005), two unspecified potato cultivars scored very low GI values of 23 and 24 respectively (Foster-Powell *et al.,* 2002). This provides evidence to suggest that different cultivars with different dry matter and starch contents, as well as those grown in different regions and under different growth conditions would have different GI values.

Previous research on South African potato cultivars indicated that there are significant differences in the nutritional composition and eating quality of potatoes, differentiated by cultivar, cultivation region and season (Gibson, 2006; Booysen, 2010). In terms of GI, the difference in HI values between the cultivars and ages of the potatoes could be considered motivation to determine individual GI values per cultivar and discarding the single GI value currently attributed to all boiled potatoes.

The current developed rapid assessment method increases the possibility to identify South African potato cultivars with a low to intermediate GI, in line with international trends. This would afford new opportunities for the South African potato industry, and other food industries, thereby growing new markets and offering consumers wider choice in South Africa. Caution should furthermore be applied to promoting all baby potatoes as having an intermediate GI of 62, as the higher HI values obtained indicated the possibility that these Mondial baby potatoes might in fact have a high GI (GI > 70). Noteworthy differences in the glycemic response were found between the two different potato-cultivars tested, which motivates further investigation.

Repeatability and reproducibility

Repeatability is the variation of outcomes of an experiment carried out in the same conditions. To determine the repeatability of the method, white bread was analyzed seven times at a given occasion (Table 1). The GI value of white bread is reported in the Glycemic Index and Load guide to be 72 (Steenkamp & Delport, 2005). The mean HI value of the seven white bread samples tested during trail one was 67.00. The standard deviation (SD) of the seven white bread samples was 3.46, with a variance of 11.94. The 95 % confidence interval indicated that there is a 95 % chance that HI values of white bread would be between 63.80 and 70.19 (Table 1), which indicated good repeatability.

Reproducibility is the variation of outcomes of an experiment carried out in conditions varying within a typical range, e.g. when measurement is carried out by the same device by different

operators, in different laboratories, at different times etc. In order to determine the reproducibility of the method maltose was tested during each trial over the three day period. Although more trials are required with more datasets in order to be able to draw solid conclusions, the SD between the AUC of the three maltose samples was 0.44 and the CV was 0.16, indicating reproducibility and good precision.

Validity of the rapid assessment method to determine glycemic index

A food is considered to have a low GI when the *in vivo* determined GI values are below 55, while a high GI has a value of 70 and above. Table 4 compares the average HI values of the samples tested with previously determined GI values as presented in The South African Glycemic Index and Load Guide. The mean HI value for bread was HI 70 (calculated as the mean of the three trials), while the GI indication for white bread is reported to be 72. Butterbeans are predicted to have an HI of 41, while the reference GI value is 31. As various factors include GI, factors such as brand, concentration of brine and cultivation methods could all play a role in actual GI. Although there seems to be a big difference in values, both HI and GI of the butterbeans suggest that the product could be labelled as low GI. The difference in HI determined for various potato cultivars, as previously mentioned possibly indicates that there could be greater difference between potato cultivars than previously recognised.

The determined HI values of pumpkin leaves (*Curcurbita*) and Taro (African potato, *Colocasia esculenta*) were both below 55, which corresponds to a low GI. Thus, for future reference, these indigenous vegetables could possibly be considered low GI food options.

CONCLUSIONS

The health consequences of different carbohydrate rich foods are reported on these categories as high, medium or low GI, and not on their specific GI values. The correlation between the determined HI values and known GI data during the preliminary exercise provides persuasive evidence that this HI method is capable of generating indicative GI values for carbohydrate rich foods. Such a rapid analysis method would be beneficial to the South African industry for both health and labelling purposes from a cost, time saving and infrastructure point of view.

RECOMMENDATIONS

It should be remembered that *in vitro* determined GI values do no always consistently correlate with in vivo determined values (Urooj & Puttaraj, 2000), but seen against the high variability observed in the results from *in vivo* methods, all obtained GI values should be presented to consumers as an indication only. It should be kept in mind that factors, such as human emotion, previous meal and other ingredients in a mixed meal, could significantly influence the actual response on blood glucose concentration in an individual. The GI should not be used as the only criterion to choose healthy foods as it is largely modulated by other factors (Laville, 2004), and should only be used as an indication of the possible glycemic effect which the ingestion of a food will have on glycemic response.

At the hand of this it could be suggested that the indication of a food into a high, intermediate or low GI range, as seems possible through the developed *in vitro* hydrolysis method, would be beneficial during product development, labelling and consumer reference purposes.

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