Development of a rapid assessment method for the glycaemic index

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DECLARATION

I, *Nicolette Gibson*, declare that the thesis/dissertation, which I hereby submit for the degree *MSc Nutrition* at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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SUMMARY

Title: Development of a rapid assessment method for the glycaemic index

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The glycaemic 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 vivo*). Rapid analysis methods (*in vitro*) are being developed and evaluated worldwide, and in many cases the values obtained have correlated well with the GI values determined by *in vivo* methods. The criticism against rapid analysis methods is that the methods do not provide numerical GI values. 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 as a screening tool during product development 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%). Further comparative trials indicated that the *in vitro* method accurately predicts the GI category of Almera potatoes *(Solanum Tuberosum L.* cultivar Almera)* and Gero fat free litchi and raspberry flavoured yoghurt, in line with results found from *in vivo* analysis. Significant inter-laboratory variability of *in vivo* analysis of GI values obtained for the Almera potato cultivar was found, and the need for future alignment of methodology and sample preparation is recommended.

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AOAC	Association of Official Analytical Chemists
ARC	Agricultural Research Council
AUC	Area under the curve
BMI	Body mass index
CCFRA	Campden and Corleywood Food Research Association
CHO	Carbohydrates
CI	Confidence interval
CV	Coefficient of variation
DRI	Daily Recommended Intake
EASD	European Association for the Study of Diabetes
EFSA	European Food Safety Authority
EUFIC	European Food Information Research Council
FAO	Food and Agriculture Organization of the United Nations
GI	Glycemic Index
GIFSA	Glycemic Index Foundation of South Africa
GIT	Gastrointestinal tract
GL	Glycemic Load
HI	Hydrolysis index
HPLC	High performance liquid chromatography
IAUC	Incremental area under the curve
IDDM	Insulin-dependent diabetes mellitus
ILSI	International Life Sciences Institute
ISO	International Organization of Standards
NIDDM	Non-insulin-dependent diabetes mellitus

NICUS   Nutrition Information Centre of the University of Stellenbosch

RAG   Rapidly available glucose

RDS   Rapidly digestible starch

SADHS   South African Demographic and Health Survey

SAG   Slowly available glucose

SANS   South African National Standards

SD   Standard deviation

SDS   Slowly digestible starch

SUGIRS   Sydney University Glycemic Index Research Service

UK   United Kingdom

USA   United States of America

WHO   World Health Organization

CHAPTER 1: INTRODUCTION AND JUSTIFICATION

In this chapter the study is introduced. A review on the practical implications of the glycaemic index (GI) is presented. This is followed by a brief summary of the health benefits, international recognition, future labelling prospects and consumer responses towards GI. The problem statement and motivation for the development of the rapid assessment method to predict GI for South Africa are also presented in this chapter.

1.1 INTRODUCTION

The term *carbohydrate* describes a family of contributing compounds, all composed of the same monosaccharide building blocks, ranging from simple sugars, or mono- and disaccharides, through sugar alcohols, oligosaccharides and dextrins, to the more complex starch and non-starch polysaccharides. With this wide array of compounds, there are many food sources which contribute to total carbohydrate intake in the human diet.

Traditionally carbohydrate-based foods have been categorised according to the structural classification of the principal carbohydrate forms present in the food (Venter, Slabber & Voster, 2003). Consequently, a carbohydrate-based food was classified as a "simple" carbohydrate when containing mostly mono- or disaccharides, or a "complex" carbohydrate when containing mainly polysaccharides or starches. Because of this categorisation it was incorrectly assumed that all "simple" carbohydrates would elicit a rapid glucose response in the human body and would thus be unsuitable for diabetics and individuals with insulin disorders, while "complex" carbohydrates were believed to cause smaller responses in blood glucose levels (Venter *et al.,* 2003).

In 1939, Conn and Newburgh already noted how different carbohydrate foods, with similar macronutrient compositions, had different glycaemic responses. In 1981 Jenkins, Wolever, Taylor, Barker, Fielden, Baldwin, Bowling, Newman, Jenkins and Goff (1981) published results confirming the hypothesis that carbohydrate exchange lists, normally used for controlling diabetes, did not adequately reflect the specific physiological effect which each food induces once

consumed. Jenkins *et al.* (1981) concluded that glucose response is not only dependent on the amount and primary structure of carbohydrate consumed at a given time, but that factors such as dietary fibre, the form of the food and the nature of the carbohydrate, all influence the extent to which glucose will be released into the blood. Thus the need arose for a simple indication or index which incorporate all these factors (Laville, 2004). As a result, the glycaemic index (GI) was developed. The GI is an empirical system for classifying carbohydrate-based foods, founded on the degree of glucose release into the blood stream once ingested (Ludwig, 2007). It is well established that blood glucose response to a food is not accurately predicted by the amount of available carbohydrates in the food (as measured by traditional food composition analysis). The need is recognised for information to complement food composition data, to enable the control of postprandial glycaemic response (Monro & Shaw, 2008).

In other words, the GI concept is based on the physiological properties of carbohydrate containing foods, which are not apparent from their chemical composition alone (Englyst, Lui & Englyst, 2007). The GI value of a food does not purely represent the degree to which carbohydrates are absorbed in the small intestine, but also reflects the combined effect of all the properties of the complete food or meal that influence the rate of glucose increase and removal from the blood stream (Englyst *et al.,* 2007; Frost & Dornhorst, 2000). In summary, it can be said that the GI concept has been developed to obtain a numerical physiological classification of carbohydrate containing foods and meals based on the rate of carbohydrate absorption into the blood, to improve nutritional advice (Laville, 2004; Jenkins *et al.,* 1981).

1.2 THE GLYCAEMIC INDEX AS A DIETARY TOOL

1.2.1 The glycaemic index concept

GI is a defined as a measurement used to classify foods according to their potential for raising blood glucose levels (Whitney & Rolfes, 2002), and is generally measured by determining the increment in blood glucose concentration accompanying the consumption of a test meal over a

set period of time and comparing it with an isoglucidic control meal (normally white bread or glucose) and is expressed as a percentage (Goni, Garcia- Alonso & Saura-Calixto, 1997).

Although the GI concept seems simple, it is difficult to measure as numerous factors influence the glycaemic response to carbohydrates. These include the intrinsic properties of the food, such as the glucose-chain length and ratio of amylase to amylopectin, and the extrinsic properties of the meal, such as total meal composition, the overall diet and variations within the host (Frost & Dornhurst, 2000; Cummings & Stephan, 2007). The nature of the carbohydrates, the type of sugars, the status of the starch, the complete food matrix and the presence of anti-nutrients all play a role in the overall glycaemic response which ingestion of the food will induce (Jenkins, Kendall, Augustin, Franceshi, Hamidi, Marchie, Jenkins & Axelson, 2002). It is the influence of these factors which makes classification of carbohydrates foods by GI rather complex (Laville, 2004). As soon as foods become part of a meal, their glycaemic response will be changed to a certain extent based on the other foods forming part of the meal. Furthermore, previous meals consumed could have an effect on the GI of the current meal. The presence of retrograded starch, formed after cooking and cooling of starch, is also known to decrease the GI value of a food, i.e. cooled maize meal porridge will have a lower GI value than warm maize meal porridge.

From a nutrition perspective, it is not particularly important to know the exact GI value of every food as this will change depending on the various factors listed. What is important is to be able to identify which foods are absorbed over a longer period of time (low GI; GI < 55), which foods ellicit a rapid surge of glucose (energy) into the bloodstream (high GI; GI > 70), and which are in between (intermediate GI; GI 55 - 70). Consequently, foods high in carbohydrates can thus be arranged into these broad categories to guide dietary choices.

1.2.2   The glycaemic index and health

A committee brought together in 1997 by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) endorsed the use of the GI method for classifying carbohydrate rich foods and recommended that GI values, together with other food

composition data, be used in the guiding of healthy food choices (FAO/WHO, 1998; Foster-Powell, Holt & Brand-Miller, 2002).

The evidence shows that high glycaemic foods are associated with weight gain and obesity (Ludwig, Majzoub, Al-Zahrani, Dallal, Blanco & Roberts, 1999; Ludwig, 2000; Liu, Willet & Stampfer *et al.,* 2000), and have been linked to an increased risk of developing non-insulin-dependent diabetes mellitus (NIDDM) (Foster-Powell *et al.,* 2002; Rondini & Bennink, 2007), certain cardiovascular diseases and cancer (Lui *et al.,* 2000; Foster-Powell *et al*, 2002). Jakobsen, Dethlefs, Joensen, Stegger, Tjonneland, Schmidt and Overvad (2010) emphasized the importance of using GI as a tool when making carbohydrate-based dietary choices, when they determined the effect of replacing saturated fats in the diet with carbohydrates. Jakobsen *et al.,* (2010) found that the GI of the carbohydrate food significantly influences the risk for myocardial infarctions. Replacing saturated fatty acids in the diet with high GI carbohydrate-based foods increased the risk for myocardial infarction, while substituting saturated fatty acids with low GI foods decreased the risk.

Ludwig and Eckel (2002) stated that since 1980 more than 100 scientific studies on the benefits of following a low GI diet on diabetes mellitus, obesity, cardiovascular disease, behavioural disorders and physical performance have been published. Since 2002, even more papers on GI and related health outcomes have been published. The main emphasise of the studies was on diabetes mellitus, cardiovascular disease, obesity and cancer (Ludwig, 2007). The GI concept has been accepted by many nutritionists, dietitians and the public as a fundamental health principle (Irwin, 2001). The FAO/WHO and numerous other international organizations with a health focus have endorsed the GI concept (Ludwig & Eckel, 2002).

1.2.3   The South African nutrition situation

Within a South African context, obesity is a growing and complex epidemic, affecting many people, particularly women, irrespective of ethnicity or education levels. It is reported that in 1998, 29 % of South African adult men and 55 % of adult women were overweight, while 9 % of adult

men and 29 % of adult women were obese (South African Department of Health Statistics (SADHS), 1998). According to Monteiro, Conde and Popkin (2004) obesity rates of women (20 to 49 years) in South Africa are relatively equal for different degrees of education, between 29.4 % to 33.7 % for the lower quartiles and 22.8 % to 30.3 % for the higher quartiles of education.

Although high obesity rates are reported in many developed countries, within a developing country, such as South Africa, obesity is increasing and often coincides with micronutrient deficiencies. According to the WHO Global Data base on Body Mass Index which included 36 countries around the globe with high stunting rates for adults, South Africa had the highest obesity prevalence among adult females (Nishada, 2009). In South Africans older than 30 years, 5.5 % are reported to have diabetes which increased with chronological age. The highest prevalence of NIDDM was reported in the Indian and coloured communities, followed by the black community (SADHS, 1998). For each gender, death rates from diabetes and obesity rose between 1997 and 2004. Female death rates were higher than male death rates, but the gap narrowed over time (Lehohla, 2006). It is widely recognized that shifts in dietary intake to a less prudent pattern are occurring with increasing momentum, particularly among the black population which comprises more than three quarters of the South African population (Bourne, Lambert & Steyn, 2002). Unfortunately, limited data is available on the obesity prevalence within different socioeconomic groups, and reference is mostly limited to ethnicity in publications related to diet and disease (Van Heerden & Schönfeldt, 2011).

Poverty may lead to both undernutrition and overnutrition, as food insecurity can result in overconsumption of low cost energy dense staples (Tanumihardjo, Anderson, Kaufer-Horwitz, Bode, Emenaker, Haqq, Satia, Silver & Stadler, 2007). Estimates suggest that approximately 43 percent of households in South Africa suffer from food poverty (Rose & Charlton, 2002) and spend approximately a third of their total food cash expenditure on starchy staple foods, dominated by maize meal followed by bread.

To combat obesity, one of the main considerations is food intake. Many factors influence food and meal choice, and these are not limited to nutrition and health considerations. Factors such as

local availability, portion size, cultural acceptability and individual likes and needs also influence ultimate food choice (FAO/WHO, 1998). It is therefore important to consider and promote locally applicable dietary guidelines and recommendations. It is also widely recognised that there is no one measure that can guide food choices in all circumstances (FAO/WHO, 1998), and any classification of foods, e.g. GI, should be used in conjunction with other information when prescribing prudent diets.

1.2.4   The glycaemic index in a South African context

In South Africa, with its diverse eating culture and socioeconomic divide between population groups, promoting healthy food choices is complex. In the low income groups, the nutritional contributions and glycaemic responses of staple foods, such as maize and bread, play a significant role in health and wellbeing, as these two staples form the basis of most meals. To improve dietary quality, strategies such as dietary diversity and the addition of other food ingredients e.g. local vegetables, would need to be incorporated as feasible solutions to increase the nutritional quality of meals (e.g. lowering GI). In the higher income groups, nutritional considerations (including GI), are the main drivers in consumers' food purchasing behaviour (Vermeulen, 2010). If GI data were readily available to the food industry, food product development prospects to target both the low and high socioeconomic groups, would increase.

Furthermore, if a larger selection of products available in the market were to indicate glycaemic response, healthy food choices, based on a wider reference than just the nutrient composition of a food, could be promoted. GI data for traditionally consumed foods in South Africa, as well as for new commodities, could add valuable guidelines to product development for industry, apart from promoting good food choices. However, there are various limitations to classifying carbohydrate foods based on their glycaemic response. These limitations include the expensive and time consuming nature of GI methodologies using human subjects (*in vivo*), which has previously restricted the generation of GI data.

In South Africa, the Glycemic Index Foundation of South Africa (GIFSA) has developed a databank of GI values on specific food commodities, which were determined through industry funding. The 4th edition of the South African Glycemic Index and Load Guide (Steenkamp & Delport, 2005) contained 468 GI values for different foods and drinks, of which 208 were determined locally by GIFSA either through *in vivo* procedures or by calculation. The other 260 values (more than 50 %) were derived from international tables. Since 2005, approximately 70 foods have been analysed by the GIFSA laboratories each year (Delport, GIFSA, Personal communication, 2010).

Although there is an International Table of Glycemic Index and Glycemic Load Values (Foster-Powell *et al.,* 2002), there are various limitations to borrowing GI data. The lack of standardisation of methodologies as well as factors influencing food characteristics, such as cultivar and region, or recipe ingredients, can have a significant impact on glycaemic response. Because of these factors, a need was identified to acquire a fast, inexpensive, yet reliable, method to predict GI for a developing country like South Africa with its dynamic food culture and staggering (growing) obesity statistics.

1.2.5   A global phenomenon characterised by contrasting opinions

Various organisations that support the GI concept, argue that GI is a robust measurement which predicts relative glycaemic response to mixed meals, is easy to implement and follow, results in consistent glycaemic control when adopted by people with diabetes and shows consistent evidence of control for many diseases including diabetes. In contrast, there are many organisations do not agree with these statements, and many researchers suggest quite the opposite (Sievenpiper, 2004).

The 1998 FAO/WHO Expert Consultation reported that the GI concept is a useful tool for selecting the most appropriate carbohydrate containing food for the maintenance of health and the prevention of certain diseases (Mann, Cummings, Englyst, Key, Liu, Ricchardi, Summerbell, Uauy, van Dam, Venn, Vorster & Wiseman, 2007; Nantel, 1999; FAO/WHO, 1998).   More

specifically, the FAO/WHO (1998) recommend that when considering carbohydrate foods, the *"...glycemic index be used to compare foods of similar composition within food groups"*.

Many guidelines for diabetes positively recommend the use of low GI foods. The European Association for the Study of Diabetes (EASD) stated that carbohydrates foods which are rich in dietary fibre and have a low GI can be recommended as part of the diabetic diet (The Diabetes and Nutrition Study Group of the European Association for the Study of Diabetes, 2000). The WHO/FAO and health agencies in Europe, Canada and Australia all acknowledge the GI concept and advocate the consumption of a controlled GI diet (Ludwig, 2007; Frost & Dornhurst, 2000).

Food-based dietary guidelines are considered one of the most effective approaches to public communication of nutrition (Englyst *et al.,* 2007). In South Africa the South African Food-Based Dietary Guidelines acknowledge the GI concept, referring to the specific healthful effects of low GI foods in preventing chronic disease and recommend the consumption of unrefined starches and fibre. The beneficial impact of consuming high GI foods in endurance sports and replenishing glycogen stores is also recognised (Department of Health, 2004). In Australia, GI is included in the national dietary guidelines. These state that all breads and cereals are economical foods that are important sources of essential macro- and micro-nutrients. The Australian dietary guidelines also recommend that wholegrain cereal choices, which are generally higher in dietary fibre, and cereals with a lower GI, should be the preferred choice (National Health and Medical Research Council, 2003).

However, the optimism surrounding GI as a dietary tool is not evident everywhere. Few governmental agencies in the United States of America (USA) have accepted the GI concept as a dietary approach and the concept is still being debated heatedly (Frost & Dornhurst, 2000; Weichselbaum, 2010). Most diabetic associations, including the Canadian Diabetic Association, Diabetes Australia, the European Association for the Study of Diabetes and Diabetes UK, include the GI concept in their recommendations. However, the American Dietetic Association maintain their position that carbohydrate quantity is more important than quality, dismissing the use of the GI in diabetes therapy (Sievenpiper, 2004). Ludwig (2007) reported that no governmental agency

or major professional association in the USA advocates the consumption of a low GI diet. He states that data inconsistency, uncertainty in measuring mixed meal effects, and the practicality of a low GI diet, are the three main points central to the GI debate in the USA.

It is evident that counselling individuals on the benefits of a controlled GI diet is still a matter of dispute (Frost & Dornhurst, 2000; Weichselbaum, 2010). Published studies have reported improved glucose tolerance and other health gains by using GI to determine food choice, but questions are still being asked regarding the practicality, clinical utility and validity of the GI concept in meal planning (Kraus, Eckel, Howard, Appel, Dasniels, Deckelbaum, Erdman, Kris-Etherton, Goldberg, Kotchen, Lictenstein, Mitch, Mullis, Robinson, Wylie-Rosett, Sachiko St.Jeor, Suttie, Tribble & Bazarre, 2000).

As previously mentioned, the FAO/WHO Expert Consultation Report (1998), recommended that both the chemical composition and physiological effect of food carbohydrates should be considered for healthy food choices. This is because the chemical nature of food components does not completely describe their physiological effect. However, the use of bioavailability factors and other physiological indexes are being increasingly considered for inclusion in food composition tables and other reference databases. Although Aston, Jackson, Monsheimer, Whybrow, Handjieva-Darlenska, Kreuzter, Kohl, Papadaki, Martinez, Kunova, Van Baak, Astrup, Saris, Jebb and Lindroos (2010) stated that there are currently no national food composition database which includes GI values, the possibilities of including the GI values in reference resources such as food composition tables, have in fact previously been considered (Schönfeldt & Holden, 2009). Towards this end, 1322 published GI values have been published in the Finnish Food Composition Database. The database expressed the GI value, the origin of the value, as well as the methods used to derive it. A further 888 GI values were calculated based on recipe formulas and included in the Finnish Database (Kaartinen, Simala, Pakkala, Korhonen, Mannisto & Valsta, 2010). The Brazilian Network of Food Composition data systems analysed the GI of 41 local foods, using FAO standards, through partnerships with and assistance from universities and local industries (Menezes, Giuntini, Dan & Lajolo, 2009). The results were included in the

Brazilian Food Composition Database as part of their initiative to reduce the use of carbohydrate values obtained by difference in food composition tables (Greenfield & Southgate, 2003; FAO/WHO, 1998). Instead, they quantify carbohydrate rich foods into fractions of available and unavailable carbohydrate, together with glycaemic response, to effectively address users' needs in terms of physiological effects after consumption (Menezes *et al.,* 2009).

From a holistic nutrition perspective, there is a key limitation to GI that needs to be considered. The GI concept alone should not be used for diet planning as it generally does not correspond to nutrient density. For example, ice cream could be considered a good choice based on its low GI, but ice cream, *per se,* is not a healthy food choice based on its high sugar and fat content. It should be remembered that only foods from the same food groups should be compared when food choices are based on GI (FAO/WHO, 1998).

Although some researchers and health professionals question the relevance and practicality or express concerns related to the validity and reliability of data, GI values in combination with food composition data seem to be a more useful source of dietary advice than the chemical classification of carbohydrates alone (Foster-Powell *et al.,* 2002; Ludwig, 2007).

1.2.6   Glycaemic index labelling

According to Venter *et al.* (2003), food labelling in terms of compositional information has two aims, firstly to inform the consumer about the composition of a food, and secondly to assist the consumer in the selection of a healthy diet. The consumer has the right to be informed about the impact that a food may have on metabolism and health (Venter *et al.,* 2003).

Consumers have been exposed to the GI concept from the 1990s, through numerous public books and media inserts. As a result consumer interest is ready for food labelling to include GI indications in a similar fashion to other nutritional components such as protein and fat content (Irwin, 2001). The GI concept has quickly become a highly sought after tool used in conjunction with other composition information to guide food choices. According to Mitchell (2008) the GI

concept can be considered the most promising form of labelling to date, however in global terms, labelling legislation has not caught up with this demand.

However, labelling of a food in terms of its GI, is a complex matter and special consideration is needed before foods are labelled with GI values as an aspect of nutritional information and healthy eating (Arvidsson-Lennor, Asp, Axelsen, Bryngelsson, Haapa, Järvi, Karlström, Raben, Sohlström, Thorsdottir & Vessby, 2004). The three common justifications for GI labelling include (1) that the GI to be assessed needs to be done in an experienced laboratory or clinic, (2) that the foods labelled with GI be high carbohydrate foods, and (3) that the comparison of GI of different foods should be limited to foods within the same food groups (Arvidsson-Lennor *et al.,* 2004; Venter *et al.,* 2003).

It is recommended by Venter *et al.* (2003) that only foods containing more than 10 g carbohydrate per 100 g portion, or supply a total of at least 40 % of energy from carbohydrate, should be labelled with GI. The labelling of low carbohydrate foods for GI is not meaningful (Venter *et al.,* 2003), but in some cases it is done for the benefit of the producer while misleading the consumer. Many consumers are unaware of the GI concept, and do not understand why certain foods are labelled for GI and others not. Often food manufacturers who supply GI on their food labels do not necessarily intend to educate consumers about the concept, but rather just want to inform the consumer that their product is superior to a competitor's product. It is strongly recommended that consumers be educated that all nutrients in a food should be considered as a whole, and that GI should not be considered as the only factor influencing food choice (Ludwig, 2007).

Although health benefits are associated with consuming low GI foods, the choice of carbohydrate rich foods should not be based solely on their GI, since low GI foods may be energy dense and contain substantial amounts of fats, sugars or undesirable fatty acids that may have a detrimental effect on health (Mann *et al.,* 2007). As a functional role of fat includes its decreased effect on the absorption of glucose into the bloodstream, high fat carbohydrate foods often have a lower GI value than their low fat counterparts. Thus many high fat carbohydrate foods could technically be

labelled as low GI food choices, and might appear "healthy" to unwary consumers, unless adequate guidelines are in place to prevent such labelling.

The proposed 2007 South African draft Regulations Relating to the Labelling and Advertising of Foodstuffs aimed to prevent the industry from taking advantage and confusing the consumer, including publication of misleading GI information. This draft label law states that only food products with a carbohydrate content providing more than 40 % of the total energy of the product can be labelled for GI (Department of Health, 2007). However, the Regulations Relating to the Labelling and Advertising of Foodstuffs (Foodstuffs, Cosmetics and Disinfectants Act, 1972, Act 54 of 1972) which came into effect on 1 March 2010 do not include any regulation relating to GI. The reason for this omission is that the Directorate: Food Control is awaiting the adoption by the South African National Standards (SANS) of an International Organization of Standards (ISO) method that has been established to determine GI (Booysen, Directorate: Food Control, Personal communication, 2010).

There are many ways of presenting the GI of a food on the product label. The way in which the GI of a food product is presented on the product label is critically important for optimal impact: the food label needs to be simple and understandable, but include enough information to educate the consumer. Irwin (2001) recommends the use of a symbol to indicate a food product's GI. By using a simple and easily recognisable symbol, Irwin (2001) states that consumers will gain a better understanding of the relationship between GI and health, dietitians, and other health professionals will be supported in their educational efforts and the food industry will make a contribution to increasing the recognition by consumers of healthy food choices.

1.2.6.1 Glycaemic index labelling in Australia

Various countries have introduced some form of GI indication on the labels of food products, with Australia being considered the leader in this context. In Australia, official dietary guidelines for healthy consumers as well as an official GI trademark, have been developed (Foster-Powell & Miller, 1995). The Dietary Guidelines for Older Australians specifically recommend: *"Eat plenty of*

cereals, bread and pasta – prefer high fibre foods and those with a lower glycaemic index"
(National Health and Medical Research Council, 2003).

The Australian market has been exposed to GI labelling of high carbohydrate foods for the longest period of time, and thus the consumer awareness in this market is also considered to be the highest (Mitchell, 2008). To address the increased demand for information surrounding the GI concept, a non-profit organisation, Glycemic Index Limited, was formed in 2001 by the Sydney University, Diabetes Australia and the Juvenile Diabetes Research Foundation (Irwin, 2001). The company aimed to implement a GI labelling program and increase awareness of GI.

The Australian GI Symbol program is not considered to be an endorsement nor a health claim (Irwin, 2001), but rather an information program giving consumers the GI indication of a specific food product. For a food product to be eligible for a GI indication and symbol, the food must contain at least 10 g carbohydrate per serving, have had GI determination done by an approved laboratory by a standard *in vivo* method, and have a nutritional composition that meets the nutrient criteria for the appropriate food category (Irwin, 2001).

Glycemic Index Limited (2006) published the improvement in consumer response to GI symbols/rating on food products. The study was done through an on-line survey questioning principle grocery buyers older than 18 years. In 2005 only 15 % of consumers could recall remembering the GI symbol on food products, while one year on, in 2006, 25 % of consumers responded positively to remembering the GI symbol on food product labels. When given a list of nutrition claims to choose what they look for on a food label 37.5 % of the respondents said they do look for the GI symbol on food products. Of the respondents, 84 % were aware of the GI concept, with the majority responding positively, being female (92 %). Furthermore, 73 % said they are likely to consider using the GI rating when choosing food, while 54 % responded that they would use the Australian developed GI symbol in food selection.

1.2.6.2 Glycaemic index labelling in the United Kingdom

In the United Kingdom (UK) many of the major supermarket chains have developed their own symbols on packaging and within stores. Some supermarkets have also determined GI of selected healthy products, and supported this with nutritional information in the form of in-store leaflets, websites, journal articles, GI diet advice and diet plans (Mitchell, 2008).

1.2.6.3 Glycaemic index labelling in other countries

The GI concept is accepted as part of medical nutrition therapy in Canada, Australia and New Zealand, and much of Europe (Woodruff, 2010). Although there is no organization in America endorsing GI classification, the Glycemic Research Institute in the United States conducts board certified human *in vivo* clinical trials. According to the Glycemic Research Institute (2010), their *Clinical Trials Program for Glycemic Claim Substantiation* is registered by the governments of the United Kingdom, United States and Canada. According to the Food and Drug Administration (FDA), the European Food Safety Authority (EFSA) and the Canadian government, human clinical trials are required before any glycaemic related claim can be made on food products.

1.2.6.4 Consumer understanding and demand for glycaemic indexed foods

The relationship between the consumer, food labels and food claims is complex. In a review study done by EdComs (2007) on consumer understanding of nutrition and health claims made on food revealed that the least accessed nutritional information was related to protein, vitamins and carbohydrates. As mentioned previously, the Glycemic Index Limited in Australia has tracked the impact of the GI Symbol Program on consumer awareness and understanding since its launch in 2002. In their 2005-2006 survey, more than 500 subjects were interviewed. The 2006 study concluded that the understanding of GI is continuing to increase, and that consumers consider the GI Symbol as a credible signpost for healthier food options (GIycemic Index Limited, 2006). More than half of the respondents reported that they would switch to brands carrying the low GI symbol. Of the consumers questioned, 37.5 % reported that they search for GI indication on the label of foodstuffs. The majority (84 %) reported that they were aware of GI, and 73 % said

they are very likely to use the GI rating when choosing foods from retail shelves (Glycemic Index Limited, 2006). This study indicated that consumer awareness and understanding is increasing in Australia, and that consumers regard the GI concept on product labels as an indication of healthier food choices.

In the United Kingdom, Dragon Brands did consumer research in 2004, on what consumers think about the GI concept (Mackenzie, 2004). The results indicated that the majority of the consumers had an accurate idea about what GI is, with the majority reporting that they got their information from women's magazines, TV and radio coverage or relatives with diabetes. The report concluded that consumers felt that the concept is in line with current trends to avoid processed and refined foods and focus on sustained energy and prolonging the feeling of fullness (Mitchell, 2008).

1.2.6.5 Problems with glycaemic index labelling

Although there has been a global surge in awareness of nutrition and the associated benefits of consuming controlled GI foods, it seems that food labelling has not been able to catch up with the science nor consumer demand in the arena (Mitchell, 2008). Furthermore legislation to control GI labelling has also fallen behind (Mitchell, 2008), as is the case in South Africa.

As mentioned before, considerations for labelling legislation should include which foods or beverages would be allowed to have a GI indication, what methodology would need to be used before the indicative value would be considered accurate, how the value should be expressed so as to be understandable and meaningful to the consumer, and which claims would be allowed to accompany high or low GI classified foods (Venter *et al.,* 2003). Another consideration that should be explored is the implication of including a meaningful range of carbohydrate containing foods that form part of the local South African diet. Food choice will only be significantly influenced by GI when most carbohydrate containing foods on the market contain GI indications to enable comparison within food groups. The complexity of determining GI values for such a variety of food products is evident.

1.3 THE GLYCAEMIC INDEX IN SOUTH AFRICA

1.3.1   Methodology

A South African task force, assembled in 2002 by the Directorate of Food Control, was created to standardise the GI methodology used in South Africa, so as to pave the way for GI labelling and consumer education. Aspects such as 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, were addressed by this task force (Pieters & Jerling, 2005). This task force established a scientifically acceptable and standardized method for GI calculations in South Africa. This method was developed to ensure that GI values generated in South Africa be comparable and compliant with global scientific principles (Badham, 2003).

The increase in the popularity of the GI concept has led to ISO, a non-governmental organization established in 1947, drafting a standard for internationally acceptable methodology. ISO members consist of the leading and recognized national standards organizations of 159 countries (including South Africa). The ISO/FDIS 26642, *Food products – Determination of the glycemic index (GI) and relevant classification*, is still being developed. In March 2010, ISO informed the Codex Alimentarius Commission that the draft would be finalised by the end of June 2010 (FAO/WHO, 2010). No final ISO/DIS 26642 has been received by the South African Department of Health at this time (October 2010). The draft ISO/DIS 264442 is attached in Addendum A.

In summary, the draft ISO/DIS states that *a minimum of 10 healthy subjects, without known food allergy or intolerance, or medications known to affect glucose tolerance should be included in a single trial. Stable doses of certain listed treatment drugs are acceptable. Exclusion criteria include known diabetes mellitus or using antihyperglycemic drugs or insulin used to treat diabetes and related conditions, major medical or surgical events requiring hospitalization within last 3 months, presence of disease or drug influencing digestion and absorption of nutrients, the use of steroids, protease inhibitors, or antipsychotics. The subjects need to consume no food or drink*

other than water for 10 or more hours prior to the test, consume no alcohol on the previous evening nor do vigorous exercise on the morning of the test.

Acceptable reference foods include anhydrous glucose powder (50 g), Dextrose (glucose monohydrate – 55 g), commercial solution used for oral glucose tolerance test containing 50 g glucose, white bread or other specific carbohydrate food of consistent composition and GI. The reference food needs to be tested in each subject at least two and preferably three times on separate days within the immediate 3-month period surrounding the testing of the product.

The amount of test food shall contain 50 g of glycemic (available) carbohydrate. The full amount should be capable of being consumed within the time frame of 12 min to 15 min. 25 g carbohydrate portion can be used for foods which have lower concentration of carbohydrates, i.e. where the bulk of food providing 50 g is unreasonably large, for example fruits. Test food shall be prepared as per instruction on the food label.

The experimental procedure entails that two blood samples are taken from each subject while in the fasting state, with the average taken as the baseline blood glucose concentration and expressed as mmol/l. Subjects then consume the test food at an even pace within 5 to 14 minutes, along with a drink of 1 or 2 cups (250 ml – 500 ml) water, coffee or tea (with 30 ml milk and/or non-nutritive sweetener if desired). Each subject should choose the drink they wish to have and be given the same type and volume of drink for all tests performed by that subject. During testing subjects shall rest. Blood samples shall be taken at 15 min, 30 min, 45 min, 60 min, 90 min and 120 min, and assayed for glucose. Testing postprandial glucose in duplicate is not necessary but is an acceptable option if the laboratory desires (Draft International Standard ISO/DIS 26642, 2008).

As this ISO method has not been finalised, a standard *in vivo* method which should be used in South Africa, was included in the proposed South African draft Regulations for the Labelling and Advertising of Foodstuffs, although no reference is made in the recently implemented regulation (Foodstuffs Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972), No. R. 146, 1 March 2010).

The standard referred to in the draft regulations was based on a method published in 2005 (Brouns, Bjork, Frayn, Gibbs, Lang, Slama & Wolever, 2005). In the draft regulation GI was defined as: *The blood glucose responses of carbohydrate foods i.e., the incremental area under the curve (IAUC) for the increase in blood glucose after the ingestion of 50 g of glycaemic (available) carbohydrate in an individual food (unless the total volume exceeds 300 ml when 25 g of glycaemic (available) carbohydrate from the individual food and the reference food will be acceptable) in the 2 hours for healthy and 3 hours for diabetic individuals from the start of the test meal, as compared with ingestion of the same amount of glycaemic (available) carbohydrate from glucose taken with 300 ml of water spread over a 10-15 minute period, tested in accordance with a defined procedure by an accredited laboratory in the same individuals under the same conditions using the fasting blood glucose concentrations*. The draft guidelines also state that no method whereby a GI value is obtained from calculations can be used for labelling purposes (Department of Health, 2007).

In South Africa, GIFSA, based in Nelspruit, evaluates GI response by means of how fast and to what extent an ingested carbohydrate containing food affects the blood glucose levels of a selected trained panel. The Nutrition Information Centre of the University of Stellenbosch (NICUS) applies a similar method to determine carbohydrate digestion rate in human subjects. The food industry, more specifically Woolworths, also demand in-house *in vivo* testing before GI indications are allowed on their product labels.

All these methods used in South Africa to determine GI generally entail the ingestion of portions of the test food and the standard food (being either white bread or glucose) containing 50g available carbohydrate, by healthy or diabetic subjects in random order after an overnight fast of between 10 and 12 hours. The same subject should repeat the standard food test at least three times from which a mean value is calculated. Blood samples are drawn during sampling at regular intervals (normally every 15 minutes) for up to two hours in healthy subjects and three hours in diabetics (Venter *et al.,* 2003).

Subjects should be trained for the trial at least three days in advance by ensuring the ingestion of a high carbohydrate diet (60 % of total energy), and with 20 % of the energy consumed derived from both protein and fat (Venter *et al.,* 2003). This type of diet is used to ensure optimal substrate induction of enzyme synthesis and activation (Vorster, Venter & Silvis, 1990).

Furthermore, subjects should consume a standard pre-evening meal with an average of 50 % of total energy obtained from carbohydrates, 30 % from fat and 20 % from protein with the aim to standardise potential second-meal effects. During the course of the trial the subjects should consume a weight maintenance diet and normal physical activity should be maintained (Venter *et al.,* 2003).

1.3.2   Labelling regulations relating to the glycaemic index in South Africa

A previously mentioned, the draft labelling regulations (Amendment Foodstuffs, Cosmetics and Disinfectants Act, 54/1972) published for comment in 2007 included recommendations for making GI claims on the labels of foods. The newly implemented Regulations Relating to the Labelling and Advertising of Foodstuffs (Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972), No. R. 146, 1 March 2010) however makes no reference to GI. Throughout the remainder of this document, reference will be made to the draft regulations which did include reference to the GI concept.

The draft regulations state that the GI of a food will be presented as a category claim. The indication of a GI on a food product will only be allowed for foods which have a carbohydrate content providing 40 % or more of the total energy value of the food, for foods with total fat contents that do not exceed 30 % of the total energy value, and with total protein contents not exceeding 42 % of the total energy value. Furthermore the GI label should not indicate a specific numerical value, but rather use low (<55), intermediate (55-70) or high GI (>70) indications. Such indications will be allowed either as a logo, or within the nutritional information table (Department of Health, 2007).

Additional wording in support of the relevant GI categories will be allowed on the product label. low GI foods may use: "*Low GI foods, when eaten in moderate portions at a time, generally provide slow release of energy and improve blood glucose control and may elicit a higher feeling of satiety*" as additional wording. "*Intermediate GI foods generally provide a moderately fast release of energy and are ideal for diabetic individuals after exercise lasting at least one hour or as a special treat*" is the wording allowed for intermediate GI foods. High GI foods may use the following wording: "*High GI foods generally provide a fast release of energy and are ideal for regular sportsmen after one hour's exercise or during and after exercise lasting more than one hour and diabetic individuals during and after exercise lasting at least two hours and more*" (Department of Health, 2007).

Despite the lack of accepted legislation, some food labels in South Africa already display a logo indicating GI (Venter *et al.,* 2003). GIFSA, the most prominent GI laboratory in South Africa, has developed a range of endorsement logos for approved foods. According to GIFSA, this logo is available at a reasonable cost to any manufacturer who wishes to use the logo for a product label, provided the product complies with certain specifications. Several food companies have already had the GI of many of their products determined by GIFSA e.g. Bokomo, Kellogg's, Sasko, Sunbake, Premier Foods, National Brands and Tradekor (GIFSA, 2010).

The procedure to have a product marked is initiated by an application to have the product endorsed by GIFSA. The GI of the product is then determined at a standard fee. This fee includes a written report for the company on the nature of the product, reasons for the test results and possible ways to alter the GI-factor in the manufacturing process. The ingredient content of a product has to be verified by an independent body/laboratory. If the product qualifies for one of the endorsement logos, the logo may be displayed together with the compulsory text, in an approved format. An annual cost, for a minimum period of three years, is applicable (GIFSA, 2010).

These logos have specific criteria, related to specific food groups, to which foods need to adhere before they qualify for endorsement. These logos are presented in Figure 1.1 to 1.4. Figure 1.1 illustrates the "green and positive" image, presented on the packaging of foods which are classified as low GI and low in fat, and are considered foods which can be consumed frequently. If the food product contains more fat, but still remains within the recommended fat content, with a low GI, it could also be considered a healthy food option with the "Often Food" logo (Figure 1.2). Foods which are low in fat, but have an intermediate GI, are labelled as special treats in orange (Figure 1.3), while low fat, high GI foods are labelled as best for exercise in red (Figure 1.4). Additionally, Diabetes South Africa has developed an endorsement logo for food products which are low GI, low in fat, sodium and caffeine and are considered to be suitable for diabetics (Figure 1.5).

In 2008 there were 36 products on the South African market that had a GIFSA endorsement logo (Mitchell, 2008), which increased to 135 products from 37 product-ranges by 2010 (GIFSA, 2010).
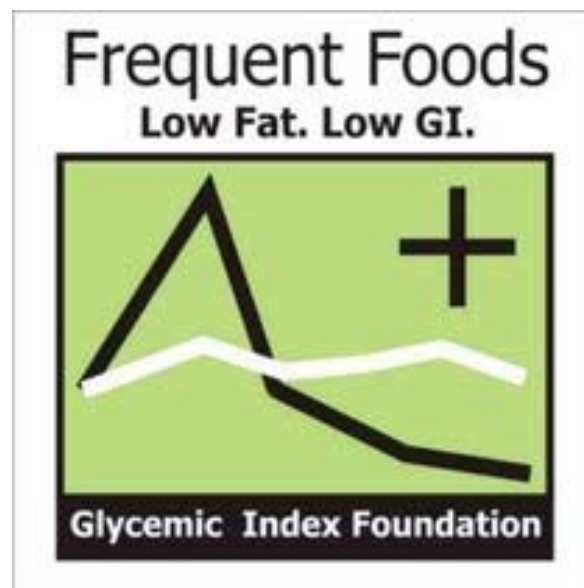
Figure 1.1: Glycemic Index Foundation of South Africa (GIFSA) endorsement logo for foods with a low fat content and a low glycaemic index (GI)

Figure 1.2: Glycemic Index Foundation of South Africa (GIFSA) endorsement logo for foods with a lower fat content and a low glycaemic index (GI)

Figure 1.3: Glycemic Index Foundation of South Africa (GIFSA) endorsement logo for foods with a lower fat content and an intermediate glycaemic index (GI)

Figure 1.4: Glycemic Index Foundation of South Africa (GIFSA) endorsement logo for foods with a lower fat content and a high glycaemic index (GI)

Figure 1.5: The Diabetes South Africa logo for foods suitable for diabetics with a low glycaemic index (GI) and a low fat, sodium and caffeine content

1.3.3 Consumer understanding of food labels

The health benefits associated with choosing foods based on blood glucose response, as part of a food's complete nutrient matrix should be well communicated to the consumer if the concept is to succeed and not be misleading (Venter *et al.,* 2003).

Consumer studies conducted by Glycemic Index Limited (Australia) have evaluated consumer awareness and understanding of their GI Symbol Program since its initiation in 2002. These studies have included telephonic surveys between 2002 and 2004 and an online survey between 2005 and 2006. In the 2005/2006 survey, more than 500 subjects were interviewed (male and female). The 2006 consumer study showed that 87 % of respondents agreed that the GI symbol is a helpful shopping tool when looking for healthy food choices. A total of 60 % of respondents indicated that they were "very likely" or "somewhat likely" to use the symbol when shopping.

Low GI was considered by most respondents to have a perceived health benefit. A total of 45 % of all respondents thought that the GI symbol was something to do with energy release, 30 % thought that it was something to do with the method of testing or rating, and 24 % thought that it was something to do with sugar metabolism. Conclusions from this consumer study suggest that awareness and understanding of GI is continuing to increase and that the GI symbol is a credible signpost for healthier food choices (Glycemic Index Limited, 2006).

A European Food Information Council (EUFIC) study (2005) found that consumers appreciate the benefits of nutritious and healthy meals, but feel the language often used on food labels is too complex and technical, and too far removed from the actual experience of eating, which is simple and immediate. Thus, it can be said that health labelling should be made user friendly, through simple language use and utilization of other simple tools. Irwin (2001) believes that consumers will gain a better understanding of the concept and the relationship between GI and health when presented with a simple and easily recognisable symbol.

In South Africa the language barriers and diverse educational levels of the population need to be taken into consideration in the development of any labelling tool. Visual aids are especially

important for low-socioeconomic groups (Consumers' Association, 2006). Studies from the UK and Europe have shown that the use of colours on food labels to indicate high or low levels of nutrients is well understood by consumers (Synovate, 2005). Most studies show that consumers are better at using visual information rather than numerical information. Studies on consumer interpretation of numerical information on food labels found that consumers found it difficult to interpret numerical information correctly (EdComs, 2007). It can thus be said that in general consumers prefer simple, non-technical claims, e.g. category claims, such as used by GIFSA in their endorsement logos. Consumers should be provided with choices within each food category, and not just between certain individual products containing current GI indications. To achieve this goal it will be necessary to determine the GI of many more food products.

1.4 RELEVANT PROBLEMS ASSOCIATED WITH THE GLYCAEMIC INDEX CONCEPT

1.4.1 Lack of standardised methodology to determine the glycaemic index

Much has been debated about the validity of the GI values given to food products. One of the main constraints of GI labelling is the lack of standardised methodology to produce reliable numerical GI values (Venter *et al.,* 2003). In many cases the variability in results is high between foods, laboratories and even between and within human subjects (Wolever, Vorster, Bjork, Brand-Miller, Brighenti, Mann, Ramdath, Granfeldt, Holt, Perry, Venter & Xiaomei, 2003; Wolever, Brand-Miller, Abernethy, Astrup, Atkinson, Axelsen, Björck, Brighenti, Brown, Brynes, Casiraghi, Cazaubiel, Dahlqvist, Delport, Denyer, Erba, Frost, Granfeldt, Hampton, Hart, Ha¨tönen, Henry, Hertzler, Hull, Jerling, Johnston, Lightowler, Mann, Morgan, Panlasigui, Pelkman, Perry, Pfeiffer, Pieters, Ramdath, Ramsingh, Robert, Robinson, Sarkkinen, Scazzina, Sison, Sloth, Staniforth, Tapola, Valsta, Verkooijen, Weickert, Weseler, Wilkie, & Zhang, 2008).

In 2003 the results from an inter-laboratory study between seven research groups around the world experienced in GI methodology (including GIFSA), reported that the standard deviation (SD) for GI values obtained from different laboratories for the same food products to be around 9. This translates into a 95 % confidence interval (CI) of about 18 (Wolever *et al.,* 2003; Wolever *et*

al., 2008). In terms of GI categories (low < 55; intermediate 55 – 70; and high GI > 70), a difference of 18 could in a worst case scenario mean a high GI food (GI > 70) could be incorrectly classified as a low GI food (GI < 55). A similar study by Van Bakel, Slimani, Feskens, Du, Beulens, van der Schouw, Brighenti, Halkjaer, Cust, Ferarri, Brand-Miller, Beuno-de-Mesquita, Peeters, Ardanaz, Dorronsoro, Crowe, Bingham, Rohrmann, Boeing, Johansson, Manjer, Tjonneland, Overvad, Lund, Skeie, Mattielo, Salvini, Clavel-Chapelon and Kakks (2009) found methodological challenges to be the greatest constraints to nutritional epidemiology, and the authors recommend that a consistent global method be implemented.

1.4.2   Lack of glycaemic index data for common foods and meals

Although variation in GI data between and within countries could be attributed to methodological problems, it should be considered that inherent differences in food products produced in different countries also contribute to differences in glycaemic response. The GI for potatoes for instance varies greatly between countries, cultivation regions, cultivars and maturity. Consequently, own GI data on local food commodities becomes increasingly important.

Chapter 3 describes the testing of two different potato cultivars grown in South Africa. Notable differences in GI were found between these two cultivars, more so than between a singular cultivar ranging from the baby to the full grown form. In addition, Chapter 4 presents a case study on a South African potato cultivar which was found to be a low GI variety. Unfortunately, differentiations are often only made between growth stage and size of potatoes in international tables and when recommending diets based on GI and little reference is made to the specific cultivar.

The 2002 edition of the International Table of Glycemic Index and Glycemic Load Values (Foster-Powell *et al.,* 2002) contains more than 1300 data entries. This table indicates both the GI when calculated using glucose as the reference food, and GI when using white bread as the reference food, the citation reference, the type and number of subjects, the reference food used and the specific time period, the serving size administered to the subjects, the amount of available

carbohydrates (grams) and the glycaemic load (GL) per serving. GL was estimated by multiplying the food's listed GI value with glucose as the reference food by the listed g carbohydrate per serving and divided by 100 (Foster-Powell *et al.,* 2002). In 2008, Atkinson, Foster-Powell and Brand-Miller (2008), systematically tabulated published and unpublished sources of reliable GI values. The table lists 2480 individual food items. The GL is becoming an increasingly popular expression of the complete glycaemic effect which the meal would induce after consumption. It is calculated by taking the percentage of the food's carbohydrate content per portion and multiplying it by its GI value. The GL gives an indication of both the quality and the quantity of the food portion or meal. The GL can only be calculated when the GI of the ingredients in the meal are known.

Although there are three times more entries in the 2002 edition than were published in the original tables (1995), and almost double this in the 2008 article, there are still numerous carbohydrate foods, many of which are staple foods, which lack GI values. Many values for foods could be expanded to include differences between cultivars, regions and countries.

The FAO/WHO Expert Consultation Report on Carbohydrates in Human Nutrition (FAO/WHO, 1998) concluded that glycaemic response data should be supplemented with values for local foods and meals, as food variety and cooking could have significant effects on glycaemic response. There is a need for an assortment of GI indications on different food products and meals, including starch based staple foods prepared in different ways, in a country like South Africa with our diverse eating culture,.

Food choice will only be influenced by GI, and accurate recommendations can only be made, if carbohydrate food products can be compared. To enable this, most of these carbohydrate foods need indications of GI. Own South African GI data, on local and cultural foodstuffs, are needed to make the GI message more comprehensive and effective.

1.4.3 Lack of access to inexpensive, reliable methodologies

Although there is a need for ample GI data, the South African food industry and government tend to neglect the determination of the GI content of foods, probably due to cost. At present, only *in vivo* methods are used to determine GI in South Africa. The use of human subjects to determine GI is currently proposed in the draft labelling regulations, although this is not yet accepted because the Department of Health, Directorate: Food Control is awaiting approval of the ISO/DIS 26642 standard method for GI determination.

Unfortunately, using human subjects is time consuming and expensive (currently, GI determination at GIFSA costs R7 500 per foodstuff (Delport, GIFSA, Personal communication, 2010)). Furthermore, data collected prior to the implementation of the regulation might not be valid due to differences in methodology.

As there are only a limited number of own South African GI data listed in The South African Glycemic Index and Load Guide (Steenkamp & Delport, 2005), and most data values are for product ranges from the larger food companies, it can be assumed that the local and smaller food industries might be hesitant to spend money to determine GI by this method. This could possibly be due to two main constraints. Firstly because of uncertainty about the outcome of the GI tests, i.e. a food might not fall into the GI category desired by the company. Secondly, there is uncertainty about the current approved methodology for determining GI. If new regulations are published which specify a different methodology, then previously determined GI values may no longer be applicable.

For consumers and the industry to have adequate exposure to a range of GI labelled food products and meals, there is a need for an alternative, cheaper and less time consuming method to predict the glycaemic response after ingestion. After GI predictions are made, further *in vivo* analysis can be done for labelling purposes if required and once such legislation is implemented. Such a rapid assessment method would be valuable from a food product development point of view and could eliminate the two main constraints listed previously.

1.5 PROBLEM STATEMENT

Information on food packaging relating to the detrimental effect on health of fat and fat fractions, has evolved over a long period of time and is understood by consumers after the right education was offered (Browne, 2006). The possibility exists that the same could be done for the GI classification of carbohydrates. The GI should not be used as a sole reference for food choice, as a healthy diet can never be selected from a single nutrient numbering system, but requires consideration of many nutritional factors (Ludwig, 2007). Although the GI concept is complex with inherent complications, it seems to be a feasible option for classifying carbohydrate rich foods based on their GI within a balanced diet derived from healthy food choices selected from all the different food groups.

Two of the main constraints of GI labelling are the lack of standardised methodology to produce reliable numerical GI values, and the limitations of borrowing GI values from other countries (Venter *et al.,* 2003). But although the variability in results is often high between foods, laboratories and human subjects, most methodologies give a relatively accurate classification of GI into one of the three categories of either high, intermediate or low GI. These categories are easier to understand and interpret as part of a healthy diet than numerical values, also when considering the impact which mixed meals could have.

Currently in South Africa only *in vivo* methods are used to determine GI. Rapid analysis methods have been evaluated worldwide. Multi enzyme hydrolysis, the determination of rapidly available glucose and amylolytic hydrolysis are some examples of methods which have been tested as rapid *in vitro* methods to predict the glycaemic index of foods (Englyst Vinoy, Englyst & Lang, 2003). In various cases these values have correlated well with the GI values determined by *in vivo* methods. The criticism against *in vitro* methods is that they do not provide a numerical GI value, but only produce an indication of the specific food's glycaemic response as high, intermediate or low. Because the health consequences of different carbohydrate rich foods are only based on their categorisation as high, intermediate or low GI, and not on their specific GI values, the *in vitro* method can make a useful contribution.

Manufacturers seldom require the precise GI of a food, and an indication of high, intermediate or low GI is usually sufficient. Such an indication can serve as a reference for additional GI research through *in vivo* methods for labelling purposes if so required (Alldrick, 2006). Taking into consideration the South African situation, Browne (2006) stated that to enable the GI message to become a primary public health communication message in South Africa, more foods identified within the GI ranges, and within appropriate price classes, for all sectors of the population, should be made available. A good example of the need for access to GI information across socioeconomic groups in South Africa is the high incidence of NIDDM, a disease of both affluence and the poor. Obesity is also prevalent across all socioeconomic groups in South Africa.

A rapid, inexpensive and reliable method of analysis to classify a food within the categories of GI could be beneficial for the South African food industry. GI category indication during product development will provide valuable indication to the food manufacturer for product formulation prior to launch, and if the anticipated GI is predicted, further *in vivo* analysis could be done to adhere to possible future labelling requirements once these are accepted. A visual summary of the development of a rapid assessment method to predict GI in South Africa is presented in Figure 1.6.

1.6 STUDY OBJECTIVE

The objective of this study was to develop an alternative, less expensive and rapid method to predict glycaemic index (GI) in South Africa. The main requirement of the method was that it should accurately and reliably predict the GI of South African foods, at a low cost, within a reduced time frame.
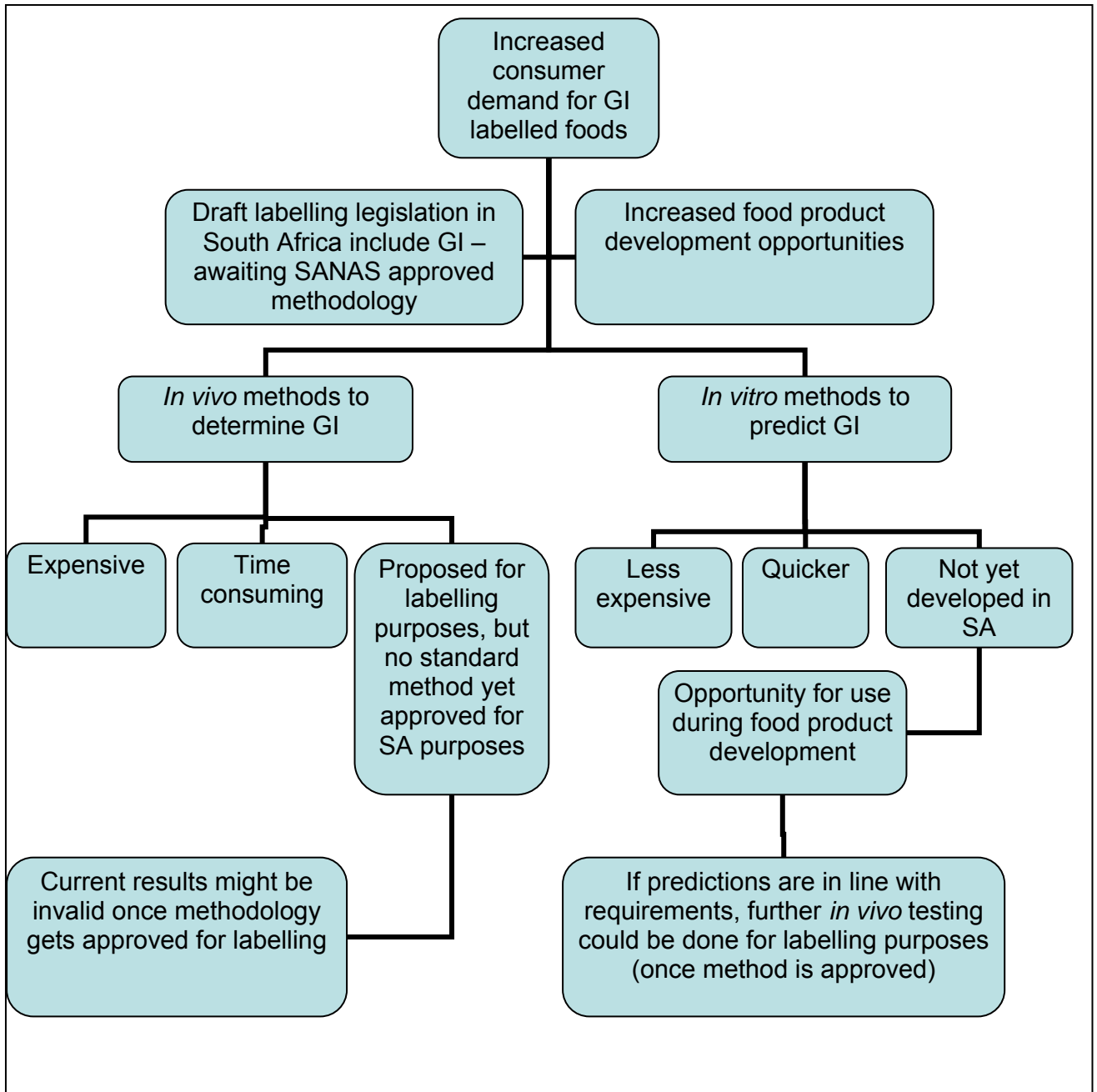
Figure 1.6: Schematic diagram to explain the context of the proposed development of a rapid glycaemic index (GI) assessment method for South Africa

1.7 THESIS LAYOUT

The thesis is presented in chapter format, each chapter focusing on a separate section of the work done.

CHAPTER 1: INTRODUCTION AND JUSTIFICATION

In Chapter 1 an introduction to the study is presented. It includes a review of the practical implications relating to the concept of the GI and presents a brief summary of the health benefits, international recognition, labelling prospects and consumer responses towards GI. The problem statement and motivation behind the development of the rapid assessment method to predict GI for South Africa are also presented in this chapter.

CHAPTER 2: METHODS USED TO DETERMINE AND PREDICT THE GLYCAEMIC INDEX

Chapter 2 presents a review of the *in vivo* and *in vitro* methods currently used to determine or predict GI.

CHAPTER 3: DEVELOPMENT OF A RAPID GLYCAEMIC INDEX ASSESSMENT METHOD

In Chapter 3 the development of the analytical procedure to determine hydrolysis index (HI) is presented. Repeatability and reliability of the method to predict GI are also presented in this chapter.

CHAPTER 4: THE APPLICATION OF THE DEVELOPED RAPID ASSESSMENT METHOD TO PREDICT GLYCAEMIC INDEX

In Chapter 4 the developed rapid assessment method was evaluated for accuracy. Results from *in vivo* GI trials on two food products were compared with the results obtained with the same food products by using the developed rapid assessment method. A theoretical inter-laboratory study is also included in this chapter.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

In this concluding chapter the main findings of the research are presented in terms of the current South African nutrition situation. The developed method is summarized and presented in a standard format which can be used in future to test samples. Recommendations are presented for future actions.

CHAPTER 6: REFERENCES

All the literature resources which were referenced in the dissertation are presented in alphabetical order in Chapter 6.

CHAPTER 2: METHODS USED TO DETERMINE AND PREDICT THE GLYCAEMIC INDEX

This chapter presents a review of the in vivo and in vitro methods currently used to determine or predict glycaemic index (GI).

2.1 INTRODUCTION

The GI is a classification of the blood glucose raising potential of carbohydrate rich foods, mostly used in dietary advice for people with diabetes, dyslipidaemia and those practising endurance sports (Wolever *et al.,* 2003). The glycaemic indices of several foods are published in international tables (Foster-Powell & Miller, 1995), and recommendations have been made to include GI values of foods together with their other nutritional components in food composition databases (Neuhouser, Tinker, Thomson, Caan, Van Horn, Snetselaar, Parker, Patterson, Robinson-O'Brien, Beresford & Shikany, 2006; Menezes *et al.*, 2009). Various methodologies have been developed to determine the GI values of foods, but for every method both positive and negative criticisms exist.

The original, and most commonly accepted, carbohydrate test for GI determination is *in vivo*, involving the ingestion of a food sample containing 50g carbohydrate and then measuring the rate at which sugars absorb through the small intestine (Brouns *et al.,* 2005). Low GI carbohydrates are classified as those that are digested and absorbed at a slow pace which leads to a low glycaemic response, while high GI carbohydrates are rapidly digested in the gastro intestinal tract (GIT), and absorbed quickly from the small intestine showing a high glycaemic response. This classification using the rate of digestion and absorption as well as glycaemic response is also tested using various *in vitro* methods, which are mostly digestion models mimicking the human GIT (Brouns *et al.,* 2005).

2.2 INTRODUCTION TO CARBOHYDRATE DIGESTION IN THE HUMAN BODY

The major energy carrying components in the human diet are starches, sugars, fats and proteins, often referred to as macronutrients. These components need to be hydrolysed into smaller molecules in the human GIT before they can be absorbed and further metabolised in the rest of the human body (Bender, 1997). The human GIT is shown in Figure 2.1. The main regions in this GIT are the mouth, the stomach, the small intestine, the large intestine and the rectum. The digestion of carbohydrates starts with the hydrolysis of the glycosidic bonds between saccharide units making up the carbohydrate, to liberate small oligosaccharides, and free mono- and disaccharides which can migrate through the wall of the small intestine. The enzymes which catalyse the hydrolysis of starch are amylases, which are secreted in both the saliva and the pancreatic juices (in the small intestine).
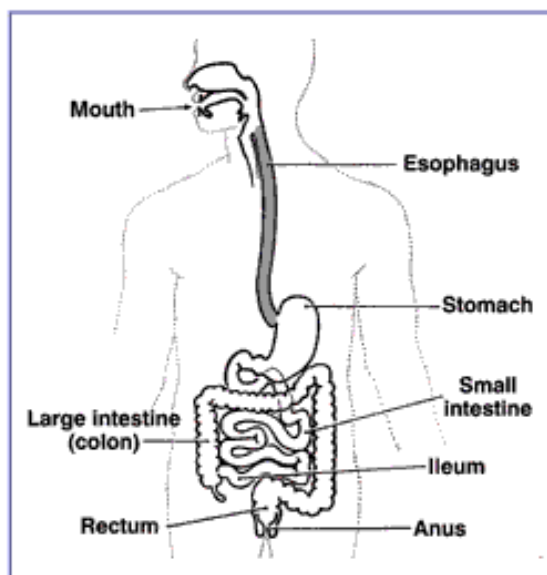
Figure 2.1: The human gastro intestinal tract (GIT) (Cameron, 2002)

The main functions of the mouth, in terms of digestion and absorption of carbohydrates, are chewing to increase the surface area of the molecules, and the initiation of starch hydrolysis catalysed by amylase enzymes in saliva. Food particles are swallowed after chewing and propelled to the stomach via the oesophagus. In the stomach proteins are hydrolysed via pepsin

enzymes, a phase which is often called the gastric phase. The acidic conditions in the stomach denature the proteins, increasing the surface area which proteolitic enzymes can hydrolyse. The acidic conditions also denature the amylase enzymes which are introduced in the mouth, yielding them inactive (Bender, 1997). Various vitamins, minerals and even some carbohydrates are released during this phase as proteins are denatured and uncoiled, which disrupt various bonds.

Once the food exits the stomach, it enters the small intestine which is responsible for most of the starch hydrolysis by amylase enzymes secreted by the pancreas. The acids from the stomach are buffered back to a neutral pH. Most of the products of digestion are absorbed through the intestinal walls of the small intestine into the rest of the body. Undigested food particles continue to the large intestine where water is absorbed and bacteria metabolise some undigested carbohydrates. The undigested gut contents are stored in the rectum prior to evacuation as faeces (Bender, 1997).

2.3 CARBOHYDRATE CLASSIFICATION

Similar to all other macronutrients, the primary classification of carbohydrates is by molecular size, as determined by degree of polymerization, the type of linkage, and the characteristics of individual monomers (FAO/WHO, 1998). This classification can be compared to that of fats, which is based on carbon chain length, number and position of double bonds and their configuration as *cis* or *trans.* Cummings and Stephan (2007) recommend a chemical approach to ensure coherent and enforceable measurements and labelling, to form the basis for terminology and an understanding of the physiological and health effects of both these macronutrients.

Chemically, carbohydrates are divided into three categories, namely sugars (one to two polymers), oligosaccharides (three to nine polymers), and polysaccharides (ten or more polymers) (McWilliams, 2009). A variety of methods are available for the measurement of the carbohydrate content of food. However, as said by Cummings and Stephan (2007) *"in classifying carbohydrate by its chemistry, the principle challenge is to reconcile the various chemical divisions with those that reflect physiology and health".* Any classification which is solely based on

chemistry, does not allow effective indication of nutritional benefits, which are specifically relevant to carbohydrates as each of the three categories previously mentioned have a variety of overlapping physiological effects. In addition to chemical analyses, terminology based on physiological properties, e.g. glycaemic response, will help to focus on the potential health effects of these macronutrients and will help to identify specific foods within a food group which are more likely to form part of a healthy balanced diet.

The GI concept is based on the distinction whether or not the carbohydrate source does or does not directly supply carbohydrates as an energy source into the blood stream following digestion and absorption in the small intestine (Cummings & Stephan, 2007). The physiological and health benefits of carbohydrates are not only dependent on their primary chemical form, but also on their physical properties including water solubility, gel formation, state of crystallization, association with other molecules and the aggregation into the complex structure of plant cell walls (Cummings & Stephan, 2007). The GI system aims to incorporate all these factors when classifying carbohydrate rich foods based on their glycaemic release of energy into the human body.

2.4 THE IMPORTANCE OF RELIABLE ANALYTICAL PROCEDURES TO DETERMINE THE GLYCAEMIC INDEX

The usefulness of the GI of a food for nutritional advice depends greatly on its reliability (Williams, Venn, Perry, Brown, Wallace, Mann & Green, 2008). A universal criticism of GI is that the measurements lack accuracy and precision (Van Bakel *et al.,* 2009; Pi-Sunyer. 2002). Accuracy, in the context of food choice, refers to the exactness with which a food value, in this case GI, can predict blood glucose control (Monro, 2005). It is important for food values to accurately predict outcomes to be successful and concerns have been expressed on the variability intrinsic to GI values (Pi-Suyer, 2002; Monro, 2005). Monro (2005) also mentioned that the accuracy of GI to predict glycaemic control becomes inaccurate when it is applied across food groups, or to mixed meals. It should however, kept in mind that the reliability of the GI to distinguish between foods

depends on the magnitude of between-food variance, and reliability will decrease as the difference in GI between foods becomes smaller (Wolever, 2010).

The European Prospective Investigation into Cancer and Nutrition Study reported ecological comparison and correlation studies from nine countries, and conducted inter-method comparisons (Van Bakel *et al.,* 2009). They observed that mean GI differences were significant for most centres, and that inter-method variations were considerable. The authors concluded that a more consistent methodology to attribute GI values to foods and validated GI values is needed to derive meaningful GI estimates for national epidemiology (Van Bakel *et al.,* 2009).

As previously mentioned, an inter-laboratory study between seven research groups reported a SD of nine for GI values. This translates into a 95 % CI of about 18 (Wolever *et al.,* 2003; Wolever *et al.,*2008). This difference of 18 can mean the difference between a food being classified as a low GI food (GI < 55) or a high GI food (GI > 70). This possibility of variation calls for additional research to designate accurate GI indications to foods.

The various variables between different methodologies are considered to be the main causes of variation in GI results obtained for the same foods products (FAO/WHO, 1998). In the past, different research institutions have used different sampling techniques (either capillary or venous blood samples drawn), times after ingestion used for calculating the glucose response curve (between one and three hours) and different reference products (glucose or white bread) (Arvidsson-Lenner *et al.,* 2004). No specific guidelines were previously available for *in vivo* methodology until the 1998 FAO/WHO Expert Consultation published a report on Carbohydrates in Human Nutrition (Arvidsson-Lenner *et al.,* 2004).

The human variability factor should also be considered as a possible reason why GI data differs between countries, laboratories and even between individuals from the same trial. Very often, when using human subjects, the GI of a food is estimated as a mean value from a small number of human subjects (n = six to twelve), and with standard errors ranging between three and 15 (Williams *et al.,* 2008). Although GI has previously been found to be unreliable to distinguish

between individuals, it should be remembered that this is not a desirable property as GI is a food-based measure. As the reliability of GI is low between individuals, it means that GI does not distinguish between individuals (Wolever, 2010).

2.5 *IN VITRO* METHODS USED TO DETERMINE GLYCAEMIC INDEX

The FAO/WHO states that published GI data should be supplemented wherever possible with tests of local foods as normally prepared because of the important impact of food variety and cooking on glycaemic responses (Nantel, 1999). The guidelines issued by the FAO/WHO recommend that GI tests should be repeated in at least six different individuals and that measurements with the reference meal should be carried out on three separate occasions. The draft ISO/DIS 26642 (2010) recommends that the reference food should be tested in each subject at least two and preferably three times on separate days within the immediate three month period prior to testing a food product. This means that ten people are often recruited per trial to compensate for any drop-outs or irregularities, which is often an expensive exercise and requires high levels of control and effort.

Because *in vivo* methods rely on human subjects and are time consuming and expensive, alternatives to the methodology have been sought. Various *in vitro* methods have subsequently have been developed in the aim to provide values representative of true GI values.

As an alternative to using only human subjects (through *in vivo* methods), *in vitro* methods have been developed to determine the rate at which carbohydrates would be released from a food, based on the hypothesis that carbohydrate release predicts GI. These *in vitro* methods include methods simulating the human GIT and determining the rate of carbohydrate hydrolysis, as well as methods calculating the degree of digestion by determining the different starch fractions including total digestible starch (Foster-Powell *et al.,* 2002).

In many cases *In vitro* methods have proven useful for product development and research on the factors influencing the rate of digestion of carbohydrates in foods, since they are regarded as less expensive and rapid alternatives (NA, 2004). Currently numerous product development activities

include the utilization of *in vitro* methodologies to predict GI. As an example, Zabidi and Aziz (2009) estimated the GI of bread substituted with different *chempedak* seed flours (indigenous seed flour from Thailand) based on starch hydrolysis procedures and concluded that *chempedak* reduces the estimated GI of bread to a great extent.

Unfortunately only a few foods have been subject to both *in vitro* and *in vivo* analysis in a single batch, thus it is difficult to assess whether *in vitro* methods accurately predict GI (Foster-Powell *et al.,* 2002). Also, various criticisms concerning *in vitro* methods have been voiced. Some of these concerns include the lack of the incorporation of variables present in humans, such as the varying rate of gastric emptying, chewing differences and pre-meal effects etc. in human subjects. Apart from these, one of the most noteworthy impediments for *in vitro* glycaemic prediction, as for *in vivo* analysis, is the lack of standardised methodology (Woolnough, Monro, Brennan & Bird, 2008).

In contrast to *in vivo* methods, results from *in vitro* methods are not considered acceptable in terms of labelling etc. as no precise value is obtained. *In vitro* methods are mostly considered to be screening tools in product development, to be followed by *in vivo* analysis if necessary. If a low cost, rapid screening tool could be developed to accurately predict the GI of a food, and the GI would fall in the same GI category as that determined by *in vivo* methodology required for labelling purposes, it would benefit both the food and health industries.

2.5.1   Basic principles of *in vitro* methodologies

In recent decades much research has been done on glycaemic carbohydrate and health, and subsequently many methods have been tried globally to determine the glycaemic effects of foods. Because *in vivo* methods are time consuming and expensive, various *in vitro* methods to obtain GI values, have been developed in different parts of the world. Most of these methods entail the simulation of oral, gastric and intestinal digestion processes (Woolnough *et al.,* 2008), and measure the rate at which carbohydrates are released. The accepted hypothesis is that this rate will predict the rate of glucose release in the human body, and thus predict GI.

The implementation of the mimicked human physiological methods can differ considerably. Differences start from the oral phase (rate, method and extent of chewing as well as the inclusion of salivary amylolitic enzymes) through to the gastric phase (inclusion and rate of gastric emptying and proteolysis) as well as differences within the small intestinal phase such as type and concentration of amylolytic enzyme, type of dialysis device, extent and type of stirring (Woolnough *et al.,* 2008). Differences in measurement of the end results also differ between authors. This will be discussed below according to each phase through which food particles travel from ingestion until absorption, namely the oral phase, the gastric phase and the small intestinal phase.

2.5.1.1 The oral phase

During human chewing, the mechanical structure of food is disrupted while exposing the carbohydrate portion to salivary amylase enzymes which causes a degree of breakdown until reaching the stomach (Gibney, Vorster & Kok, 2002). This process can be mimicked with ease, and various techniques for duplicating the human oral phase are being used. The chewing process has been simulated by using food processors (Brennan, Blake, Ellis & Shofield, 1996), mincers (Englyst, Englyst, Hudson, Cole & Cummings*,* 1999; Araya, Conteras, Alvina, Vera & Pak, 2002), and sieves with various gauges (Brighenti, Casiraghi & Baggio, 1998). Some authors, instead of using *in vitro* methods to duplicate the oral phase, have also employed volunteers to chew the food under standardised conditions before subjecting the food samples to further *in vitro* analyses (Muir, Birkett, Brown, Jones & O'Dea, 1995; Akerberg, Liljeberg, Granfeldt, Drews & Bjork, 1998).

When food is chewed, it is to a certain extent exposed to salivary amylase enzymes. Some authors have included brief exposure to amylase enzymes (Brighenti, Pellegrini, Casiraghi & Testolin*,* 1995; Mishra, Monro & Hedderley, 2008), while others have excluded the salivary amylase phase due to the minimal effect it may have on digestion during the short period of exposure before becoming deactivated by acidic conditions in the stomach. As monetary issues

are prime motivators for *in vitro* methods, limiting unnecessary expenses should continually be considered.

Chewing is a subjective process which varies between individuals and between foods. Woolnough *et al.* (2008) performed a study using various methodologies to simulate chewing. They found that different mechanisms significantly influenced the hydrolysis curve profiles for foods containing robust structures such as wheat, because *in vitro* methods such as mincing, chopping and sieving, overestimated carbohydrate digestibility compared to those foods which where chewed by human subjects. Foods with less robust structures, such as chick peas and bread, did however show little difference in hydrolysis when chewed by *in vitro* or *in vivo* methodologies.

To represent the oral phase during the development of the rapid assessment method, 40 ml buffer solution (pH 6.9; 37 °C) was added to a food sample containing 2 g carbohydrate, and homogenized with an Ultra Turrax for a maximum period of two minutes.

2.5.1.2 The gastric phase

Within the stomach the protein component of the meal is digested with the aid of peptic enzymes within an acidic environment (Gibney *et al.,* 2002). The rate of emptying of the mixed food (called chyme) from the stomach into the small intestine is controlled by the pylorus. This process happens periodically, depending on various factors, including food quantity, viscosity and pH (Bender, 1997).

Analysts differ in how they execute this proteolytic step. Holm, Bjork, Drews and Asp (1986) as well as Brighenti *et al.* (1995) incubated samples with pepsin for 60 minutes, while Granfeldt, Bjork, Drews and Tovar (1992), Muir and O'Dea (1992) and Englyst *et al.* (1999) incubated their samples for only 30 minutes.

Although Englyst *et al.* (1992), Brighenti *et al.* (1998), Urooj and Puttraj (1999) and Araya *et al.* (2002) did not include a pepsin step in their methodologies, this proteolytic step is usually

included in *in vitro* methodology. The main reasons for including this step are the role that protein digestion plays in disrupting the protein-dependent food structures and the starch-protein interactions which will enhance the accuracy of further treatments and analyses (Woolnough *et al.,* 2008). Woolnough *et al.* (2008) tested the effects of different proteolytic methodologies on hydrolysis. They evaluated the effect of the omission of the proteolytic step, as well as the effect of ten minute, 30 minute and 60 minute pepsin incubation periods on the hydrolysis of starch. Most foods showed little difference in the rate and extent of sugar release over time, thus proving to be independent of proteolysis. However, certain foods, such as pasta showed small differences in rate of starch hydrolysis over time due to their dense protein matrix (Woolnough *et al.,* 2008). It should be noted that although pasta is not considered a high protein food, it contains a continuous protein matrix which entraps starch granules and/or limits starch hydrolysis by alpha amylase, which affects GI. The number of times pasta is passed through sheeting rollers during preparation, has been found to play an important role in the structural and physicochemical properties of this protein matrix. Proteins seem to dissociate more from the starch granules with increase in sheeting passes, thereby allowing an increased degree of digestion of starch and a subsequent increase in GI (Kim, Petrie, Motoi, Morgenstern, Sutton, Mishra & Simmons, 2008).

To represent the gastric phase during the development of the rapid assessment method, the homogenized sample was adjusted to pH 2.5 with 5 % orthophosphoric acid and 2 ml of a pepsin enzyme solution was added. The sample was then placed in a shaking water bath (37°C) for one hour.

2.5.1.3 Small intestinal phase

After emptying from the stomach through the pylorus the food chyme enters the small intestine (Gibney *et al.,* 2002). The food chyme is mixed with the amylase, and the starch fractions are hydrolysed as the chyme travels through the small intestine with the aid of peristaltic movements (Gibney *et al.,* 2002). The starch fractions are hydrolysed into their monosaccharide fragments. These are absorbed through the small intestinal wall into the portal blood where they contribute to glycaemic response.

As stated by Woolnough *et al.* (2008), "*during the intestinal phase of any in vitro scheme the fundamental occurrence is hydrolysis of sample by alpha amylose within the constraints of physiological parameters*". Depending on the required result, various authors measure the hydrolysate at different times. For the determination of available carbohydrate, measurements are taken only at the end of the incubation period (Jenkins, Wolever, Jenkins, Thorne, Lee, Kalmusky *et al.,* 1983). The rate of carbohydrate digestion is measured by taking samples of hydrolysate at different time points, as was done by Englyst *et al.* (1992) who took samples at 20 minutes and at 120 minutes. Rapidly digestible starch were those samples measured at 20 minutes, while slowly digestible starch was those samples measured at 120 minutes, and the starch which was not digested by 120 minutes was termed resistant starch. Goni *et al.* (1997) measured hydrolysed starch fractions every 30 minutes for a period of three hours which measured the rate of starch digestion, in similar fashion to what is done when drawing blood glucose samples from human subjects during *in vivo* trials. The amount of starch digested at each time point was expressed as a percentage of total starch, and the area under the concentration-over-time curve (AUC) was calculated and compared against a reference food (white bread), which is similar to the current accepted *in vivo* methodology. In fact, Goni *et al.* (1997) compared the obtained *in vitro* values of hydrolysis with obtained *in vivo* GI values and found a significant correlation at 90 minutes of digestion.

The method used to simulate the peristaltic movement in the small intestine, as well as the extent of mimicking the small intestinal process also differs between analysts. Englyst *et al.* (1992) added glass balls in screw top tubes containing the mixture and placed them horizontally into a shaking water bath. Samples for analysis were directly drawn out of these tubes. Goni *et al.* (1997) did not add glass balls and only placed tubes into a shaking water bath. Goni *et al.* (1997) also drew samples directly from the mixture for analysis. Akerberg *et al.* (1998) used magnetic stirrers to simulate peristalsis. Brighenti *et al.* (1995) transferred the mixture into dialysis bags which were submerged into a buffer solution from where the amount of reducing sugars were measured from the dialysate every 30 minutes. Mixing was achieved by adding glass balls to the dialysis tubes and inverting the tubes every 15 minutes. Brighenti *et al.* (1995) measured a sugar

diffusion index, based on the amount of maltose diffusion across the dialysis tube, and compared this against equivalent reference values from white bread.

To represent the small intestinal phase during the development of the rapid assessment method, the acidic samples from the gastric phase were removed from the shaking water bath and adjusted to pH 6.8 ± 0.2 with 50 % KOH. An Alpha amylase solution was added, and the mixture was transferred into dialysis tubes. The tubes were inserted into 500 ml flasks containing 400 ml buffer solution (37°C), and placed in the shaking water bath (37°C). 40 ml of the buffer solution was extracted every 30 minutes for a total period of three hours. The sugar concentration which diffused into the buffer solution over time was determined.

2.5.2   Determining the amount of rapidly available carbohydrates in foods

Determining the amount of rapidly available sugars is based on the classification of dietary carbohydrates in a food taking into account the site, rate and extent of digestion, as opposed to the classification based on degree of polymerization (Figure 2.2) proposed by the FAO/WHO report on Carbohydrates in Human Nutrition (1998). According to Cummings and Stephen (2007), the main challenge when classifying carbohydrates on chemistry alone, is to reconcile the various chemical divisions with those reflecting physiology and health; they point out that each of the major chemical classes has a variety of overlapping physiological functions (Table 2.1).

Classifying foods according to the amount of rapidly available sugars divides dietary carbohydrates into sugars, starch fractions, and non-starch polysaccharides. Furthermore, it groups them into rapidly available glucose (RAG) or slowly available glucose (SAG), depending on what amount of glucose is likely to be available for rapid and slow absorption in the human small intestine (Gibney *et al.,* 2002). Englyst *et al.* (1999) predicted that RAG is an important food related determinant of the glycaemic response of the food.
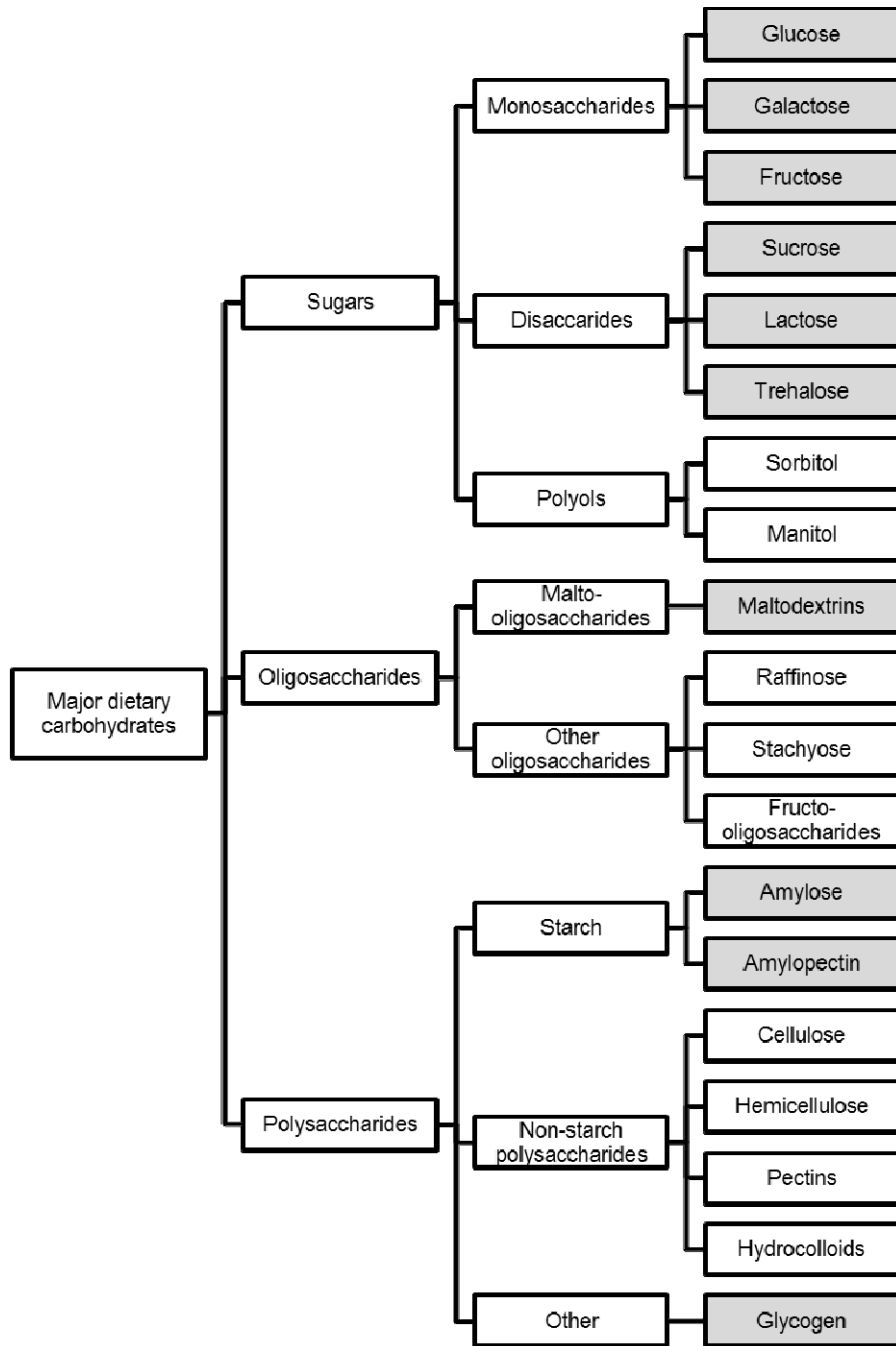
Figure 2.2: Classification of the major dietary carbohydrates (adopted from Nantel, 1999).

Glycaemic carbohydrates (available for absorption in the small intestine) are presented in shaded

blocks

Table 2.1: Principle physiological properties of dietary carbohydrates (adopted from Cummings and Stephan, 2007)

	Provide energy	Glycaemic carbohydrate	Cholesterol lowering	Increase calcium absorption	Source of short chain fatty acids	Increase stool output
Monosaccharides	X	X				
Disaccharides	X	X		X		
Polyols	X				X	X
Maltodextrins	X	X				
Oligosaccharides	X			X	X	
Starch	X	X			X	X
Non-starch polysaccharides	X		X		X	X

Englyst *et al.* (1999) determined the RAG, SAG and starch fractions by High Performance Liquid Chromatography (HPLC). They measured the amount of glucose released from a test food during timed incubation with digestive enzymes under standardized conditions. According to Englyst *et al.,* (1999) the RAG component of a food explained 70 % of the variance in glycaemic response. The HPLC method to determine rapidly available glucose can be divided into two categories, firstly the amounts of free sugars, glucose and fructose, and secondly the RAG, SAG, total glucose and starch fractions.

Englyst *et al.* (1999) determined the amounts of glucose and fructose by weighing samples of the food (as eaten) containing less than 0.6 g carbohydrate to the nearest milligram, in 50 ml polypropylene centrifuge tubes. Five millilitre internal standard (40 g arabinose/L in water with 50 % standard benzoic acid), 20 ml water and five glass balls were added to each tube. The tubes were capped and vortex mixed. The tubes were then placed into a boiling water bath and left for 30 minutes. The tube contents were then vortex mixed vigorously to completely disrupt the structure. The tubes were cooled to 37 °C at which point 0.3 ml invertase enzymes were added and the tubes placed in a 37 °C shaking water bath for 30 minutes. Then 0.2 ml of the sample

was added to 4 ml absolute ethanol and vortex mixed. This was the free sugar glucose portion (FSG). Values for FSG (sum of free glucose and glucose from sucrose) and for free sugar fructose (FSF; the sum of free fructose and fructose from sucrose) were obtained from this portion (Englyst *et al.,* 1999).

To determine the RAG, SAG, total glucose and starch fractions, Englyst *et al.* (1999), weighed samples of food containing less than 0.6 g carbohydrate to the nearest milligram, into 50 ml polypropylene centrifuge tubes. Then 5 ml of the internal standard solution (40 g arabinose/L in water with 50 % standard benzoic acid) and 10 ml freshly prepared pepsin-guar gum solution (5 g pepsin/L and 5 g guar gum/L in 0.05 mol HCl/L) were added. The tubes were capped, vortex mixed and placed in a 37°C water bath for 30 minutes to allow hydrolysis of the proteins by the pepsin. Five millilitre of 0.5 mol sodium acetate/L was added to each tube to form a buffer at pH 5.2. Each tube was capped and shaken gently to disperse the contents and then placed in the 37°C water bath to equilibrate for a few minutes, after which 5 ml enzyme mixture was added and placed back into the water bath. The shaking was started at this time and not interrupted until all portions were collected. Each tube was removed exactly 20 minutes after the enzyme mixture was added, and 0.2 ml of the contents was added to 4 ml absolute ethanol and vortex mixed to stop the hydrolysis. This portion was considered to contain the RAG fraction. The tube was returned to the shaking water bath immediately after the sample was taken. After another 100 minutes, another 0.2 ml was taken and added to 4 ml absolute ethanol and vortex mixed. The amount of carbohydrates in this portion, minus the RAG amount, was considered to be the SAG fraction.

When all the portions had been collected, the tube contents were vigorously vortex mixed to break up any large particles and the tubes were placed together into a boiling water bath and collected after 39 minutes. The tubes were vortex mixed again and cooled in ice water for 15 minutes. Potassium hydroxide (10 ml of 7 mol/L) was added, the tubes were capped and the contents mixed by inversion. The tubes were secured horizontally in a shaking water bath with ice water and shaken for 30 minutes. Tubes were removed from the ice water and 0.2 ml of the

contents was added to 1 ml of 1 mol acetic acid/L. Amyloglucosidase solution (40 µL amyloglucosidase diluted 1:7 with water) was added, the contents mixed and the tubes placed in a 70 °C water bath for 30 minutes, followed by a boiling bath for 10 minutes. The tubes were cooled to room temperature before the addition of 12 ml absolute ethanol. This final portion represented the total glucose portion.

Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were calculated from these portions. To determine RDS, the RAG amount minus the FSG amount was multiplied by 0.9. Similarly in determining SDS, Englyst *et al.* (1999) multiplied the SAG amount by 0.9. Englyst *et al.* (1999) found the correlation between RAG and *in vivo* glycaemic response to be highly significant ($p < 0.0001$).

Englyst, Veenstra and Hudson (1996) used a similar method to predict GI, but used a colorimetric endpoint. They found a similar correlation between the GI and the RAG. Van Kempen, Regmi, Matte and Zijlstra (2010) investigated the correlation of a modified Englyst-assay to portal glucose appearance in pigs and found that *in vitro* starch digestion can, after corrected for gastric emptying, predict portal glucose appearance up to eight hours after ingestion.

2.5.3   Multi - enzyme hydrolysis

Brighenti *et al.* (1995) developed a method based on multi-enzyme hydrolysis, the last stage of which involved the incubation of the test material with pancreatic (alpha) amylase and diffusion through a dialysis tube. This simulates the effect of enzymatic breakdown of food in the human body, as well as the ability of reducing sugars to diffuse through the contents of the dialysis tube and across a membrane into surrounding fluids. The rate at which reducing sugars are released can be compared with that of a reference material and a hydrolysis index (HI) value can be determined for each food. In many cases the HI correlates with the GI value of a given food (Alldrick, 2006).

Similarly, Giacco, Brighenti, Parillo, Capuano, Ciardullo, Rivieccin, Rivellese and Riccardi (2001) employed the same method which is based on multi-enzyme digestion in a dialysis tube, followed

by analysis of the reducing sugars released into the surrounding buffer solution. Giacco *et al.,* (2001) stated that although this method is less sophisticated than the rapidly available glucose measurement it produces a good estimate of the GI of foods containing low amounts of mono- and disaccharides.

In the study performed by Giacco *et al.* (2001), after simulated chewing, two grams of each food product was placed in a dialysis tube to undergo breakdown by salivary alpha amylase, pepsin and pancreatic alpha amylase, to simulate digestion. Analysis on each food product were repeated three times, of which one included the addition of active enzymes, one with deactivated enzymes (blank), and one with deactivated enzymes plus a known amount of maltose to allow for measurement of sugar diffusability through the dialysis tube in the presence of food. The amount of reducing sugars was measured at 0 minutes, and thereafter every 30 minutes for five hours by means of colorimetric analysis. The digestion results, minus the blank, were then expressed as a percentage to those of white bread to calculate a digestion index. The diffusion results were related to those obtained through the diffusion of pure maltose in the absence of food to calculate the sugar diffusion index.

Germaine, Samman, Fryirs, Griffiths, Johnson and Quail (2008) compared six such *in vitro* starch digestibility methodologies for predicting GI of grain foods. Differences between the tests included methods for simulating the oral phase, adding or omitting amylase during the oral phase, and using a dialysis tube during the small intestinal phase. Of the six methods tested, five ranked all foods in the same order as obtained from *in vivo* analysis. Germaine *et al.* (2008) demonstrated a significant correlation ($r = 0.93$; $p > 0.01$) between one of the methods (mincing without amylase in the oral phase) and the reported GI responses.

Alldrick (2006) also developed a similar method based on simulating the human digestive tract and applying the various intestinal enzymes as they are present in the human body. Alldrick has registered this method at the Campden and Corleywood Food Research Association (CCFRA) as a method used to determine the hydrolysis index (HI) of a food. The HI is then used as an indication of the predicted GI of the specific foodstuff. This specific HI method developed by

Alldrick (2006) was used as a guide for the rapid analysis method developed in the present study which is described in Chapter 3.

According to Alldrick (2006) the cost per HI analysis method is approximately one tenth to one twentieth of the cost of a clinical *in vivo* GI trial. This rapid assessment method cannot simulate the rate of gastric emptying or the rate of flow through the small intestine, thus no specific value can be allocated; however this method can be effective as a screening tool to classify foods according to the three previously mentioned categories of high, intermediate and low GI foods. Further clinical testing can then later be done on the foods if the need for a specific value arises, e.g. if the product fell within the desired GI range, for labelling purposes.

2.5.4   Variables not represented by *in vitro* methodology

Although *in vitro* carbohydrate digestion rates have been proven to mimic the GI for some foods, such as legumes and grains (Trout, Behall & Oslilesi, 1993), the rate of gastric emptying after food consumption is a determinant of glycaemic response, but in *in vitro* analysis this in not taken into consideration. Factors that influence stomach emptying include acidity, osmolality, volume and concentration of sugar or soluble fibre (Foster-Powell *et al.,* 2002).

Although human related variables such as pre-meal effects are not represented, it should be noted as previously mentioned that *in vivo* obtained GI values also do not significantly vary between individuals, which means that GI does not predict human variation. This is desirable because GI, and values obtained from *in vitro* methods, should predict variation between foods, and not between humans (Wolever, 2010).

2.6 SUMMARY

Generally speaking, there is a need in South Africa for a rapid assessment method to predict the GI of foodstuffs at a reduced cost and within a reduced timeframe. Although various *in vivo* methods are already being used in South Africa, with no method yet approved for regulatory

purposes, only a limited number of food products currently include GI indications on their product label, or have GI levels which were determined by accredited laboratories.

Determining the amount of rapidly available carbohydrates and other multi-enzyme hydrolysis procedures have been tested worldwide to predict glycaemic response of food products. The development of a reliable rapid assessment method to predict GI, based on simulating the human GIT, will provide a valuable, less expensive and / or time consuming, tool for the South African food industry to predict the GI value of their foods (either during or after the product development phase). After *in vitro* determination, *in vivo* analysis could be done as required by legislation, if the food falls within the desired GI range.

CHAPTER 3: DEVELOPMENT OF A RAPID GLYCAEMIC INDEX ASSESSMENT METHOD

In this chapter the development of the analytical procedure used to determine hydrolysis index (HI) is presented. Repeatability and reliability of the method to predict glycaemic index (GI) are also presented, by comparing obtained HI values to published GI values. The content of this chapter was submitted and accepted as a peer-reviewed research article in the Journal of Food Composition and Analysis.

3.1 INTRODUCTION

A committee brought together in 1997 by WHO and FAO endorsed the use of the GI method for classifying carbohydrate rich foods (FAO/WHO, 1998). They recommended that GI values together with other food composition data be used in the guiding of healthy food choices (Foster-Powell *et al.,* 2002).

As a result of the associated health benefits, the GI classification of foods is receiving increasingly more attention in both medical and nutritional fields. Food manufacturers and retailers are showing more and more interest in determining and publishing indications of their products' GI values (NA, 2004). Although GI values have been published for various foods (Foster-Powell *et al.,* 2002) the need and demand for GI data continue to increase. The FAO/WHO Expert Consultation Report on Carbohydrates in Human Nutrition (FAO/WHO, 1998) concluded that glycaemic response data should be supplemented with values for local foods and meals, as food variety and cooking could have significant effects on glycaemic response. Furthermore, the new South African draft labelling regulations include the recommendation that the labelling of all carbohydrate rich foods should show an indication of the product's GI, indicating if the product has a high, intermediate or low GI (Booysen, 2007). Unfortunately, the South African Department of Health's Directorate: Food Control is still awaiting the adoption by SANS of an international ISO method that has been established to determine the GI of a product or foodstuff.

Although the legislation has not been enforced yet, there is a need for variety and amount of GI indications in a country like South Africa with its diverse eating culture and dynamic food industry. In South Africa only *in vivo* methods are currently used to determine the GI of a food. This current *in vivo* method, which is expensive and time consuming, might not be accessible to all in the food industry. Due to limited funds, the local and smaller food industries may be hesitant to spend money to determine GI by this method, especially during the product development phase. Furthermore, the future prospects of the implementation of a SANS approved *in vivo* methodology for labelling purposes, which might differ from current *in vivo* methods used, also motivates the need for a predictive, yet less expensive, method.

Rapid analysis methods to determine GI have been evaluated worldwide (Goni *et al.,* 1997; Englyst *et al.*, 2003), and *in vitro* carbohydrate digestion rates have been proven to mimic the GI for certain foods, such as legumes and grains (Trout *et al.,* 1993). For consumers and the industry to have adequate exposure to the GI of a range of food products and meals, an alternative, more economical and less time consuming method to predict the glycaemic response after ingestion is needed. After GI predictions are made, further *in vivo* analyses can be done for labelling purposes if required once, and if, such legislation is implemented.

In South Africa no such rapid assessment method has been developed. The purpose of this study was to investigate and develop such a method, and once developed, determine its reliability to predict a food's GI classification into one of the three GI categories, namely high, intermediate or low GI.

3.2 HYDROLYSIS INDEX METHODOLOGY

The *in vitro* method for evaluating starch digestibility has previously been developed by CCFRA. It involves mechanical disruption and multi-enzymatic digestion based on proteolysis, followed by incubation with pancreatic alpha 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 Alldrick (Campden and Chorleywood Food Research Association, U.K.), Prof

Neitz (Biochemistry, University of Pretoria) and the Agricultural Research Council (ARC) analytical laboratory (Irene).

3.2.1 Materials and preparation

The following materials used in developing the method were obtained from Sigma-Aldrich, Johannesburg, SA. These materials include the following:

- Flat Cellulose Membrane dialysis tubing (D9652)
- Porcine stomach pepsin powder (77160)
- Alpha amylase (A9972)
- Maltose Grade 1 monohydrate.

Other chemicals used in the development of the rapid assessment method were obtained from the ARC-Irene Analytical Services, and included the following:

- Dipotassium phosphate ($K_2HPO_4.3H_2O$, 28.23 mM) and Potassium dihydrogen phosphate (KH_2PO_4, 136.09 mM) which were used to make up a 0.1 M Potassium phosphate buffer solution (pH 6.9).
- Orthophosphoric acid (H_3PO_4)
- Hydrochloric acid (HCl, 0.2 M) and Potassium chloride (KCl, 0.2 M), which were used to make up a HCL.KCL buffer (pH 1.68)
- 50 % Potassium hydroxide solution (KOH).

The equipment used during the method belongs to the ARC-Irene Analytical Services and included the following:

- Ultra Turrax
- Shaking water bath
- Single channel pipettes
- 200 ml Erlenmeyer flasks
- 20 ml, 50 ml and 2000 ml volumetric flasks
- 100 ml and 500 ml volumetric cylinders
- 200 ml glass beakers

- 500 ml glass bottles with lids
- Various sized funnels.

3.2.1.1 Potassium phosphate buffer (0.1 M, pH 6.9)

Potassium phosphate buffer (0.1 M) was used as the surrounding liquid into which the carbohydrates would diffuse through the dialysis tubing, as well as the buffer used during liquidizing of the food. Two litres of this buffer solution were made up by adding 18.13 g KH_2PO_4 (136.09 mM) and 15.23 g $K_2HPO_4.3H_20$ (228.23 mM) to a 2000 ml volumetric flask and filling it with distilled water to the 2 L level. The pH was adjusted to pH 6.9 with 50 % KOH.

3.2.1.2 Orthophosphoric acid

For decreasing the pH to simulate the gastric phase, 5 % orthophosphoric acid was used. 5 ml orthophosphoric acid was added to 100 ml distilled water.

3.2.1.3 HCl.KCl buffer

A HCl.KCl (0.2 M) buffer was used to dissolve the pepsin enzyme before addition to the samples during the gastric phase. KCl (0.2 M) was prepared by adding 1.4 g KCl to 100 ml distilled water. HCl (0.2 M) was prepared by adding 1 ml 375 N HCl to 18.75 ml distilled water. 25 ml KCl (0.2 M) was added to 5 ml HCl (0.2 M), which was made up with distilled water to 100 ml.

3.2.1.4 Potassium hydroxide solution

A 50 % potassium hydroxide solution was used to increase the pH to 6.9 ± 0.2 after the acidic gastric phase had been completed and before the addition of the amylase enzymes. The solution was made by adding 50 ml potassium hydroxide to 50 ml distilled water.

3.2.1.5 Pepsin enzyme solution

To prepare the pepsin enzyme solution, 2.5 g of the porcine pepsin powder was dissolved in 10 ml HCl.KCl (0.2 M) buffer.

3.2.1.6 Alpha amylase solution

As alpha amylase is heat sensitive, the amylase solution was kept in the refrigerator at a temperature below 4°C until just before adding it to the samples. A quantity of 31 µl had to be added to each sample. For each sample tested 31 µl was made up to 1 ml with the potassium phosphate buffer solution, e.g. when eight samples were tested 310 µl amylase were made up to 10 ml, and 1 ml of the solution was administered to each sample.

3.2.1.7 Dialysis tube

Different length dialysis tubes (between 20 and 30 cm, depending on the volume of the sample) were cut in advance and submerged in distilled water to soften the tubing. One end was folded back and tied off with string. Once samples were transferred into the tube, the other end was also tied off after expelling most of the air. Care was taken to ensure at least 3 cm empty space above the mixture to enable bending of the tube when inserting into the flasks during the small intestinal phase.

3.2.1.8 Samples and repetitions

Based on published GI values, food samples were selected 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 period. The analysis took place over three trial days. Two composite samples of each food product tested, except for maltose and white bread, were analysed.

During the first trial, seven 4.4 g samples of white bread (containing 2 g carbohydrate each) was analysed to determine the repeatability of the method. A 2 g maltose sample was included in the trial.

During the second trial, two potato cultivars, namely Mondial (15.7 g) and Darius (14.7 g), were used together with baby potatoes from the Mondial cultivar (15.7 g) (containing 2 g carbohydrate each). All samples were cooked and weighed without skin. Potatoes are considered to have a

high GI (baked 85; microwaved 82) and baby potatoes an intermediate GI (baked 62), irrespective of cultivar (Steenkamp & Delport, 2005). Maltose (2 g) and bread (4.4 g) were analysed as control samples.

During trial three canned butterbeans (16.7 g), boiled young pumpkin leaves (*Curcurbita*) (59 g) and boiled Taro (African potato, *Colocasia esculenta*) (5.8 g) were analysed together with maltose (2.0 g) and white bread (4.4 g) as control samples. Two composite samples of each food product, except for maltose and white bread, were analysed during the second and third trials.

Samples were procured from various places. Baby potatoes, canned butterbeans and white bread were purchased from retail outlets. Mondial and Darius potatoes were supplied by distributors. Pumpkin leaves (*Curcurbita*) and Taro (African potato, *Colocasia esculenta*) were harvested fresh at the ARC, Roodeplaat, South Africa. In Table 3.1 the carbohydrate contents of the food products tested are presented together with the sample sizes (g) which contained 2 g carbohydrates each.

Table 3.1: The carbohydrate content (per 100 g) of maltose, white bread, Mondial potatoes, Mondial baby potatoes, Darius potatoes, canned butterbeans, pumpkin leaves (*Curcurbita*) and Taro (African potato*, Colocasia esculenta*) used to calculate sample sizes

	Carbohydrate (CHO) (g)	Sample (g) to represent 2 g CHO
Maltose	100	2.00
White bread*	45.9	4.40
Mondial potato[#]	12.7	15.7
Darius potato[#]	13.6	14.7
Mondial baby potato	ND	15.7
Canned butterbeans[&]	12.0	16.7
Pumpkin leaves^	3.39	59.0
Taro^	34.6	5.80

*Kruger, Sayed, Langenhoven & Holing, 1998.
[#]Own laboratory data determined at ARC-Irene Analytical Services
[&]Product label
^USDA Food Composition Database for Standard Reference. http://www.nal.usda.gov/fnic
[ND]Not determined

3.2.2 Method

3.2.2.1 Oral phase

A sample of each food as eaten, containing 2 g of carbohydrate, was sliced, ground and placed in an Erlenmeyer flask with 20 ml of a buffer solution at 37 °C. The samples were further homogenized with an Ultra Turrax at 13 500 rpm until liquid or for a maximum time of two minutes. The Ultra Turrax was rinsed with an additional 20mL buffer solution to ensure that most of the carbohydrates remained in the sample. The Erlenmeyer flasks containing samples which had undergone this oral phase are presented in Figure 3.1.

Figure 3.1: Samples of white bread (1A), pumpkin leaves (*Curcurbita*) (2B) and Taro (African potato, *Colocasia esculenta*) (3A) which were homogenized by Ultra Turrax during the oral phase

3.2.2.2 Gastric phase

The pH of each sample was decreased to pH 2.5 with 5 % orthophosphoric acid, after which 2 ml of the pepsin enzyme solution was added. The samples were swirled. All the samples were placed in a 37 °C shaking water bath for one hour to simulate the time that food would be

churned in the human stomach, a phase which is often labelled the gastric phase. In Figure 3.2 the samples can be seen in the shaking water bath during the gastric phase.

Figure 3.2: Samples in the shaking water bath at 37°C for one hour during the gastric phase

3.2.2.3 Small intestinal phase

After the gastric phase, each sample was buffered back to pH 6.9 ± 0.2 with 50 % KOH. Alpha amylase solution (2 ml) was added to each sample and swirled to distribute the enzyme throughout the sample. The content of each flask was then transferred into a 20 to 30 cm dialysis tube (Figure 3.3). The tube was closed and placed in bottles containing 500 ml buffer solution (Figure 3.4). The flasks were placed in the stirring water bath and 40 ml of the buffer solution were extracted every 30 minutes to determine the rate of hydrolysis of carbohydrate from the dialysis tube into the buffer solution (Figure 3.5).

The summary of the methodology followed from the oral phase, through the gastric phase and ending in the small intestinal phase is presented in Figure 3.6.

Figure 3.3: Dialysis tubes containing the food samples, representing the small intestinal phase

Figure 3.4: Dialysis tubes surrounded by buffer solution during the small intestinal phase

Figure 3.5: Transfer of buffer solution from a flask to containers for the analysis of amount of carbohydrate which diffused through the dialysis tube into the surrounding fluid.
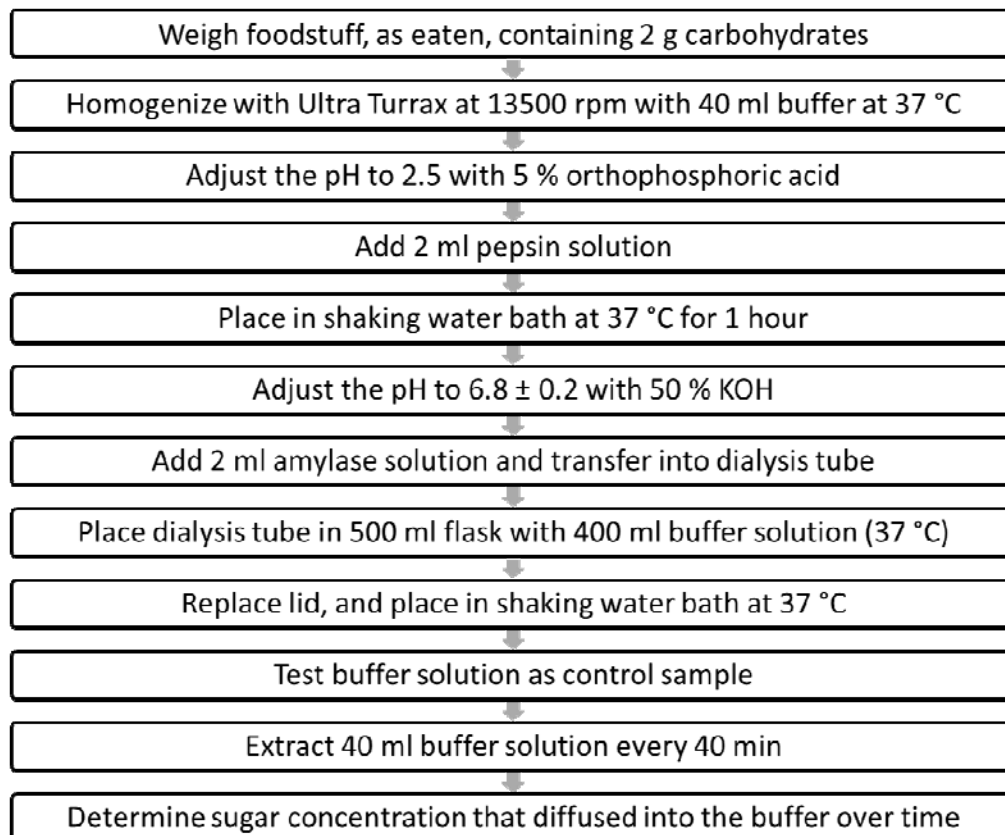
Figure 3.6: Summary of the method used to simulate the human gastro intestinal tract (GIT) and predict the rate of absorption of carbohydrates

3.2.2.4 Determining amount of diffused carbohydrate

Reduced sugars were determined using Infra-red spectrophotometry (Milkoscan). The Milkoscan works on the principle of an Infrared Spectrophotometer. The infrared energy produced by a wide range source is filtered through infra-red filters of specific wavelengths and focused through the samples under test. This energy passes through the samples and strikes a detector which converts it into a corresponding electrical signal. This signal after amplification is processed by a microprocessor which displays the results on a LCD panel. All the experimental work was performed at the ARC-Irene Analytical Services, Agricultural Research Council: Animal Production Institute.

3.2.2.5 Interpretation of results

The carbohydrate concentration values were plotted on a graph and the AUC was determined. The 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 used:

AUC of food tested ÷ AUC of reference food (maltose) X 100 = HI of food tested

3.2.3   Statistical analysis

The data obtained from each experimental procedure was entered into a spread sheet in Microsoft Excel (2000). Data was analysed by the using the statistical computer program GenStat for Windows (2003). Mean values, SD's and CV's were calculated to determine the repeatability and reproducibility of the method.

3.3 RESULTS AND DISCUSSION

The amount of carbohydrates which diffused through the dialysis tube and into the surrounding fluid was tabulated and graphically presented as a concentration-over-time curve. In Figures 3.7, 3.8 and 3.9 the concentration-over-time curves of the samples tested during the three trials are presented. From these curves, the mean areas under the AUC for each food product tested were

determined. The HI values were then determined using the calculation above. The GI values as presented in The South African Glycemic Index and Load Guide (Steenkamp & Delport, 2005) are included in the Figure footers for comparison.

In Figure 3.7 the concentration of sugars which diffused into the buffer solution over time is presented. From the graphs it can be seen that the seven bread samples show good repeatability, as similarity can be seen in the tempo of carbohydrate release. From these graphs AUC values were determined and compared to the AUC of the maltose sample to obtain the HI of each bread sample. In Table 3.2 the HI of the seven white bread samples, together with one maltose sample, are 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 a reported GI value of 72.
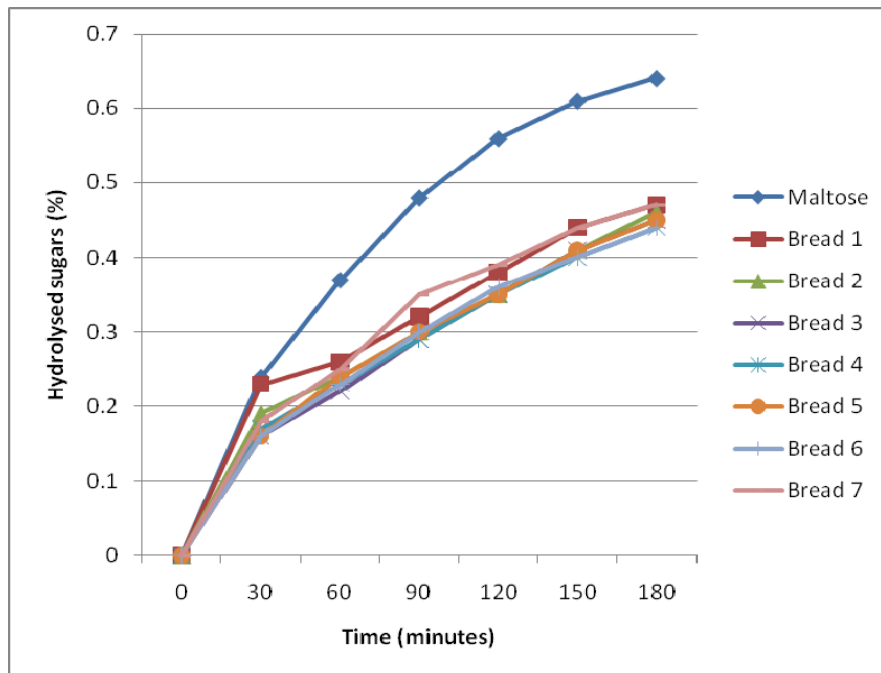
Figure 3.7: The hydrolysis of carbohydrate from maltose (GI = 100) and seven samples of white bread (GI = 72) through the dialysis tube into the surrounding buffer solution over time (Trial 1)

Table 3.2: Area under the concentration-over-time curve (AUC) and hydrolysis index (HI) values for maltose (GI = 100) and seven samples of white bread (GI = 72) (Trial 1)

	Area under the concentration-over-time curve (AUC$^\$$)	Hydrolysis index (HI)*	Glycaemic index (GI)$^\#$
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	64.3	-
Sample 7	55.4	71.5	-

$^\$$AUC determined by geometric calculation by applying the trapezoid rule
*HI determined as the AUC of sample / AUC of maltose X 100
$^\#$GI values obtained from The South African Glycemic Index and Load Guide (Steenkamp & Delport, 2005)

While the determined HI value of white bread during trial two (HI 72.5) 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 from the reported GI values was observed (Figure 3.8; Table 3.3). In Figure 3.8 it can be seen that the Darius cultivar had a lower release of carbohydrates into the surrounding buffer solution than the Mondial baby potatoes. The one sample of the Mondial cultivar had a release rate similar to the Mondial baby potatoes, while the second sample released carbohydrates into the fluid at a similar rate as the Darius potato cultivar.
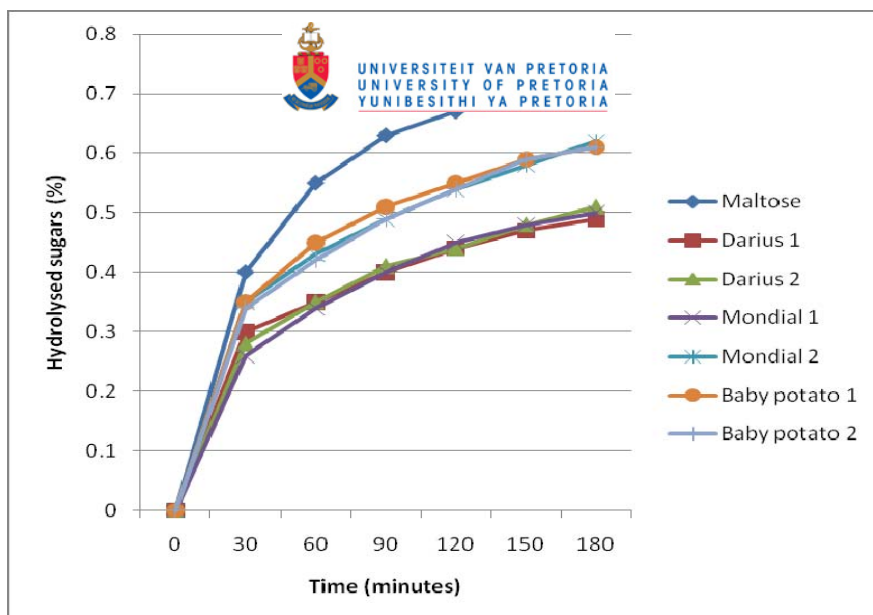
Figure 3.8: The hydrolysis of carbohydrate from maltose (GI = 100), Darius potatoes, Mondial potatoes (GI (baked potatoes) = 85) and Mondial baby potatoes (GI = 62), through the dialysis tube into the surrounding buffer solution over time (Trial 2)

Table 3.3: Area under the concentration-over-time curve (AUC) and hydrolysis index (HI) values for maltose, white bread, Mondial, Darius and Mondial baby potatoes (Trial 2)

	Area under the concentration-over-time curve (AUC$^\$$)	Hydrolysis index (HI)*	Glycaemic index (GI)#
Maltose	3.33	100	100
White bread	2.42	72.5	72
Mondial mean	2.44	73.3	85
SD	0.37	11.0	
Darius 1	2.18	65.5	-
Darius 2	2.7	81.1	-
Darius mean	2.21	66.4	85
SD	0.007	0.21	
Mondial 1	2.21	66.2	-
Mondial 2	2.22	66.5	-
Baby potatoes mean	2.72	81.7	62
SD	0.05	1.49	
Baby potatoes 1	2.76	82.7	-
Baby potatoes 2	2.69	80.6	-

$^\$$AUC determined by geometric calculation by applying the trapezoid rule
*HI calculated as the AUC of food sample expressed as a percentage of the AUC of maltose
#Obtained from The South African Glycemic Index and Load Guide (Steenkamp & Delport, 2005)

Baked potatoes are reported to have a GI of 85 according to the South Africa Glycemic Index and Load Guide (Steenkamp & Delport, 2005). The determined mean HI of the Mondial potatoes was 66.4 (SD 0.21), while Darius potatoes had a mean HI of 73.3 (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 in the South African Glycemic Index and Load Guide (Steenkamp & Delport, 2005). The determined mean HI value of the Mondial baby potatoes was 81.7 (SD 1.49) which indicates that it has a high GI rather than an intermediate GI.

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 *et al.,* 1981) and high GI values of 87 to 100 (Soh & Brand-Miller, 1999). In the International Table of Glycemic Index and Glycemic Load values (2002), 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 could 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 as a motivation to determine individual GI values per cultivar and to discard 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 South African consumers a 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 glycaemic response were found between the two different cultivars tested, which motivates further investigation.

As a result of these findings, a potato cultivar, cultivated in Australia to have low to intermediate glycaemic properties, was sourced for analysis. The cultivar underwent testing with the developed rapid assessment method (HI), together with *in vivo* analysis at two recognized GI laboratories in South Africa to determine GI and compare the validity of the developed rapid assessment method to predict GI. The findings from this study are presented in Chapter 4.

The amounts of carbohydrates which diffused over the dialysis membrane into the buffer solution during trial three (pumpkin leaves (*Curcurbita*), Taro (African potato, *Colocasia esculenta*) and canned butterbeans) are presented in Figure 3.9 and Table 3.4. It can clearly be seen that pumpkin leaves had a significantly lower carbohydrate release over time than the other samples. Canned butterbeans and the cooked Taro samples had similar qualities in terms of the tempo of carbohydrate release into the surrounding buffer solution.
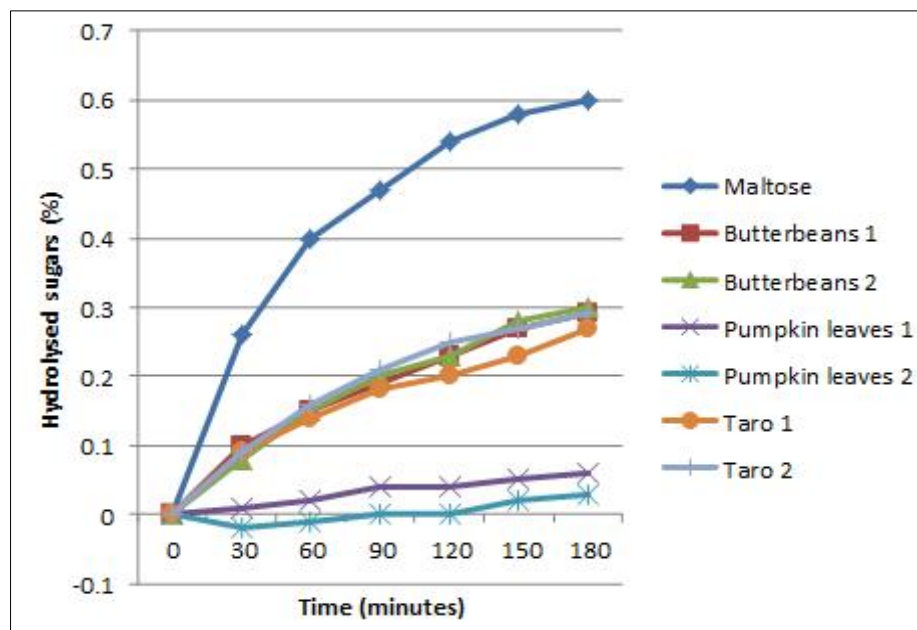
Figure 3.9: The hydrolysis of carbohydrate from maltose (GI = 100), butterbeans (GI = 31), pumpkin leaves (no GI determined) and Taro (no GI determined) through the dialysis tube into the surrounding buffer solution over time (Trial 3)

Table 3.4: Area under the concentration-over-time curve (AUC) and hydrolysis index (HI) values for maltose, white bread, canned butterbeans, pumpkin leaves (*Curcurbita*) and Taro (African potato, *Colocasia esculenta*) (Trial 3)

	Area under the concentration-over-time curve (AUC$^\$$)	Hydrolysis index (HI)*	Glycaemic index (GI)$^\#$
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$^\&$
SD	0.13	5.13	-
Sample 1	0.19	7.45	-
Sample 2	0.005	0.20	-
Taro Mean	1.05	41.2	ND$^\&$
SD	0.11	4.16	-
Taro 1	0.98	38.2	-
Taro 2	1.13	44.1	-

$^\$$AUC determined by geometric calculation by applying the trapezoid rule
*HI calculated as the AUC of the sample expressed as a percentage of the AUC of maltose
$^\#$Obtained from The South African Glycemic Index and Load Guide (Steenkamp & Delport, 2005)
$^\&$ND: No GI has yet been determined in South Africa

The HI value for canned butterbeans indicated that butterbeans have a low GI (Table 3.4), a result that is in line with the reported GI values. An HI value of 42.7 (SD 0.14) was determined, compared to a low GI value of 31 which is reported in the South African Glycemic Index and Load guide (Steenkamp & Delport, 2005). Although these values differ considerably (11.7), in terms of GI classification, both values fall within the low GI range (GI < 55). The HI values of pumpkin leaves (*Curcurbita*) (HI 3.82) and Taro (African potato, *Colocasia esculenta*) (HI 41.2), two indigenous vegetables consumed by many rural South Africans, both fall within the low GI range (GI < 55). Although no GI values for these commodities have been determined before, the HI results provide good indication that both these foods possibly have low glycaemic responses

once ingested. Thus, for future reference, these indigenous vegetables could possibly be considered as low GI food options, and *in vivo* GI testing could be recommended.

3.3.1 Repeatability

Repeatability is the variation of outcomes of an experiment carried out under the same conditions. To determine the repeatability of the method, white bread was analysed seven times on a given occasion (Table 3.2). 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 trial one was 67.00. The SD of the seven white bread samples was 3.46, with a variance of 11.94. The 95 % CI indicated that there is a 95 % chance that HI values of white bread would be between 63.80 and 70.19 (Table 3.2), which indicated good repeatability.

3.3.2 Reproducibility

Reproducibility is the variation of outcomes of an experiment carried out under 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. To determine the reproducibility of the developed method, maltose was tested during each of the three trials (Table 3.5). Although more data are required to draw solid conclusions, the SD between the AUC of the maltose samples was 0.44 and the CV was 0.16, indicating reproducibility and good precision.

Table 3.5: Reproducibility of the rapid assessment method

	Mean AUC*
Day 1	2.58
Day 2	3.33
Day 3	2.55
Mean AUC	2.82
SD	0.44
CV	0.16
Variance	0.20

*AUC determined by geometric calculation by applying the trapezoid rule

3.3.3 Validity of the rapid assessment method to determine the glycaemic 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. Foods with intermediate GI values lie between these two points. Table 3.6 compares the mean HI values of the samples tested with previously determined GI values as presented in The South African Glycemic Index and Load Guide (2005). The mean HI value for bread was HI 70, while the GI indication for white bread is reported to be 72. Butterbeans are predicted to have an HI of 42.7, while the reference GI value in the Guide is 31. As various factors influence GI, factors such as brand, concentration of brine and cultivation methods could all play a role in determining the actual GI. Although there seems to be a big difference in values, both the HI and the 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.

Table 3.6: Correlating calculated hydrolysis index (HI) values with previously reported glycaemic index (GI) values

	Hydrolysis index (HI)			Glycaemic index (GI)[#]
	HI*	SD	Variance	
Maltose	100	-	-	100
Bread	70.0	3.45	11.9	72
Darius	66.4	0.21	0.05	85
Mondial	73.3	11.0	122	85
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[^]
Taro	41.2	4.16	17.3	ND[^]

*HI calculated as the AUC of sample expressed as a percentage of the AUC of maltose
[#]Obtained from The South African Glycemic Index and Load Guide (Steenkamp & Delport, 2005)
[^]ND: No GI has yet been determined in South Africa

In Figure 3.10 the HI data are visually compared to the GI data tabulated in the South African Glycemic Index and Load Guide (Steenkamp & Delport, 2005). A clear correlation can be drawn between the HI values and previously determined GI values with high GI foods receiving high HI scores, and low GI foods obtaining lower HI scores.
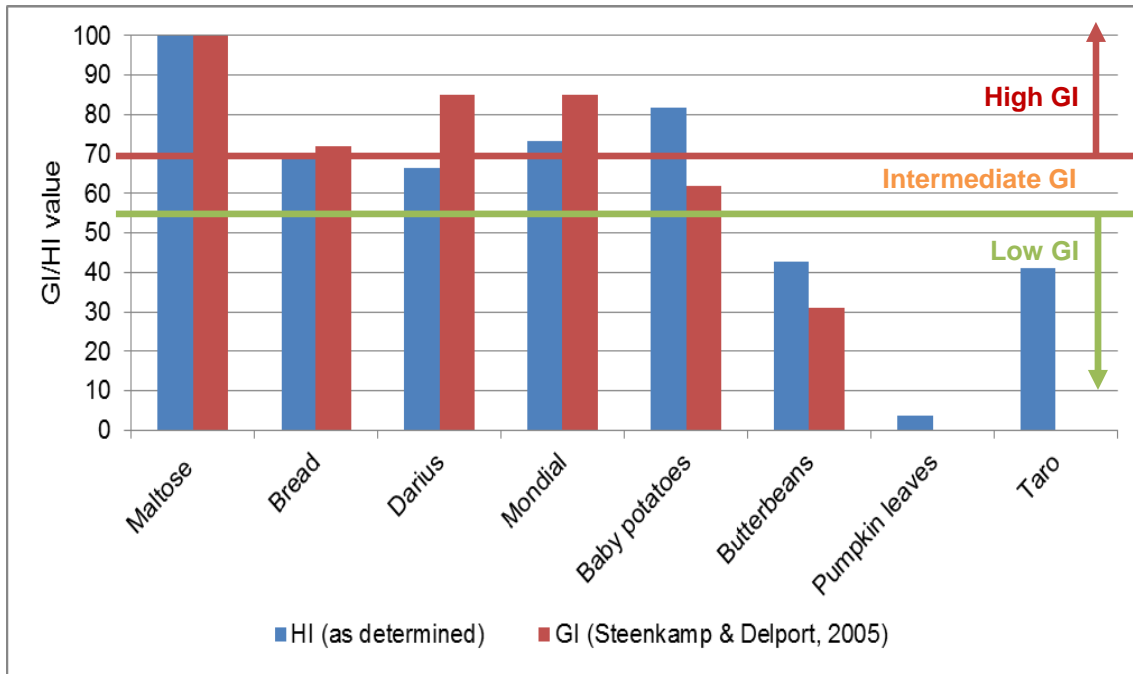
Figure 3.10: Visual presentation of calculated hydrolysis index (HI) values of food products tested compared to glycaemic index (GI) values tabulated in the South African Glycemic Index and Load Guide (Steenkamp & Delport, 2005)

3.4 CONCLUSIONS

The HI values calculated for white bread, Mondial potatoes and butterbeans predict that these foods fall in the same GI categories (high, intermediate or low GI) as previous *in vivo* methodologies have shown, which indicates that HI correlates reasonably well with the GI of these foods.

The correlation between the determined HI values and known GI data during the development of this rapid assessment method provides evidence that the method is capable of generating

indicative GI values for carbohydrate rich foods. Such a rapid method to predict GI would be beneficial to the South African food industry, and in particular during food product development from a cost, time saving and infrastructure point of view. The developed method unlocked the possibility of isolating South African potato cultivars with low or intermediate GI, and possibly also other vegetables and fruits with high starch contents which are commonly considered high GI foods.

The developed rapid assessment method was tested for accuracy in predicting GI in a case study format which is presented in Chapter 4.

3.5 ACKNOWLEDGEMENTS

CHAPTER 4: THE ACCURACY OF THE DEVELOPED RAPID ASSESSMENT METHOD TO PREDICT GLYCAEMIC INDEX

In this chapter the developed rapid assessment method was evaluated for accuracy. Results from in vivo glycaemic index (GI) trials on two food products (potatoes and yoghurt) were compared with the hydrolysis index (HI) results obtained on the same food products through the rapid assessment method (in vitro). A comparative inter-laboratory study was also performed on the in vivo GI values obtained on the potato cultivar tested in duplicate by two different laboratories in South Africa (Glycemic Index Foundation of South Africa (GIFSA) and the Nutrition Information Centre of the University of Stellenbosch (NICUS)), and the Sydney University Glycemic Index Research Service (SUGIRS) in Australia.

4.1 MOTIVATION

To determine the accuracy of the rapid assessment method, the *in vitro* method which was developed and is described in Chapter 3 needed to be tested against *in vivo* methods for accuracy as no other *in vitro* method exists in South Africa. Towards this end, two food products were selected to undergo both *in vivo* (GI) analysis at the GIFSA, and *in vitro* (HI) analysis by means of the developed method. The two products selected included a potato cultivar *(Solanum Tuberosum L.* cultivar Almera*)* with a potentially low GI which was chosen as a high carbohydrate food. As the second product tested, Gero raspberry and litchi flavoured fat free yoghurt was chosen as a food sample containing a protein and carbohydrate matrix. The GI and HI results obtained from the different methodologies used for the determination of the GI of these two products were compared.

Furthermore, the analysis for the potato cultivar (*Solanum Tuberosum L.* cultivar Almera*)* was sent for *in vivo* analysis at two different GI laboratories in South Africa, namely GIFSA and NICUS. Both laboratories performed the *in vivo* GI analysis of the Almera potato cultivar on two different occasions. GIFSA also performed the analysis at both of their two laboratories, namely in Durban and Nelspruit. Comparisons were drawn between the methodology and results

obtained from these four trials, as well as GI data obtained from the Syndey University Glycemic Index Research Service (SUGIRS) in Australia on the specific potato cultivar.

The objective of this trial was to determine the accuracy of the HI of a food, obtained by the rapid assessment method, to predict the GI of a food. Comparisons were drawn between data obtained on the same two food products (a potato cultivar with a potentially lower GI and a fat free yoghurt product) using the developed *in vitro* (HI) method and *in vivo* (GI) analysis. In addition, *in vivo* (GI) results obtained on the same potato cultivar, analysed by different *in vivo* laboratories, were compared.

4.2 THE GLYCAEMIC INDEX OF ALMERA POTATOES

4.2.1 Nutritional Analysis

To determine the GI of any food, the amount of carbohydrate such a food contains needs to be determined. As Almera is a new cultivar on the South African market and no nutritional data are available for this specific cultivar, nutrient analysis of selected nutrients (energy, carbohydrate (by difference), protein, fat and ash) was done at the ARC-Irene Analytical Services. The laboratory holds SANAS accreditation (Association for Official Analytical Chemists (AOAC), 2005).

4.2.1.1 Total fat

AOAC method 960.39 (2005) was used to determine the total fat content of the samples. Samples were freeze-dried to ensure that all the moisture had been removed from the samples. The fat extraction was performed on 2 g freeze-dried samples with the Tecator Soxtec System 1034 extraction unit using petroleum ether (40-60 °C).

4.2.1.2 Moisture

For determination of moisture content the weight loss of a 5 g sample was measured in triplicate (AOAC, 2005).

4.2.1.3 Total ash

The total ash is the inorganic matter of a sample and it was analysed according to AOAC method 920.153 (2005). The organic matter of a sample is removed by heating at 550 °C overnight. The remaining residue is inorganic matter (ash).

4.2.1.4 Protein

The analysis is based on the Dumas Combustion method, AOAC 992.15 (2005). The sample is combusted at $\pm$ 1100 °C – 1350 °C and 10 cm^3 of the sample gas is analysed. A thermal conductivity cell detects the difference in thermal conductivity caused by the presence of Nitrogen. A conversion factor of 6.25 was used in the calculation of the protein content. Duplicate samples were analysed.

4.2.1.5 Food energy content

The energy content was calculated from the percentage protein, carbohydrate and fat making use of the following factors:

Energy (kJ / 100 g) = 37 (% fat) + 17 (% protein) + 17 (% carbohydrates) (Atwater & Bryant, 1900)

4.2.1.6 Results and discussion

The nutritional data was expressed on a wet, raw basis (the form in which the samples were received by the laboratory). In Table 4.1 the nutritional composition of Almera potatoes as analysed, is presented together with values tabulated for potatoes in the National Medical Research Council (MRC) Condensed Food Composition Tables for South Africa (2010). The analysed Almera potatoes were notably higher in moisture (8 % higher) and protein content (49 % higher) than the values tabulated in the National Food Composition Tables for raw potatoes (flesh and skin). Almera potatoes are also notably lower in carbohydrate (34 % lower) per 100 g raw product. It should be noted that the values for potatoes presented in the MRC tables were

determined in 1998 and were most probably derived from the BP1 cultivar *(Solanum Tuberosum L.* cultivar BP1*).*

Table 4.1: The nutritional composition of raw Almera potatoes expressed as a percentage (%)

	Moisture	Fat	Protein	Carbohydrates	Fibre	Ash
Almera (raw with skin)*	86.3	0.07	2.24	10.5	1.02	0.93
Potatoes (raw with skin)^	80.2	0.10	1.50	15.9	1.50	0.90

*Own data obtained at ARC-Irene Analytical Services
^Kruger, Sayed, Langenhoven & Holing, 1998.

4.2.2 Glycaemic index determined by the Glycemic Index Foundation of South Africa (GIFSA) Trail 1

4.2.2.1 Materials and methods

The GI of the carbohydrate rich Almera potato cultivar was determined by the GI Foundation of South Africa at the laboratory in Durban, South Africa. The study was conducted according to international protocol (as recommended by an International Expert Consultation on Carbohydrates in Human Nutrition, FAO/WHO, 1998), the recommendations of the International Life Sciences Institute (ILSI) appointed International Committee for the Standardization of GI Testing Methodology (Brouns *et al.,* 2005) and the draft regulation of the South African Department of Health pertaining to GI testing methodology (Department of Health, 2007). Ethical approval was obtained from the Faculty of Health Sciences Research Ethics Committee (University of Pretoria) (Ethics number: 8/2006) and all subjects signed informed consent. The subjects who partook in the trial included seven healthy subjects and three subjects with NIDDM.

The Almera potatoes were grown in the Western Cape under controlled conditions, harvested when the tubers reached medium size, couriered to the investigator within three days after harvesting, and stored at room temperature until needed. Potatoes were peeled, weighed and cubed into 50 to 60 mm cubes. These cubes were cooked in salted water until done (approximately 15 minutes), cooled overnight, and reheated before consumption the next

morning. Ten pre-screened panellists each received 522.3 g cooked potatoes, which each panellist divided into ten portions and consumed within ten and 15 minutes together with 250 ml water (Brouns *et al.,* 2005). Blood glucose readings were taken every 15 minutes starting immediately after ingestion, until the blood glucose reading fell below the initial reading, or for a maximum total time of 180 minutes. The results from the subjects were used to determine the GI of the product by using the formula: GI = (AUC of the test food X 100) ÷ (AUC of the subjects' reference glucose value).

4.2.2.2 Results and discussion

Table 4.2 presents the AUC and the mean GI values of each panel member, together with the SD. The CI indicates that the researcher can be 95 % confident that the GI of the specific product will lie between the bottom and the top values presented i.e.: for Almera potato between 33 and 52.4 GI. The mean GI value of Almera potatoes was found to be 43, which classifies this product as a low GI food (GI < 55). Baked Mondial potatoes are recorded as having a GI of 85 (high GI) in the South African Glycemic Index and Load Guide (Steenkamp & Delport, 2005).

Figure 4.1 graphically presents the blood glucose concentrations effects which the Almera potato samples had on the ten human subjects who consumed them. It can clearly be seen that the AUC of subjects 6, 7 and 10 were greater than for the other subjects as they are NIDDM. However, subjects with NIDDM also have greater AUC values for glucose (with which AUC of Almera is compared), which in general compensates for the higher AUC values, and produces a representative GI on calculation.

According to the ISO/DIS 26642 standard on GI methodology, subjects with NIDDM, or those using insulin, should not be included as participants in trials. As this standard is yet to be accepted, modifications to these recommendations could still be made. In future it is recommended that all diabetic subjects (both insulin dependent and non-insulin dependent) be excluded from *in vivo* GI trials to adhere to the recommendations within the ISO/DIS 26642 standard.

Table 4.2: Area under the concentration-over-time curve (AUC) and calculated glycaemic index (GI) values of Almera potatoes as determined by the Glycemic Index Foundation of South Africa (GIFSA) (Trail 1)

Subjects	Area under the concentration-over-time curve (AUC[#])		Glycaemic index (GI)^
	Glucose mean	Almera potatoes	
GI Mean	-	-	43
SD	-	-	14
95 % CI (lower)	-	-	33
95 % CI (higher)	-	-	52
Subject 1	439	201	46
Subject 2	304	85.0	28
Subject 3	311	89.7	29
Subject 4	315	149	47
Subject 5	287	154	54
Subject 6*	1248	661	53
Subject 7*	1283	788	61
Subject 8	406	110	27
Subject 9	220	121	55
Subject 10*	684	187	21

[#]AUC determined by geometric calculation by applying the trapezoid rule
^GI calculated as the AUC of Almera expressed as a percentage of the AUC of glucose
*Non-Insulin Dependent Diabetes Mellitus (NIDDM)
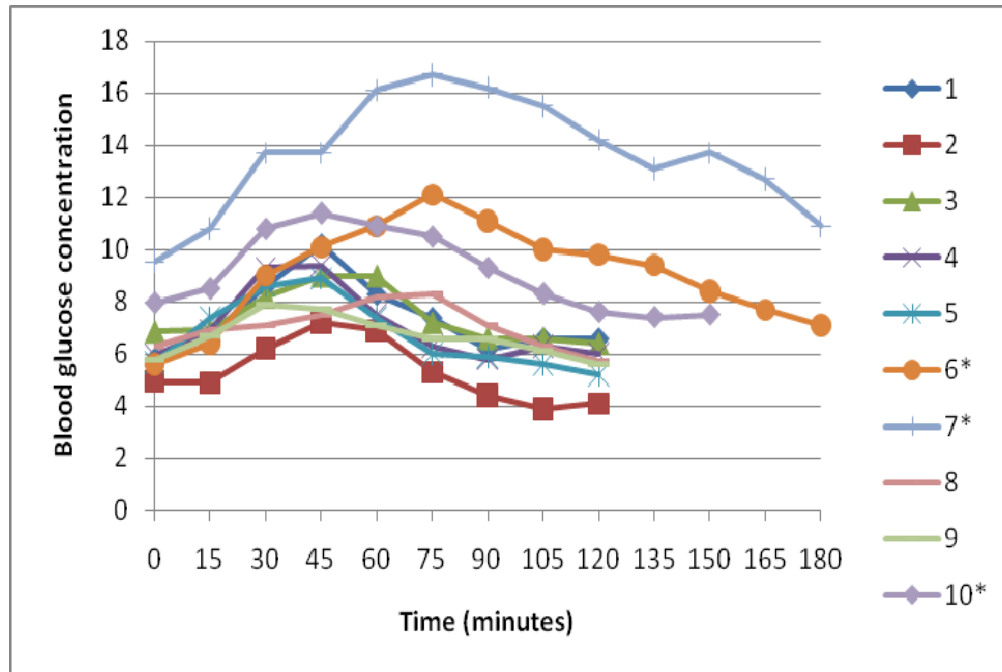
* Non-insulin-dependent diabetes mellitus

Figure 4.1: Capillary blood glucose response to Almera potatoes as tested in ten human subjects over time (minutes) at the Glycemic Index Foundation of South Africa (GIFSA) (Trail 1)

4.3 THE GLYCAEMIC INDEX OF GERO LITCHI AND RASPBERRY FAT FREE YOGHURT

4.3.1 Materials and methods

The GI of the Gero litchi and raspberry fat free yoghurt was determined by GIFSA at their Nelspruit laboratory, South Africa in October 2004. All subjects who took part in the trial were trained to ensure that they knew how to use glucometers accurately. Subjects performed a reference glucose test on at least two, but preferable three different occasions to establish a reference value. According to Wolever *et al.* (2003) the margin of error in GI estimates decreases significantly from one to two measurements of the reference foods, but no benefit is derived from taking more than three reference measurements.

A standard food portion was calculated using the nutritional analyses which were supplied to the laboratory by the farmer (Table 4.3). An amount of 847.5 g yoghurt was determined to present 50 g glycaemic carbohydrate. Ten subjects were required to consume the full portion, as is, within a

period of 15 minutes. Of the eight subjects that took part in the trail, four were NIDDM subject and one was an insulin-dependent diabetic mellitus (IDDM) subject. Blood glucose readings were taken every 15 minutes starting immediately after ingestion, until the blood glucose reading fell below the initial reading, or for a maximum total time of 180 minutes. The results from the subjects were used to determine the GI of the product by using the formula: GI = (AUC of the test food X 100) ÷ (AUC of the subjects' reference glucose value).

Table 4.3: Nutritional content of Gero litchi and raspberry fat free yoghurt expressed as a percentage (%)*

	Fat	Protein	Carbohydrates	Fibre
Gero litchi and raspberry yoghurt	0.5	5.0	6.0	0.73

*Obtained from product label

4.3.2   Results and Discussion

Table 4.4 presents the AUC and the mean GI values of each panel member, together with the SD. The CI indicates that the researcher can be 95 % confident that the GI of the specific product will lie between the bottom and the top values presented i.e. for Gero litchi and raspberry flavoured yoghurt between eight and 14.4.

The mean GI value of Gero litchi and raspberry flavoured yoghurt was 25 which classify this product as a low GI food (GI < 55). The SD was 15, which according to analytical report by GIFSA is adequate to consider the results as trustworthy.

Table 4.4: Area under the concentration-over-time curve (AUC) and calculated glycaemic index (GI) values of Gero litchi and raspberry flavoured yoghurt as determined by the Glycemic Index Foundation of South Africa (GIFSA)

| Subjects | Area under the concentration-over-time curve (AUC$^\$$) | | Glycaemic index (GI)^ |
	Glucose mean	Gero litchi & raspberry yoghurt	
GI Mean	-	-	25
SD	-	-	15
95 % CI (lower)	-	-	14.4
95 % CI (higher)	-	-	35.5
Subject 1	170	61.5	36
Subject 2	410*	108	26
Subject 3	1059$^\#$	513	49
Subject 4	550*	194	35
Subject 5	184	42.0	23
Subject 6	727*	13.0	2
Subject 7	620*	128	21
Subject 8	125	10.3	8

$^\$$AUC determined by geometric calculation by applying the trapezoid rule
^GI calculated as the AUC of yoghurt expressed as a percentage of the AUC of glucose
*Non-insulin-dependent diabetes mellitus (NIDDM)
$^\#$Insulin-dependent diabetes mellitus (IDDM)

In Figure 4.2 the glycaemic responses which the yoghurt samples elicited in each of the ten human subjects, are presented in the form of glucose response curves. It can be seen that subjects 1, 5 and 8 did not draw blood samples for the entire test period of 180 minutes, because their blood glucose levels returned to the fasting level or below more rapidly after consumption. A non-linear pattern, or scattered pattern, in the glucose response after the consumption of the Gero yoghurt samples is noted for most of the subjects.
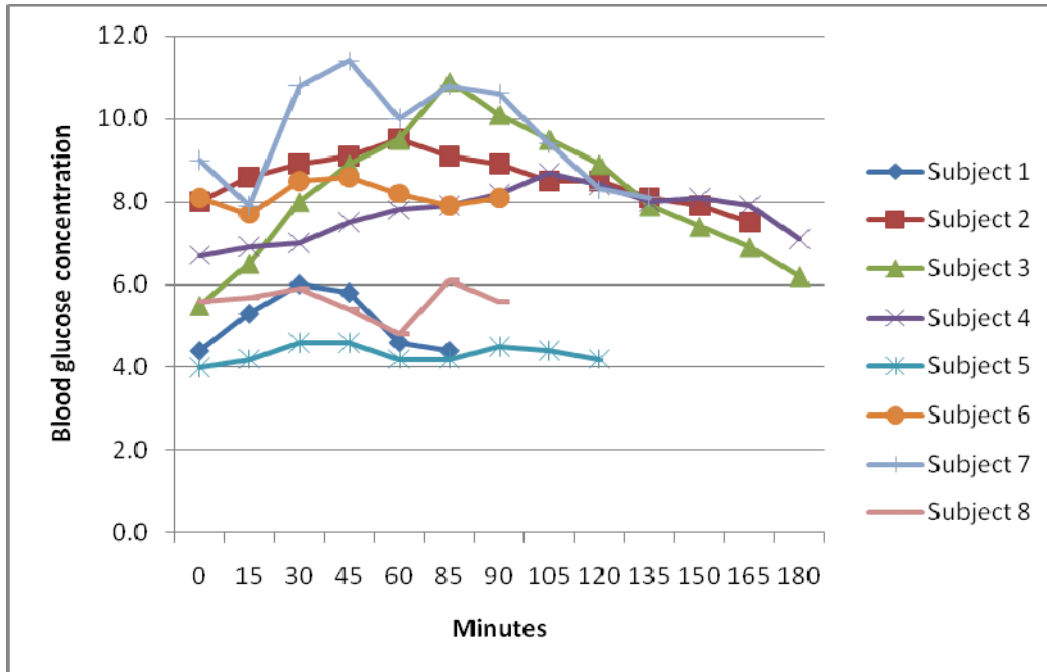
Figure 4.2: Capillary blood glucose response to Gero litchi and raspberry flavoured yoghurt as tested in eight human subjects over time (minutes) at the Glycemic Index Foundation of South Africa (GIFSA)

4.4 THE HYDROLYSIS INDEX OF ALMERA POTATOES AND GERO LITCHI AND RASPBERRY FLAVOURED YOGHURT DETERMINED BY THE RAPID ASSESSMENT METHOD

4.4.1 Materials and methods

4.4.1.1 Samples

Maltose and defrosted white bread (from the same batch as used when developing the method described in Chapter 3) were used as control samples during the trial. Samples to represent 2 g carbohydrates were weighed for each food, i.e. 2 g maltose and 4.4 g white bread.

A 3 kg bag of Almera potatoes was couriered from the cultivation area to Pretoria, South Africa, within three days post-harvest. Four potatoes were randomly selected from the bag to represent the batch. The potatoes were peeled and cut into blocks after which they were cooked,

uncovered, in boiling water on a stove top until soft (ten minutes). Three samples of 19.0 g, each containing 2 g of carbohydrate calculated from the nutritional composition previously obtained by analysis (Table 4.1), were used for HI determination. The samples were tested at room temperature, approximately five hours after cooking.

Three 100 ml litchi and raspberry flavoured fat free Gero yoghurts were purchased from retail outlets representing two separate batches (with different batch numbers). Samples weighing 34.0 g, containing 2 g of carbohydrate based on the nutritional composition provided on the product label, were used for the analysis.

4.4.1.2 Materials and preparation

Flat cellulose membrane dialysis tubing, porcine stomach pepsin powder, alpha amylase, and maltose monohydrate were obtained from Sigma-Aldrich, Johannesburg, SA. The ARC-Irene Analytical Services supplied dipotassium phosphate ($K_2HPO_4.3H_2O$, 28.23 mM) and potassium dihydrogen phosphate (KH_2PO_4, 136.09 mM) which were used to make up a 0.1 M potassium phosphate buffer solution (pH 6.9); orthophosphoric acid (H_3PO_4); hydrochloric acid (HCl, 0.2 M) and potassium chloride (KCl, 0.2 M), which were used to make up a HCl.KCl buffer (0.2 M, pH 1.68); and a 50 % potassium hydroxide solution (KOH).

The equipment used during the method belonged to the ARC-Irene Analytical Services, and included an Ultra Turrax, shaking water bath, single channel pipettes, Erlenmeyer flasks, volumetric flasks, volumetric cylinders, glass beakers, glass bottles with lids and the various funnels required.

To prepare the pepsin enzyme solution, 2.5 g of the porcine pepsin powder were dissolved in 10 ml HCl.KCl (0.2 M0) buffer. As alpha amylase is heat sensitive, the amylase solution was kept in the refrigerator at a temperature below 4°C until just before adding to the samples. A quantity of 31 µl was administered to each sample. For each sample tested 31 µl was made up to 1 ml with the potassium phosphate buffer solution, e.g. when eight samples were tested 310 µl amylase were made up to 10 ml, and 1 ml of the solution was administered to each sample.

4.4.1.3 Methodology

A sample of each food as eaten, containing 2 g of carbohydrate, was sliced, ground and place in an Erlenmeyer flask with 20 ml of a buffer solution at 37°C. The samples were further homogenized with an Ultra Turrax at 13 500 rpm until liquid or for a maximum time of two minutes. The Ultra Turrax was rinsed with an additional 20 ml buffer solution to ensure that most of the carbohydrates remained in the sample.

To simulate the gastric phase, the pH of each sample was decreased to pH 2.5 with 5 % orthophosphoric acid, after which 2 ml of the pepsin enzyme solution was added. The samples were swirled. All the samples were placed in a 37 °C shaking water bath for one hour to simulate the time that food would be churned in the human stomach.

After the gastric phase, each sample was buffered back to pH 6.9 ± 0.2 with 50 % KOH. Alpha amylase solution (2 ml) was added to each sample and swirled to distribute the enzyme throughout the sample. The content of each flask was then transferred into a dialysis tube, 20 to 30 cm in length. The tube was closed and placed in flasks containing 500 ml buffer solution. The flasks were placed in the stirring water bath and 40 ml of the buffer solution was extracted every 30 minutes to determine the rate of hydrolysis of carbohydrate from the dialysis tube into the buffer solution. In Figure 3.5 (Chapter 3) the methodology is summarized.

Reduced sugars were determined using Infra-red spectrophotometry (Milkoscan). All the experimental work was performed at the ARC-Irene Analytical Services, Agricultural Research Council: Animal Production Institute, Irene, South Africa.

The values were plotted on a graph and the AUC was determined. The 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 used:

AUC of food tested ÷ AUC of reference food (maltose) = HI of food tested

4.4.2 Results and discussion

The amount of carbohydrates which diffused through the dialysis tube of each sample into the surrounding fluid was tabulated and graphically presented as concentration-over-time curves (Figure 4.3). From these curves, the mean areas under the concentration-over-time curves for each tested food product were determined. The HI values were calculated as the relation between the AUC of the specific food compared to the AUC of maltose as the reference food, using the calculation indicated previously.
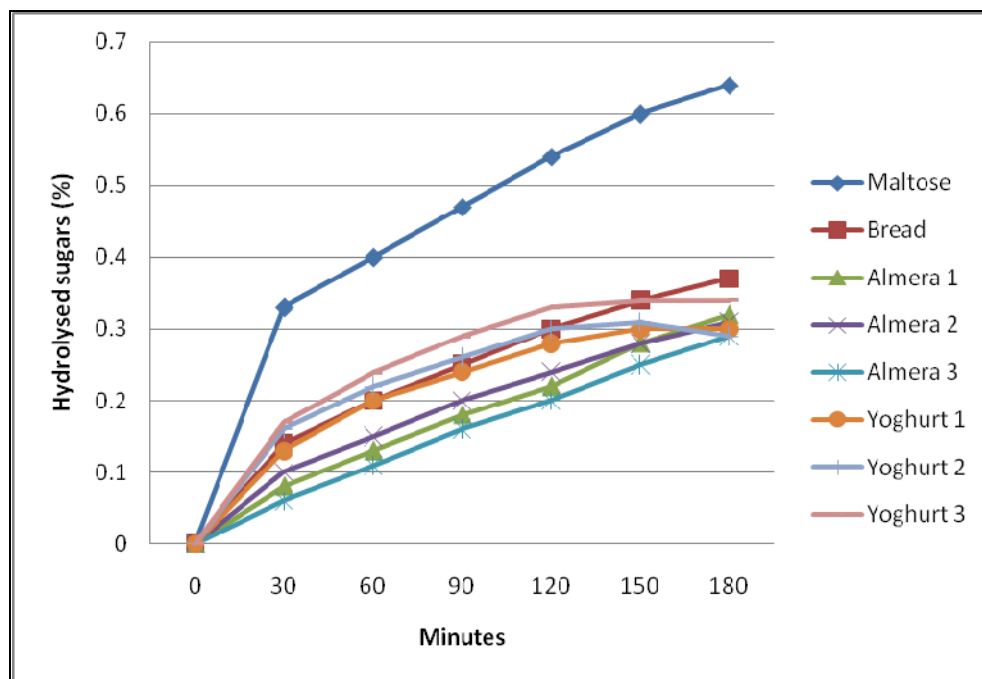
Figure 4.3: The concentration-over-time curves used for the determination of the area under the curve (AUC) of each sample tested

In Table 4.5 the AUC and calculated HI values are presented. White bread had a HI value of 53, Almera potatoes had a mean HI value of 38.8, while the Gero yoghurt had a mean HI value of 53. The low HI value of white bread could be attributed to the fact that the same bread was used for testing throughout the experimental period of twelve months, during which slices of bread were kept frozen until used for testing. According to a study done by Burton and Lightowler (2008) freezing significantly decreases the glycaemic response of white bread. This highlights the need

to define and maintain storage of white bread if used as a reference food. Within this research project maltose was used as the reference food, however white bread was included in each trial to determine repeatability. In future, fresh white bread, preferable of the same brand should be used.

Table 4.5: Area under the concentration-over-time curve (AUC) and hydrolysis index (HI) values of the maltose, white bread, Almera potatoes and Gero yoghurt

	Area under the concentration-over-time curve (AUC$^\$$)	Hydrolysis index (HI)^
Maltose	79.8	100
White bread	42.5	53.0
Almera potato Mean	31.0	38.8
SD	-	3.81
95 % CI (lower)	-	35.0
95 % CI (higher)	-	43.2
Sample 1	31.5	39.0
Sample 2	33.8	42.0
Sample 3	27.8	35.0
Gero yoghurt Mean	42.4	53.0
SD	-	4.55
95 % CI (lower)	-	48.5
95 % CI (higher)	-	57.6
Sample 1	39.0	49.0
Sample 2	41.9	52.0
Sample 3	46.2	58.0

$^\$$AUC determined by geometric calculation by applying the trapezoid rule
^HI calculated as the AUC of food tested expressed as a percentage of the AUC of maltose

4.5 COMPARING OBTAINED GLYCAEMIC INDEX VALUES WITH DETERMINED HYDROLYSIS INDEX VALUES

To establish if the rapid assessment method would be accurate in predicting the GI of foods, the determined HI were compared with the analysed GI values (Table 4.6). It should be noted that the SD of the HI method (3.81 potatoes; 4.55 yoghurt) was significantly smaller than that obtained from GI analysis (14 potatoes; 15 yoghurt). However, the limited number of samples used for each food product in HI determination could also be responsible for the low SD values.

Although it is advisable to use a sample size greater than five for a reliable t-test, a 95 % CI for the HI value determined for Almera potatoes (range between 29.4 and 48.3) indicates that the mean HI (38.8) does not differ significantly (p = 0.1999) from the GI value of 43. The 95 % CI for the mean HI value of Gero yoghurt (range between 41.8 and 64.4) indicates that the mean HI (53.1) differs notably from the mean GI value (25), although both values group Gero yoghurt in the low GI category (GI < 55).

The HI value of Almera potatoes (38.8 ± 9.47) predicts that this potato cultivar falls within the low GI range of less than 55. This was in line with results found with *in vivo* GI analysis which was conducted at GIFSA Durban on the same batch of potatoes, that established a GI value of 43 ± 9.6.

The HI value of Gero litchi and raspberry flavoured fat free yoghurt (53.0±11.3) predicts that this yoghurt would fall within the low GI range, with a probability of an intermediate GI response due to the large CI observed (range between 41.8 and 64.4). The *in vivo* GI analysis, conducted on a different batch two years prior to the HI trial, indicated the GI value of the yoghurt to be 25 ± 10.6, indicating a low GI with a relatively large CI (range between 14.4 and 35.5).

Table 4.6: Correlating calculated hydrolysis index (HI) values with obtained *in vivo* glycaemic index (GI) values

	Hydrolysis index (HI)				Glycaemic index (GI)			
	HI	SD	95 % CI (lower)	95 % CI (higher)	GI	SD	95 % CI (lower)	95 % CI (higher)
Maltose	100	-			100*	-		
Bread	53.0	-			72*	-		
Almera	38.8	3.81	29.4	48.3	43	14	33	52.4
Gero yoghurt	53.1	4.55	41.8	64.4	25	15	14.4	35.5

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

The individual values obtained from the rapid assessment method are visually compared to the GI values obtained from the GIFSA laboratory in Figure 4.4 for the Almera potato cultivar, and in Figure 4.5 for the Gero yoghurt, to display variation in results. A significantly greater variation is observed within the *in vivo* group (GI) than is observed between the samples which underwent *in vitro* analysis by means of the rapid assessment method (HI). Once again it should be noted that to accurately draw conclusions, a larger sample size for *in vitro* testing is advised.
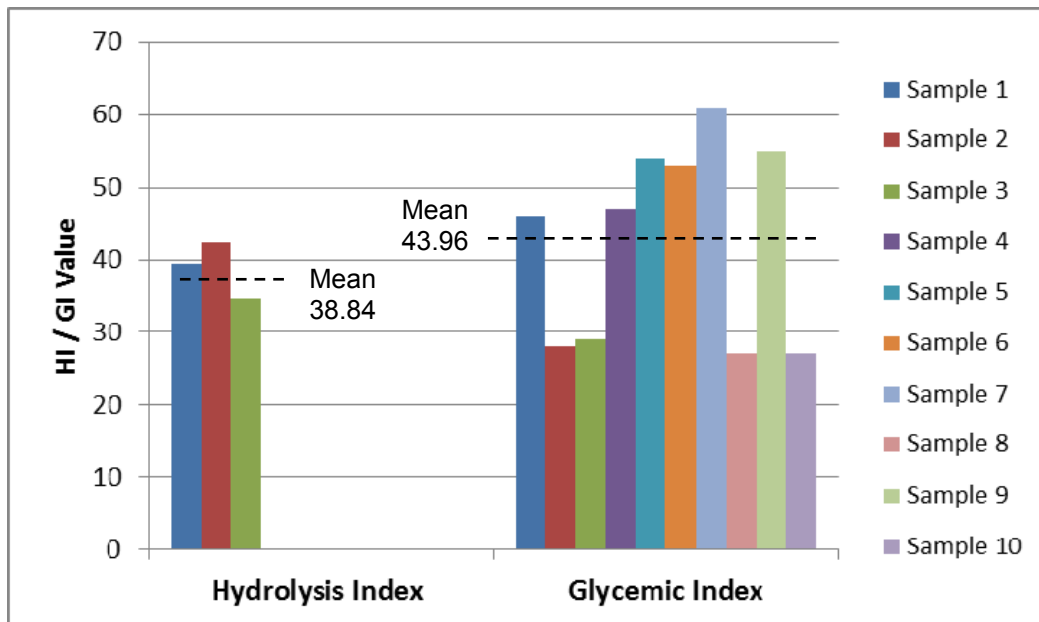
Figure 4.4: Individual hydrolysis index (HI) values (95 % CI 9.47) and glycaemic index (GI) values (95 % CI 9.6) for Almera potatoes to visualise variation in results
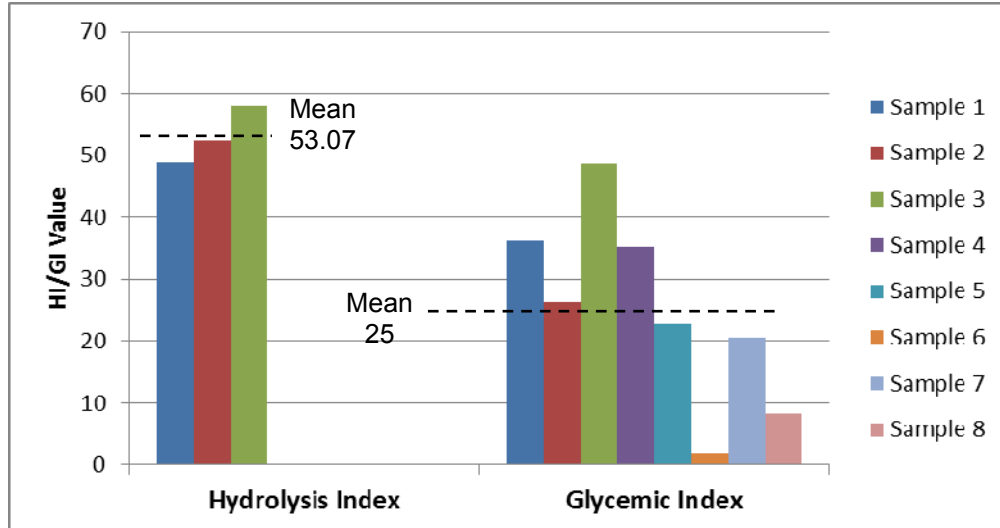
Figure 4.5: Individual hydrolysis index (HI) values (95 % CI 11.3) and glycaemic index (GI) values (95 % CI 10.6) for Gero litchi and raspberry flavoured yoghurt to visualise variation in results

4.6 A THEORETICAL INTER-LABORATORY STUDY BETWEEN *IN VIVO* LABORATORIES IN SOUTH AFRICA

As the test results from both GIFSA and the HI methodology indicated that the GI of Almera potatoes appears to be low, the producer intended to market the product as such within a retail chain. The retailer, according to protocol, had the GI of all products marketed as such analysed at NICUS on two different occasions. GI analyses were also repeated by GIFSA Nylstroom and the results found from these four trials were compared with results found by SUGIRS in Australia.

4.6.1 The glycaemic index of Almera potatoes determined by the Nutrition Information Centre of the University of Stellenbosch (NICUS)

GI determination at NICUS was repeated twice, once during June 2010 and once during August 2010, both in the same laboratory, but using different panel members. Both these trials are presented in this section.

4.6.1.1 Materials and methods

The objective of the studies was to determine the GI values of the Almera potato cultivar. The studies were approved by the Committee for Human Research of the University of Stellenbosch. During the first trial 13 healthy volunteers, and during the second trial 14 healthy volunteers, participated.

The potatoes were cultivated under controlled conditions, harvested when the tubers reached medium size, couriered to the laboratory within three days after harvest, and stored in a cool, dry place until needed. On the day of testing the products were peeled and cubed into squares of approximately 20 X 30 mm. These cubes were boiled until soft (approximately 20 minutes) and a portion to represent 25 g glycaemic carbohydrate (264 g) was weighed for each panellist during both trials. The samples were consumed by the volunteers and blood glucose was measured every 15 minutes for the first 60 minutes, and every 30 minutes for a second 60 minutes, adding up to a total period of two hours.

4.6.1.2 Results and discussion

The blood glucose concentration-over-time curves from the first trial are presented in Figure 4.6. Using the AUC values to determine GI, the results from the first trial indicated that the mean GI value was 84 (SD 21) (Table 4.7), which indicated that the product falls within the high GI category. These results are in direct contrast to the GIFSA results which had indicated that the Almera potato cultivar falls within the low GI category. Statistically there were no outliers identified within the NICUS group of volunteers, but it was recommended that the test be repeated by a new group of volunteers because the NICUS results differed so significantly from the GIFSA results.

During the second trial, 14 healthy volunteers ingested 264 g of cooked Almera potatoes to represent 25 g glycaemic carbohydrate. The mean GI for the second evaluation was even higher, namely 96 (SD 21) (Table 4.8), which is higher than white bread and close to the GI for glucose (the reference food). The recognised mean GI for baked potatoes as presented in the South African Glycemic Index and Load Guide is 85 (Steenkamp & Delport, 2005). In Figure 4.7 the concentration of blood glucose measured over time can be seen. It should be noted that during the trial candidates were not prompted to halt blood glucose withdrawal once a value lower than the fasting glucose value was obtained and all participants continued the trial for a total period of two hours.
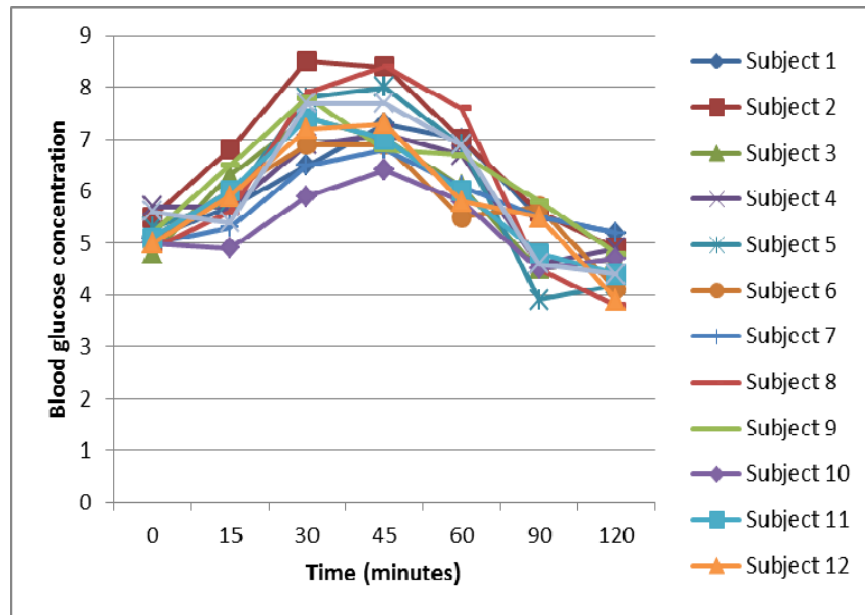
Figure 4.6: The concentration-over-time curves used for the determination of the area under the curve of each sample tested by volunteers at the Nutrition Intervention Centre of the University of Stellenbosch (NICUS) after consumption of the Almera potato sample (Trial 1)
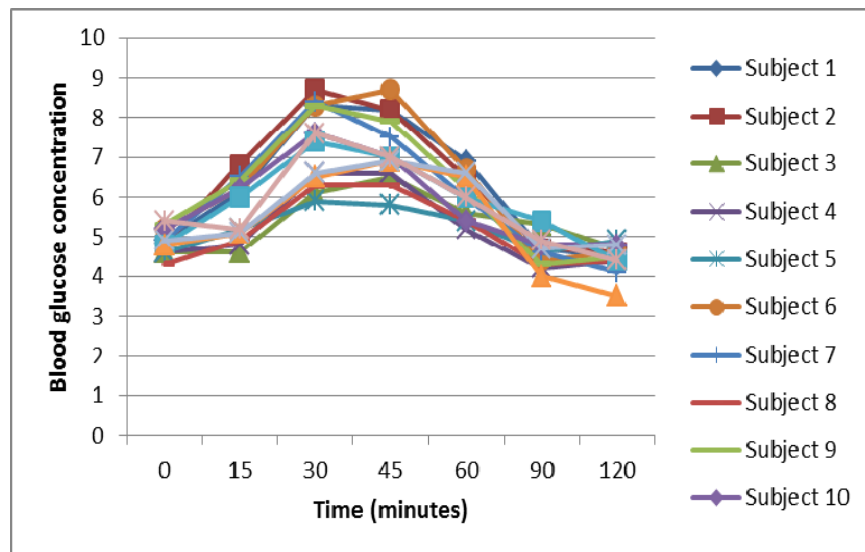
Figure 4.7: The concentration-over-time curves used for the determination of the area under the curve of each sample tested by volunteers at the Nutrition Intervention Centre   of the University of Stellenbosch (NICUS) after consumption of the Almera potato sample (Trial 2)

Table 4.7: Area under the concentration-over-time curve (AUC) and glycaemic index (GI) values of the Almera potato cultivar obtained from 13 healthy volunteers at the Nutrition Information Centre of the University of Stellenbosch (NICUS) (Trail 1)

| Subjects | Area under the concentration-over-time curve (AUC*) | | Glycaemic index (GI)^ |
	Glucose mean	Almera potatoes	
GI Mean	-	-	84
SD	-	-	21
Subject 1	96	119	125
Subject 2	145	144	99
Subject 3	95	120	126
Subject 4	80	53.6	67
Subject 5	214	110	51
Subject 6	124	88.9	72
Subject 7	99	96.8	98
Subject 8	192	164	85
Subject 9	152	131	86
Subject 10	83	47.2	57
Subject 11	154	93.4	60
Subject 12	137	109	80
Subject 13	101	82.4	82

*AUC determined by geometric calculation by applying the trapezoid rule
^GI calculated as the AUC of Almera expressed as a percentage of the AUC of glucose

Table 4.8: Area under the concentration-over-time curve (AUC) and glycaemic index (GI) values of the Almera potato cultivar obtained from 14 healthy volunteers at the Nutrition Information Centre of the University of Stellenbosch (NICUS) (Trial 2)

| Subjects | Area under the concentration-over-time curve (AUC*) | | Glycaemic index (GI)^ |
	Glucose mean	Almera potatoes	
GI Mean	-	-	96
SD	-	-	21
Subject 1	163	161	98
Subject 2	146	176	121
Subject 3	74	96	130
Subject 4	97	72.9	75
Subject 5	89	69	77
Subject 6	142	140	98
Subject 7	142	137	97
Subject 8	121	95.3	79
Subject 9	83	114	137
Subject 10	110	80.5	74
Subject 11	158	131	83
Subject 12	105	91.6	87
Subject 13	105	94.1	90
Subject 14	93	65	70

*AUC determined by geometric calculation by applying the trapezoid rule
^GI calculated as the AUC of Almera expressed as a percentage of the AUC of glucose

4.6.2 The glycaemic index of Almera potatoes determined by the Glycemic Index Foundation of South Africa (GIFSA) Trial 2

Due to the discrepancies in the results between the initial GIFSA trials and the results found by NICUS, it was decided to repeat the trial at the GIFSA laboratory. During the second trial it was decided to boil the potatoes the morning before consumption and consume warm, similar to the method followed by NICUS, rather than the evening before and consumed the next morning at room temperature. This method was followed for the samples which underwent the first GIFSA trial. It should be noted that this cooking and cooling method used in the first GIFSA trial was

repeated when using the developed rapid assessment method to predict the GI of the Almera potatoes. This was done to compare the results obtained by these two different methods.

The development of retrograded starch during cooling could have decreased the GI of the potatoes. It is considered that the GI of potatoes is not significantly lower after cooling when compared to the effect of cooling on the GI of other foods such as maize meal porridge (Delport, GIFSA, personal communication, 2010). However, a study conducted by Fernandes, Velangi and Wolever (2005) and Kinear and Wolever (2010) found that cooling had a different effect on GI between different potato cultivars. The general conclusion of both publications was that the effects of cooking, cooling and reheating on the GI depends on potato variety.

4.6.2.1 Materials and methods

The second trial was conducted according to the draft ISO/DIS 26642 which is based on the joint FAO/WHO Expert Consultation on Carbohydrates in Human Nutrition (FAO/WHO, 1998), the recommendations of the ILSI appointed International Committee for Standardisation of GI Testing Methodology (Brouns *et al.,* 2005), and the two inter-laboratory studies on the meaurement of GI of foods (Woelever *et al.,* 2003; Wolever *et al.,* 2008).

Eleven trained, healthy volunteers took part in the trial (no subjects were NIDDM or IDDM). Subjects took a fasting blood glucose reading as well as a second reading as a control, the mean of which was used in the calculations (as is recommended in the draft ISO/DIS 26642).

The potatoes were peeled and cooked in salt water until done (approximately 15 minutes). Portions weighing 528.5 g, representing 50 g available carbohydrate, were consumed after cooking while still hot, by each individual within a time frame of 15 minutes. A drink of water, rooibos tea or decaffeinated coffee (250 – 500 ml) was allowed to be consumed together with the food portion. Blood glucose readings were taken immediately after ingestion (fasting reading) and then every 15 minutes for a total period of two hours, or until the blood glucose reading fell below the fasting level.

4.6.2.2 Results and discussion

The Almera potatoes appeared to have an intermediate GI of 63, with a 95 % CI of between 49.6 and 76.0 (Table 4.9). According to the results, this means that the consumer can be 95 % confident that the potatoes will have a GI value of between 49.6 and 76. As previously mentioned, the GI of the samples tested during the first trial at GIFSA could have been lower due to the cooling of the samples overnight. As the samples in this test were consumed warm, carbohydrates were more readily available for absorption and had a more pronounced effect on blood glucose.

According to the GIFSA test report (2010), the amylose to amylopectin ratio could have a significant influence on GI. Because the GI of potatoes is generally high, it is possible that the Almera cultivar has a higher amylose content, which was shown to decrease the GI in rice. In contrast, a higher amylopectin ratio increases GI (Van Amelsvoort & Weststrate, 1992).

The 50 g carbohydrate portion (528.5 g cooked potatoes) consumed by each panelist was double the amount consumed by each panelist during the NICUS trail (25 g carbohydrate portion; 264 g cooked potatoes). Both these portion sizes are acceptable according to the ISO/DIS 26642 standard, as this should not significantly influence the GI. The larger portion size (50 g carbohydrate portion; 528.5 g cooked potatoes) is a very large portion to consume. However, based on the complications (including time and financial constraints) of determining 25 g glucose reference values for each panel member, GIFSA decided to use the greater 50 g food portion. Glucose reference values after the consumption of 50 g glucose were already available for all panel members.

Table 4.9: Area under the concentration-over-time curve (AUC) and glycaemic index (GI) values of the Almera potato cultivar obtained with eleven healthy volunteers at the Glycemic Index Foundation of South Africa (GIFSA) (Trail 2)

Subjects	Area under the concentration-over-time curve (AUC*)		Glycaemic index (GI)^
	Glucose mean	Almera potatoes	
GI Mean	-	-	63
SD	-	-	18
Subject 1	316	160	51
Subject 2	244	197	81
Subject 3	179	152	85
Subject 4	173	105	61
Subject 5	296	254	86
Subject 6	265	118	45
Subject 7	220	77.3	35
Subject 8	252	115	46
Subject 9	321	205	64
Subject 10	356	272	76

*AUC determined by geometric calculation by applying the trapezoid rule
^GI calculated as the AUC of Almera expressed as a percentage of the AUC of glucose

4.6.3 The glycaemic index of Almera potatoes determined by the Sydney University Glycemic Index Research Service (SUGIRS)

SUGIRS was established in 1995 to provide a commercial GI testing laboratory for the international food industry. The standard protocol at the laboratory involves feeding ten or more healthy volunteers a portion of test food containing 50 g glycaemic carbohydrate, and measuring the effect on blood glucose concentration over a period of two hours. Each person, at another stage, consumes a standard glucose portion containing 50 g glucose and the response of blood glucose concentration is also measured. A GI value for the test food is calculated, as in the South African laboratories, by dividing each individual's glucose AUC of the test food by their glucose AUC of the reference food. The final GI value is then calculated as the mean GI for all participants that took part in the trial.

According to Dr Alan Barkley of SUGIRS, their laboratory tested the GI of the Almera potato cultivar on eight different occasions. The results from these trials varied from 40 to 69, with a mean GI value of 55. For each test, the potatoes used for the samples were grown under slightly different conditions. In Dr Barkley's opinion the Almera cultivar, when grown under the correct conditions and cooked appropriately, would have a low GI (Barkley, SUGIRS, Personal communication, 2010).

4.6.4   Comparing the glycaemic index values of Almera potatoes determined by different laboratories

As significant differences were observed between the GI results obtained with Almera potatoes between the different laboratories in South Africa, it was decided to investigate the possible reasons for discrepancies. It can be clearly seen that the GI values from the NICUS laboratory were significantly greater than the values obtained from GIFSA and SUGIRS (Figure 4.8). Furthermore, the data obtained in the second trial at GIFSA produced results which were similar to the result published on the electronic database of the glycemic index website (www.glycemicindex.com).

The mean GI, calculated by averaging all the available values, comes to an intermediate GI value of 67, which also groups the cultivar in the same category as did the second GIFSA trial. According to these results it could be said that the Almera potato cultivar should be classified as an intermediate GI food.
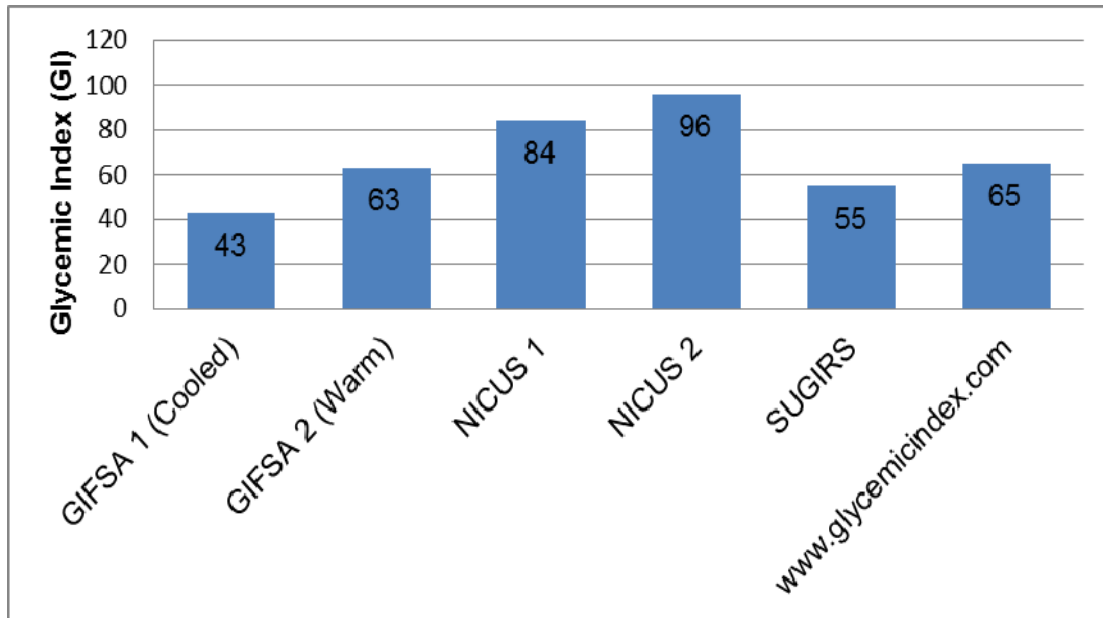
Figure 4.8: GI values for cooked Almera potatoes obtained from *in vivo* testing at the Glycemic Index Foundation of South Africa (GIFSA) (n = 2), the Nutrition Intervention Centre of the University of Stellenbosch (NICUS) (n = 2), Sydney University Glycemic Index Research Service (SUGIRS) (mean of eight tests), and the data available from the electronic database at www.glycemicindex.com

As previously stated, various factors influence the GI of a food product including product characteristics such as cultivar, preparation method, etc. Furthermore, the method used to determine GI could also impact significantly on the GI reading which is obtained.

4.6.4.1 Inter- and intra-individual variability

Firstly it is essential to note that human subjects vary. Secondly, many factors, including emotional and stress factors, which cannot be controlled, may also play a significant role in influencing glycaemic response (Cummings & Stephen, 2007).

Concerning subject variability, a study conducted to determine the inter-individual and intra-individual variability of GI values of white bread found a high degree of variation both between individuals (CV = 94 %), and within individuals over a period of time (CV = 17.8 %, with a 42.8 %

variability) (Vega-López, Ausman, Jalbert & Lichtenstein, 2006). Wolever, Nuttall, Lee, Wong, Josse, Csima *et al.* (1985) found a significant coefficient of variation (CV) (25 %) in glucose response within individuals forming part of an eleven member panel on eight separate occasions. Another study evaluated the intra-individual reproducibility (within the same individual, when repeatedly measured) and inter-individual variability (among individuals) of GI value determinations for white bread relative to glucose in 56 volunteers who differed by sex, age (18 to 85 y) and body mass index (BMI) (18.5 to 24.9, 25 to 29.9, 30 to 35 kg/m^2). Each volunteer underwent three sets of food challenges in random order, after abstaining from strenuous physical activity and alcohol intake for at least 72 hours prior to each session. Each set involved ingestion of glucose and white bread (each containing 50 g available carbohydrate). An intra-individual CV range from 2 to 77 % and an inter-individual CV of 25 % were found. An association was observed between the GI value for white bread and age (r=0.30, p=0.028), but not for sex (63 ± 15 vs. 63 ± 18; males vs. females, p=0.96) or BMI (67 ± 18, 62 ± 14 and 58 ± 15 for BMI categories 18.5 to 24.9, 25 to 29.9 and 30 to 35, respectively, p=0.17) (Matthan, Ronxhi, Ausman, Griffith, Pittas & Lichtenstein, 2010). These high CV values within subjects suggest that no one individual can be expected to give accurate glycaemic values at all occasions.

There is also a large variability in glycaemic response between different individuals (Frost & Dornhurst, 2000). In a study which included healthy individuals, non-insulin treated NIDDM, insulin-treated NIDDM and IDDM subjects, it was found that the CV values between individuals from each group were 26 %, 34 %, 23 % and 34 % respectively (Wolever & Jenkins, 1986). This adds up to a mean inter-individual CV of 29 %.

Earlier work by Coulston, Hollenbeck, Liu, Williams, Starich, Mazzaferri *et al.* (1984), noted that by expressing glycaemic response of a test food as a comparison of the response to a reference food, the variation in GI that occurs for age, sex, body composition, ethnicity and medical conditions should be accounted for. Similarly Jenkins *et al.* (1983) found that by expressing glycaemic results this way reduced inter-individual CV from 40 % to 10 %.

Although expressing values as a percentage compared to response to a control food reduced inter-individual variation, the GI measurements of the same food have been seen to vary greatly between individuals (Frost & Dornhurst, 2000). Although GI was calculated as stated above, the study by Matthan *et al.* (2010) indicate that variability in GI values can still in part be explained by differences in age. Another study done by Hollenbeck, Coulston and Reaven (1986) found that the GI values of lentils range between 23 and 70 for different subjects. It is furthermore suggested that this variation in results obtained between individuals can be reduced when both the food to be tested and the control food are measured in triplicate by each panellist (Frost & Dornhurst, 2000; Wolever, Jenkins, Josse, Wong & Lee, 1987).

In this study, as a high degree of variation was observed between individuals within the initial GI tests at both laboratories, it was prudently decided to repeat the analysis in both laboratories. However, including an additional 14 individuals in the second NICUS trial did not alter the GI of the test food in such a way as to change the GI category into which the food would be classified (Table 4.6; Table 4.7).

During the initial GIFSA trial, three NIDDM subjects were included in the test group. According to the draft ISO/DIS 26642 standard, only healthy subject should be included in trials. NICUS used only healthy subjects with BMI's ranging from normal to obese. No data was available on the individuals included in the trials performed by the SUGIRS. Both GIFSA and NICUS each recruited a total of more than 20 individuals to determine the GI of the Almera cultivars, yet the GI results remained significantly different between the two laboratories. It is therefore possible that differences in methodologies used by the different laboratories may have resulted in the significant variation in the results.

4.6.4.2 Between laboratory variation and control measures

Differences often exist between the methodologies used in different laboratories (size of the carbohydrate load, reference food, time of follow-up food, different ways of calculating value, etc.). It is believed that many of these differences do no influence the results significantly.

a) Number of subjects

According to the FAO/WHO Expert Consultation Report on Carbohydrates in Human Nutrition (1998) no less than seven subjects should be used in any one GI trial. Subjects should be studied on separated days in the morning after a ten to twelve hour fast. A standard drink of water, tea or coffee is allowed with each test meal (FAO/WHO, 1998). The draft ISO/DIS 26642 standard recommends that no less than ten subjects be included in each trial. In both laboratories more than ten subjects were included during each trial, and a total number of more than 20 individuals volunteered and successfully completed the *in vivo* GI testing in each of the laboratories.

b) Amount of test food

Testing requires that both the reference food and test food contain the same amount of available carbohydrate, i.e. either 25g or 50g. According to the FAO/WHO Expert Consultation Report, the test portion should contain 50 g glycaemic (available) carbohydrate, while the ISO/DIS 26642 states that both 25 g or 50 g samples can be used, provided that the reference food dose corresponds to the amount of test food administered.

One of the main differences of the methodology used by GIFSA and NICUS was the amount of test sample included in each trial. GIFSA included a test food sample representing 50 g glycaemic carbohydrate (528.5 g), whereas NICUS included a test food samples representing 25 g glycaemic carbohydrate (264 g) because a sample exceeding 500 g was considered excessive for consumption.

Although both sample sizes are considered adequate by the ISO/DIS 26642 standard, a study by Wolever and Bolognesi (1996) determined the effect which source and amount of available carbohydrate had on postprandial glucose. They determined the effect which samples containing 0, 25, 50, 75 or 100 g available carbohydrate would have on blood glucose. Through regression analysis they found that the amount of carbohydrate administered was responsible for 47 to 57 % of the variation in the results. As mentioned, calculating a food's GI in terms of a comparison of the test food's response to that of a control food decreases the possible variability which the

decrease in portion size could have. It is still recommended that the possible effect which different portion sizes could have had on glycaemic response be further investigated.

c) *Carbohydrate determination*

According to Foster-Powell *et al.* (2002), one of the main reason why GI values for similar foods often vary is because of the method used for determining the carbohydrate content of the test foods. Composition tables are often used for determining the carbohydrate content of the test food portion, as opposed to direct analytical measurement of available carbohydrates (Foster-Powell *et al.,* 2002). The available carbohydrate should not include resistant starch. This can be difficult as resistant starch is difficult to measure, and often no values are available in food composition tables (Foster-Powell *et al.,* 2002). This glycaemic carbohydrate is calculated as the total carbohydrates minus dietary fibre as determined by the AOAC method (FAO/WHO, 1998).

Although nutritional data were analysed and used to calculate the sample size of the test foods, carbohydrate by difference, together with analytically determined dietary fibre, were used to calculate the glycaemic carbohydrate value. In future, as recommended by the ISO/DIS 26642, it will be considered compulsory to analytically determine total individual glycaemic carbohydrates (starches and sugars).

d) *Reference food*

According to the FAO/WHO Expert Consultation Report (1998) either white bread or glucose can be used as a reference food. Acceptable reference foods according to the ISO/DIS 26642, include anhydrous glucose powder (50 g), dextrose (55 g), commercial solutions used for oral glucose tolerance tests containing 50 g glucose, or white bread or other specific carbohydrate food of consistent composition and GI. Both laboratories used glucose as the reference food.

e) *Previous meal and consumption time*

The glycaemic response to the same food or meal may be influenced by the time, composition and GI of the previous meal (Arvidsson-Lenner *et al.,* 2004). Studies have shown that consuming

a low GI food with a prolonged glycaemic response for breakfast results in improved glucose tolerance at lunch time (Arvidsson-Lenner *et al.,* 2004). A similar tendency is seen with breakfast after a low GI supper the night before. This effect has been termed the "second meal effect" (Wolever, Jenkins, Ocana, Rao, Collier, 1988). A possible explanation of this effect might be due to some low GI foods producing prolonged insulin responses, and providing sustained insulin levels during the next meal. Also the colonic fermentation of dietary fibre (decreasing the GI of a food), results in elevated short chain fatty acid serum levels. This in turn reduces serum-free fatty acid levels and glucose output from the liver (Arvidsson-Lenner *et al.,* 2004).

According to the report received from GIFSA, individuals received two information sheets containing information related to the code of conduct required to eliminate possible variations. All subjects were required to consume one of three prescribed pre-test meals on the evening before the tests. All individuals also recorded the specific meal consumed the previous night on the test form. No specifications regarding the meal which individuals in the NICUS test groups consumed are stated in the reports. Further information was requested, however, no feedback was received.

According to the ISO/DIS 26642, subjects should arrive at the testing site in the fasting state, two blood samples should be taken in the fasting state and the average of these used as the baseline blood glucose concentration. The GIFSA test reports noted that all participants were requested to fast for a period of at least ten hours before the test, during which sips of water were allowed. No specifications are indicated in the information received from NICUS. The reports indicated that the testing complied with the recommendations of the ISO/DIS 26642, and it is thus anticipated that subjects were informed to arrive in the fasting state, and that they complied.

The ISO/DIS 26642 states that subjects should consume the food portion in a period of five to 14 minutes, and consume a drink of one or two cups water, coffee or tea (sweetened with non-nutritive sweetener) in this same period. GIFSA reported that participants consumed the samples within a 15 minute time frame and were allowed to consume one cup (250 ml) of water, rooibos tea or decaffeinated coffee (sweetened with artificial sweetener if desired).

Furthermore, all subjects are required to rest during testing (ISO/DIS 26642). GIFSA reported that participants were required to refrain from physical activity and not smoke during the duration of the test. No specifications were reported in the NICUS test report, however, as previously stated, they did note that they adhere to the recommendations of the ISO/DIS26642 standard.

f) Analysis procedure and blood sampling

Blood glucose response is normally measured by drawing capillary whole blood, however in some cases venous blood is also drawn. An inter-laboratory trial involving seven experienced GI laboratories found that the five laboratories that used finger prick capillary blood sampling obtained similar GI values, with less inter-individual variation (Foster-Powell *et al.,* 2002). According to Foster-Powell *et al.* (2002) the reason for this is that after food ingestion the glucose concentrations change to a greater degree in capillary blood than in venous blood, and could thus also be a more accurate reflector of glycaemic response after consumption of high GI foods. Capillary blood is also preferred in the FAO/WHO Expert Consultation Report (1998), as it is easier to obtain and the rise in blood glucose is greater and less variable than in samples drawn from venous blood.

During the trials performed at both laboratories (GIFSA and NICUS) capillary blood was drawn. During GIFSA testing, analysis ceased as soon as blood glucose dropped below the fasting level. NICUS continued testing for a total period of two hours.

g) Methods used to calculate glycaemic index

According to Monro (2005) there is still no agreement on the best method to calculate the incremental area under the blood glucose response curve (AUC). A number of different methods have been used to determine the AUC, but the FAO/WHO (1998) stated that the most often used method involves geometric calculation by applying the trapezoid rule (FAO/WHO, 1998; Monro, 2005). Prior to the standardized methodology presented in the FAO/WHO Expert Consultation Report on Carbohydrates in Human Nutrition (1998), different groups used different techniques to calculate the area under the blood glucose response curve. To avoid this problem, most

international tables have provided conversion factors or indicate the different methods used (Frost & Dornhurst, 2000).

In the method proposed by the FAO/WHO (1998) and ISO/DIS 26642 the net AUC over the baseline value at zero time is determined by adding positive trapezoids, in most cases ignoring the area beneath the fasting concentration baseline (when blood glucose falls below the baseline, only the area above the baseline is used in the calculations). GIFSA indicated that they used the calculation recommended in the ISO/DIS 26642, and NICUS only stated that the international protocol was followed in the test reports supplied. Additional information requested was not made available at the time of submission of this thesis.

4.7 CONCLUSIONS

The results from the rapid assessment method classified the Almera cultivar in the same category (low GI) as the first GIFSA trial results (low GI), which used the same batch of potatoes and where samples were evaluated after cooking and cooling, prior to consumption. The rapid assessment method results for Gero yoghurt also classified the yoghurt within the same category (low GI), in a similar fashion to the results found in the GIFSA trial. These results provide persuasive evidence that the rapid assessment method can accurately predict the GI of food products. Future testing of the accuracy of the rapid assessment method to predict GI of food products with a more complex food matrix (containing fat, protein and carbohydrate) is recommended.

Significant differences were found between the GI values and more specifically, GI categories (low, intermediate and high GI), obtained from the different laboratories executing *in vivo* analysis on the Almera potato cultivar. The laboratories indicated that they used the same methodology recommended in the draft ISO/DIS 26642. Although the differences between methodologies used, are generally not considered to affect GI and are allowed according to the ISO/DIS 26642, further investigation and clarification are recommended.

Although the laboratories in South Africa grouped the Almera cultivar into the low, intermediate and high GI categories based on the results from the different tests, the mean GI category when combining all available values for Almera potatoes indicated that the cultivar falls within the Intermediate GI category (GI 67). It was also determined that the Almera cultivar falls within the intermediate GI category according to the second GIFSA GI test (GI 63) (performed on warm samples served immediately after cooking), the mean value from studies by the SUGIRS (GI 55), and the value from the electronic database at www.glycemicindex.com (GI 65). GIFSA has endorsed the Almera potato cultivar in South Africa to be marketed as an intermediate GI option, and labelling with the orange "lower GI" GIFSA logo (Figure 1.3) will be allowed. The Diabetes SA (Figure 1.5) endorsement logo will also be allowed to be displayed. The words "Special Treats" will need to be printed below the logo, together with the GL per meal serving.

4.8 ACKNOWLEDGEMENTS

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

In this concluding chapter the main findings of the research are presented in relation to the current South African nutrition situation. The developed method is summarized and presented in a standard format for future testing of samples. Recommendations are presented for future actions.

5.1 INTRODUCTION

The joint FAO/WHO Expert Consultation on Carbohydrates in Human Nutrition held in Geneva in September 1979 was the first to focus on the topic of carbohydrates (FAO/WHO, 1980). This meeting aimed to review the role of carbohydrates as determinants of human health and disease. Carbohydrate based foods, and especially simple sugars with rapid glycaemic responses in the human body, were increasingly regarded as responsible for weight gain and obesity, increasing risk of developing non-insulin-dependent diabetes mellitus (NIDDM), certain cardiovascular diseases and even certain types of cancer. In 1997, eighteen years later, the joint FAO/WHO Expert Consultation on Carbohydrates in Human Nutrition added the role which classifying foods according to GI and GL could play in making healthy food choices. Furthermore, they recommended that both the chemical composition as well as the physiological effects of food carbohydrate should be considered in healthy food choices (Nantel, 1999; FAO/WHO, 1998). The concept of GL is becoming increasingly relevant as researchers realise that diets that all low GI do not neccasarily ensure weightloss if the total energy content of the diet is not taken into consideration. The GL of a food or meal can only be calculated if the GI of most ingredients are available, a situation which has not yet been achieved in South Africa.

In the developing world, where the emphasis has traditionally only been on undernutrition, the prevalence of obesity is also increasing at a rapid rate (Steyn and Temple, 2008). Most developing countries, such as South Africa, are faced with the consequences of both nutritional deficiencies and excesses, and are subjected to the double burden of persisting under-nutrition in the midst of the growing epidemic of obesity and non-communicable diseases (Schönfeldt &

Gibson, 2009). For South Africa, the National Food Consumption Survey (NFCS) (1999) showed that one out of two children had energy intake less than two thirds of their energy needs. Nationally the mean intakes of calcium, iron, zinc, vitamins A, D, E, C, B6, riboflavin and niacin were also very low compared to the Daily Recommended Intake (DRI). In 1999, one in ten children between the ages of one and nine years were underweight, and just more than one in five were stunted (NFCS, 1999). In contrast to the nutrient deficiencies in South Africa, 56.2 % of the adult population was recorded as overweight or obese in 2003 (SADHS, 2003).

To combat malnutrition, it is thus important to consider both under- and overnutrition. The most commonly consumed foods in South Africa, as reported by the 2003 South African Demographic and Health Survey, are maize meal porridge, government subsidised brown bread, full cream fresh milk, white sugar and tea (SADHS, 2003). Furthermore, the very low socioeconomic groups spend about a third of their total food cash expenditure on bread and cereals, dominated by maize meal followed by bread. Maize meal and bread are both carbohydrate sources which are regarded as high GI, providing rapid energy, and not sustaining satiety.

Rapid urbanization is continuous in South Africa, with people increasingly moving from rural areas into urban settlements in search of better work opportunities and income generation potential. A study done in an urbanised informal settlement, found that the majority of these households (60 percent) had a monthly income of less than ZAR500/month (US$64.56). Of the income available to these households, up to 71 percent was allocated to the purchasing of food, which consisted mainly of maize meal (Amuli, 2006). In terms of food product development prospects, significant challenges exist for the food industry, as well as for the government, to present these low income consumers with healthy and affordable food choices. Furthermore, the existing health trend within the higher socioeconomic groups (Vermeulen, 2010) creates an opportunity to increase the number of food products that have a GI classification to enable healthy food choices.

Until recently, the only way to determine the GI of a food was by determining the increment in blood glucose concentration in a human subject 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.). This is a process which is often considered out of reach for many food companies based on cost and time expenditures, especially during the product formulation phase. The developed rapid assessment method aims to provide the industry with a rapid screening tool which could be used during product development to identify formulations with desired glycaemic responses.

5.2 SUMMARY OF FINDINGS

During the development of the rapid assessment method in this study, it was found that the results from the method correlated well with GI data tabulated in the South African Glycemic Index and Load Guide (Steenkamp & Delport, 2005). The values which differed significantly were for different potato cultivars and growth stages, motivating the need to include cultivar (species level) when classifying foods according to GI. This is in line with the increasing focus on biodiversity, including describing food at species level (FAO, 2010).

Using the same sample preparation, the method is able to group food products in the same category as the *in vivo* GI assessment by GIFSA. Signifcant differences were however observed between the laboratories in South Africa testing the same food (Almera potatoes), indicating the need for future alignment of methodology. Furthermore, significant differences were found when sampes were prepared differently, indicating the need to identify cooking method and other preparatory directions on food packaging to ensure accurate classification of the food as consumed.

The developed method produced good repeatability (CV 0.78 %), reproducibility and precision (CV 3.5 %), and it can thus be stated that the method can be used with confidence to predict the GI category of food products.

5.3 HYDROLYSIS INDEX METHODOLOGY

The following method was developed and validated in this study.

5.3.1 Principle of the method

Carbohydrate based foods are subjected to *in vitro* simulation of the human GIT to determine the rate of digestion and absorption of carbohydrates from the intestinal tract into the surrounding fluids. This is done by determining the rate at which the carbohydrates hydrolyse through a permeable membrane. The three different phases which food particles undergo, are the oral phase, the gastric phase and the intestinal phase. These phases are simulated by adjusting pH to represent the pH in the human body within the different phases, adding appropriate enzymes, and subjecting samples to peristaltic movements using a shaking water bath at 37 °C.

Maltose is used as a reference standard to determine the rate of hydrolysis of the carbohydrates in the specific food, in comparison to the rate of hydrolysis of maltose as the reference food. The amount of carbohydrates absorbed, is measured by means of infrared spectrophotometry (Milkoscan).

5.3.2 Reagents

The following reagents are used:

- Potassium phosphate buffer solution (0.1M, pH 6.9)
- Orthophosporic acid
- HCl.KCl buffer (pH 1.68)
- Potassium hydroxide solution (50 %)
- Porcine stomach pepsin powder (77160)
- Alpha amylase (A9972)
- Maltose Grade 1 monohydrate.

5.3.3 Solutions

The following solutions are used:

- Pepsin enzyme solution

To prepare the pepsin enzyme solution, 2.5 g of pepsin are dissolved in 10 ml HCl.KCl buffer solution, 2 ml of which are added to each food sample.

- Alpha amylase solution

To prepare the amylase solution, 310 µl refrigerated enzyme (kept refrigerated until just prior to usage) is made up to 10ml with potassium phosphate buffer, 1 ml of which is administered to each food sample.

5.3.4 Apparatus

The following apparatus is used:

- Flat Cellulose Membrane dialysis tubing (D9652)
- Ultra Turrax
- Shaking water bath at 37°C
- Single channel pipettes
- 200 ml Erlenmeyer flasks
- 20 ml, 50 ml and 2000 ml volumetric flasks
- 100 ml and 500 ml volumetric cylinders
- 200 ml glass beakers
- 500 ml glass bottles with lids
- Various sized funnels

5.3.5 Procedure

The procedure followed is described in a step-by-step format:

5.3.5.1 Oral phase

A sample of each food as eaten, containing 2 g of carbohydrate, is sliced and ground and transferred to an Erlenmeyer flask with 20 ml of potassium phosphate buffer solution at 37 °C, and further homogenized with an Ultra Turrax at 13 500 rpm until liquid or for a maximum time of two minutes. It is subsequently rinsed with an additional 20 ml buffer solution to ensure that most of the carbohydrates remained in the sample.

5.3.5.2 Gastric phase

Decrease pH of each sample to pH 2.5 with 5 % orthophosphoric acid. Add 2 ml of the pepsin enzyme solution. Swirl samples. Place samples in a 37 °C shaking water bath for one hour to simulate the time that food would be churned in the human stomach.

5.3.5.3 Intestinal phase

Buffer samples back to pH 6.9 ± 0.2 with 50 % KOH. Add 2 ml of the alpha amylase solution and swirl to distribute the enzyme throughout the sample. Transfer content of each flask into a 20 to 30 cm dialysis tube. Close tube with string, and place in flasks containing 500 ml potassium phosphate buffer solution. Close lids and place flasks in the stirring water bath. Extract 40 ml buffer solution every 30 minutes to determine the rate of hydrolysis of carbohydrate from the dialysis tube into the buffer solution.

5.3.6 Determination

Determine the amount of reduced sugars in the buffer solution by means of Infra-red Spectrophotometry (Milkoscan).

5.3.7 Calculation

The carbohydrate concentration values are plotted on a graph and the AUC is determined. The HI values are calculated as the relation between the AUC of the specific food compared to the AUC of maltose as the reference food, using the following formula:

AUC of food ÷ AUC maltose x 100 = HI of food

5.3.8 Classification

If the food product has a HI value of less than 55, it predicts that the GI of the food product would be in the low GI category. If the HI of the food product falls in the range between 55 and 70, it is predicted that the food falls within the Intermediate GI category. An HI value above 70 predicts a high GI.

5.4 CONCLUSIONS

A need was identified for a rapid assessment method to accurately predict the GI of foodstuffs at a reduced cost and within a reduced time frame, which motivated this research project. The developed hydrolysis method adheres to these requirements and predicts the GI of foodstuffs by determining the HI of the food. The results obtained indicate that the determined HI values correlate well with determined GI values if sample preparations are similar.

The rapid assessment method requires no pre-preparation prior to testing. The complete test procedure from sample collection until results are available could be performed within one working day. *In vivo* GI analysis requires panel members to control dietary intake up to 24 hours prior to analysis, analysis takes up to three hours, after which the measurement of blood glucose concentration needs to be done.

The cost involved to execute one *in vitro* trial is much lower than *in vivo* analysis. Basic apparatus and chemicals, commonly available in analytical laboratories, are used. The enzymes used are

more expensive than the other chemicals, yet the total cost for *in vitro* analysis remains significantly less than the R7500+ required for one food product tested at an *in vivo* laboratory.

5.5 RECOMMENDATIONS

Urooj and Puttaraj (2000) state that it should always be considered that *in vitro* methods do not consistently correlate with *in vivo* determined values. However, seen against the high variability observed in *in vivo* results, all GI values should be presented to consumers as an indication only. Seemingly negligible factors often have significant impacts on the ultimate glycaemic response which the food product may have in the individual, i.e. human emotions, cooking method of food, previous meal, or other products consumed with the food.

Classifying a food into the categories of high, intermediate or low GI, provides the consumer with a general indication, based on the traffic light system, of healthy choices. The developed rapid assessment method could play a significant role in presenting the food industry, and thus the consumer, with the possibility of having more food items classified into these categories.

Based on the high variability between the laboratories in South Africa determining the GI of the Almera potatoes, a workshop is proposed to align methodology, not only to comply with the ISO/DIS 26642, but also to eliminate additional seemingly negligible differences which caused these disparities. Further examination of the validity of the current *in vivo* methods used by the different laboratories in South Africa is proposed, as well as the reliability of testing GI of foods using these *in vivo* methods.

5.6 REFLECTIONS

The developed rapid assessment method provides a less expensive and less time consuming alternative to predict GI of foods than *in vivo* methods. Because GI remains a growing global health trend, the most significant benefit of the method is that it provides an opportunity for food companies to screen new food products (formulations, cultivars, etc.) during the development phase. Final food products could also be tested by the *in vitro* method prior to sending samples to

in vivo laboratories to ensure that the results meet the desired requirements for labelling purposes.

Based on the persuasive evidence presented in this thesis, it is proposed that that the use of *in vitro* methodologies for labelling purposes (when using the non-numerical, category based, classification system) could be further investigated.

CHAPTER 6: REFERENCES

Akerberg AKE, Liljeberg HGM, Granfeldt YE, Drews AW & Bjork IME. 1998. An *in vitro* method, based on chewing, to predict resistant starch content in foods allows parallel determination of potentially available starch and dietary fibre. *Journal of Nutrition*, 128, 651-660.

Alldrick, A. 2006. Dietary Fibre and glycemic index of foods. Campden and Chorleywood Food Research Assocaition (CCFRA). [internet reference] http://www.campden.co.uk Accessed: 30 / 06 / 2007

Amuli DJ (2006) Purchasing patterns of major plant staples in low-income households in the Vaal Triangle. MTech dissertation. Vaal University of Technology.

AOAC, 2005. Official methods of analysis, Association of Official Analytical Chemists, Washington, DC (2005).

Araya H, Contreras P, Alvina M, Vera G & Pak N. 2002. A comparison between an *in vitro* method to determine carbohydrate digestion and the glycemic response in young men. *European Journal of Clinical Nutrition*, 56, 735-739.

Arvidsson-Lenner R, Asp NG, Axelsen M, Bryngelsson S, Haapa E, Järvi A, Karlström B, Raben A, Sohlström A, Thorsdottir I & Vessby B. 2004. Glycaemic Index – Relevance for health, dietary recommendations and food labelling. *Scandanavian Journal of Nutrition*, 48(2), 84-94.

Aston LM, Jackson D, Monsheimer S, Whybrow S, Handjieva-Darlenska T, Kreutzer M, Kohl A, Papadaki A, Martinez JA, Kunova V, Van Braak MA, Astrup A, Saris WHM, Jebb SA & Lindroos AK. 2010. The Diogenes Study: Developing a methodology for assigning glyceamic index values to foods consumer across Europe. *Obesity Reviews*, 11, 92-100.

Atkinson FS, Foster-Powell K & Brand-Miller JC. 2008. International Tables of Glycemic Index and Glycemic Load Values: 2008. Diabetes Care December, 31(12) 2281-2283

Atwater WO & Bryant AP. 1900. The availability of fuel value of food materials. *In 12ᵗʰ Annual Report Connecticut Agricultural Expt. Stationary Storrs*, 73 – 110.

Badham J. 2003. Calculating glycaemic index. *South African Journal of Clinical Nutrition*, 16(4), 114.

Bender DA. 1997. Introduction to nutrition and metabolism. Taylor & Francis Ltd. London.

Booysen L. 2010. Comparison of the eating quality of selected potato cultivars from two production regions in South Africa and a proposed classification system for the consumer. Master's Thesis. Department of Consumer Sciences. University of Pretoria. South Africa.

Bourne LT, Lambert EV & Steyn K. 2002. Where does the black population of South Africa stand in the nutrition transistion? *Public Health Nutrition,* 5(1A), 157-162.

Brennan CS, Blake DE, Ellis PR & Schofield JD. 1996. Effects of guar galactomannan on wheat bread microstructure and on the *in vitro* and *in vivo* digestability of starch in bread. *Journal of Cereal Science*, 24, 151-160.

Brighenti F, Casiraghi C & Baggio C. 1998. Resistant starch in the Italian diet. *British Journal of Nutrition*, 80, 333-341.

Brighenti F, Pellegrini N, Casiraghi MC & Testolin G., 1995. *In vitro* studies to predict physiological effects of dietary fibre. *European Journal of Clinical Nutrition,* 49, S81–S88.

Brouns F, Bjork I, Frayn KN, Gibbs AL, Lang V, Slama G & Wolever TMS. 2005. Glycemic Index Methodology. *Nutrition Research Reviews*, 18, 145-171.

Browne C. 2006. Glycemic Index & Glycemic Load. Great Inspiration or just good luck? Paper presented at SAAFoST Symposium: Ingredients with a functional twist. Cape Town.

Burton P & Lightowler HJ. 2008. The impact of freezing and toasting ont eh glycaemic response of white bread. *European Journal of Clinical Nutrition*, 62(5), 584-499.

Cameron AJ. 2002. Epidemiology of Barrett's esophagus and adenocarcinoma. *Diseases of the Esophagus.* 15, 106–108.

Conn JW & Newburgh LN. 1939. The glyceamic response to isoglucogenic quantities of protein and carbohydrate. *Journal of Clinical Investigation,* 15*,* 665-671.

Coulston AM, Hollenbeck CB, Liu GC, Williams RA, Starich GH, Mazzaferri EI *et al.* 1984. Effect of source of dietary carbohydrate on plasma glucose, insulin and gastric inhibitory polypeptides responses to test meals in subjects with non-insulin dependent diabetes mellitus. *American Journal of Clinical Nutrition,* 40, 965-970.

Cummings JH & Stephan AM. 2007. Carbohydrate terminology and classification. *European Journal of Clinical Nutrition*, 61(S1), S5-S18.

Demographic and Health Survey. (2003). Department of Health, online. Available online at http://www.doh.gov.za/facts/index.html/. Accessed June 2009.

Department of Health. 2007. Draft guidelines for regulation governing the labelling and advertising of foodstuffs, R643 of 20 July 2007. Government Gazette No 30075. Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972). *National Department of Health, South Africa*

EdComs. 2007. Review and analysis of current literauter on consumer understanding of nutrition and health claims made on food. COI for FSA.

Englyst HN, Kingman SM & Cummings JH. 1992. Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition.* 46(S2), S33-50.

Englyst HN, Veenstra J & Hudson GJ. 1996. Measurment of rapidly available glucose (RAG) in plant foods: a potential *in vitro* predictor of the glycemic response. *British Journal of Nutrition*, 75, 327 – 337.

Englyst KN, Englyst HN, Hudson GJ, Cole TJ & Cummings JH. 1999. Rapidly available carbohydrates in foods: an *in vitro* measurement that reflects the glycemic response. *American Journal of Clinical Nutrition*, 69, 448 – 454.

Englyst KN, Lui S & Englyst HN. 2007. Nutritional characterization and measurement of dietary carbohydrates. *European Journal of Clinical Nutrition*, 61(1), S19-S39.

Englyst KN, Vinoy S, Englyst HN & Lang V. 2003. Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. *British Journal of Nutrition*, 89, 329–339.

European Food Information Council (EUFIC). 2005. FOOD TODAY 02/2005. What's new in obesity research? Available online, accessed 10 December 2010.: http://www.eufic.org/article/en/page/FTARCHIVE/artid/new-obesity-research/

FAO. 2010. Biodiversity and Nutrition A Common Path. Food and Agriculture Organization of the United Nations. Nutrition and Consumer Protection Division. Available online, accessed 10 December 2010, www.fao.org/infoods/biodiversity

FAO/WHO. 1980. Carbohydrates in human nutrition. Report of a joint FAO/WHO expert meeting. FAO Food and Nutrition Paper 15. Food and Agriculture Organization of the United Nations. Rome.

FAO/WHO. 1998. Carbohydrates in Human Nutrition. Report of a joint FAO/WHO Expert Consultation (FAO Food and Nutrition Paper 66). Food and Agriculture Organization of the United Nations, Rome.

FAO/WHO. 2010. Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission: Communication from ISO (report of activities relevant to Codex work). Thirty-third session, Geneva, Switzerland, 5 – 9 July 2010.

Fernandes G, Velangi A & Wolever TMS. 2005. Glycemic index of potatoes commonly consumed in North America. *Journal of the American Dietetic Association,* 105, 557-562.

Foster-Powell K & Miller JB. 1995. International tables of glyceamic index. *American Journal of Clinical Nurtrition*, 62, 871S-893S

Foster-Powell K, Holt SAH, Brand-Miller CB. 2002. Internation table of glycemic index and glycemic load values:2002. *American Journal of Clinical Nutrition,* 76, 5-56.

Frost G, Dornhurst A. 2000. The relevance of the glycaemic index to our understanding of dietary carbohydrates. *Diabetic Medicine*, 17, 336-45.

Germaine KA, Samman S, Fryirs CG, Griffiths PJ, Johnson SK, Quail KJ. 2008. Comparison of *in vitro* starch digestibility methods for predicting the glycaemic index of grain foods. *Journal of the Science of Food and Agriculture*, 88(4), 652-658.

Giacco R, Brighenti F, Pariillo M, Capuano M, Ciadullo AV, Rivieccio A, Rivellese Aa & Raccardi G. 2001. Characteristics of some wheat-based foods of the Italian diet in realtion to their influence on the postprandial glucose metabolism in patients with type 2 diabetes. *British Journal of Nutrition,* 85, 33 – 40.

Gibney MJ, Vorster HH, Kok FJ. 2002. *Introduction to human nutrition*. Oxford, Blackwell Science. London.

Gibson N. 2006. Development of objective methods for the classification of potatoes. Honours thesis. BSc Nutrition and Food Sciences. Department of Food Science. University of Pretoria.

GIFSA – Glycemic Index Foundation of South Africa. 2010. Available online at www.gifoundation.co.za, accessed October 2010.

Glycemic Index Limited. 2006. Glycemic Index Symbol Program. Australia. April 2006 Consumer Research. Available online at: www.gisymbol.com.au. Accessed 13 November 2008.

Glycemic Research Institute. 2010. Available online at http://www.glycemic.com/, accessed December 2010.

Goni I, Garcia-Alonso A & Saura-Calixto F. 1997. A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research,* 17, 427-437.

Grabfeldt Y, Bjorck I, Drews A & Tovar J. 1992. An *in vitro* procedure based on chewing to predict metabolic response to starch in cereal and legume products. *European Journal of Clinical Nutrition*, 46, 646-660.

Greenfield H & Southgate DAT. 2003. *Food composition data: production, management, and use*. FAO Publishing Management Service, Rome.

Hollenbeck CB, Coulston AM & Reaven GM. 1986. Glyceamic effects of carbohydrates: a different perspective. *Diabetes Care*, 9, 641-647

Holm J, Bjorck I, Drews A & Asp NG. 1986. A rapid method for analysis of starch. *Starch*, 38, S224-S226.

Irwin T. 2001. A new glyceamic index labelling programme for Australia. *Nutrition Bulletin*, 26, 317-318.

ISO/DIS 26642. 2008. Food products – Determination of the glycemic index (GI) and relevant classification. Draft international standard. International organization for Standardization.

Jacobsen MU, Dethlefsen C, Joensen AM, Stegger J, Tjonneland A, Schmidt EB & Overvad K. 2010. Intake Of carbohydrates compared with intak of saturated fatty acids and risk of myocardial infarction: importance of the glycemic index. *American Jounral of Clinical Nutrition*, 91, 1764-1768.

Jenkins DJ, Wolever TM, Jenkins AL, Thorne MJ, Lee R, Kalmusky J *et al.* 1983. The glycaemic index of foods tested in diabetes patients: a new basis for carbohydrate exchange favouring the use of legumes. *Diabetologia*, 24, 257-264

Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL & Goff DV. 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. *American Journal of Clinical Nutrition*, 34, 362-366.

Jenkins DJ, Kendall CWC, Augustin LSA, Franceschi S, Hamidi M, Marchie A, Jenkins AL & Axelsen M. 2002. Glycemic index: overview of implications in health and disease. *American Journal of Clinical Nutrition*, 76(1), 266S-273S.

Kaartinen NE, Simila ME, Pakkala H, Mannisto S & Valsta LM. 2010. Glycaemic index values in the Finnish food composition database: an approach to standardised value documentation. *European Journal of Clinical Nutrition*, 64, s68-s72.

Kim EHJ, Petrie JR, Motoi L, Morgenstern MP, Sutton KH, Mishra S & Simmons LD. 2008. Effect of Structural and Physicochemical Characteristics of the Protein Matrix in Pasta on *In vitro* Starch Digestibility. *Food Biophysics*, 3, 229–234.

Kraus RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW, Kris-Etherton P, Goldberg IJ, Kotchen, Lictenstein TA, Mitch WE, Mullis R, Robinson K, Wylie-Rosett J, St.Jeor S, Suttie J, Tribble DL & Bazarre TL. 2000. AHA Dietary Guidelines.

Revision 2000: A statement for Helahtcare Profesionals from the Nutrition Committee of the American Heart Association. *Stroke,* 31, 2751-2766.

Kruger M, Sayed M, Langenhoven M & Holing F. 1998. Composition of South African Foods: Vegetables and Fruit. UDMS, Cape Town.

Laville, M. 2004. Could glycemic index be the basis of simple nutritional recommendations? Invited Comment. *British Journal of Nutrition.* 91, 803-804.

Lehohla P. 2006. Knowing causes of death is crucial for planning. Business Report. Statistics South Africa. Available online at http://www.statssa.gov.za/news_archive/ 14September2006_1.asp, accessed 1 October 2010.

Liu S, Willet W, Stampfer M *et al.* 2000. A prospective study of dietary glycemic load, carbohydrate intake, and risks of coronary heart disease in US women. *American Journal of Clinical Nutrition*, 71, 1455-1461.

Ludwig DS & Eckel RH. 2002. The glycemic index at 20y. *American Journal of Clinical Nutrition,* 76, S264-S265.

Ludwig DS, Majzoub JA, Al-Zahrani A, Dallal GE, Blanco I & Roberts SB. 1999. High glycemic index foods, overeating and obesity. *Pediatrics,* 103(3), 26.

Ludwig DS. 2000. Dietary glycemic index and obesity. *Journal of Nutrition*, 130, 280-283.

Ludwig DS. 2007. Clinical Update: the low-glycaemic-index diet. Comment. *The Lancet*, 369, 890-892.

Mackenzie D. 2004. Glycemic index: another complicated word or the next big idea? What do consumers think? Presented at a seminar hosted by the Food and Drink Innovation Network, Daventry, UK, September 2004. Available online at: www.fdin.co.uk. Accessed 13 November 2008.

Mann J, Cummings JH, Englyst HN, Key T, Liu S, Ricchardi G, Summerbell C, Uauy R, van Dam RM, Venn B, Vorster HH & Wiseman M. 2007. FAO/WHO Scientific Update on carbohydrates in human nutrition: conclusions. *European Journal of Clinical Nutrition*, 61(1), S132-137.

Matthan NR, Ronxhi J, Ausman LM, Griffith JL, Pittas AG & Lichtenstein AH. 2010. Age but not Body Mass Index (BMI) or Sex Affect Glycemic Response to a Single Food in Healthy Subjects. *Circulation*, 122, Abstract 19680

McWilliams M. 2009. Foods: Experimental Perspecitves. 6th Edition. Prentice Hall Publishers. London.

Menezes EW, Giuntini EB, Dan FM & Lajolo. 2009. New information on carbohydrates in the Brazilian Food Composition Database. *Journal of Food Composition and Analysis,* 22, 446-452.

Mishra S, Monro JA & Hedderley D. 2008. Effect of processing on slowly digestable starch and resistant starch in potato. *Starch/Sturke*, 60, 500-507.

Mitchell HL. 2008. The glycemic index concept in action*. American Journal of Clinical Nutrition*, 87, 244S-246S.

Moneteiro CA, Conde WL, Lu B & Popkin BM. 2004. Obesity and inequities in health in the developing world. *International Journal of Obesity*. 28, 1181-1186.

Monro J. 2005. Expressing the glyceamic potency of foods. *Proceeding of the Nutrition Society*, 64, 115-122.

Monro JA & Shaw M. 2008. Glycemic impact, glycemic glucose equivalent, glycemic index, and glycemic load: definitions, distinctions, and implications. *American Journal of Clinical Nutrition,* 87, 237S-243S.

Muir JG & O'Dea K. 1992. Measurement of resistant starch: factors affecting the amount of starch escaping digestion *in vitro. American Journal of Clinical Nutrition*, 56, 123-127.

Muir JG, Birkett A, Brown I, Jones G & O'Dea K. 1995. Food processing and maize variety affect amount of starch escaping digestion in the small intestine. *The American Journal of Clinical Nutrition,* 61, 82-89.

NA. Author Unknown. 2004. Testing glyceamic index of foods: *in vivo*, not *in vitro*. Letter. *European Journal of Clinical Nutrition,* 58, 700-701.

Nantel G. 1999. Carbohydrates in human nutrition. *FNA/ANA,* 24, 6-10

National Food Consumption Survey (NFCS): Children aged 1 – 9 years in South Africa, NFCS group, (1999). Accessed 22 August 2008. Available on line: http://www.sahealthinfo.org/nutrition/foodconsumption.htm

National Health and Medical Research Council. 2003. Dietary Guidelines for Children and Adolescents in Australia incorporating the Infant Feeding Guidelines for Health Workers. Commonwealth of Australia. ISBN: 1864961538. AusInfo, Canberra, Australia.

Neuhouser ML, Tinker LF, Thomson C, Caan B, Van Horn L, Snetselaar L, Parker LM, Patterson RE, Robinson-O'Brien R, Beresford SAA & Shikany JM. 2006 Development of a glycemic index database for food frequency questionnaires used in epidemiologic studies. *Journal of Nutrition*, 136, 1604-1609.

Nishada C. 2009. Adressing the Double Burden of Malnutrition in Developing Countries a Global Perspective. Symposium; Africa Highltghts Part II. 19TH International Congress on

Pieters M & Jerling JC. 2005. Measuring the glycemic index – consensus and issues of debate. *South African Journal of Clinical Nutrition*, 18(3), 232-236.

Pi-Suyer, F.X. 2002. Glycemic index and disease. *American Journal of Clinical Nutrition*, 76, 290S-298S

Rondini EA & Bernink MR. 2007. Ontaria White Bean Producers. Nutrition and health. Available online at: http://www.ontariobeans.on.ca/health4_beans_diabetes.php. Accessed June 2009.

Rose D & Charlton KE. 2002. Prevalence of household food poverty in South Africa: results from a large nationally representative survey. *Public Health Nutrition,* 5(3), 383-389.

Schönfeldt HC & Gibson N. 2009. Healthy eating guidelines in a South African context. Journal of Food Composition and Analysis, S22, 68-73.

Schönfeldt HC & Holden J. 2009. Food Composition data in nutrition. In *Introduction to Human Nutrition,* edd Gibney MJ, Vorster HH and Kok FJ. The Nutrition Society Textbook Series. Blackwell Science Ltd., Oxford.

Sievenpiper JL. 2004. Glycemic index in the treatment of diabetes: The debate continues (Commentary). *Journal of the American College of Nutrition*, 23(1), 1-4.

Slabber M. 2005. Complexities of consumer understanding of the glycaemic index concept and practical guidelines for incorporation in diets. *The South African Journal of Clinical Nutrition,* 18(3), 252-257.

Soh NL & Brand-Miller J. 1999. The glycaemic index of potatoes: the effect of variety, cooking method and maturity. *European Journal of Clinical Nutrition*, 53(4), 249-54.

South African Department of Health, Statistics (SADHS). 1998. Available online at http://www.doh.gov.za/facts/sadhs-f.html. Accessed October 2010.

Steenkamp G & Delport L. 2005. The South African Glycemic Index and Load Guide. 4th Edition. GIFSA Publication. www.gifoundation.com

Steyn NP & Temple N. 2008. *Community Based Nutrition Textbook for South Africa: A Rights-Based Approach.* South African Medical Research Council. Chronic Diseases of Lifestyle Unit, FAO/MRC, pp. 101–160.Synovate. 2005. Quantitative Evaluation of Alternative Food singposting concepts. COI for FSA.

Tanumihardjo SA, Anderson CA, Kaufer-Horwitz M, Bode L, Emenaker NJ, Haqq AM, Satia JA, Silver HJ, Stadler D (2007). Poverty, obesity, and malnutrition: An international perspective recognizing the paradox. *Journal of the American Dietetic Association,* 107, 1966-1972.

Trout DL, Behall KM & Osilesi O. 1993. Prediction of glyceamic index for starchy foods. *American Journal of Clinical Nutrition*, 46(2), S91-S101.

United Stated Department of Agriculture Database for Standard Reference (USDA). 2010. Available online at: http://www.nal.usda.gov/fnic/foodcomp/search/, accessed December 2010.

Urooj A & Puttraj S. 1999. Digestibility index and factors affecting rate of starch digestion *in vitro* in conventional food preparation. *Nahrung*, 43, S42-S47.

Van Amelsvoort JMM & Weststrate JA. 1992. Amylose-amylopectin ratio in a meal affects postprandial variables in male volunteers. *The American Journal of Clinical Nutrition,* 992(55), 712-8

Van Bakel MM, Slimani N, Feskens EJM, Du H, Beulens JWJ, Schouw YT, Van Der Brighenthi F, Halkjaer J, Cust AE, Ferrari P, Brand-Miller J, Bueno-de-Mesquita HB, Peeters P, Ardanaz E, Dorronsoro M, Crowe FL, Bingham S, Rohrmann S, Boeing H, Johansson I, Manjer J, Tjonneland A, Overvad K, Lund E, Skeie G, Mattiello A, Salvini S, Clavel-Chapelon F & Kaaks R. 2009. Methodological chalenges in the application of the glcemic index in epidemiological studies using data from the European prospective investigation into cancer and nutrition. *Journal of Nutrition*, O: jn.108.097121v1-jn.108.097121.

Van Kempen TATG, Regmi PR, Matte JJ & Zijlstra RT. 2010. In Vitro Starch Digestion Kinetics, Corrected for Estimated Gastric Emptying, Predict Portal Glucose Appearance in Pigs. *Journal of Nutrition,* 140(7), 1227 - 1233.

Van Heerden I & Schönfeldt HC. 2011. The lack of food intake data and the consequences thereof. *South African Journal of Clinical Nutrition. Accepted.*

Vega-López S, Ausman LM, Jalbert SM & Lichtenstein AH. 2006. Inter- and Intra-Individual Variability in Glycemic Index Values for White Bread Determined Using Standardized Procedures. Circulation. 114, I900

Venter CS, Slabber M. & Vorster HH. 2003. Labelling of foods for glyceamic index – advantages and problems. *South African Journal of Clinical Nutrition,* 16(4), 118-126.

Vermeulen H. 2010. *Presentation*: *Consumer trends and Analysis*. Bureau of Food and Agricultural Policy (BFAP). August 2010. National Agricultural Marketing Council General Meeting, Pretoria, South Africa.

Vorster HH, Venter CS & Silves N. 1990. The glycemic index of foods: a critical evaluation. *South African Journal of Food Science and Nutrition*, 2, 13-17.

Weichselbaum E. 2010. An overview of the role of potatoes in the UK diet. *Nutrition Bulletin*, 35, 195-206.

Whitney EN & Rolfes SR. 2002. *Understanding Nutrition,* 9th Edition. Wadsworth, Australia

WHO Global database on body mass index. Available online at http://apps.who.int/bmi/index.jsp, accessed July 2010.

Williams SM, Venn BJ, Perry T, Brown R, Wallace A, Mann JI & Green TJ. 2008. Another approach to estimating the reliability of the glycaemic index. *British Journal of Nutrition,* 100, 364-372.

Wolever TM, Jenkins DJ, Josse RG, Wong GS & Lee R. 1987. The glycemic index: similarity of values derived in insulin-dependent and non-insulin dependent diabetic patients. *Journal of American Coll Nutrition,* 6, 295-305.

Wolever TM, Jenkins DJ. 1986. The use of the glycemic index in predicting blood glucose response to mixed meals. *American Journal of Clinical Nutrition*, 43, 167-172.

Wolever TM, Nuttall FQ, Lee R, Wong GS, Josse RG, Csima A *et al.,* 1985. Prediction of the relative blood glucose response of mixed meals using white breag glycemic index. *Diabetes Care*, 8, 418-428.

Wolever TMS, Brand-Miller JC, Abernethy J, Astrup A, Atkinson F, Axelsen M, Björck I, Brighenti F, Brown R, Brynes A, Casiraghi MC, Cazaubiel M, Dahlqvist L, Delport E, Denyer GS, Erba D, Frost G, Granfeldt Y, Hampton S, Hart VA, Ha¨tönen KA, Henry CJ, Hertzler S, Hull S, Jerling J, Johnston KL, Lightowler H, Mann N, Morgan L, Panlasigui LN, Pelkman C, Perry T, Pfeiffer AFH, Pieters M, Ramdath DD, Ramsingh RT, Robert SD, Robinson C, Sarkkinen E, Scazzina F, Sison DCD, Sloth B, Staniforth J, Tapola N, Valsta LM, Verkooijen I, Weickert MO, Weseler AR, Wilkie P & Zhang J. 2008. Measuring the glycemic index of foods: Interlaboratory study. *American Journal of Clinical Nutrition,* 87, 247S-257S.

Wolever TMS, Jenkins DJ, Ocana A, Rao V & Collier G. 1988. Second-meal effect: Low-glycemic-index foods eaten at dinner improve subsequent breakfast glycemic responce. *American Journal of Clinical Nutrition,* 48, 1041-1047.

Wolever TMS, Vorster HH, Bjork I, Brand-Miller J, Brighenti F, Mann JI, Ramdath DD, Granfeldt Y, Holt S, Perry TL, Venter C & Xiaomei Wu. 2003. Determination of the glycemic index of foods: interlaboratory study. *European Journal of Clinical Nutrition*, 57, 475-482.

Wolever TMS. & Bolognesi C. 1996. Prediction of glucose and insulin responses of normal subjects after consuming mixed meals varying in energy, protein, fat, carbohydrate and glycemic index. *Journal of Nutrition*, 126, 2807-2812.

Wolever TMS. 2010. Letter to the editor: Another approach to estimating the reliability of the glyceamic index: a different interpretation. *British Journal of Nutrition*, 103, 1695-1696

Woodruff S. 2010. Florida State University, Department of Health and Nutritional Sciences. Available online: http://www.tshc.fsu.edu/he/nutrition/nutrition_specialneeds/glycemic_index.htm, accessed 22 October 2010.

Woolnough JW, Monro JA, Brennan CS & Bird AR. 2008. Simulating human carbohydrate digestion in vitro: a review of methods and the need for standardisation. *International Journal of Food Science and Technology*, 43(12), 2245–2256.

www.glycemicindex.com. Available online: http://www.glycemicindex.com/ Accessed 10 December 2010.

Zabidi MA & Aziz NAA. 2009. *In vitro* starch hydrolysis and estimated glycaemic index of bread substituted with different percentage of chempedak (Artocarpus integer) seed flour. *Food Chemistry,* 117(1), 64-68.

ISO/DIS 26642: Draft International Standard: Food Products – Determination of glycemic index (GI) and relevant classification.