PhD Synopsis

Determination of Glycemic Index of Selected Foods and Formulation of Low Glycemic Index Food Products

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1. BACKGROUND AND RATIONALE

The rising rates of non-communicable diseases (chronic diseases and their associated metabolic disorders) are due to two factors namely: the demographic change and the increasing prevalence of overconsumption and inactivity associated with the Western lifestyle. These have public health and economic implications, and continue to be a matter of great concern. With the world’s population aged 60 years or more increasing at more than three times the overall population growth rate and rising to about 1200 million in 2025, the importance of lifelong health promotion and disease prevention activities that can prevent or delay, the onset and severity of noncommunicable diseases and promote healthy ageing should be considered (WHO, 2012)

This has resulted in continued interest in both diet and lifestyle modifications in prevention and treatment of chronic diseases. Carbohydrates are the most important nutrient of our diet, in terms of fulfilling energy requirements and other metabolic functions. They represent 45–55% of our daily energy intake, 10–15% being simple carbohydrates or sugars, the remainder being starches and oligosaccharides (Blaak et al., 2012). Carbohydrate intake has been a fairly neglected area until recently, even though carbohydrate accounts for most calories in most diets.

There is increasing evidence that both the amount and type of carbohydrate play an important role in weight management and risk of chronic diseases. Classifying carbohydrates according to their post-prandial glycemic effect (ie, the glycemic index of foods) has yielded more useful insights than the historical distinctions of simple versus complex chemical structure. Diets based on carbohydrate foods that are more slowly digested and absorbed (ie, low glycemic index diets) have been independently linked to reduced risk of type 2 diabetes, cardiovascular disease, and some types of cancer (Marsh, 2008). Glycemic index (GI) is a physiological classification of the available carbohydrate content in foods, first proposed in 1981 (Jenkins et al., 1981). The current findings, together with the fact that there are no demonstrated negative effects of a low glycemic index diet, suggest that the glycemic index should be an important consideration in the dietary
management and prevention of obesity and chronic diseases (Liu et al., 2000; Salmeron, Ascherio, et al., 1997; Salmeron, Manson, et al., 1997).

By definition, the GI compares equal quantities of carbohydrate and provides a measure of carbohydrate quality but not quantity. In 1997 the concept of GL was introduced by researchers at Harvard University to quantify the overall glycemic effect of a portion of food. Thus, the GL of a typical serving of food is the product of the amount of available carbohydrate in that serving and the GI of the food. The higher the GL, the greater the expected elevation in blood glucose and in the insulinogenic effect of the food. The long-term consumption of a diet with a relatively high GL (adjusted for total energy) is associated with an increased risk of type 2 diabetes and coronary heart disease (Liu et al., 2000).

Rice is the most important staple and a major source of carbohydrate in the Asian diet. Rice is being widely used in human nutrition as a source of energy due to its high starch level (approximately 90% in polished white grains). However, the level of starch can vary among grains of different varieties due to genetic and environmental factors. The values of total starch can vary and the rate and extension of starch digestion can be influenced by different factors, including variation in the amylose:amylopectin ratio, grain processing, physicochemical properties (particularly gelatinization characteristics), particle size and the presence of lipid–amylose complexes.

The main differences in starch composition that influence physicochemical and metabolic properties of rice are caused by variation in the proportions of its two macromolecules, amylose and amylopectin. Amylose is essentially a linear molecule in which D-glucose units are linked by α-1,4 glucosidic bonds, while amylopectin, a branched polymer, contains both α-1,4 and α-1,6 bonds. The amylose: amylopectin ratio is inversely correlated to GI. Rice has given a wide range of results in glycemic index (GI) studies around the world. The GI of white rice has ranged from as low as 54 to as high as 121 when bread (GI = 100) is used as the reference food (Jenkins et al., 1988; Jenkins et al., 1988; Brand, 1985). This makes it difficult to classify rice as a high- or low-GI food and advice to individuals with diabetes may be incorrect if the product has not been specifically tested first. It is likely that much of the variation in
the GI of rice is due to differences in the proportion of starch present as amylose, i.e. amylose: amylopectin ratio. Most rice contains 20% amylose but varieties that contain a higher proportion of amylose (e.g., 28%) have been shown to have a slower rate of digestion and produce lower glycemic and insulin responses. The classification of rice as a high- or low-GI food may therefore depend on the amylose content of commercial varieties, but the consumer has no way of determining this from the food label.

Currently for regulatory and labelling purposes, the Glycemic Index (GI) of foods has to be determined by in-vivo testing which is expensive and time consuming. Thus, we looked at exploring novel and highly sensitive technology like High Resolution Melting (HRM) technology that was developed recently. HRM is used for the genetic analysis which can detect mutations, epigenetic differences and polymorphisms in double-stranded DNA. In this project, HRM is used to detect polymorphisms such as single base changes (SNPS) in different rice samples.

While it may present challenges for food manufacturers to develop low glycemic index foods, it is well worth to develop these products because of the prevalence of diabetes and pre-diabetes in the region and beyond. It is estimated that by 2030, more than 16 percent of the global population will have a blood sugar problem. "Most of the risk factors are things that can be managed and modified." "We can reverse pre-diabetes and prevent it from becoming diabetes. Food has become the reason for what's ailing us, but it can actually be a solution in a number of different ways." (IFT, 2012)

During the last few years, there has been a large body of data that suggests a diet composed of low GI foods has a role to play in the prevention or treatment of a number of chronic diseases including type 2 diabetes mellitus, cardiovascular disease and cancer. Despite the existence of these supporting data the utility of glycemic index as a tool in the daily diet is still not well utilised due to lack of awareness and educational resources about the concept. Existing data also support that there is a perceived deficiency in reliable glycemic index education available to the public and the nutrition educators (Grant & Wolever, 2011). The application of the
low GI concept in the prevention and management of diet related chronic diseases continues to be a topic of debate due to the following factors:

- In-vivo determination of glycemic index (GI) – methodological issues in available carbohydrate analysis and glycemic index testing. There are very few accredited glycemic index laboratories in the world providing credible data on glycemic index values for foods
- Lack of data on the glycemic index of commonly consumed foods
- In-vivo determination of glycemic index is very expensive and time consuming
- There are no locally manufactured low glycemic index products available to the consumers
- Lack of awareness of glycemic index concept

2. **OBJECTIVES**

In order to address the above mentioned issues, the research study was carried out with the following objectives: To

i) determine the glycemic index and glycemic load of commonly consumed foods

ii) analyse the amylose and amylopectin content of selected foods and ascertain its correlation to the glycemic index values

iii) explore novel and highly sensitive technology like High Resolution Melting (HRM) technology to detect polymorphisms such as single base changes (SNPS) in different rice samples

iv) develop commercially viable low glycemic index foods products

v) determine the awareness of glycemic index concept and develop educational tools on glycemic index.

3. **METHODOLOGY**
PHASE A  Determination of the glycemic index and glycemic load of commonly consumed foods

3A.1 Reference, Test Foods and Reagents

Thirty different varieties of test foods were tested. A 25g or 50-g dextrose anhydrous load was used as the reference. All the test food samples were prepared as they are usually prepared for consumption. The portion size of each test food contained 25g or 50g available carbohydrate (ACHO) defined as total carbohydrate minus dietary fibre.

3A.2 Methods

3A.2.1 Determination of Glycemic Index and Glycemic Load

Healthy volunteers who met the inclusion and exclusion criteria with normal glucose tolerance took part in the study. Ethics approval was obtained and all the subjects signed an informed consent form. The subjects took 25 or 50 g available carbohydrate portions of each of the reference or test food samples in random order on separate mornings after an overnight fast. Foods were consumed over 12 -15 minutes. Finger-prick capillary blood samples were taken at -5, 0 (fasting), 15, 30, 45, 60, 90, and 120 minutes after the meal began. Safe-T-Pro lancets were used to puncture the finger sites and capillary blood was drawn and transferred into minicollect tubes which were coated with an anticoagulant. The blood samples were analysed immediately for blood glucose using YSI2300 glucose/lactate analyser. 25g or 50 g dextrose anhydrous dissolved in 250ml was used as the reference food.

The reference food was tested at least two to three times and the test food was tested only once. The Incremental area under the curve for test and reference foods were analysed and the GI values were calculated by expressing each subject’s AUC of the test food as a percentage of the same subject’s mean reference AUC. The mean of the resulting values was the GI of the test food. If any individual subject’s GI value for a food sample fell outside the group mean (average) GI value plus or minus two standard deviations the value was classified as an outlier (unusual observation) and removed from the dataset. The group mean was expressed as mean GI. The final GI of the test food was expressed as GI ± S.E.M; where GI is the mean GI value of all subjects, excluding outliers and S.E.M is the standard error of the mean.
The Glycemic Load (GL) of the foods tested was calculated as follows:

$$GL = \frac{GI \times \text{ACHO present in one serving of the food item}}{100}$$

In addition to in-vivo GI testing of composite meals, the GI of the composite meals was also calculated using the formula proposed by (Wolever, 2006). The GI of individual foods was weighted according to the amount of carbohydrate each food contributed to the composite meal, to estimate the GI of composite meal.

**PHASE B  Determination of amylose and amylopectin of selected rice varieties**

3B.1 **Reference, Test Foods and Reagents**

Fourteen different varieties of rice (test foods) were tested. A 50-g dextrose anhydrous load was used as the reference. All the rice samples were prepared as per instructions in a rice cooker. Amylose and amylopectin was analysed using the K-AMYL 07/11 enzyme assay kit (Megazyme, 2011a).

3B.2 **Methods**

3B.2.1 **Determination of amylose and amylopectin**

A modification of Concavalin A (Con A) method was used (S.H.Yun, 1990). The rice samples were completely dispersed by heating in dimethyl sulphoxide (DMSO). Lipids were removed by precipitating the starch in ethanol and recovering the precipitated starch. After dissolution of the precipitated sample in an acetate/salt solution, amylopectin was specifically precipitated by the addition of ConA and removed by centrifugation. Amylose in the supernatant was enzymatically hydrolysed to D-glucose, which is analysed using glucose oxidase/peroxidase reagent. The total starch in a separate aliquot is similarly hydrolysed to D-glucose and measured colorimetrically by glucose oxidase/peroxidase. The concentration of amylose in the starch sample is estimated as the ratio of GOPOD absorbance at 510nm of the supernatant of the Con A precipitated sample, to that of the total starch sample.
Calculation of amylose content (％)

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\text{Amylose, } \% \text{ (w/w)} = \frac{\text{Absorbance (Con A supernatant)}}{\text{Absorbance (Total Starch Aliquot)}} \times 66.8
\]

**PHASE C Profiling of glycemic index of rice samples using High Resolution Melting (HRM) technology**

**3C.1 Methods**

In HRM analysis, a PCR product was generated through amplification and then subjected to a gradual temperature increase. This was done in the presence of a dye that fluoresces when bound to double-stranded DNA. This method clearly differed from standard melt curve analysis. To perform HRM, the temperature was increased from low to high. The fluorescence of third-generation intercalating dyes (e.g., EvaGreen) was measured continuously and was plotted against increasing temperature. Increased fluorescence was only measured as long as the dye was bound to dsDNA. As the temperature increased, the DNA started to disassociate into two single strands, that resulted in DNA melting (Montgomery, Wittwer, Palais, & Zhou, 2007). The melting temperature \(T_m\) of the DNA sample under analysis occurred at the point of the melt phase where the rate of change in fluorescence was greatest. When the DNA completely melted, only some background fluorescence was detected. Genome sequences of rice were sourced from gene bank, conserved sequences were located and specific primers were designed to distinguish low, medium and high GI of rice samples.

**PHASE D Development of low glycemic index food products.**

**3D.1 Product concept**

The product concept was based on the following criteria:

i. Products that are suitable for all age groups.

ii. Products that are healthier than their market counterparts

iii. Products that are most suited for commercialization (this also includes cost effectiveness)
3D.2 Methods

The low GI products that were developed included: Multigrain cookie, Muffins, Fried rice and a Beverage. A thorough search for low GI ingredients was done using the existing international GI database and as well as sourcing from ingredient suppliers. The ingredients were purchased from reliable suppliers, with certificate of analysis (COA).

The glycemic index value of the product formulations were calculated using the method proposed by (Wolever, 2006). Nutrient analysis of the trial formulations were computed using nutrition information from the Food works nutrient database. Product development trials were conducted if the calculated GI values were below 55 and the computed nutritional information confirmed the formulations to be healthier than their market counterparts. Preliminary sensory analysis and in vivo Glycemic Index testing was conducted to ascertain that the developed products are lower in GI and are also palatable. Many product trials were conducted until the product formulations were finalized. Sour dough technology was used to develop the muffins and modified atmospheric packaging was used to produce a shelf-stable product,

PHASE E Assessment of Knowledge and Awareness of Glycemic Concept and Development of Educational Tools on GI

3E.1 Materials and Methods

3E.1.1 Construction and administration of survey questionnaires

Three different types of questionnaires namely: one for the general public, one for health professionals and another one for food industry representatives were constructed and administered during 2009/10 and 2012/13. All the questionnaires were pre-tested to check for glitches in wording of questions, clarity of instructions and the order of presentation of questions before administration. These questionnaires were administered to gather information on the knowledge and awareness of the GI concept among respondents. The questions relating to knowledge and awareness about the GI concept, preferred low GI information sources were some of the questions that were kept the same for all the three questionnaires administered. In addition to the common questions, the
questionnaires constructed for the health professionals and food industry representatives included specific questions related to specific areas.

The survey questionnaires to the public were administered face-to-face and through online survey mode. The online survey was shared in various social media platforms such as Facebook and Twitter. The survey questionnaires were sent by snail mail to the health professionals and food industry representatives.

Survey conducted among the general public included 2139 participants who were randomly selected to complete the survey. The first phase of the survey was conducted among 1000 respondents during 2009/2010 and the second phase of the survey was conducted between September 2012 and February 2013. Convenience and stratified sampling were used to collect the data. Stratified sampling was one of the sampling methods that were used to divide the entire target population into different subgroups.

3E.1.2 Development of Educational Tools on GI

Appropriate evidence based information about GI was delivered in form of print material (pamphlets), booklet, talks, workshops, cooking demonstration and recipe book.

Findings of the study

- A wide range of GI values from moderate to high glycemic index among the foods tested was noted.
- The rice values had a wide range of GI values and certain unpolished varieties were higher in GI. The parboiled basmati rice was lower in GI compared to the polished varieties
- None of the rice varieties tested in this study had amylose content more than 25%. The basmati rice (Type 3), which had the highest amylose content, fell under the medium GI category.
- The results show that the amylose content of the rice samples has an influence on the GI values, with the exception of parboiled basmati rice.
The HRM technology is a novel, sensitive, cost-effective, high throughput technique, which can be used as a screening tool to characterise various rice samples, which can be beneficial to farmers, traders and end users.

All the developed products were low in GI and also palatable.

In conclusion, the findings of the survey conducted among the general public, health professionals and food industry representatives indicate that awareness on GI needs to be stepped up.

Educational tools on GI such as pamphlet, booklet, composite/mixed meal recipes compiled into a recipe book were developed. Cooking demonstration sessions on low GI recipes, talks and workshops were also conducted to increase the awareness.

REFERENCES


