Profiling of The Glycaemic Index of Under-utilised Vegetables

By

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Abbreviations

CHD	Coronary heart disease
CHO	Carbohydrate
CVD	Cardiovascular disease
DNS	3,5-dinitrosalicylic acid
GI	Glycaemic Index
Hcy	Homocysteine
HMG-CoA	β-hydroxy-β-methylglutaryl-coenzyme A
IAUC	Incremental Area Under the Curve
IGT	Impaired Glucose Tolerance
LDL	Low Density Lipoprotein Cholesterol
NIDDM	Non-insulin Dependent Diabetic Mellitus
STZ	Streptozotoxin-induced diabetic
T2DM	Type 2 Diabetes Mellitus
tHcy	Total plasma Homocysteine
WHO	World Health Organisation

ABSTRACT

Glycaemic Index is a term used to describe the effect on blood glucose of consuming a carbohydrate rich meal. It is a measure of the rise in blood glucose following consumption of a test food relative to a reference food (glucose). Various associations have been found between the GI value of the food and a number of chronic diseases, notably diabetes mellitus (DM) which is linked to high blood glucose and to diets high in unrefined carbohydrates.

Whilst there has been considerable research into the GI values of commonly consumed foods, very little is known about the GI values of plants that fall into the category of underutilised species. These include numerous fruit, vegetables, legumes, herbs, spices and nuts that are generally grown and consumed by subsistence and small-scale farmers and their communities. Anecdotal evidence suggests that some of these underutilised plants have particular medicinal properties, in particular can be used to control diabetes mellitus.

The total starch and reducing sugar content of white rice plus four selected underutilised species (ginger, turmeric, bitter gourd and fenugreek) were analysed using the DNS method to determine the levels of reducing sugars after acid hydrolysis. The effects of cooking method (raw, boiled or fried) on the rate of starch digestibility were also investigated using in vitro and in vivo digestibility studies.

White rice had the highest starch content (78.47g/100g DM) and lowest (0g) level of reducing sugars present.

Out of the underutilised plants, both ginger and turmeric had high levels of starch (57.54-68.11 g/100g DM) and reducing sugars (2.71-3.12 g/100g DM). The total starch and reducing sugars in bitter gourd were very low (3.52-4.16g/100g DM and 0.86-1.2 g/100g DM respectively) across all three treatments. Mean values for starch and reducing sugars in turmeric and fenugreek respectively were 64.78 g/100g DM and 51.94g/100g DM: and 2.7g/100gDM and 0.7 g/100g DM respectively.

The glucose response of a single meal (individual species) and a mixed meal (rice plus one of the test species) was estimated using an *in vitro* model of digestion. The samples underwent digestion with α amylase followed by protease and pancreatic amylase. Aliquots of the digest were removed at 30 minute intervals over a 3 hour period and tested for reducing sugars (using the DNS

method) to evaluate the rate of digestion. This was used to estimate the GI response.

There was a significant effect of cooking process (raw, boiled or fried) on the rate of glucose release from all four test samples ($p<0.001$). The highest values for bitter gourd were in the boiled sample (0.187 mg/ml at 60 minutes) followed by the fried (0.163 mg/ml at 90 minutes) and the raw sample (0.167 mg/ml at 120 minutes). The peak of glucose release from ginger was at 90 minutes in both the boiled and fried samples (0.442 mg/ml glucose and 0.307 mg/ml glucose respectively). Raw ginger responded more slowly, with a peak glucose concentration (0.170 mg/ml) at 180 minutes. Boiled fenugreek seeds had a peak in glucose release (0.128 mg/ml) at 150 minutes.

To evaluate the response to feeding these four underutilised species in a mixed meal based on white rice, *in vivo* glucose tolerance tests were carried out on a small number (8) of volunteers.

Eight healthy participants (21-33 years old) were recruited from the University of Nottingham Malaysia Campus (UNMC). Ethical approval for the study was granted by the UNMC ethics committee. Volunteers were asked to observe an overnight fast. They took part in the study on two consecutive days. Boiled white rice was used as a reference food and was consumed (3.5g dry weight to provide 1g available carbohydrate) by all participants on day one. Capillary blood glucose was measured at 0 time (before eating) and at 30 minute intervals for 3 hours post prandial. This glucose tolerance test provided the baseline against which the test samples were evaluated for their capacity to moderate the glucose response.

On the second day, a boiled test sample was added to the rice to provide 1g available carbohydrate. The procedure above was repeated. Due to time constraints, only two replicates per sample were taken.

All four underutilised vegetables, when consumed with rice, had the effect of slowing down the digestion and related glucose release into the blood stream. This study did not investigate the mode of action by which this works. Based on these results, the four underutilised species could be classified as low GI foods. This pilot study warrants a larger study to investigate the effects more thoroughly.

Key words

Glycaemic index, bitter gourd, turmeric, ginger, fenugreek, underutilised.

CHAPTER 1: INTRODUCTION

The term glycaemic index (GI) was introduced around 40 years ago (Jenkin *et al*., 1981). It is used to describe the effect on blood glucose of consuming a carbohydrate rich meal. It is accepted knowledge that the digestion and absorption of carbohydrate leads to an increase in the level of blood glucose. The extent and rate of elevation of blood glucose depends on a number of factors including the rate of digestion and absorption, enzyme activity, insulin production from the pancreas and sensitivity of the insulin receptors. The rate at which blood glucose is elevated is an indication of the glycaemic index. The GI is a scale from 0 to 1, with pure glucose having a GI value of 1.

Dietary carbohydrates can broadly be classified into two types – simple and complex. Simple carbohydrates include the monosaccharides – glucose, galactose and fructose. Complex carbohydrates include the long chain polysaccharides (starches), hemicelluloses and β-glucans. The complex carbohydrates are found in staple foods- grains, pulses and tubers and form the large part of a healthy diet. Simple sugar, especially glucose, is added to foods as sweeteners and flavour enhancers, and is mainly found as an added ingredient in processed foods. Fructose and galactose are present in fruit and milk respectively.

During digestion, complex carbohydrates are broken down into the monosaccharides for absorption into the body. The preferred fuel for the body is glucose and it is this form which is most easily absorbed from the intestine into the blood stream. The ease with which carbohydrates are digested and absorbed depends to a large extent on their structure, the type of starch, the presence of dietary fibre and any anti-nutritional components that affect enzyme activity

Foods are described as low, medium or high GI according to the speed with which they are digested and absorbed into the blood stream. After absorption from the small intestine, there is an increase in blood glucose concentration. For optimum health, it is essential that the blood glucose is regulated and maintained between strict limits (5-10mmol).This is achieved by two pancreatic hormones, insulin and glucagon, that work synergistically to control the levels of circulating glucose. In conditions where the pancreas or insulin receptors are damaged, this system is compromised and blood glucose levels cannot be

controlled. This leads to the development of type II diabetes mellitus. A diet in high GI foods has been associated with increased risk of non-communicable diseases such as obesity, cardiovascular disease (CVD), type II diabetes (Hingginbotham *et al.,* 2004a; Salmeron *et al*., 1997a; 1997b). However, a low GI diet has shown to be beneficial in reducing disease markers of certain diseases; including T2DM (Jenkin *et al*., 2008; De Natale *et al*., 2009).

Type II diabetes mellitus can be a large extent of controlling by dietary intervention and, in cases of overweight, obesity or weight loss. Avoiding foods that result in a sudden peak in blood glucose is advised. Foods that contain slow release complex carbohydrates, rather than simple carbohydrates (sugars) are recommended. These slow release carbohydrates are also termed low GI foods, since they have a low glycaemic value. Foods are divided into high, medium and low GI foods based on the type of carbohydrate present, their digestibility and effect on blood glucose. Labelling foods as such should help consumers select foods that are beneficial to health. It would be useful if all foods could be given a GI value according to their effect on blood glucose levels.

Because of the health implications of consuming excess sugar and refined carbohydrates (high GI foods), there has been considerable research into the GI values of most commonly consumed foods. However, very little is known about the GI values of plants that fall into the category of underutilised species. These include numerous fruit, vegetables, legumes, herbs, spices and nuts that are generally grown and consumed by subsistence and small-scale farmers and their communities. Anecdotal evidence suggests that some of these underutilised plants have particular medicinal properties, in particular can be used to control diabetes mellitus.

In recent years, the GI has been transformed from a potentially useful tool in planning diets for diabetic patients to a main solution for the prevention of type II diabetes, cardiovascular disease and even certain cancers in the general population, to a public health initiative for the improvement of health and prevention of diseases such as obesity and type II diabetes. With the current interest in dietary diversification and the search to find alternatives to the common staples of wheat, rice and maize, there is a need to understand the nutritional value and quality of a range of less well known foods. Hence this study was conducted to evaluate the GI value of a few underutilised vegetables and herbs.

Rather than testing the glycaemic response of all foods on individuals, we need an *in vitro* model that simulates digestion. Several studies have been carried out to find a robust *in vitro* model with varying results.A study by Wolever *et al* (1985) indicated a good correlation between *in vitro* digestion models and *in vivo*digestion for the predicted and observed glycemic response of mixed meals of bread and white pea beans. However, other researchers reported a poor correlation between the two models tested (Flint *et al*., 2004). This is attributed to the fact that several other (dietary and non-dietary) factors affect the glycemic response (Jenkin *et al.,* 1984b). These include the presence of other nutrients (in particular fat and protein content of the diet which can affect digestion and absorption of carbohydrates), transit time through the gut (linked to the type of meal, the presence of dietary fibre and individuals' physiology), processing methods (the impact of processing on the development of resistant and refractive starch and the breakdown of anti-nutritional factors), varietal differences in the composition of species of fruits and vegetables.

This study aims to assess the GI values of four underutilised species (bitter gourd, ginger, turmeric and fenugreek),by assessing the predicted (*in vitro*) and observed (*in vivo*) responses. In addition to assessing the GI value of each test species, the GI response to consuming mixed meals (white rice plus one of the test foods) was evaluated by the *in vitro* and *in vivo* methods. The potential of each species to control (or moderate) T2DM was investigated by reviewing existing literature.

1. Objectives of research

- To review existing evidence of the role of selected under-utilised species in controlling T2DM
- To create GI profilesby determining the effect of under-utilised species on the GI value of rice-based diet

2. Hypotheses

The consumption of various under-utilised plant species is beneficial in controlling blood glucose and hence in the control of type II diabetes mellitus. The method of cooking affects the response.

CHAPTER 2: LITERATURE REVIEW

2.1. The potential roles of under-utilised species

2.1.1. What are under-utilised species?

On a global scale, there are over 7,000 plant species that they have been found to be used for food and 21,000 plants used as medicinal plant (Patil *etal*., 2011). However, we rely on a very few species to meet our nutritional (and non-food) needs. Of the thousands of species grown, many are only utilised in the immediate area of production. They have not benefited from formal research or from commercialisation. A lot of the knowledge of the production, uses and values of these species lies with the indigenous people who are the producers. Examples of a few of the under-utilised species found in Malaysia include Ceri (*Lepissanthesalata)*, Kedondong (*Spondiascytherea),* Salak *(Salaccazalacca)*. In general, these plants do not bring commercial interest; therefore, they have not been domesticated. They demonstrate in quality, taste, fruit size that do not meet the demand in the market (Schieber *et al.,* 2008).So, they are called under-utilised species. There are numerous terms to describe these species such as minor crops, neglected species, orphan crops, under-developed species, underexploited or promising species (Padulosi *et al.,* 2003). These species are not only highly adapted to stressful conditions, can yield with low fertiliser input (Lazaroff, 1989). They are grown for local consumption and not ready for commercialisation (Themelis *et al.,* 2003).

In terms of contributing to global food supply, the under-utilised species cannot compete with the more common commercialised crops. They do; however, have the potential to contribute to food and nutritional security especially in the volatile and changing climates of the future.

Table 2.1: Under-uti*No table of figures entries found.****lised species for multipurpose in many countries***

Countries	Underutilised plants
China	5000 medicinal
India	2500 medicinal
Malaysia	800 fruit trees
Kenya	800 food species
Mediterranean	137 vegetables

Sourced: IPGRI, 2002

According to the statistic of Ethnobotanical survey in 2002, there are a thousand under-utilised plant species, are used in different purposes. In particularly, the largest plant source was found in China, where has 5000 various under-utilised species for medicinal used, followed by India (Table 2.1.1). The under-utilised fruits in Malaysia as high as 800 species and in Mediterranean region, there are over 137 under-utilised vegetables species. With focused and targeted research, some of these species have the potential to provide nutrition and medicinal compounds in the climates of the future.

The potential of under-utilised species to contribute to food and nutritional security is of global interest. There is evidence that some of under-utilised fruits and vegetables contain nutrients and phytochemicals that could be beneficial to health (Moore *et al.,* 2005). It helps to maintain the normal physiological functions in the body and also can be used to control non-communicable diseases; especially, diabetes (Nair *et al*., 2013).

2.1.2. Improving nutritional security

It is evident that a large consumer proportion of people sufficient calories without attention to nutritional quality; therefore, they might be lack of essential vitamins and micronutrients in their diet. Globally, over 200 million people suffer from vitamin A deficiency and over 1,600 million people were reported in iron deficiency (WHO, 2008). It has been shown that death caused by zinc deficiency was estimated over 400,000 children per year (Angelova *et al.,* 2013). With a great source of vitamins and micronutrients, under-utilised fruits and vegetables may have a role to play in reducing micronutrient deficiency diseases.

The fruits and vegetables are a good source of vitamins (vitamin A, C, E, B vitamins) and trace minerals (Manganese, Potassium, Zinc, Iron, and Copper). The powerful of natural antioxidant is an excellent source for the immune system and slows the aging process. It has been associated with a decreased risk of type 2 diabetes (Hamer *et al*., 2008; Larsson *et al.,* 2007). Vegetables and fruits are also a major source of dietary fibre (McKee *et al.,* 2000), which has been shown to improve insulin sensitivity and insulin secretion (Liese *et al.,* 2003).

According to the FAO database and other food composition tables, some under-utilised species (fruits, vegetables, spices and herbs) (Table 2.2) are good source of essential micronutrients. For example, bitter gourd is an excellent amount of minerals and vitamins such as 20mg calcium, 260mg potassium, 70mg phosphorus, 126mg of carotene (Vitamin A), 88mg of vitamin C and B complex vitamins. All these minerals and vitamins are very important to improve immune system, promote normal growth and development. They can be not only used to improve human health but also prevent the risk of non-communicable diseases. Another example is fenugreek seed, which is used as an important ingredient for culinary purposes in India. It contains high levels of micronutrients such as 160mg calcium, 370mg phosphorus, 96mg carotene per 100gm edible portion. These data indicate that the under-utilised species are potential source of micronutrients for human nutrition and contribute to improved health and nutritional status.

In addition, folate is a member of the B vitamin family, which is need for energy production and required for the synthesis of nucleic acids. Folate is also the most vital nutrients in regulating homocysteine (Hcy) level. High levels of Hcy have been associated with an increased risk of atherosclerosis in high levels of Hcy

(Raymon *et al.*, 2010). The total plasma homocysteine (tHcy) has been suggested as a potentially modifiable risk factor for coronary heart disease (CHD) and stroke (Verhoef *et al.,* 1999). The meta-analysis of eight randomized trials from UK found that participants who consumed folic acid ranging from 0.8 to 5.0 mg per day lead to a 25% reduction in homocysteine levels. From that, 25%-lower than usual Hcy level was associated with an 11% lower risk of CHD and 19% lower risk of stroke (JAMA, 2002). Hence, the increasing of folate intake is generally considered an effective means of lowering tHcy levels. Folic acid is not only reported in commercially processed foods (where is added as a fortificants) but also is naturally present in under-utilised food sources such as fenugreek, jackfruits, whole wheat, dates and star fruits. Bitter gourd is also reported to be a good source of folic acid, there are 72ug folic acid per 100g was presented in fresh bitter gourd juice to reduce the incidence of neutral tube defects (Salbaum *et al*., 2010).

Dietary fibre from whole grains has been found to play a role in moderating blood cholesterol levels (Kendall *et al.,* 2006). The effects of dietary fibre are attributed to the less atherogenic lipid on the carbohydrate diet. Especially, LDL cholesterol is a cause to develop cardiovascular disease (CVD), heart disease or stroke (Willet *et al.,* 2002). Many under-utilised species have high levels of dietary fibre. For example, turmeric contains around 53% dietary fibres or high fibre content from fenugreek seed contains 65% dietary fibre. These two species may play a role in lowering blood LDL cholesterol levels (USDA, 2001). According to the data of meta-analysis study, which summarised from 184 clinical studies reporting the effect of soluble fibre from oatmeal products, psyllium, pectin and guar fibre on blood cholesterol. As a result, these soluble fibres significant reduced both total cholesterol and LDL cholesterol. The tests for heterogeneity were highly significant with cholesterol changes at $P < 0.001$ (Lisa *et al.*, 1999). The reason for reducing cholesterol and lipid storage in the body might be a consumption of dietary fibre (Turley *et al.*, 1998). To give an example, a rich dietary fibre source of jackfruit occupies 1.5g per 100g (4%RDA) fresh jackfruits leading to protect colon mucous membrane lead to preventing colon cancer in the long term (USDA, 2000).

Phyto-nutrients are complex chemicals; including phenolic compounds, phytoestrogens, antioxidants and thousands of other compounds (Rui, 2007). The phyto-nutrients are able to neutralize the concentration of free radicals

which have antioxidant functions; therefore, they may have the potential to protect against the development of cancer (Oakes *et al.*, 1997). For cereal and whole wheat flour, the bran and germ fraction contributed 83% of total phenolic content, 79% of total flavonoid content, 51% of total lutein (Seges *et al.*, 2010). The Black Women's Health Study, a cohort study of over 50,000 women was followed-up in 12 years, suggested that a diet containing more whole grains, vegetables and fruits decreased risk of breast cancer when compared to Western diet containing refined grains, processed meat and sweets (Tanya *et al*., 2009).
Moreover, the essential oils in some herbs and spices can be used for medicinal purposes. For instance, gingerol and zingerone that are found in fresh ginger extract are essential oils that have been shown to improve the intestinal motility, against diarrhoea and pathogenic bacteria (Akintobi *et al*., 2013). In addition, the poly-phenolic compound such as curcumin, which create a deep orange pigment for turmeric, presents in the controlling of LDL cholesterol. And it plays role as an antioxidant to scavenge free radical in inhibiting tumour as well as inflammatory (Suresh *et al*., 2013).

Table 2.2: *The nutritional value of under-utilised fruits, vegetables, spices and herbs. All values in 100gm edible portion*

Name of underutilised species	**Minerals**				**Vitamins**				
	Ca (mg)	P (mg)	Fe (mg)	K (mg)	Carotene (mg)	B1 (mg)	B2 (mg)	Niacin (mg)	C (mg)
Fruits and vegetables									
Star fruit *(Averrohoa Carambola)*	3	12	0.08	133	-	0.01	0.02	0.37	34.4
Soursop *(Annona Muricata)*	12	20	0.5	294	-	0.09	0.09	-	26.9
Salak*(Zalacca Edulis)*	38	18	3.9	-		-	-	-	8.4
Rambutan (*NepheliumLappaceum*)	7.9	16.5	0.48	179	-	0.02	0.06	0.78	39.5
Mangosteen (*GardiniaMangostana*)	5.49	9.31	0.17	48	0.03	0.05	0.05	0.29	7.2
Bitter gourd (*MomordicaCharantia)*	20	70	0.61	260	126	0.07	0.09	0.5	88
Ginger root (*ZingiberOfficinale)*	20	60	3.5	415	40	0.06	0.03	0.6	6
Spices and herbs									
Fenugreek seed (*TrogonellaFoenum-graecum)*	160	370	6.5	-	96	0.34	0.29	1.1	-
Turmeric (*Curcuma Longa)*	150	282	67.8	-	30	0.03		2.3	-
Drumstick leaves (*MoringaOleifera)*	185	112	4	337	0.38	0.26	0.66	2.2	51.7

2.1.3. Poverty reduction

The under-utilised species not only provide food for local consumption but also raise income for farmers in both rural and urban area (Chadha et al., 2007). For example, little millet (Panicum Sumaerense) is the best second grain in India after barley, which was used to produce malt. Here, the income of farmer is not only increased threefold but also employment in the region, especially women (Vijayalakshmi et al., 2010).

Many under-utilised species have not received any research interest and as a consequence have not been developed. On the other hand, many have attracted some research interest and show signs of development potential. For instance, velvet tamarinds (*Dialum Indum*), horse mango (*Mangifera Feotida),* tampoi (*Baccaurea Macraipa*) are species that show potential*.* Moreover, fruit plantations help in employment generation, especially rural labour. The role of under-utilised cultivation is central of reducing poverty and improving livelihoods. For increasing rural incomes target crops, the competitiveness of selected crops is enhanced to develop the value of strategies with marketing or commercialisation (Kruijssen, 2010). Many under-utilised species have a short shelf life; therefore, the processing for long term storage includes canning, freezing or pickling will help to improve income generation. Besides, the demand of local, national and international market is prerequisite for development of markets (Markelova *et al.,* 2009).

2.1.4. Environmental sustainability

A great potential of under-utilised species not only support to rural communities by improving incomes but also for stability of the ecosystem. Moreover, the genetic resources address to present and future environmental challenges. There are noextension services, policy and decision markers, donors, technology providers and consumers. Therefore, fresh underutilised fruits are sold soon after harvest or following a storage period of several weeks.

On the other hand, global warming threatens agriculture productivity, temperature, rainfall affect to the growth of crops due to climate change (Frison *et al*., 2011). Although under-utilised plants can grow in harsh condition such as poor soil, degraded vegetation, drought, they produce amount of yield annually.

For instance, cowpea is a drought-tolerant crop that also fixes nitrogen in poor soils and is consumed in Africa and Asia (Padulosi *et al.,* 1987). The local people had no proper training regarding harvesting, post-harvest care, storage and marketing of these species (Mohamedien *et al.,* 1995). Meeting with local community revealed that they were interested in an agency that could train people in cultivation of these medicinal species.

At the same time, the agriculture is confronting the effects of climate change, loss of productive land, increasing competition for water, migration from rural to urban areas and the growing social concerns about the nature of the food production system. The highest priority objectives are food and nutritional security in the long term. Therefore, this can be achieved with an enhanced local productivity and yield stability strategy that fully embraces the benefits of both between and within crop diversification (Padulosi *et al.,* 1999).

2.1.5. The potential in agricultural development

The minor crops are also presented as new crops, but are now starting to receive more research and possible commercial attention (Vietmeyer, 1990). They have been neglected because they are not as productive as the major crops and therefore not as attractive to famers to produce (Hugles *et al.,* 2009). For instance, sorghum is the fifth major cereal crop in the world after wheat, rice, maize and barley in a global area of about 47million hectares. Sorghum is an important staple in Africa, the Middle East and Asia. On the other hand, spice trade such as cinnamon, pepper, cloves and ginger in Asian countries become high demand in added ingredients to increase flavour of foods (Morales, 2008).

Besides, the loss of local knowledge and ignorance of traditional generation leading to these crops may erode the genetic base. The food supply from major crops may be exhausted leading to hunger risk in the future. Minor crops not only play a big role in food security but also support for the nutritional security. To increase the awareness of population about under-utilised species, the Convention on Biological Diversity (Anon, 2000) and the Global plan of action for the conservation were prompted to sustainable utilization of plant genetic resources for food and agriculture in the future (Anon, 1996).

2.2. Glycaemic Index

Carbohydrate is one of the macronutrients that provide the body with energy. Current health recommendations indicate that an individual should consume at least 50% of their daily calories from carbohydrates. Simple carbohydrate have only one or two glucose molecules; however, complex carbohydrates which are composed of long chain of sugars (Janice *et al.*, 2007), in particular from complex carbohydrates from whole grains and vegetables. Starch is an example of a complex carbohydrate and glucose is a simple carbohydrate. Starch is composed of long chains (some branched) of glucose molecules. The digestion of complex polysaccharides such as starch results in the production of simple sugars – glucose – which is absorbed into the blood stream and is available for metabolic functions in the body. Complex carbohydrates cannot be directly utilised by the body without being digested into smaller monosaccharides.

The digestion and absorption of complex carbohydrates takes longer than simple sugars such as glucose. After digestion, glucose passes into the bloodstream and is transported to metabolic sites (liver, brain, muscles) within the body. The speed of digestion and absorption is reflected in the increase in blood glucose following a meal. The slower the rate of digestion (from complex carbohydrates), the longer it takes for the blood sugar level to increase. Likewise, the faster the breakdown (from simple sugars), the blood sugar level peaks more quickly. Insulin is released from the pancreas in response to increasing levels of blood glucose. The effect of carbohydrates on blood glucose content is termed the glycaemic index (GI). A high GI food is one made of carbohydrates that are relatively easy to digest, resulting in a rapid increase in blood sugar. A low GI food is one that takes longer to digest and absorb the carbohydrates. GI is measured on a scale from 1 to 100. Pure glucose has a value of 100. Its range is indicated that it is less than 55 means low GI, the value of GI from 56 to 69 is medium and greater than 70 is high GI content (Wolever, 2013*)*.However, high carbohydrate intake can have an effect on the glucose response. Thus, the quality of carbohydrate is measured by Glycaemic Index.

The Glycaemic Index (GI) is defined as a value to show how much glucose appears in the blood after the consumption of a single food (Jim *et al.,* 2007). After consuming, carbohydrate is broken down into glucose for absorption. The rate of glucose appears in the blood stream is a measurement of foods GI. It is

measured as the incremental area under the curve (IAUC) of 2 hours glucose response (Jenkins, 1981).

***Table 2.3**: Food items with high and low glycaemic index value*

Food items	Serving size (grams)	GI value (Glucose =100)
High GI foods		
White rice	150	89
Vanilla wafers	25	77
Rice cakes	25	82
Watermelon	120	72
Raisin	60	64
Low GI foods		
Brown rice	150	50
Spagetti wholemeal	180	42
Grapefruit	120	25
Chickpeas	150	10
Lentils	150	29
Carrots	80	35

Sourced: Atkinson et al., 2008.

Table 2.3 shows the GI value of different foods per serving size in grams. It is clear that the high GI foods are processed foods or baked foods which contain high sugar content per serving. In particularly, a serving of white rice has very high GI level (GI=89), while brown rice has GI of 50 in the same amount of 150gm serving size. On the other hand, grapefruits has significant lower GI value (GI=25) compare to watermelon (GI=72) per 120gm serving. Therefore,

the low GI diet should be considered because it has shown the beneficial in the management and prevention of non-communicable diseases.

In addition to the type of carbohydrate present, the GI value of a food is affected by several other factors including the chemical structure of starch, cooking method, the rate of gastrointestinal motility, the presence of protein, fat and fibre in the diet. Therefore, all were results of the differences of GI level (Krezowki *et al.,* 1986; Thorne *et al.,* 1983).

For example, Perceval *et al.* (2011) studied the effect of different cooking methods (boiling, frying, baking and roasting) on GI and glycaemic response of 10 sweet potato cultivars. As a result, baked and roasted sweet potatoes had high GI values (87±2 and 93±4), while fried sweet potatoes reached intermediate GI level (70±3). The boiled sweet potatoes were found to have the lowest value (46±5). During the boiling process, starch gelatinisation occurs, which affects the ease with which it is digested. It is likely that after gelatinisation there is some retrogradation or production of starch that is resistant to hydrolysis, hence the lower GI value. It is evident from this study that the boiling method of cooking sweet potatoes presented lower glycaemic response compared to frying, baking and roasting. From that, this study also indicated that boiling sweet potato might be the preferable method to minimise the risk of T2DM and other chronic diseases (Perceval *et al*., 2011).

Additionally, the relationship between GI and satiety is complex. In case, the low and high GI carbohydrate may have an impact on satiety through the different time of hormonal responses. In a study, it was concluded that low GI carbohydrates have a satiating effect over a 2-3hour period, whereas high GI carbohydrates are associated with a more immediate reduction in appetite and food intake in the short term (Anderson *et al*., 2003). Therefore, the high GI foods should be avoided, which can lead to blood glucose spikes and eventually cause insulin resistance, which result in the development of type 2 diabetes mellitus (T2DM) and other chronic diseases.

The positive effects of consumption of low GI carbohydrate on glucose and insulin response were reported in the intervention studies in different subject conditions. In particularly, the Maki studies found that consumption of foods containing β-glucan from oats had favourable effects on postprandial insulin levels in the entire study sample and blood pressure in obese subjects. Additionally, dietary fibres in tested meal form viscous gels in the

gastrointestinal tract have been shown to reduce glycaemic response via the attribution to mechanical slowing of glucose absorption (Maki *et al.*, 2007).
Moreover, the consumption of high and low GI diet was studied of 28 women with or without the history of coronary heart disease. For 3 weeks, the high GI diet consumption increased production of free fatty acids in the late postprandial state lead to hypoglycaemia. And it is mediated by increasing in regulatory hormones; including cortisol, glucagon and growth hormone (Frost *et al*., 1998). According to the metabolic studies, it has been indicated that high carbohydrate intake is a cause of insulin resistance, lower high-density lipoprotein (HDL) cholesterol concentration, increase fasting triglyceride concentration compared to low GI diet (Jenkins *et al*., 2008).

2.3. The role of diet in development of Diabetes Mellitus

Diabetes mellitus (DM) is one of the chronic non-communicable diseases that is on the increase on a global scale. It affects about 1% of the Western countries and 5-10% of the world population. By the year 2010, the total prevalence of type 2 diabetes mellitus (T2DM) patients worldwide was estimated at 239million (Vats *et al.,* 2002). According to the statistic of International Diabetes Federation (IDF), the number of T2DM was indicated 8.2% (72.1million) of population in South-East Asia region by 2011; however, this number will be predicted up to 10.2% (123million) in 2030 (Idf, 2013). From that, diabetes mellitus becomes a major threat to global public health as well as the prevalence of diabetes is rapidly increasing. Amongst of South-East Asia countries, India is the largest contributor to regional mortality with 983,000 deaths attributable to diabetes (Idf, 2013). However, in developed countries such as Europe, the prevalence of T2DM is estimated at around 6% and predicted to rise over 8% in 2030 (Idf, 2013). There have been dramatic increases in prevalence associated with, either migration or the way of life of many Western countries by increasing energy-dense foods, high in fat and sugars.
T2DM is also known as complex metabolic disorder of carbohydrates. It is closely linked to other non-communicable disease states, in particular to overweight or obesity. Both genetic and lifestyle factors are also involved in the development of T2DM. Simple lifestyle adjustments such as increasing physical activity, reducing food intake, modifying the diet and losing weight are associated with a

reversal of the symptoms of T2DM. Similarly, the lifestyle interventions have been shown to increase insulin sensitivity in insulin-resistant individuals prior to the development of impaired glucose tolerance (IGT) (Tuomilehto *et al.,* 2001). High carbohydrate diet may be associated with increased insulin resistance by contributing to energy density of the diet lead to the development of being overweight and obesity, the principle risk determinant of non-insulin dependent diabetic mellitus (NIDDM). Besides, T2DM patients need to achieve adequate intakes of non-starch polysaccharides through regular consumption whole grain, cereals, fruits and vegetables.

2.4. Potential of under-utilised species in controlling and regulating T2DM

According to the World Health Organisation (WHO) in 2002, up to 90% of thepopulation in developing countries uses plants for primary health care. A plant-based diet could play a role in the prevention and treatment of T2DM, principally by improving blood glucose control, lowering blood pressure and reducing body weight. Among 21,000 medicinal plants, there are over 800 species which have been found to play a role in the control type 2 diabetes (Patil *et al*., 2011). Some examples include bitter gourd (*Momordica Charantia*), ginger *(Zingiber Officinale Rosc.)*, holy basil (*Ocimum Tenuiflorum)*, curry leaves (*Murraya Koenigii)* and several others.

Moreover, some under-utilised species have shown excellent positive outcomes in controlling T2DM. Both *in vitro* and *in vivo*studies have been carried out to investigate the effect of these species on the control of diabetes. The following under-utilised species have all been shown to have 'anti-diabetic' properties.

2.4.1. Bitter gourd

Bitter gourd (*Momordica Charantia*) is a tendril bearing vine belonging to the Cucurbitaceae family; it has many local names such as bitter melon, karela or balsam pear (Ram *et al*., 2002). It is cultivated wildly throughout Asia, grows well in hot weather as well as can develop in rich soils with high water properties. Depending on the cultivar, bitter gourd differ in their growth habits, maturation period and various fruits characteristics, including size, shape, colour and surface texture. In general, bitter gourd fruits have a knobby surface and are ready to harvest from 1-3 week after flowering. The fruits weigh from 80-120g for each (Tindall, 1983). The mature fruits taste is very bitter; therefore, the fruits are usually harvested before maturity. This bitterness is created by momordicosides compound. The fruits of bitter gourd are served in various ways such as boiled, fried, sliced or dried. However, their seeds should not be eaten because they contain alkaloids, which can be fatal. Alkaloids, triterpenes, a saponin-like substance and other biodynamic compounds present in various parts of the plant (Morton, 1967).

Moreover, the primary constituents of bitter gourd are charantin, cucurbutanoids, momordicin and oleanolic acids, which are responsible for the hypoglycaemic principle or anti-cancer properties (Cunnick *et al.,* 1993). During the past decade, the medicinal properties of bitter gourd were investigated by many medical researchers. Bitter gourd extracts have been shown to inhibit growth and proliferation of various types of cancer cells in animal studies and in *in vitro.* This may be attributed to the identification of a potent inhibitor of guanylate cylase, which is an enzyme treat in many types of tumour cells (Lee *et al*., 2003).

Anti-diabetic properties were also carried out based on bitter gourd components. Ethanolic extract of bitter gourd is reported to show anti-hyperglycaemic effect in normal and streptozotoxin-induced diabetic rats (STZ) which might be due to inhibition of glucose-6-phosphatase as well as stimulation of the activity of hepatic glucose-6-phosphate dehydrogenase (Gibbetal *et al.,* 2009). Studies have shown that the hypoglycaemic and lipid-lowering properties of bitter gourd can repair damaged β-cells, thereby stimulating insulin levels and improve signalling of insulin. And triterpenoids compound present in bitter gourd could be responsible for activation of AMP-activated protein kinase (Huang *et al.,* 1992). The mechanisms proposed for the hypoglycaemic effect of bitter gourd have

been attributed to an inhibitory effect on glucose absorption in the intestine to enhance insulin release from β-cells (Higashino *et al.,* 1992).

For another example, a study was investigated that there was a significant ($p<.004$) increase β cell in the pancreas of the streptozotocin-induced diabetes rats. After 9 weeks of bitter gourd fruit extract treated, the percentage of β-cells in treated rats was increased to 50.22%, compared to untreated rat (27.04%). The result of this study have demonstrated marked changes in the pattern of distribution of insulin-positive cells in pancreatic tissue of diabetic animals compared to normal animals (Ahmad *et al*., 2000). In a study conducted on type 2 diabetes patients, the extract of bitter gourd fruit was reported to show hypoglycaemic effect on T2DM patients (100 patients). After 75g of bitter gourd juice was given, the mean of blood glucose response was significant decreased at $p<.001$ in 86% patients out of 100 cases (Ahmad *et al*., 2000).

2.4.2. Ginger

Ginger *(Zingiber Officinale Rosc.)* in the Zingiberaceae family is an underground rhizome, whose originating in South-East Asia (Purseglove *et al.,* 1988). Ginger root is not only used in food as a spice but also a medicinal plant that has been used in Chinese, Ayurvedic herbal medicines. Its harvest depends on the cultivar and varies from 7-9months for annual crops. The volatile oil constitutes odour of ginger, which occupies from 1% to 3%, depending on the origin of cultivars. Among of 70 oil components, sesquiterpene hydrocarbon-α-zingiberene predominate accounts for 20-30% of the oil obtained from dry ginger (Purseglove *et al.,* 1981). The aroma and flavours of fresh ginger will be different from dry ginger by evaporation volatile oil during drying (Purseglove *et al.,* 1981). Moreover, the most abundant compound in fresh ginger is gingerol, which is homologous series of phenols in structure. The concentration of gingerol in the dry ginger was slightly decreased compared to fresh ginger (Afzal *et al*., 2001). Fresh ginger and ginger powders are used widely for culinary purpose, baking and confectionary.

At present, it is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs. Herbs and spices are generally considered as safe and have been proved effective against diseases (Kapadia *et al.,* 2002). The interested potential of ginger is investigated for the treatment of diabetes. In particularly, an aqueous extract of raw ginger

was fed to STZ-induced diabetic rats at a dose of 500mg/kg body weight. After 7weeks of feeding, their blood was collected to analyse glucose, cholesterol and triacylglycerol levels. Using raw ginger resulted that in a significant lowering of serum glucose, hypo-cholesterolemic and hypolipidemic potential compared to control diabetes rats group (Amin *et al.,* 2006). The identification of active compounds of ginger on anti-diabetic properties, STZ-induced diabetic rats were fed by oral ingestion of ginger juice (4mL/kg body weight) during 6weeks. As a result, the serum insulin concentration was significantly induced in these diabetic rats (Akhani*et al.,* 2004). Thus, the value of ginger is indicated in managing the effects of diabetic complications in human subjects.

2.4.3. Fenugreek

Fenugreek *(Trigonella Foenum-graecum L.)* belongs to the family of Fabaceae. It is a native of South-Eastern Europe and the Mediterranean region (Petropoulos, 2002), where it is found wild growing and cultivated for a long time. Fenugreek is well known as a strong aroma, bitter taste and increase flavour in food with small quantity added. It presents in various forms of seeds such as whole seeds, powdered or paste in the spice store. The seeds are usually golden yellow colour and hard seed (Abha *et al*., 2004). Besides, they have been used widely for medicinal purposes including inflammation, cardiovascular diseases and metabolic disorders (Araee *et al.,* 2009).

The effect of an ethanolic extract of fenugreek on STZ-induced diabetic rats was investigated. The study was followed up 14 days via oral administration of fenugreek extract at different concentration of extract (0.1, 0.25 and 0.5g/kg body weight). After 2 weeks, the result was significant effect on weight loss in diabetic rats. From that, the serum glucose, cholesterol profile, urea, uric acids was reduced, whereas insulin level was inclined in only diabetic rats, $p<.05$ (Akram *et al*., 2007). Moreover, in the study of Gupta *et al*., 2001, the seed fibres of fenugreek reduces the rate of glucose absorption and may also delay gastric emptying, thereby preventing the rise in blood sugar levels following a meal.

An amino acid, 4-hydroxyisoleucine, was detected in fenugreek seeds and the anti-diabetic properties were studied in an *in vivo* study. It effects on liver function and blood glucose in diabetes rats at dose 50mg/kg body weight after administration 8weeks. As a result, 4-hydroxyisoleucine improved insulin

resistance, glucose tolerance, reduced hyperglycemia and did increased HDL cholesterol levels to 31% at p<.05 (Haeri *et al*., 2009). Another study about amino acid 4-hydroxyisoleucine, it stimulates powerfully insulin secretion at all levels of cellular organization. And the cells are more sensitive to insulin and increase in the number of insulin receptor sites to burn cellular glucose at high fibre diet (Broca *et al.,* 2000). The combination between amino acid 4-hydroxyisoleucine and total dietary fibre in fenugreek seed showed a highly significant effect on hyperglycaemic (Ribes *et al.,* 1986).

2.4.4. Turmeric

Turmeric *(Curcuma longa L.)* has the same family with ginger root, Zingiberaseae.It is a perennial herb, which is known as an important commercial crop and develops in Southern Asia, India and other tropical countries. It presents in dark brown skin on the exterior and deep orange yellow flesh. Turmeric is a rhizome that has been use in different purposes. Curcumin and other essential oils such as turmerone, zingiberins present in turmeric root, which display in deep orange flesh of turmeric.

For medicinal used, it is reported that curcumin decreases blood glucose, HbA1c in diabetic rats, in addition, curcumin also decreased oxidative stress in diabetic rats. The beneficial effect of curcumin has been suggested in diabetic dyslipidemia without altering the hyperglycemic status in diabetic rats. It is observed that the reduction in blood cholesterol in curcumin fed rats (Babu*et al*., 1997). Curcumin also increased the activity of β-hydroxy-β-methylglutaryl-coenzyme A (HMG-CoA) reductase and hepatic cholesterol-7α-hydroxylase enzymes in diabetic rat livers. Curcumin also has an antioxidant property; it not only inhibits lipid peroxidation significantly in rat liver but also reduces the increased accumulation of advanced glycation end products (AGEs) and cross-linking of collagen in tail tendon and skin of diabetic animals, thereby preventing AGEs induced complications of diabetes (Sajithlal*et al*., 1998, Reddy *et al*., 1992).

2.4.5. Ficus religiosa

Ficus religiosa *(Religiosa Linnaeus)* commonly known as sacred fig belonging to thefamily of Moraceae, which are used in the traditional system for the treatment of diabetes (Simmonds *et al*., 2006). Fruits of *Ficus religiosa* are small, circular in shape and compressed. It has green colour at raw stage and turns black when it is ripe. It contains a good source of antioxidant; including tannins, saponins, polyphenolic compounds, flavonoids and sterols. These bioactive components present in *Ficus religiosa* have been shown in*in vitro* and *in vivo*of pharmacological studies. The effectiveness of *Ficus religiosa* has been investigated in anti-hyperglycemic study. It was observed during 3weeks of alloxan-induced diabetic mice by oral administration in aqueous extract. Consequently, there was a significant lowering blood glucose level as well as increased insulin levels (Deshmukh *et al*., 2006). To manage T2DM, the hypercholesteremia and hypertriglyceridemia were decreased through significantly reduced serum triglycerides as well as the total cholesterol levels in STZ-diabetes rats at dose of 500mg/kg body weight.

The oxidative stress and oxidative damaged tissues are the major etiologies in the complications of T2DM. Oxidative stress in diabetes coexists with a reduction in the antioxidant status, which can increase further free radicals. From that, the aqueous extract of *Ficus religiosa* brought the benefits to STZ-induced rats by relatively less weight compared to control rats. As a result, the uptake of glucose, free fatty acids from circulation was declined, while β-oxidation in adipose tissue was accelerated lead to weight loss in diabetic rats (Nicklas *et al*., 2006).

2.4.6. Ivy gourd

Ivy gourd *(CocciniaIndica)* grows wildly and cultivated in Malaysia, Africa, Southeastern Asia and other tropical areas. This species are a dioeciously perennial with glabrous stems. They are green with white stripes and turn scarlet at maturity stage. Leaves, young shoot and immature fruits are cooked as vegetables. Various plant parts are used medicinally. Based on its nutrient content, it was used for diabetes treatment. On the one hand, oral feeding of 500mg/kg body weight of ivy gourd leaves extract showed not only significant hypoglycaemia in alloxan-diabetic dogs but also increased glucose tolerance (Singh *et al.,* 1985). In addition, the ethanol extract of leaves at dose of

200mg/kg affected on fasted rats and STZ-induced diabetic rats. After 18hr oral feeding, blood glucose levels decreased to 23% for fasted rats and 27% for STZ-induced diabetic rats, hepatic glucose-6-phosphatase was declined to 19% and 32% for two groups, respectively (Shibib *et al.,* 1993). On the other hand, ivy gourd was also used in vivo via clinical study. Oral administration of dried *Cocciniaindica* extract on thirty on set diabetic patients (500mg/kg) for 6 weeks. As a single oral dose, the extract has been shown to exert beneficial in the significantly raised of lipoprotein lipase and the levels of glucose-6-phosphatase, which involved in glucose metabolism (Kamble *et al.,* 1998).

2.4.7. Others

Several other herbs and spices are also reported to have anti-diabetic properties. These include Tulsi leaves (*Ocimum Sanctum),* Amla (*Emblica Officinalis)*, Gurmur leaves (*Gymnema Sylvestre),* Jamun fruits (*Syzygium Cumini)* and curry leaves (*MurrayaKoenigii)*. In particularly, eugenol in Basil leaf *(Ocimum Sanctum)* is an active constituent that has been found to be responsible for its therapeutic potential. The major bioactive constituents present in leaves and stems of holy basil; including flavonoids, saponins, tannins and also rich in vitamin, minerals, chlorophyll and many other phytonutrients. In controlling non-communicable diseases, holy basil properties weredetected in a significant reduction in blood glucose with a simultaneous increase in glycogen, hemoglobin and protein in STZ-induced diabetic rats, which has been observed in ethanolic extract of holy basil (Kumari *al.,* 2014). Moreover, diet containing 1% of leaf powder fed to normal and STZ-induced diabetic rats for 4 weeks lead to reduced fasting blood glucose, total amino acids, total cholesterol, triglyceride and uronic acid, hypolipidemic effect (Rai *et al*., 1997).

To evaluate the anti-hyperglycaemic efficacy of curry leaf, STZ-induced diabetic rats were fed at 200mg/kg dose. After 30days oral administration, there was a significant decreased ($p<0.05$) at the level of glucose, plasma insulin, uric acid and creatinine in treated group of rats. Moreover, a rich antioxidant sources in curry leaf extract was presented in decreasing oxidative stress and against pancreatic β-cell damage (Palanisamy *et al.,* 2006).

In short, the under-utilised species show their potential in controlling and regulating T2DM in many studies. Moreover, they also have a high nutritional value and contain bioactive compounds; therefore, they are ideal to use to

control T2DM and to improve nutritional status. Amongst the neglected species, four have been selected, namely Bitter Gourd, Ginger, Turmeric and Fenugreek for further study to determine the effect of consumption on the Glycaemic Index

CHAPTER 3: MATERIALS AND METHODS

3.1. Underutilised species sources

The four underutilised species that were used in this study are bitter gourd (*Momordica Charantia*), ginger (*Zingiber Officinale*), turmeric (*Curcuma Longa*), and fenugreek (*Trigonellafoenum-graecum*). These raw materials were purchased from local supermarket in Semenyih, Malaysia.

3.2. Preparation of plant materials

Bitter gourd was selected in unripe stage with a dark green skin colour. The fruits were small, firm and free form blemish or mould. Selected bitter gourd fruit had many ridges and warts on the surface. Ginger and turmeric samples were selected based on their skin colour, yellow brown skin for mature ginger and dark brown skin for turmeric. The roots were heavy in weight, all of a similar size and maturity, firm with smooth skin and a spicy fragrance. Ginger roots that were light in weight, soft and with a wrinkled skin were avoided because they might have lost of nutrient content or poor of postharvest quality. Fenugreek seeds were selected based on colour and size of seeds. See figures 3.1 to 3.4.
Depending on variety of under-utilized species, the physicochemical characteristics of four species were assessed such as colour of fruit or rhizome, shape, length, weight and flavour is described in Table 3.1.

Table 3.1: *The description of physicochemical features of four selected species*

Variety	**Physicochemical characteristics**				
	Colour	Shape	Length (cm)	Weight (g)	Flavour
Bitter gourd	Pale yellow to dark green	Oblong fruit with jagged surface	6-18	100-120	Strongly bitter
Ginger	Yellow brown skin and pale yellow flesh	Irregularly shaped	10-18	100-120	Smell pungent, sharp peppery favour
Fenugreek	Golden yellow seed	Rectangular to rounded shape	0.35		Bitter taste, strong flavour and aroma
Turmeric	Dark brown skin and deep orange yellow flesh	Finger-shaped	7-9	30-50	Peppery, warm and bitter flavour, slightly pungent

Figure 3.1: Bitter gourd

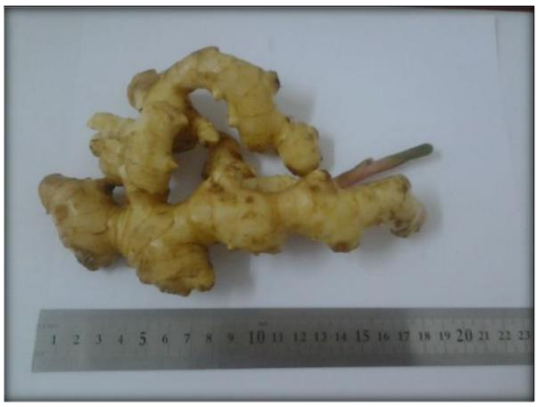

Figure 3.2: Ginger

Figure 3.3: Fenugreek

Figure 3.4: Turmeric

3.3. Preparation of sample materials

The four selected under-utilised species were prepared according to different cooking methods (Table 3.2). The methods selected were based on normal consumption and preparation methods. These include uncooked, boiled and fried for bitter gourd, ginger, turmeric and fenugreek. Boiled white rice was used as a reference to compare with the samples.

Table 3.2: *The different cooking method of four selected species*

Samples	Bitter gourd	Ginger	Turmeric	Fenugreek	White rice
Raw	✓	✓	✓		
Boiled	✓	✓	✓	✓	✓
Fried	✓	✓		✓	

The fresh bitter gourds were washed under tap water and dab dried with dry tissue to prevent the spread of dust or microorganisms. Their seeds were removed manually using a sharp knife. The flesh of bitter gourd was cut into small species, and then divided into three portions, 150g for each cooking method. The first portion of fresh cutting bitter gourd, it was wrapped with aluminium foil which was used as a raw sample. To prepare boiled sample, the flesh of bitter gourd of second portion was boiled in 300ml of water for 10minutes in a stainless steel pan and cooled in water to prevent further cooking from residual heat. The third portion of flesh was stir-fried with the addition of 15ml oil (Naturel cooking oil) for 10minutes and no additive added. They were wrapped in aluminium foil round for the next step. Following cooking, samples were kept in the freezer at -80^{0}C for one day before freeze drying.

Ginger and turmeric are rhizomes; therefore, they were prepared in a different way. These two species are a common spice for the multiple use. To prepare fresh ginger and turmeric, their tough skin was removed using a sharp knife and then washed under tap water to remove dirt. Then, they were dried using tissue. Both ginger and turmeric were chopped into small cubes. For ginger roots, they were prepared similarly to bitter gourd; including uncooked, boiled and fried

form. It was also repeated for turmeric rhizome; however, only uncooked and boiled form was prepared. Because turmeric is quite sticky when it stirs fried with oil. Following processing, they were wrapped carefully in aluminium foil and stored in the freezer at -80^{0}C.

The fenugreek seeds were sorted to remove stones as well as black seeds. A sample (150g) of fenugreek seeds were weighed for each portion of cooking process. After 2 hours of soaking in water, the cooking steps were repeated for boiling and frying, similar to bitter gourd process. After processing, they were stored in the freezer at -80^{0}C for a day.

To boil white rice (Jasmine Sunwhite AAA fragrant rice), 150g of long grain rice was washed twice in tap water and cooked using a rice cooker. Boiled white rice was kept cool at the room temperature and wrapped with aluminium foil for freezing.

All samples were weighed before and after freeze drying to determine water lost during drying as well as to calculate how much water loss after freeze-drying.

3.4. Freeze-drying procedure for samples fractions

Freeze-drying method is used as a method in foods as it can retain nutrients as well as bioactive compounds in fruits and vegetables. It was took 20 minutes to warm up before the main drying started. Frozen samples were placed in the freeze-dryer (Christ alpha 1-2/LD plus) at -20^{0}C under 1 mbar of pressure for three days, according to the method of Marques *et al.*, 2006. The sample packages were punctured to create small holes with a knife on the entire package surface. The purpose of this was to facilitate the drainage of water during freeze drying process.

When the freeze drying process was complete, the dry weights were recorded to calculate the percentage of water loss. In addition, the dried products were ground into a powder using a mortar and pestle. The powder was kept in plastic zipper bags and stored in the freezer at -20^{0}C prior to analysis.

3.5. Determination of dry matter

Dry matter (%) was calculated according to the following equation:

$$\textbf{Dry matter (\%) = (W2-W1) / W2 x100}$$

Where W1 is the weight of selected samples after freeze drying, W2 is the weight of fresh samples. All samples were not replicated because the weight of dry matter after freeze drying was sufficient for the study.

3.6. Chemicals

Sodium hydroxide, Hydrochloric acid, Sodium nitrite, Phenol, Monopotassium phosphate, Disodium phosphate and Maltose were obtained from Sigma, Germany. Potassium Sodium tartrate (Rochelle salt), 3,5-dinitrosalicylic acid, Human salivary amylase enzyme (type XIII-A), α-amylase from porcine pancreas (type I-A) were purchased from Sigma-Aldrich, US. D(+)-glucose monohydrate were obtained from Darmstadt, Germany. Pepsin from human gastric mucosa was purchased from Bio Basic Int, Canada.

3.7. Determination of carbohydrates

Total carbohydrate was measured by acid hydrolysis followed by estimation of the reducing sugars (glucose) by the DNS (3,5-dinitrosalicylic acid) method. DNS (3,5-dinitrosalicylic acid) is an aromatic compound that reacts with the free carbonyl group of a reducing sugar under alkaline conditions. During the DNS reaction (Figure 3.5) one mole of DNS acid will react with one mole of reducing sugar. The carbonyl group (C=O) in reducing sugar is oxidised to a carboxyl group (O=C-OH) under alkaline conditions. Simultaneously, the reduction of DNS acid produces the reduced form of 3-amino,5-nitrosalicylic acid. The colour change of solution, from yellow to a red-brown colour is measured by a spectrophotometer at 540nm (Cole *et al*., 1933; Doner *et al*., 1992).

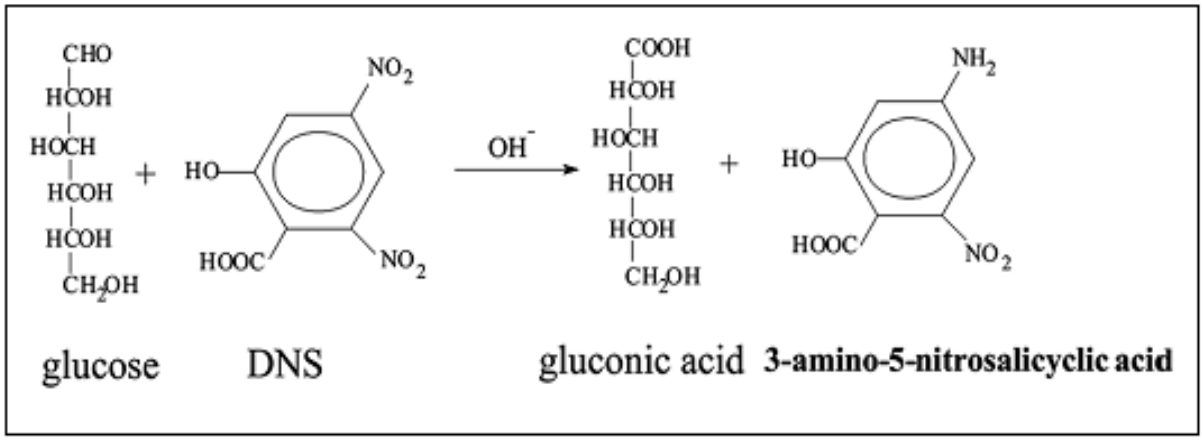

Figure 3.5: *DNS reaction under alkaline condition (*Meng-lei *et al., 2015)*

3.7.1. Advantages and disadvantages of the DNS method

It is a convenient and relatively cheap method of estimating reducing sugar content, however the method has low specificity; some of the colour change seen may be due to decomposition of the sugar. Each reducing sugar has a different specificity and will yield a different colour, therefore to improve the accuracy of the method; it should be calibrated for each reducing sugar.

The DNS method is accurate, but is non-specific – it will only measure reducing sugars and cannot be used to identify which reducing sugars are present. It will not measure non-reducing sugars, for example sucrose

3.7.2. Precaution steps

To protect reducing sugar during processing, it must be absolutely worked in low light conditions. And when placing the test tube with reagent in the boiling waterbath, it must be folded with aluminum foil to prevent the evaporation. All of the samples should be stored at 4^0C during analysis. However, samples powder as well as DNS reagents should not be used after 2 weeks.

3.7.3. Preparation of DNS reagents

The 1% DNS reagent contained 0.2% phenol, 0.05% sodium sulfite, 40% Rochelle salt (potassium sodium tartrate) and 1% sodium hydroxide (Miller, 1959). 1g of 3,5-dinitrosalicylic acid was dissolved in 50ml distilled water. After that, 0.2g of phenol and 20ml of sodium hydroxide 2M were added and made up to 100ml with distilled water. The yellow-orange colour of 1% DNS reagent was obtained. Sodium sulfite (0.05g) the 1% DNS reagent before using to get the maximum colour intensity.

To make 40% Rochelle salt by dissolving 40g Rochelle salt in 100ml distilled water.

3.8. Determination of total carbohydrate in sample

3.8.1. Preparation of the standard curve

To prepare the glucose standard solution, dissolve 0.1g D(+)- glucose monohydrate in 100ml of distilled water to create 0.1% glucose standard.

Standard glucose (0, 0.2, 0.4, 0.6, 0.8, 1.0 ml) was transferred into 6 labelled test tubes. Each sample made up to 2ml with the addition of distilled water. The test tubes were shaken well. 1ml of Rochelle salt was added after the colour had developed in 5 minutes in the boiling water bath at 98^0C. The tubes were cooled in an ice bath for few seconds. After that, 1ml of aliquot was removed to measure the absorbance at 540nm using UV/Vis spectrophotometer (Libra S12). For blank, the glucose solution was replaced with distilled water. The calibration curve was plotted with the absorbance against the glucose concentration (mg/ml). Glucose was used as a standard to produce calibration curve.

For the maltose standard curve, all the procedures were repeated similar to the glucose standard curve.

3.8.2. Determination of starch

The starch of selected species was evaluated by acid hydrolysis (Myers *et al.,* 1921) (McCready *et al.,* 1950). Each sample powder (1g) was weighed into a 50ml centrifuge tube and added 5ml of hydrochloric acid 2.5M. All the tubes are mixed well by using vortex mixer. Then, they were left in boiling water bath at 98^0C and shaking in every 30minutes. After 3 hours, a small amount of sodium carbonate was added into the tube until no longer effervescence and made up to 100ml with distilled water. After 20min of centrifugation at 7000rpm, the supernatant was collected and discarded residue.

An aliquot (250ul) and distilled water (750ul) were transferred to new test tube. This was followed by adding 2ml of distilled water and 1ml of DNS reagent. The colour of aliquot had developed in 5minutes in boiling water bath.40% Rochelle salt (1ml) was added to stabilise the developed colour and kept in an ice bath. When the aliquot was cooled, 1ml of aliquot was removed into cuvette. The absorbance at 540nm was measured against water blank using UV/Vis spectrophotometer. The recorded absorbance of glucose was multiplied by 0.9 to convert to starch. The amount of total starch was expressed as gram per 100g of dry matter (g/100g DM).

3.8.3. Determination of reducing sugar

Reducing sugar is a type of carbohydrate that can be oxidised by mild or weak oxidising agents. It contains a free aldehyde or ketone group in its structure and donates electrons to another molecule. Maltose is one of reducing sugar, which is produced when starch molecules are broken down. Therefore, reducing sugar was measured using the 3,5-dinitrosalicylic acid (DNS) assay (Miller, 1959).

The reducing sugar was determined by ethanol hydrolysis using spectrophotometric method (McCready *et al.,* 1950) (Lee *et al.,* 1970). Weighed 1g of each powdered sample in 50ml centrifuge tube and extracted twice with hot ethanol 80% (5ml each time). The transparent supernatant was collected by using Whatman filter paper No1 after placing in water bath at 80^0C for 20minutes. The removal of ethanol was done by evaporating of the aliquot by rotary evaporator (Buchi Rotavapor, R-200) at 80^0C. It was made up with 10ml distilled water to dissolve the remaining of reducing sugar.

To do this, 1ml of aliquot was withdrawn and mixed with 2ml of distilled water. Then 1ml of DNS reagent was added into the test tube and placed them in

boiling water bath for 5minutes. To stabilise the colour under these conditions, 1ml of 40% Rochelle salt was added prior to cooling and left them in ice bath. The absorbance was recorded at 540nm by UV/Vis spectrophotometer. Water blank was used as a reference. Otherwise, the sample blank also was prepared, which consists of reducing sugar extraction aliquot and distilled water. It was used to avoid the bias due to the colour and turbidity of sample. The amount of reducing sugar present in the sample was expressed as gram per 100g of dry matter (g/100g DM).

3.9. *In vitro* model of digestion

The *in vitro* model of digestion was developed to simulate the physiological conditions of human digestion. It included three phases – oral, gastric and intestinal phase (Goni *et al.* (1997), Englyst *et al*. (1992) and Germaine *et al.* (2008)). The purpose of the *in vitro* digestion was to measure the rate of carbohydrate breakdown and glucose release from the test samples. This was to determine the GI value of each of the foods under investigation.

Single samples (test samples) and mixed meal samples (test sample plus white rice) underwent the *in vitro* digestive process. This was in order to estimate the GI value of each individual food and also to ascertain if consumption of the test food together with white rice had any moderating effect on the glucose release from rice in the mixed meal. Boiled white rice was used as a reference food in both single and mixed meals (Table 3.3). Due to time constraints, only the boiled test samples were evaluated in this system.

Table 3.3: *The test meal for in vitro analysis*

Single meal	**Mixed meal**
Raw bitter gourd	White rice + Raw bitter gourd
Boiled bitter gourd	White rice + Boiled bitter gourd
Fried bitter gourd	White rice + Fried bitter gourd
Raw ginger	White rice + Raw ginger
Boiled ginger	White rice + Boiled ginger
Fried ginger	White rice + Fried ginger
Raw turmeric	White rice + Raw turmeric
Boiled turmeric	White rice + Boiled turmeric
Boiled fenugreek	White rice + Boiled fenugreek
Fried fenugreek	White rice + Fried fenugreek
Boiled white rice	

3.9.1. Single meal digestion

A sample of material, equivalent to 1g of available carbohydrate, was weighed into a volumetric flask. The carbohydrate content of each sample (% Dry Matter) varied considerably between samples (3.7-71.61 g/100gDM) hence the amount used for *invitro* analysis varied to reflect this difference (Table 3.4). An aliquot (184U) of salivary α-amylase (human, type XIII-A, Sigma, A-1031; 300-1500unit/mg) was added to the sample flask and vortex mixed for 2 minutes to represent the oral phase. After adding 100U of pepsin (human, Bio Basic Int, 3000NFU/mg) in 6ml sodium potassium phosphate buffer (pH 1.5), the sample flask was placed in a water bath at 37˚C and stirred every 10 minutes to simulate gastric digestion. After 30 minutes, pancreatic α-amylase (110U) (porcine, type I-A, Sigma, A-4268; 700-1400unit/mg) in 100ml of sodium potassium phosphate buffer (pH 6.9) was added into the sample flask. The intestinal digestion phase was simulated by incubating the flask at 37˚C in a shaking water bath for 180 minutes with slowly shaking at 120rpm.

Triplicate aliquots (2ml) were removed at 0, 30, 60, 90, 120 and 180 minutes into a micro-centrifuge tube to assay for glucose content. The tubes were placed in a hot water bath at 98˚C for 5minutes to halt enzyme activity and then cooled in an ice bath. After centrifuging at 15,000rpm for 5 minutes, the supernatant was collected and transferred into a labelled test tube. These test tubes were used for reducing sugar determination by DNS colourimetry test.

To each test tube containing 1ml of sample aliquot, distilled water (1ml) and followed by DNS reagent (1ml) were added. All test tubes were placed in a boiling water bath at 98˚C for 5minutes, then removed and placed on ice to reduce heat. To prevent further colour development, 40% Rochelle salt (1ml) was added and gently shaken. It was followed by transferring into cuvettes. The absorbance of reducing sugar was recorded at 540nm wavelength by using UV/Vis spectrophotometer. The reducing sugar concentration was expressed as milligram per milliliter of food sample (mg/ml).

Table 3.4: *The sample powder weight provide 1g available CHO in single meal test*

Samples	Amount of CHO in 100g dry matter (g)	Amount of sample powder for 1g available CHO(g)
Bitter gourd	3.7	27
Ginger	71.61	1.4
Turmeric	67.14	1.5
Fenugreek	58.35	1.7
White rice	27.17	3.5

3.9.2. Mixed meal digestion

To provide 1g of available carbohydrate for the assessment of the mixed meal, the weight of powder were mixed between white rice powder and sample powder (Table 3.5). The procedure for *in vitro* digestion of a single meal was used for the mixed meal assessment (see section 3.9.1).

Table 3.5*: The sample powder weight provide 1g available CHO in mixed meal test*

Samples	Amount of sample powder for 1g available CHO (g)
White rice + Bitter gourd	15.27
White rice + Ginger	2.47
White rice + Turmeric	2.51
White rice + Fenugreek	2.63

3.10. *In vivo* model of digestion

The *in vivo* evaluation of glucose liberation (and assessment of GI of each food) from the single and mixed meals was carried out in human volunteers. Boiled white rice (which is a high GI food) was used as a reference test food against the glycaemic responses of test foods was measured.

3.10.1. Study design

The glycaemic response of consuming four underutilised species together with boiled white rice were evaluated using four volunteers. Boiled white rice was consumed alone in a single meal to serve as a baseline reference. Test samples were added to the rice to provide 1g available carbohydrate (Table 3.6). Blood glucose measurements, taken by finger pin prick, were made at 30 minute intervals for a duration of 3 hours after consumption of the experimental meal (Table 3.6).

Table 3.6: *The test meal of in vivo analysis*

Single meal	Mixed meal
White rice	White rice + bitter gourd
	White rice + ginger
	White rice + turmeric
	White rice + fenugreek

3.10.2. Subjects

The ethical approval for this study was approved by The University of Nottingham Malaysia Campus and granted permission to recruit participants (TNO030715-A) (Appendix 1). The healthy participants were recruited at The University of Nottingham Malaysia Campus via flyers and posters. Subjects included females and males (18 years old and above) of Malaysian nationality (Appendix 4). These participants were committed to join in two sessions of measurement on two consecutive days.

Participants were required to fast overnight (10-12 hours) before taking the test. All participants were asked questions about their general health and to declare if they were a smoker, drinker or diabetic. If they answered positively to these

three questions, they were excluded from the study. All provided an informed consent before participation.

3.10.3. Test meals

All meals were prepared in the kitchen of Nutrition department, the University of Nottingham Malaysia Campus. Three of the test samples (bitter gourd, ginger and turmeric) were washed under tap water and the skin removed from ginger and turmeric. All three were cut into thin strips. Fenugreek seeds were soaked in hot water for 30 minutes before cooking. The four test samples plus white rice were boiled separately. Due to time constraints, only the boiled test samples were evaluated. The cooked weight of test food required for each meal is presented in Table 3.7, which was calculated based on the carbohydrate in fresh weight of samples.

Fasting blood glucose (mmol/l) was measured for each participant using the finger prick test using the Accu-Check Active kit (Roche Diagnostics, Germany). Disposable 0.4mm lancets (Accu-Chek Softclix lancets) were used to prick fingers by pressing firmly on the side of the fingertip. Finger was gently massaged to form a droplet of blood which was placed on the test strip (Accu-Chek Active test strip) to record the blood glucose concentration. After fasting glucose concentrations were measured, all participants consumed the test food.

On the first day of testing, participants consumed white rice alone. This was served as a reference meal. Blood glucose concentration was read over a 3 hour period at 0, 30, 60, 90, 120, 150, 180 minutes after consumption of the test meal.

The number of participants was divided into four groups, each containing two people. The following day, participants consumed one type of selected vegetables with white rice as a mixed meal. Blood glucose concentration was measured at 30 minute intervals from 0 to 180 minutes after consumption of the meal. After 180 minutes of the test, participants were given fruits and beverages.

***Table 3.7**: The portion size of samples in mixed meal in vivo analysis*

Test food	Portion size (g)
White rice	160
Bitter gourd	25
Ginger	10
Turmeric	10
Fenugreek	10

3.11. Statistical analysis

The replicated results were expressed as mean± standard deviation (mean±SD). Data on glucose concentration (mg/ml) of 4 selected species (bitter gourd, ginger, turmeric and fenugreek) was subjected to two-way ANOVA in randomised blocking design in Genstat Statistical Software, 17th edition. Treatment of samples was set as a factor containing three levels (raw, boiled and fried). Time was converted as a factor with six levels (0, 30, 60, 90, 120, 150 and 180 minutes). Significant difference in the F test were further analysed using Fisher's Protected LSD test at 5% level. The graph was plotted using Microsoft Excel 2010.

CHAPER 4: RESULTS

4.1. Determination of dry matter

Table 4.1 shows the percentage of dry matter (%DM) of selected samples under three different cooking methods (raw, boiled and fried). It was determined by freeze drying method. Overall, there was 5.08% to 98.43% DM obtained after freeze drying as well as the water content (%) were shown in Table 4.1. And the amount of water loss was ranged from 2.36g to 142.38g per 150grams of fresh weight of samples. In particularly, the highest dry matter was collected from fenugreek seeds, 85.68% and 98.43% DM of boiled fenugreek and fried fenugreek, respectively.

Moreover, there was not much water content in the fenugreek with 14.32% for boiled fenugreek and 1.57% for fried fenugreek. There was 91%-95% of water content in fresh weight of bitter gourd. Therefore, the percentage of dry matter of bitter gourd had the lowest percentage from 5.08%DM to 9.22%DM for all three cooking treatments. It was followed by the dry matter of ginger in various cooking methods, raw, boiled and fried was 9.30%DM, 12.17%DM and 20.56%DM. Raw turmeric and boiled turmeric were presented in 18.0%DM and 10.61%DM, respectively. With 31.8% DM and 68.2% water content presented in boiled white rice after freeze drying.

Table 4.1: *Dry matter of selected species in different cooking method determined by freeze-drying*

Materials	Fresh weight of sample (g)	Weight of freeze dried sample (g)	Water loss (g)	Water content (%)	Dry matter (%)
1. Raw Bitter Gourd	150	11.42	138.58	92.39	7.61
2. Boiled Bitter Gourd	150	7.62	142.38	94.92	5.08
3. Fried Bitter Gourd	150	13.83	136.17	90.78	9.22
4. Raw Ginger	150	13.95	136.05	90.70	9.30
5. Boiled Ginger	150	18.26	139.39	87.83	12.17
6. Fried Ginger	150	30.84	119.16	79.44	20.56
7. Raw Turmeric	150	27.00	123.00	82.00	18.0
8. Boiled Turmeric	150	15.92	134.08	89.39	10.61
9. Boiled Fenugreek	150	128.52	21.48	14.32	85.68
10. Fried Fenugreek	150	147.64	2.36	1.57	98.43
11. Boiled White Rice	150	47.7	102.3	68.2	31.8

4.2. Determination of starch and reducing sugar

The total starch and reducing sugar, as determined by chemical hydrolysis, varied in different species. The amount of total starch and reducing sugar were calculated by glucose the standard curve equation; $y=0.7051x+0.0158$, $R^2=0.9876$ (Figure 4.1). All data expressed as mean±SD of three replicates.

As is presented in table 4.2, total starch of bitter gourd in different cooking treatment (raw, boiled and fried) was 3.52, 4.16 and 3.80g/100g DM, respectively. While the total starch in each treatment of ginger was significant higher than bitter gourd. With 57.54, 68.11 and 64.11g/100g DM were measured for raw, boiled and fried of ginger. It was followed by the total starch of turmeric, which was given 60.95 and 68.60g/100gDM for raw form and boiled form of turmeric. Boiling of fenugreek (56.80g/100g DM) resulted in much higher starch compared with fried form (47.08 g/100g DM). Moreover, only the boiled white rice was double amount of total starch content, 78.47 g/100g DM. It is clearly seen that boiled form of all species had a significantly higher in total starch, followed by the fried form and raw form.

In term of reducing sugars, all forms of fenugreek was much lower than others species. With 0.78 and 0.62g/100g DM were presented for boiled and fried fenugreek, respectively. Next is bitter gourd, which demonstrated at 1.2 g/100g DM for raw form, 0.86g/100g DM for boiled form and 1.00g/100g DM for fried

form. Following by turmeric, the reducing sugar was no significant different between two treatments. It was presented in 2.78 g/100g DM for raw turmeric and 2.63 g/100g DM for boiled turmeric. Besides, the species had the highest reducing sugars amount was ginger. As is shown, raw ginger was 3.12 g/100g DM, 2.71 g/100g DM for boiled ginger and 3.00 g/100g DM for fried form of ginger. Especially, white rice was no reducing sugar in the same condition with others. In short, boiled form of all species was a significant higher in total sugar, compared to other form. And, the higher total sugar of species in different cooking method contained, the lower reducing sugar it had.

***Table 4.2**: The value of total starch and reducing sugar of selected species expressed as mean±SD (g/100g DM).*

Materials	**Total starch** ***(g/100g DM)***	**Reducing sugar** ***(g/100g DM)***
1. Raw Bitter Gourd	3.52±0.043	1.20±0.004
2. Boiled Bitter Gourd	4.16±0.031	0.86±0.009
3. Fried Bitter Gourd	3.80±0.052	1.00±0.004
4. Raw Ginger	57.54±0.018	3.12±0.016
5. Boiled Ginger	68.11±0.015	2.71±0.004
6. Fried Ginger	64.11±0.042	3.00±0.008
7. Raw Turmeric	60.95±0.007	2.78±0.005
8. Boiled Turmeric	68.60±0.013	2.63±0.008
9. Boiled Fenugreek	56.80±0.021	0.78±0.004
10. Fried Fenugreek	47.08±0.084	0.62±0.002
11. Boiled White Rice	78.47±0.028	0.00±0.000

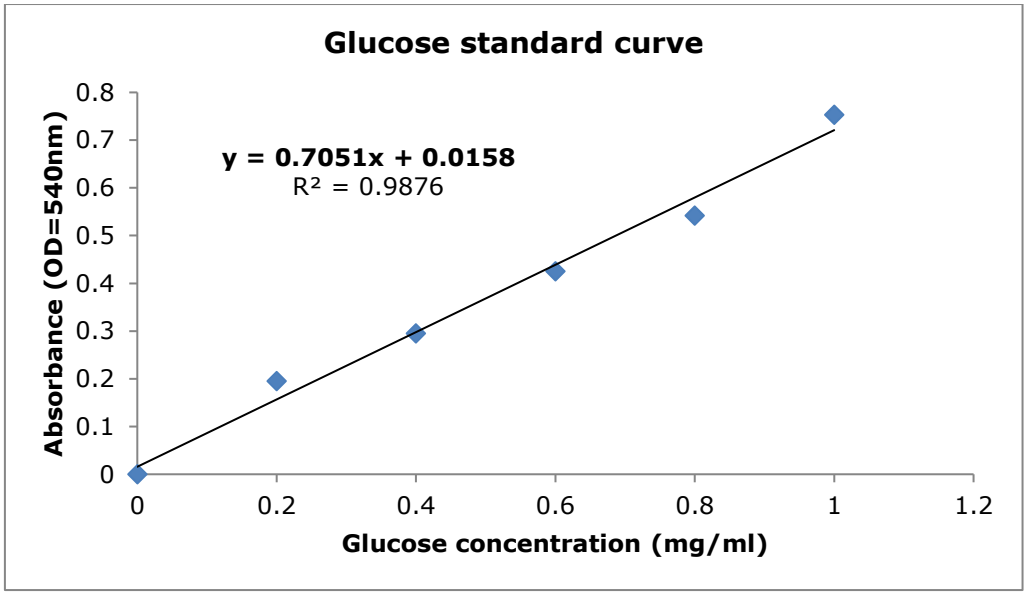

Figure 4.1: *The glucose standard curve for starch and reducing sugar determination*

4.3. *In vitro* digestion model

All samples (white rice, bitter gourd, turmeric, ginger and fenugreek) were digested either alone (single meal) or in combination with white rice (mixed meal). The glycaemic response was given slowly or rapidly depending on reducing sugar content. And it was determined by using a maltose standard curve. Aliquots (1ml) of the digestion were withdrawn at 30 minutes intervals according to the DNS method (section 3.6).

The glycaemic response was dependent on the rate of digestion and the reducing sugar content of the sample. .A maltose standard curve was used to determine the rate of starch breakdown since maltose is one of the early breakdown products of starch – it is likely that this disaccharide is present in the digestion before it is fully converted to glucose.

The maltose concentration (mg/ml) was calculated under the standard curve of maltose ($y=0.9997x-0.0192$, $R^2=0.998$) (Figure 4.2). All data was presented in mean±standard deviation (mean±SD) of three replicates.

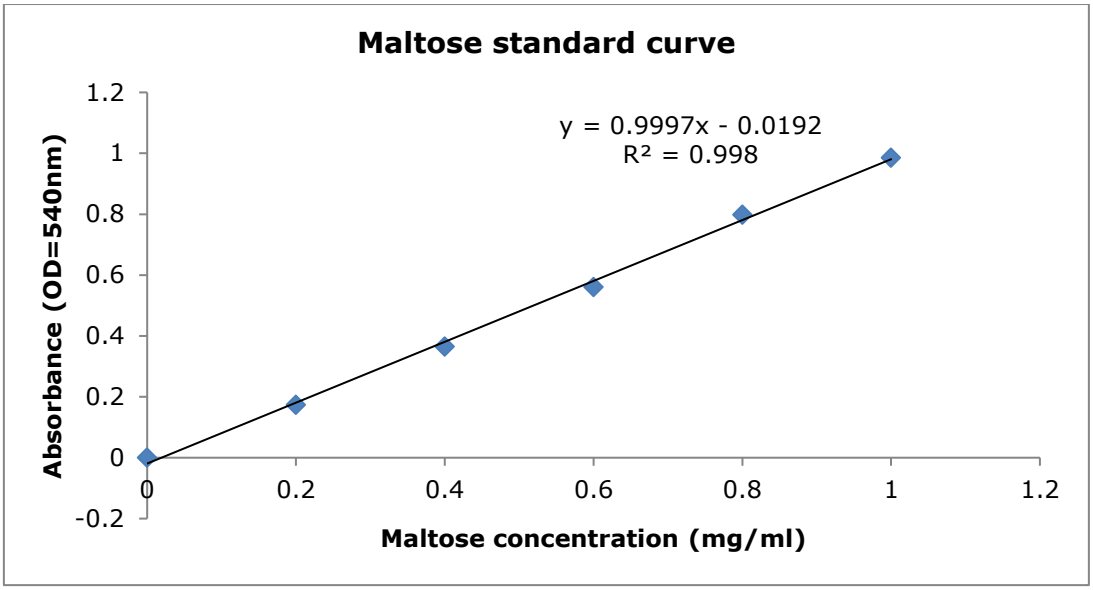

***Figure 4.2**: The maltose standard curve for the enzymatic digestion*

4.3.1. The enzymatic digestion of selected species in single meal

4.3.1.1. White rice

Table 4.3 illustrates the glucose concentration (mg/ml) of boiled white rice during 3 hours of the enzymatic digestion. The maltose concentration increased rapidly after the first 30 minutes (0.647 mg/ml). After 1 hour of digestion, it concentration decreased sharply to 0.147mg/ml. This suggests that the maltose is then further hydrolysed to glucose. The maltose concentration continued to decline throughout the test period of 3 hours, indicating that the majority of digestion had taken place quickly, within the first hour. The salivary α-amylase enzyme speeds up the breakdown of starch molecules lead to increase concentration of maltose in hydrolysis process.

Table 4.3: *Glucose concentration (mg/ml) of white rice in single meal over 3 hours (mean± SD)*

Time (min)	Glucose concentration (mg/ml) White rice
0	0.019±0.000
30	0.647±0.006
60	0.147±0.005
90	0.088±0.004
120	0.073±0.009
150	0.045±0.004
180	0.040±0.004

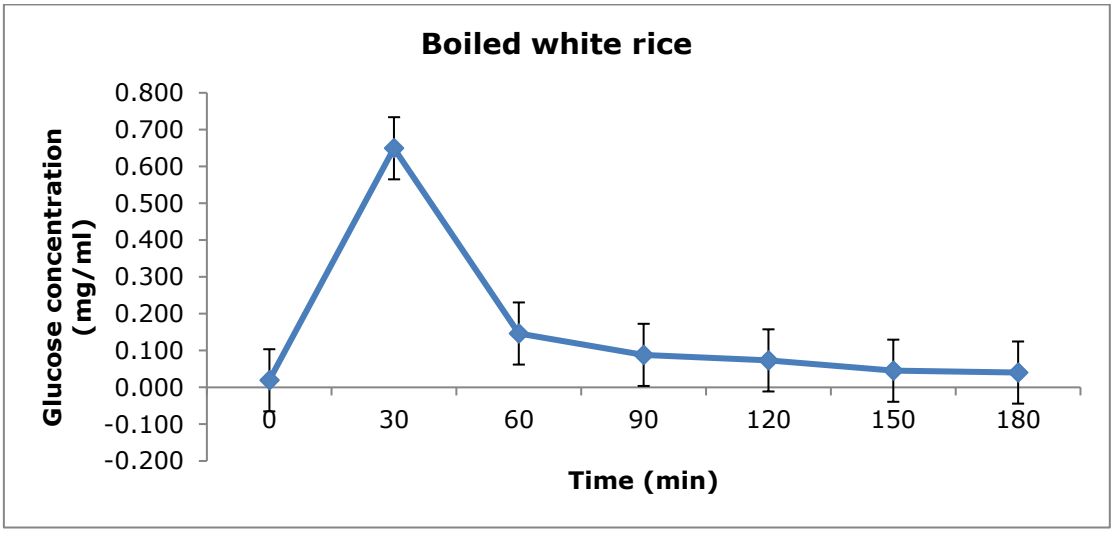

Figure 4.3*: The digestion of white rice in single meal over 3 hours*

4.3.1.2. Bitter gourd

Table 4.4 represents the maltose concentration (mg/ml) of three treatments (raw, boiled and fried) of bitter gourd and figure 4.4 depicts the digestion of each treatment in a single meal over a 3 hour period. All three samples of bitter gourd showed a similar trend in the way they were digested. The maltose concentration continued to increase over a prolonged period, peaked and then declined over 3 hours.

With regards to three lines of bitter gourd, boiled form had the fastest maltose response at 60 minutes to reach a peak of maltose concentration, 0.187mg/ml. It was followed by fried form (0.163mg/ml, 90 minutes) and raw form (0.167mg/ml, 120 minutes). After reaching that peak, the maltose concentration gradually going decreased until 180 minutes for boiled, fried and raw form of bitter gourd (0.136mg/ml, 0.123mg/ml and 0.144mg/ml) respectively.

The two-way ANOVA analysis shows the significant value ($p<.001$) of maltose concentration (mg/ml) between three different treatments of bitter gourd (Appendix 6, Table 4.5). From the significant difference between three treatments ($F(2,62)=112.02$, $p<.001$), it was concluded at least one of the treatment means was different from the other. By using Fisher's LSD test, it is clearly seen there was a significant difference between each treatment. And the boiled bitter gourd had the highest mean of maltose concentration (0.115mg/ml), followed by raw bitter gourd (0.101mg/ml) and fried bitter gourd (0.098mg/ml) (Appendix 6, Table 4.6).

Table 4.4: *Glucose concentration (mg/ml) of bitter gourd in single meal over 3 hours (mean ±SD)*

Time (min)	Glucose concentration (mg/ml)		
	Raw bitter gourd	**Boiled bitter gourd**	**Fried bitter gourd**
0	0.019±0.000	0.019±0.000	0.019±0.000
30	0.087±0.003	0.127±0.003	0.097±0.003
60	0.126±0.003	0.187±0.003	0.144±0.003
90	0.143±0.005	0.171±0.006	0.163±0.005
120	0.167±0.005	0.155±0.005	0.143±0.003
150	0.156±0.006	0.143±0.004	0.130±0.004
180	0.144±0.005	0.136±0.003	0.123±0.007

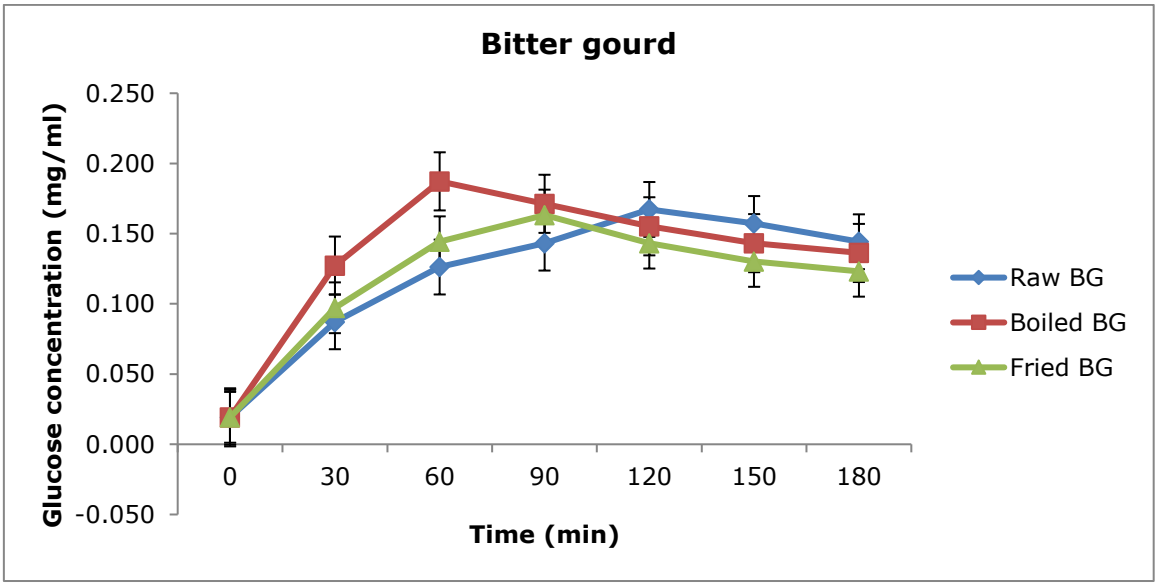

Figure 4.4: *The digestion of bitter gourd in single meal over 3 hours*

4.3.1.3. Ginger

As shown in table 4.7, the highest maltose concentration for each type of ginger was 0.170, 0.442, 0.307 mg/ml for raw, boiled, fried form, respectively. Looking at the release of glucose during digestion, the raw ginger had the slowest rate of digestion (peak glucose concentration at 180 minutes). It was followed by the fried form of ginger, which peaked at 90 minutes and boiled ginger at 60 minutes. The trend of 3 different treatments of ginger was plotted in the graph from zero time to 180 minutes (Figure 4.5). It can be seen that all three treatments were gradually increased to reach the peak after starting the period at 0minutes. Both boiled and fried ginger rose to a peak at around 90 minutes after digestion started (0.442mg/ml, 0.307mg/ml) and then decreased until 180 minutes (0.248mg/ml, 0.193mg/ml), respectively. Raw ginger showed a completely different pattern – the glucose concentration continued to increase slightly throughout the 180 minutes of testing. However, it never reached the same high values as boiled or fried ginger.

To compare the different means, the two-way of analysis of variance was carried out (Appendix 7, Table 4.8). The glucose concentration of 3 treatments was positively affected by the different time period ($F_{(2,62)}$=149.87, p<.001). It was followed by Fisher's LSD test (Appendix 7, Table 4.9); the glucose concentration mean level of raw ginger was an optimum at 0.1272 to get the best effect on T2DM management.

Table 4.7: *Glucose concentration (mg/ml) of ginger in single meal over 3 hours (mean ± SD).*

Time (min)	Glucose concentration (mg/ml)		
	Raw ginger	**Boiled ginger**	**Fried ginger**
0	0.019±0.000	0.019±0.000	0.019±0.000
30	0.125±0.006	0.277±0.024	0.211±0.003
60	0.126±0.006	0.343±0.010	0.309±0.062
90	0.130±0.011	0.442±0.047	0.307±0.034
120	0.142±0.001	0.363±0.042	0.232±0.063
150	0.165±0.040	0.264±0.035	0.202±0.006
180	0.170±0.005	0.248±0.004	0.193±0.005

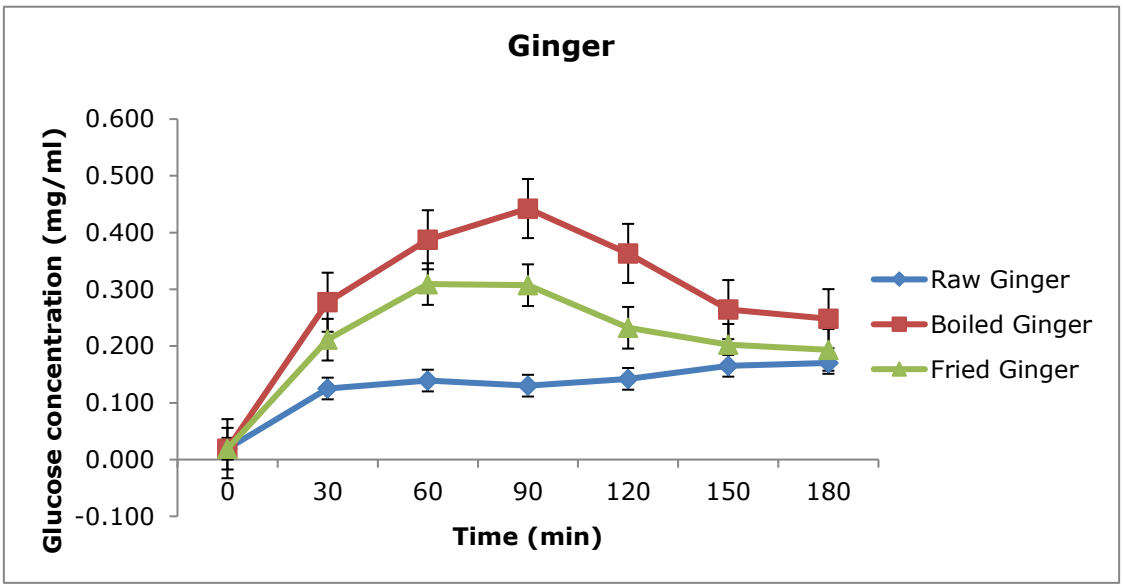

Figure 4.5: *The digestion of ginger in single meal over 3 hours*

4.3.1.4. Turmeric

In term of starch digestion and maltose production, turmeric was examined in only two forms of treatments; raw and boiled. As can be seen from Table 4.10 and Figure 4.6, there was higher release of maltose from boiled turmeric than from the raw form. Glucose response of boiled turmeric was increased from 0.019mg/ml at zero time to 0.360mg/ml at 120 minutes. While raw turmeric had a later response from 0.019 mg/ml at 0 minute to 0.121mg/ml after 180 minutes. The line graph is shown in figure 4.6, which presents the glucose concentration of two treatments against time over a 3 hours testing periods.

The maltose response of the two type of treatments was significantly different ($F_{(2,62)}$=112.78, p<.001); therefore, they are different effect on blood glucose response (Appendix 8, Table 4.11). It was followed by Fisher's LSD test (Appendix 8, table 4.12); the mean level of raw turmeric and boiled turmeric was expressed in different category. From that, the optimal glucose concentration was presented in raw turmeric at 0.0843 to get a slower effect on glucose response.

Table 4.10: *Glucose concentration (mg/ml) of turmeric in single meal over 3 hours (mean ± SD)*

Time (min)	Glucose concentration (mg/ml)	
	Raw turmeric	**Boiled turmeric**
0	0.019±0.000	0.019±0.000
30	0.044±0.004	0.199±0.056
60	0.050±0.025	0.288±0.013
90	0.054±0.010	0.314±0.006
120	0.052±0.222	0.360±0.046
150	0.117±0.006	0.339±0.008
180	0.121±0.005	0.233±0.006

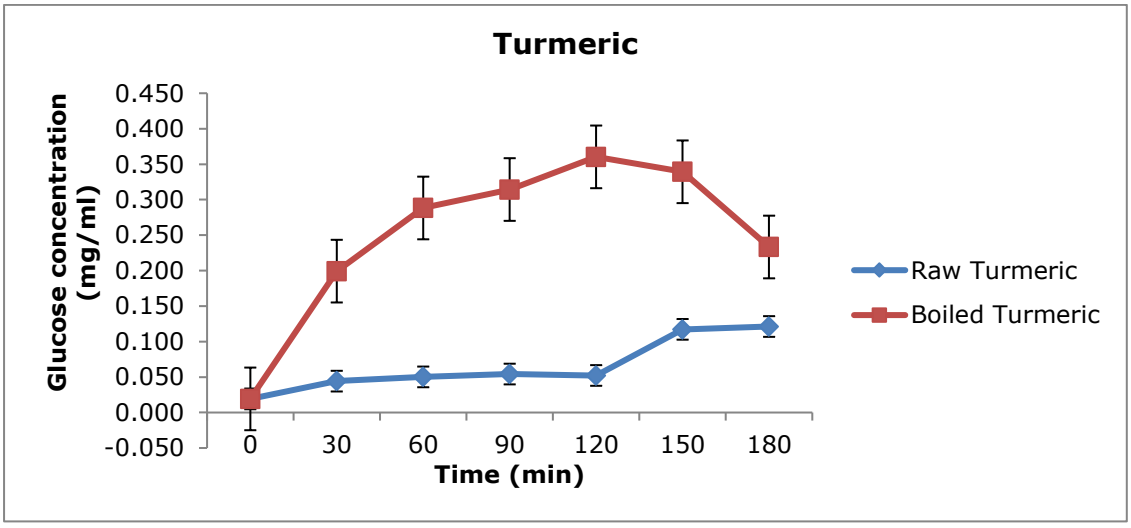

Figure 4.6: *The digestion of turmeric in single meal over 3 hours*

4.3.1.5. Fenugreek

The maltose concentration of two treatments of fenugreek seed (boiled and fried) is shown in Table 4.13. Raw fenugreek was not measured since it is not usually consumed in the raw state. Both types of fenugreek, boiled and fried, showed a similar trend in the pattern of glucose release following digestion. Both samples had a gradual increase of glucose concentration throughout the 3 hour testing period with a levelling off during the final thirty minutes for the boiled sample. In particular, the boiled form of fenugreek had a peak at 150 minutes; 0.128mg/ml. While fried form of fenugreek was inclined continuously from 0.019 mg/ml (0 minutes) to 0.126 mg/ml (180 minutes). And the line graph (Figure 4.7) also illustrates clearly of the glucose concentration (mg/ml) in different period of time (min). As is observed, boiled fenugreek was decreasing whereas fried form was going up at the last 30 minutes.

The ANOVA result shows that the glucose concentration (mg/ml) was different significant ($F_{(2,62)}$=748.74, p<.001) between two different treatments in table 4.14 (Appendix 9). According to the Fisher's LSD test, the mean of glucose concentration of fried fenugreek was 0.07343, compared to 0.08324 of boiled fenugreek. It concluded fried fenugreek was a good suggestion for the effect of glucose response on T2DM patients.

Table 4.13: *Glucose concentration (mg/ml) of fenugreek in single meal over 3 hours(mean ± SD)*

Time (min)	Glucose concentration (mg/ml)	
	Boiled fenugreek	**Fried fenugreek**
0	0.019±0.000	0.019±0.000
30	0.062±0.016	0.062±0.016
60	0.066±0.012	0.059±0.009
90	0.079±0.007	0.064±0.010
120	0.079±0.009	0.075±0.005
150	0.128±0.003	0.122±0.005
180	0.122±0.005	0.126±0.004

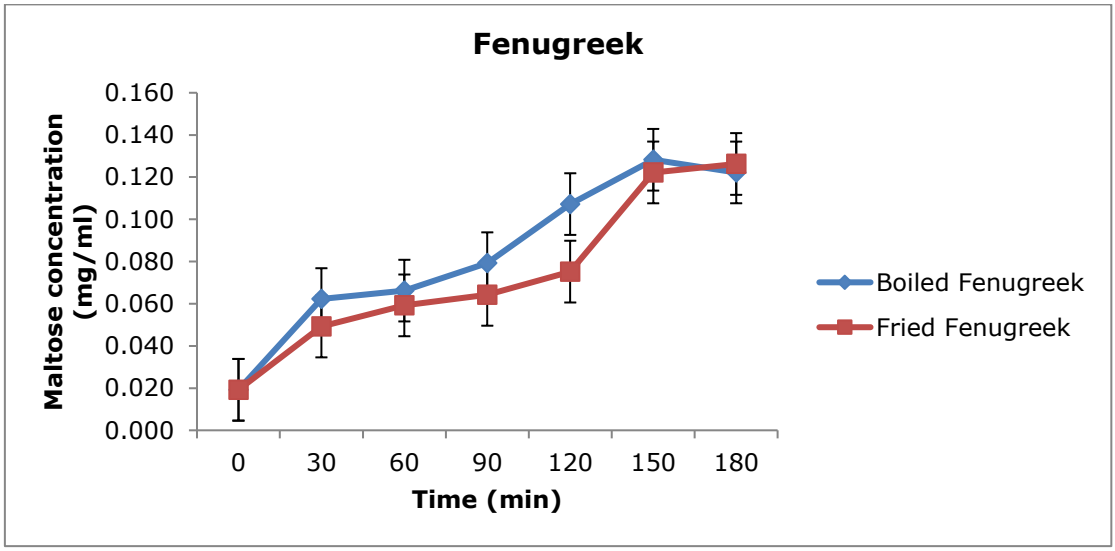

Figure 4.7: *The digestion of fenugreek in single meal over 3 hours*

4.3.2. The enzymatic digestion of species in mixed meal

In order to determine whether the four samples under investigation (bitter gourd, ginger, turmeric and fenugreek) could moderate the glucose release response from white rice, the in vitro digestion of mixed meals was carried out. The results of glucose production were compared with those for white rice alone.

4.3.2.1. Bitter gourd

In the mixed meal of bitter gourd with white rice, all three types of bitter gourd (boiled, fried and raw) showed a similar pattern of digestion; a gradual increase in glucose production with time, a peak in glucose concentration and then a decline over the 180 minutes testing period. The main difference between the three samples was the speed at which the glucose concentration reached its maximum value (Table 4.16). In particular, the raw bitter gourd combined with white rice had the latest response at 120 minutes with 0.203mg/ml of glucose concentration. Following by fried bitter gourd with white rice was reached a peak of digestion at 90 minutes (0.237mg/ml) and boiled bitter gourd with white rice was at 60 minutes (0.262mg/ml). From that, it is clearly seen in Figure 4.8 that all three samples of bitter gourd, when combined with rice, had an effect on the digestion of white rice – they all delayed the peak of glucose production significantly from the 30 minutes seen with pure white rice alone (Figure 4.8).

Moreover, the comparison between mean of treatments in different time was carried out by 2-way ANOVA analysis. Data on the table 4.17 (Appendix 10) indicates that there was a significant difference between treatments at $F_{(2,62)}=37.04$, $p<.001$. And therelationship of treatment and time also had a highly significant ($F_{(12,62)}=323.05$, $p<.001$). Therefore, when bitter gourd combined with white rice had a lower effect on glucose released compared to white rice only in single meal.

Based on the result of Fisher's LSD test (Appendix 10, table 4.18), it depicts that the smallest mean level of raw bitter gourd with white rice was 0.1079 as a good diet in the blood glucose management.

***Table 4.16**: Glucose concentration (mg/ml) of bitter gourd in mixed meal over 3 hours (mean ± SD)*

Time (min)	Glucose concentration (mg/ml)		
	Raw bitter gourd_white rice	**Boiled bitter gourd_white rice**	**Fried bitter gourd_white rice**
0	0.019±0.000	0.019±0.000	0.019±0.000
30	0.077±0.003	0.156±0.004	0.113±0.003
60	0.105±0.005	0.262±0.006	0.182±0.001
90	0.141±0.005	0.125±0.005	0.237±0.006
120	0.203±0.002	0.093±0.005	0.128±0.003
150	0.121±0.003	0.072±0.006	0.090±0.010
180	0.090±0.007	0.061±0.007	0.075±0.005

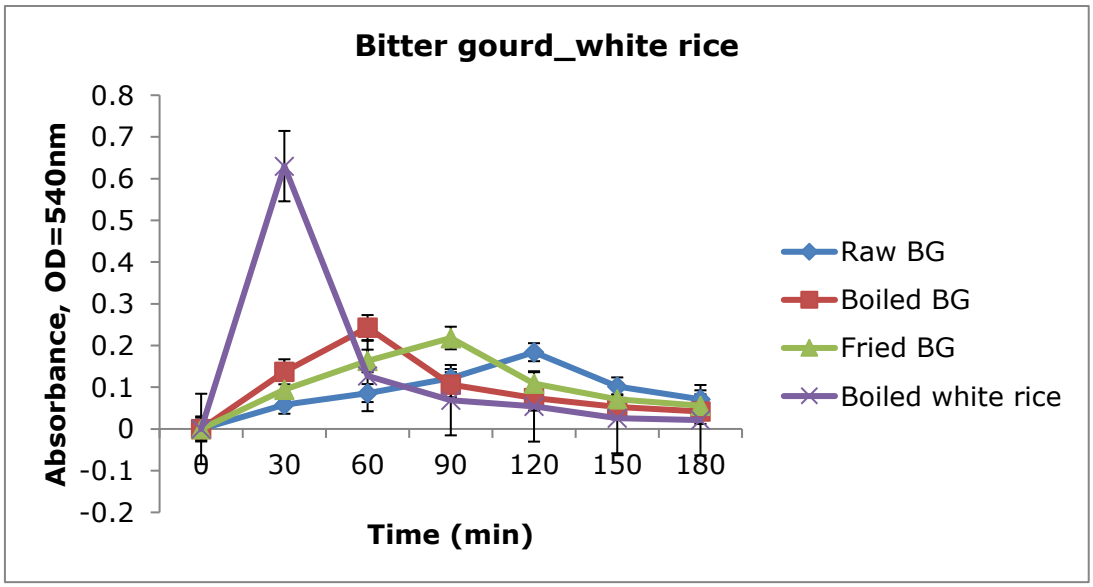

***Figure 4.8:** The digestion of bitter gourd with white rice in vitro analysis over 3 hours*

4.3.2.2. Ginger

The maltose concentration over time of three treatments of ginger (raw, boiled, fried) combined with white rice are presented in Table 4.19. Boiled ginger with white rice presented a rapidly digestion to get a peak at 60minutes with 0.371mg/ml. However, fried ginger with white rice was gradually reached a peak of digestion at 90 minutes (0.295mg/ml) of glucose concentration and 120 minutes (0.252mg/ml) of glucose concentration for raw ginger with white rice. After getting a maximum digestion, the glucose concentration of raw, boiled and fried ginger with white rice was decreased dramatically to 0.102mg/ml, 0.145mg/ml and 0.115mg/ml at 180 minutes, respectively (Figure 4.9).

Besides, the result of 2-way ANOVA analysis shows a significant different at $F_{(2,62)}=338.53$, $p<.001$ in the comparison mean between 3 treatment of ginger combined with white rice (Appendix 11, Table 4.20). The glucose concentration of ginger with white rice has a difference compared to white rice in single meal.

According to the Fisher's LSD test, the mean value of each treatment was compared. As can be seen, raw ginger with white rice not only had a slowly glucose response but also the smallest mean of glucose concentration, 0.1291. It was followed by 0.1548 for fried ginger with white rice and 0.1792 for boiled ginger with white rice (Appendix 11, Table 4.21).

Table 4.19: *Glucose concentration (mg/ml) of ginger with white rice in mixed meal over 3 hours (mean ± SD)*

Time (min)	Glucose concentration (mg/ml)		
	Raw ginger_white rice	**Boiled ginger_white rice**	**Fried ginger_white rice**
0	0.019±0.000	0.019±0.000	0.019±0.000
30	0.091±0.005	0.153±0.002	0.116±0.006
60	0.137±0.005	0.371±0.007	0.192±0.010
90	0.180±0.049	0.236±0.005	0.295±0.005
120	0.252±0.009	0.172±0.003	0.202±0.007
150	0.122±0.010	0.157±0.003	0.144±0.003
180	0.102±0.004	0.145±0.005	0.115±0.012

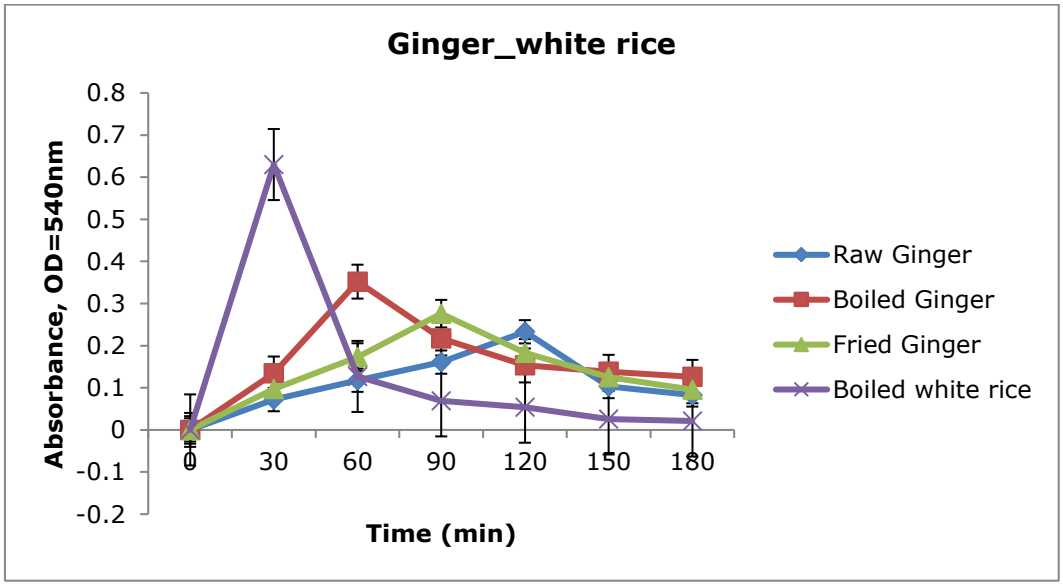

Figure 4.9: *The digestion of ginger with white rice in vitro analysis over 3 hours*

4.3.2.3. Turmeric

In the mixed meal, the glucose concentration of two treatments of turmeric (raw and boiled) combined with white rice was determined in Table 4.22. The highest glucose concentration as well as the fastest response was with boiled turmeric with white rice at 90 minutes (0.374mg/ml). Raw turmeric with white rice had a peak 30minutes later at 120 minutes after the start of digestion with 0.150mg/ml of glucose concentration. As is observed in Figure 4.10, the glucose production form boiled turmeric with white rice was much higher than raw turmeric with white rice. Both samples declined after the peak glucose concentration.

It is clearly seen that there was a statistically significant ($F_{(2,62)}$=2534.95, p<.001) of two treatments of turmeric with white rice from the ANOVA test (Appendix 12, table 4.23). It means the 2 treatments of turmeric with white rice had a totally difference on glucose response during 3 hours of digestion. Furthermore, the Fisher's LSD test indicates the mean level of two treatments was different with letters (Appendix 12, table 4.24). The mean of glucose concentration presents as 0.08510 for raw turmeric with white rice and 0.17429 for boiled turmeric with white rice. From that, the mean of raw turmeric with white rice was lower than boiled form of turmeric with white rice.

Table 4.22: *Glucose concentration (mg/ml) of turmeric with white rice in mixed meal over 3 hours (mean ± SD)*

Time (min)	Glucose concentration (mg/ml)	
	Raw turmeric_white rice	**Boiled turmeric_white rice**
0	0.019±0.000	0.019±0.000
30	0.064±0.007	0.161±0.005
60	0.098±0.007	0.245±0.003
90	0.110±0.022	0.374±0.010
120	0.150±0.004	0.201±0.007
150	0.072±0.003	0.122±0.016
180	0.067±0.005	0.097±0.005

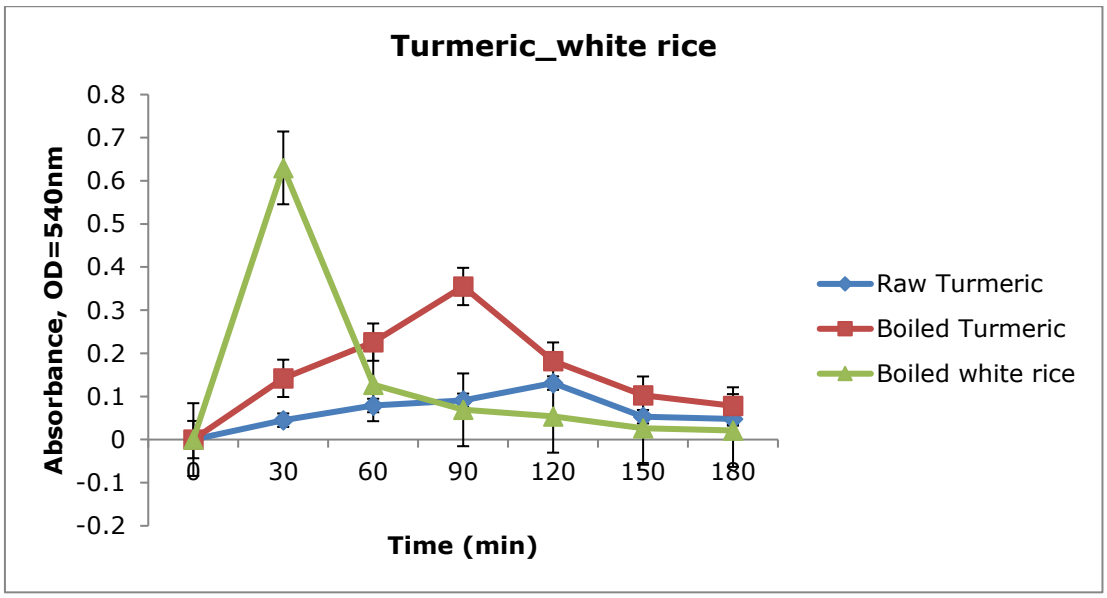

Figure 4.10: *The digestion of fenugreek with white rice in vitro analysis over 3 hours*

4.3.2.4. Fenugreek

The combination of two treatments of fenugreek (boiled and fried) with white rice is presented in table 4.25. The trend for both types was very similar, showing an increase in glucose concentration at 120 minutes after the start of digestion. Boiled fenugreek had a higher glucose response at (0.146mg/ml) than fried fenugreek (0.130mg/ml). The line graph was gradually inclined from zero time to 120 minutes for both treatments of fenugreek with white rice (Figure 4.11). After that, these lines went downward in the next 30minutes. The line of boiled fenugreek with white rice was continuously going down; however, the fried fenugreek with white rice had a stable trend at the last 30 minutes.

ANOVA analysis indicates a significant difference between the two treatments of fenugreek with white rice ($F_{(2,62)}$=819.80, p<.001) (Appendix 13, Table 4.26); therefore, they are different effect on glucose response. Moreover, the effect between treatment and time was shown statistically significant ($F_{(12,62)}$=49.81, p<.001). It was followed by the Fisher's LSD test; the mean level of fried fenugreek with white rice was 0.07319 as a good effect on glucose response (Appendix 13, Table 4.27).

Table 4.25: *Glucose concentration (mg/ml) of fenugreek with white rice in mixed meal over 3 hours (mean ± SD)*

Time (min)	Glucose concentration (mg/ml)	
	Boiled fenugreek_white rice	**Fried fenugreek_white rice**
0	0.019±0.000	0.019±0.000
30	0.070±0.005	0.055±0.005
60	0.091±0.007	0.077±0.006
90	0.122±0.002	0.092±0.008
120	0.146±0.007	0.130±0.05
150	0.081±0.008	0.072±0.008
180	0.056±0.015	0.067±0.006

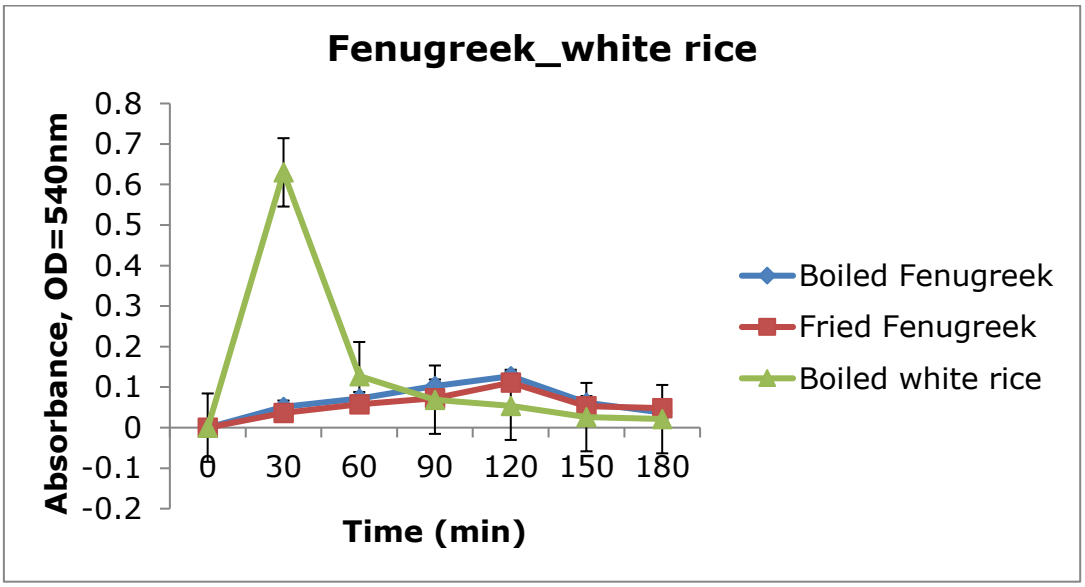

Figure 4.11: *The digestion of fenugreek with white rice in vitro analysis over 3 hours*

4.4. *In vivo* model

4.4.1. Participants characteristics

Eight healthy participants aged between 21-33 participated in this study. Participants consisted of three males and five females. Mean overall fasting glucose was 4.8±0.40mmol/l. All data expressed as mean±standard deviation (mean±SD).

4.4.2. Bitter gourd

Table 4.28 presents the data for a mixed meal of bitter gourd plus white rice, compared with consumption of white rice alone. In general, the blood glucose of white rice consumption was higher than a mixed meal of bitter gourd with white rice until 90 minutes after consumption, when the two values were similar.

Consumption of white rice alone produced a rapid increase in blood glucose concentration (to 6.9mmol/l) within 30 minutes of consuming the meal. Blood glucose remained elevated at this level for up to 60 minutes and then started to decline gradually over the 3 hours of digestion to a final value of 4.9mmol/l. The combination of bitter gourd with white rice resulted in a slower increase in blood glucose levels, reached a peak 6.3mmol/l at 90min, after which it showed a gradual decline to 3.6mmol/l at 180min (Figure 4.12).

In brief, the consumption of bitter gourd together with white rice produced a slower release of glucose, compared to consumption of white rice alone. Therefore, it concludes adding bitter gourd to a meal could be beneficial in moderating the glucose response.

Table 4.28: *Blood glucose concentration (mmol/l) of bitter gourd in a mixed meal in vivo measurement*

Time (min)	Blood glucose (mmol/l)	
	Boiled white rice	**Boiled bitter gourd _white rice**
0	5.2±0.67	4.4±0.78
30	6.9±0.79	4.8±0.14
60	6.8±0.45	5.5±0.71
90	6.2±1.06	6.3±1.27
120	5.6±0.68	6.0±0.14
150	4.9±0.45	4.8±0.21
180	4.9±0.77	3.6±0.35

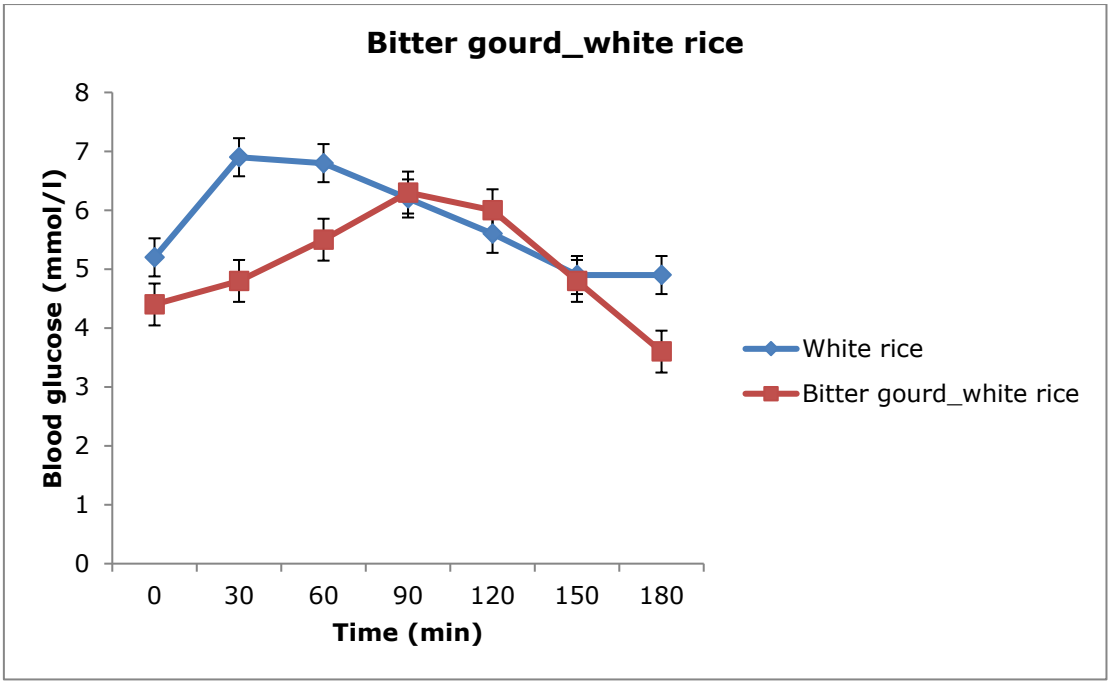

Figure 4.12: *The digestion of bitter gourd in mixed meal over 3hours in vivo analysis*

4.4.3. Ginger

Figure 4.13 compares the effects of ginger consumed with white rice on the blood glucose response with the consumption of white rice alone over a 3 hour time. It is clearly seen that the combination of boiled ginger with white rice was produced a lower glucose response than white rice alone.

Boiled ginger with white rice showed a slight increase in blood glucose levels after 30 minutes (much lower than rice alone) that was sustained throughout the 3 hour test period. It started at zero time with 4.8mmol/l; reached a peak of 5.1mmol/l at 60min and slowly decreased to 4.2mmol/l at 180min. This would infer that the consumption of boiled ginger together with white rice can moderate the glucose response

Table 4.29: *Blood glucose concentration (mmol/l) of ginger in mixed meal in vivo measurement*

Time (min)	Blood glucose (mmol/l)	
	Boiled white rice	**Boiled ginger_white rice**
0	5.2±0.67	4.8±0.28
30	6.9±0.79	5.0±0.35
60	6.8±0.45	5.1±0.92
90	6.2±1.06	4.8±0.07
120	5.6±0.68	4.7±0.49
150	4.9±0.45	4.6±0.99
180	4.9±0.77	4.2±0.49

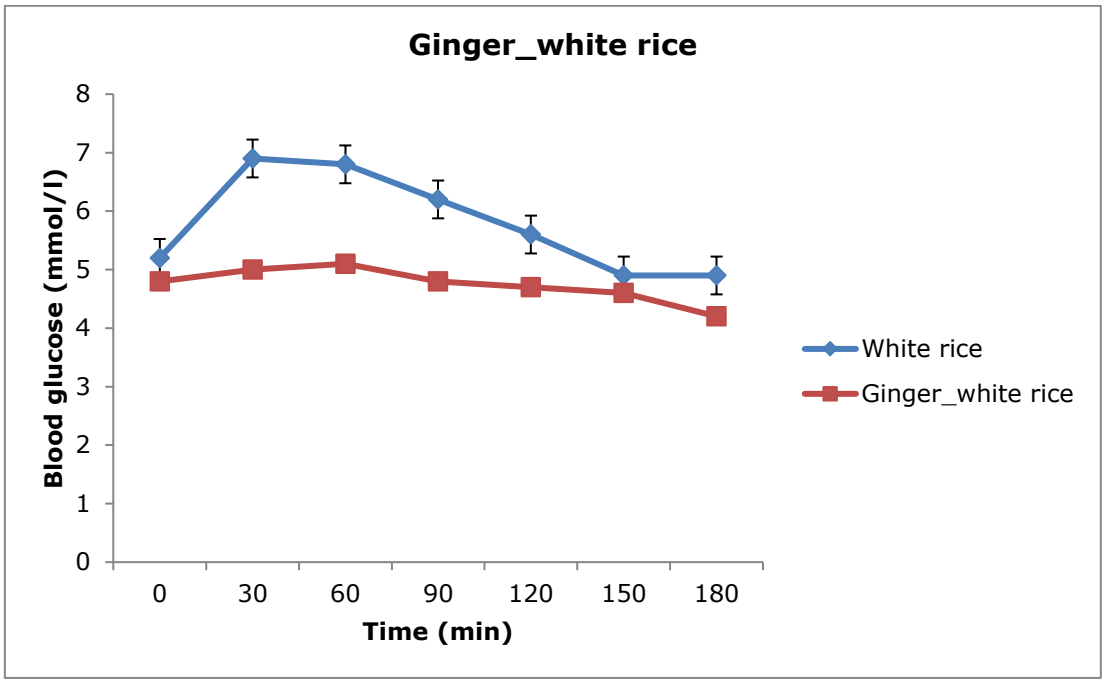

Figure 4.13: *The digestion of ginger in mixed meal during 3hours in vivo analysis*

4.4.4. Turmeric

Figure 4.14 compares the blood glucose response of consuming boiled turmeric and white rice with white rice alone during 3 hours of digestion. In general, the blood glucose levels following consumption of turmeric fluctuated over the 3 hours and showed a very similar trend to the white rice profile. Blood glucose concentration following the mixed meal reached a peak at 90 minutes (6.7mmol/l) and then decreased to 4.2mmol/l at 180 minutes (Table 4.30).

Table 4.30: *Blood glucose concentration (mmol/l) of turmeric in mixed meal in vivo measurement*

Time (min)	Blood glucose (mmol/l)	
	Boiled white rice	Boiled turmeric_white rice
0	5.2±0.67	4.7±0.71
30	6.9±0.79	6.2±1.41
60	6.8±0.45	6.4±0.64
90	6.2±1.06	6.7±0.49
120	5.6±0.68	5.2±0.49
150	4.9±0.45	4.9±0.42
180	4.9±0.77	4.2±0.14

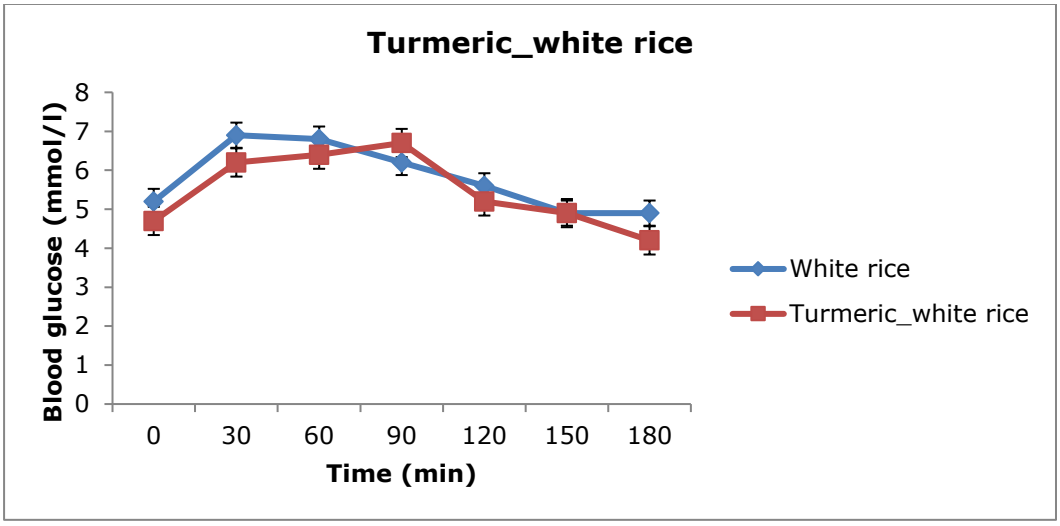

Figure 4.14: *The digestion of turmeric in mixed meal over 3hours in vivo analysis*

4.4.5. Fenugreek

The comparison of glucose response of consuming fenugreek in a mixed meal with white rice and white rice in a single meal is shown in Figure 4.14. The glucose response of boiled fenugreek with white rice showed a gradual increase over 90 minutes, reaching a peak of 6.8mmol/l, followed by a gradual decline to 3.9 mmol/l.

This suggests that boiled fenugreek consumed together with white rice may be beneficial in moderating the glucose response by delaying the breakdown of starch from white rice.

Table 4.31: *Blood glucose (mmol/l) of fenugreek in a mixed meal in vivo measurement*

Time (min)	Blood glucose (mmol/l)	
	Boiled white rice	**Boiled fenugreek_white rice**
0	5.2±0.67	4.5±1.13
30	6.9±0.79	5.8±0.57
60	6.8±0.45	6.6±0.42
90	6.2±1.06	6.8±0.07
120	5.6±0.68	4.6±0.99
150	4.9±0.45	4.4±0.64
180	4.9±0.77	3.9±0.42

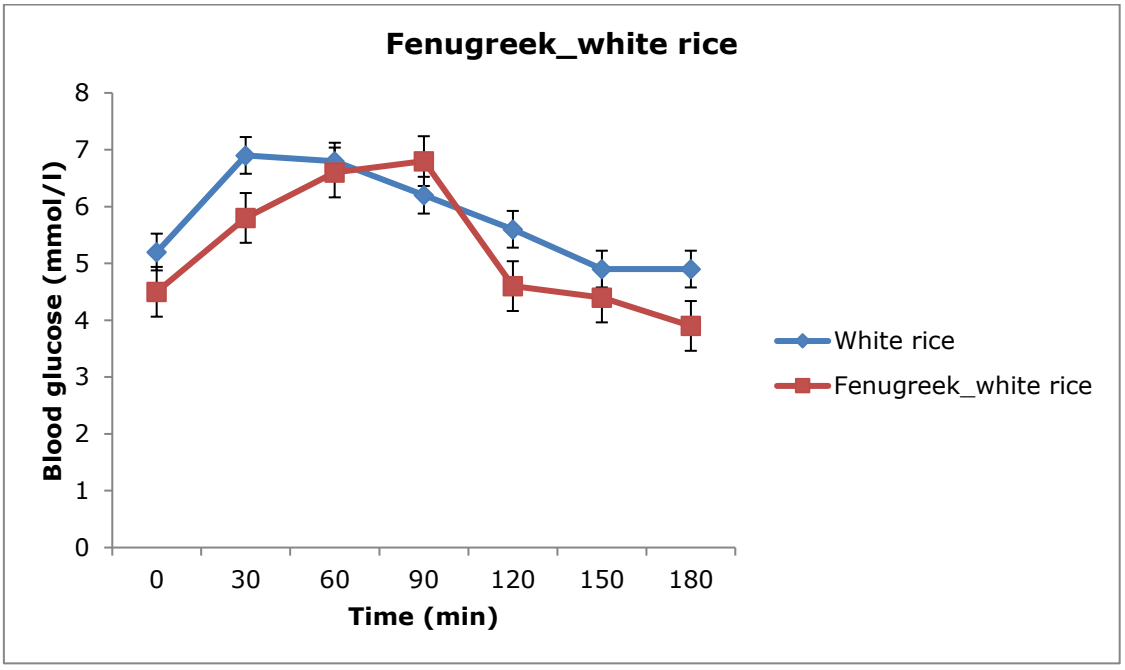

Figure 4.15: *The digestion of fenugreek in a mixed meal over 3hours in vivo analysis*

CHAPTER 5: DISCUSSION

Carbohydrate metabolism is a key factor in the type 2 diabetes mellitus (T2DM).Carbohydrate digestion begins with the production of salivary amylase, which starts the breakdown of starch in the mouth (the oral phase of digestion). It continues in the small intestine via the action of pancreatic amylase, to break down complex carbohydrates into maltose and then glucose. As blood glucose levels rise, the pancreas produces insulin that prompts cells to absorb blood sugar, thus reducing the blood sugar levels back to normal. If the pancreas is not functioning (not producing insulin) or the insulin receptors are not responding to increased levels of circulating insulin, the glucose homeostasis will be affected, with the result that blood glucose levels remain elevated. The blood glucose response can be moderated by controlling the diet, in particular by consuming foods with a low glycaemic index (GI) value. These foods are digested more slowly in the body and result in a slower release of glucose into the blood stream. Simple sugars, such as glucose and sucrose are digested rapidly and lead to a sudden increase in blood sugar, which is not desirable or advisable, particularly in diabetics. For the management of T2DM, and for promotion of general health, it is advisable to consume foods with a low GI value. This study aimed to evaluate the GI value of a number of underutilised vegetables that are reputed to be useful in controlling T2DM.

The gold standard for assessing the effects of different foods on blood glucose levels is *in vivo* studies that involve the direct measurement of blood glucose following the consumption of test foods. However, it is not practical to use this method for all foods of interest. An *in vitro* system which simulates human digestion can be used to predict the rate of digestion and the blood glucose response of the test foods.

The *in vitro* system used in this study, based on the method of Goni *et al.* (1997), is a robust system that could be used to predict the GI values of other foods of interest.

Among published literature, there was over 90% studies had used either glucose or white bread as the reference food (Foster-Powell *et al.,* 2002) for both *in vitro* and *in vivo* study. White rice is consumed as a staple food in South East Asia countries; and the test foods we are interested in are consumed as part of a rice based meal, we chose to use rice as the reference food in this study.

White rice is easily and quickly digested and absorbed in the small intestine. It is a high GI food that should be consumed in moderation by people with T2DM. Various studies have shown that the GI of white rice can be altered (lowered) by simple techniques such as cooking method. It also varies according to particle (grain) size and variety. By using different cooking method or rice variety (steamed, boiled, fried), GI value of white rice (GI=38-94, glucose=100) was investigated from diabetics to non-diabetics subjects (Foster-Powell *et al.,* 1995). Amylose content in starch also makes an effect on GI value. For example, instant rice and low-amylose rice were presented a higher GI value (GI=88-91) than brown rice (GI=55), long grain white rice (GI=56) and parboiled rice (GI=47) (Behall *et al.,* 1988).

The GI value of white rice can be decreased by adding it into a mixed meal. In particular, if rice is consumed with foods that are high in fat or protein, this can slow down the digestion and absorption of sugars. Adding dairy products such as milk or cheese to rice can reduce the GI value. The GI of rice can be decreased because of fat and protein content in dairy product. It will effect on lengthening of the absorption process lead to lowering GI of rice (Collier *et al.,* 1983).

The GI value of foods is used in a clinical setting to prescribe therapeutic diets and by health practitioners to promote low GI diets as a factor to reduce disease risk.

In this study, a small pilot study was carried out to examine the glycaemic response of a test meal on 8 healthy participants. Because the number of volunteers was low and there are only two replicates of each test, it is impossible to draw conclusions from the *in vivo* study. Rather it serves as an indicator for whether a large scale trial would be beneficial. Initial results look promising, with some impacts of the test foods on moderating (slowing down) the glucose response of consuming boiled white rice. Time was a limiting factor in this study. If more time had been available, the *in vivo* study could have been carried out with more volunteers, and on the whole range of test foods (raw, boiled and fried samples) to determine the effects of cooking on the GI response.

Using the *in vitro* model of predictive response, there was a noticeable and significant difference in the glucose response of different foods and of different cooking methods. The boiled form of all four test foods showed a significant

difference to the other treatments (raw and fried). Boiled vegetables produced a faster glucose response than the fried or raw forms. This is most likely due to the gelatinisation of starch that takes place during boiling, making it easier to digest. Moreover, it also might be affected by the molecular structure of starch. The ratio of amylose and amylopectin (amylose:amylopectin ratio) is one of the main factors affecting starch digestibility and physiological response (Lehmann *et al.,* 2007). Amylose is a more complex molecule that slows digestion and insulin response time, providing a lower glycaemic index, which opposed to amylopectin. The amylose molecule provides a high fibre source with a low glycaemic index (Behall *etal.,* 1989). And T2DM may benefits from the diet high in amylose due to the slowerinsulin response and prevent quick spikes in glucose levels (Behall *et al.,* 1989).

In addition, the measurement of glycaemic response in mixed meal indicated that the glycaemic impact of boiled foods can affect the glycaemic response elicited by the earlier release of glucose after two hours postprandial. When the glucose profile is considered, the differences in glucose concentration between treatment (raw, boiled and fried) after the consumption of mixed meal (3 hours of digestion) may explain why a lower or higher glycaemic response. As is shown in the result, the glucose concentration of boiled was greater than the concentration of raw and fried form at the same time points.

Frying adds some fat to the food, which can slow down the rate of digestion. Raw foods take longer to digest since the starch is more inaccessible The recommendations from this would be that fried foods (bitter gourd being one example) is better than boiled bitter gourd for management of blood glucose levels.

In a single meal, the glucose concentration of all sample species was released slower than single boiled white rice due to the different in total starch and reducing sugar. A report concluded the consumption 3g of raw ginger powder in 30 days had a significant effect on reducing blood glucose, triglyceride, total cholesterol, was levels in diabetic patients (Andallu *et al.,* 2003). Thus, the raw consuming of test food is consumed alone as a single meal, which depict a positively effect on the glucose management.

Combining the test foods with boiled white rice in a mixed meal had a moderating effect on the glucose response in all cases. It can be inferred from this that the selected under-utilised species can be added to rice based diets to

reduce the GI value. The intervention studies have been conducted over the past decade to examine the benefits of low GI diets. It concludes low GI diet had positive effect in blood glucose control among diabetes patients (Luscombe *et al.,* 1999; Fontvieille *et al*., 1992).

When lower GI foods are consumed with a high GI food, the meal GI is lowered appreciably. For instance, the GI of pasta (GI=58) consumed alone was higher than the GI of rice taken alone (GI=48). However, it was the same range of GI level (GI=38) when these foods were consumed within meal (Bornet *et al.,* 1987). Similarly, it would be delayed of glycaemic response when white rice (high GI) was combined with underutilised vegetables (low GI) as a mixed meal compared to taken white rice alone. Besides, the proportional contribution of available carbohydrate is an important factor to consider when predicting meal glycaemic responses. The FAO/WHO, 1998 states that to obtain an accurate estimate of meal GI all carbohydrate sources should be accounted for. However, it has been suggested that only the main carbohydrate sources need to be used to predict a composite GI (Wolever, 2002). In comparison to this, it included all foods that contributed >0.1g carbohydrate to the diet in the dietary GI prediction (Aston *et al*., 2010).

A mixed meal of four samples could be considered as low GI diet, which may be helpful in a clinical setting where low GI meals are advised. To achieve good control of blood glucose in diabetes, it focuses on consuming low GI foods. For example, using high dietary fibre with a low GI rather than cornflakes products (which have a high GI). The recommendations that advise avoiding the consumption of white rice may be adjusted. This adjustment could be that a reasonable amount of white rice within a typical mixed meal is suitable for those following a low GI diet, so long as the white rice is consumed together with raw or fried vegetables that themselves have a low GI value.

If the scientists provide a support in low GI vegetables (bitter gourd, ginger, turmeric and fenugreek) are healthy foods for diabetes patient with high positive effect; including low GI which can be control blood glucose, rich in nutrient values. When the concept of low GI food gets a response from the public via the marketing, the food manufacturers will provide a new product with health benefits. However, the new GI foods need to fit the demand of consumers. A new low GI product will be more successful brand and marketing is appreciated.

In term of labelling foods, it needs to provide enough provision of information about GI on product.

Diabetes mellitus is known as a clinical syndrome; therefore, the application of underutilised vegetable on the diet is a suggestion for diabetic population. The carbohydrate rich meal (high GI) should be avoided in their daily diet because it raises blood glucose concentration. Hence, the low GI food is the best choice for them to control their blood glucose response. According to the result, the raw form of selected underutilised vegetables in this study had a lower glucose response compare to boiled form and fried form; is recommended to them.

CHAPTER 6: CONCLUSION

6.1. General conclusion

In summary, the investigation of four types of under-utilised vegetables; bitter gourd, ginger, turmeric and fenugreek, has demonstrated that the different species have a various glycaemic response over 3 hours of digestion time. They were determined in three different cooking methods (raw, boiled and fried) based on the cooking of daily diet. The various preparations of four samples in different way were a significant effect on glucose response. It might be caused by varying in nutrient composition, physiochemical properties or variety.

Both *in vitro* and *in vivo* (although with limited data) studies support our hypothesis that the combination of low GI vegetables with boiled white rice can moderate the glucose response of white rice. The method of preparation of vegetables is also important in determining the rate at which the carbohydrate is digested and absorbed. Amongst the four vegetables we studied, raw vegetables are more effective than fried, which are more effective than boiled, which is in line with other research findings. Consumption of the raw form of all samples may minimise the risk of postprandial blood glucose spikes, thereby reducing diabetic and other chronic diseases. Thus, it may prove to be more efficacious in the management of type II diabetes mellitus.

6.2. Recommendations for further study

Based on the initial findings of the *in vivo* study, it would be beneficial to carry out a bigger, well designed *in vivo* study to determine the impacts of different cooking methods on the blood glucose response in volunteers. More interestingly, it would be useful to measure the impacts in diabetic as well as non-diabetic volunteers. This would allow us to make recommendations about food preparation and give dietary advice to control blood glucose levels.

Regarding the *in vitro* work, it would be useful to continue using this method to profile a range of other underutilised fruit and vegetables, to determine their GI values. The rate of digestion and absorption also depends on the type of starch present, in particular the levels of amylose and amylopectin. This study did not look into the physico-chemical properties of the carbohydrate fractions of each of the test vegetables. It would be interesting to carry out a profile analysis of the carbohydrate fraction of each vegetable.

The nutritional analysis is required for all test foods to get a better understanding of the effective mechanism on glucose response. The nutrient composition of selected under-utilised species should be determined to know the amount of nutrients present in addition to the anti-nutritional factors.

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APPENDICES

1. Ethics application
2. Participant information sheet
3. Consent form
4. Participant questionnaire
5. Data collection sheet
6. ANOVA test of bitter gourd in single meal
7. ANOVA test of ginger in single meal
8. ANOVA test of turmeric in single meal
9. ANOVA test of fenugreek in single meal
10. ANOVA test of bitter gourd in mixed meal
11. ANOVA test of ginger in mixed meal
12. ANOVA test of turmeric in mixed meal
13. ANOVA test of fenugreek in mixed meal

Appendix 1

Ethics application

UNMC Research Ethics Committee
Application Form (version 1, Oct 2011)

Application identification number:

TNO030715-A

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Application Form: University of Nottingham Malaysia Campus
Application for approval of research study involving human participants

PART A

Applicant details

The applicant must be the person who will conduct the investigations; each application must be made by ONE applicant. The applicant should be:

- Student (or project supervisor) – in the case of taught or research courses,
- Research staff (a member of university research or academic staff) – the individual who will conduct the study in the case of funded research projects,
- Principal investigator – in the case of applications for ethics approval in advance of the submission of a research proposal.

Student Applicant

If the applicant is an undergraduate or postgraduate taught or research student please complete the section below. The application must be approved by a supervisor in Part C and submitted via the supervisor's University of Nottingham email account.

Note: Research Assistants who are registered on a postgraduate programme should complete the Student Applicant section.

Student name:	Thai Ngoc Ro	Student ID #:	014476
Course:	C90R-MRS1 Master of Research	Email address:	khbx4tno@nottingham.edu.my
Supervisor:	Dr. Susan Azam Ali		

Staff Applicant

If the applicant is a member of university research or academic staff, please complete the section below: For research staff, the application must be approved by the principal investigator (PI) and submitted via the PI's University of Nottingham email account.

Name of applicant:		Name of PI (if different)	Click here to enter text.
Applicant email address:	Click here to enter text.	PI email address:	Click here to enter text.

Project details

Title of project:	Profiling of the glycaemic index of underutilised fruits and vegetables

Application identification number:	TNO030715-A
Study start date (planned):	3rd Mar 2014
Study completion date (planned):	End October 2015

Please state whether this application is:	This is a request for an extension of the original approval (due to expire on 3 July)

Note: the application identification number should consist of the applicant's initials followed by the date of submission. E.g. If John Wong submitted a proposal on 1st December 2011 then the application identification number would be JW011211. This number should also be inserted in the header of every page of the application.

Previous applications:

Has a similar study been approved by the UNMC Research Ethics Committee? If YES, state the title and the submission date in the boxes below. This may speed up the review process. Also, state how this application differs from the previous study.

Title of similar study:	Profiling of the glycaemic index of underutilised fruit and vegetables
Submission date of similar study:	3 March to 3 July 2015
How application differs:	This is a request for an extension to the previous application. Through no fault of her own (extenuating circumstances due to visa issues (4 months in Vietnam) and subsequently the delay in arrival of chemicals(from December 2014 until June 2015) for the research) have led to a delay in her carrying out the research.

Appendix 2

Participant information sheet

Participants Information Sheet

<u>Research Project</u>: Profiling of Glycaemic Index of under-utilised fruits and vegetables

Invitation paragraph

You are being invited to take part in a research project. Before you decide it is important for you to understand why the research is being done and what it will involve. We would like you to take a few minutes to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this

1. Who will conduct the research?

My name is Thai Ngoc Ro and my supervisor's name is Dr. Susan Azam Ali. We are in school of Biosciences

2. What is the aim of the research?

In this study, we propose to review evidences of the role of selected underutilised fruits and vegetables, which effect on type II diabetes mellitus (T2DM). Moreover, we also carry out the profile of glycaemic index (GI) of under-utilised species. The investigation of blood glucose response in single and mixed meals containing species is studied in *in vivo* experiment.

3. Do I have to take part?

Your participation in this research is completely voluntary. We would like you to consent in this study as we believe that you can make an important contribution to the research. Moreover, you are at liberty to withdraw at any time without prejudice or giving reasons.

4. What will I do if I take part?

If you decide to take part, we will ask you to read information sheet carefully. After that, you will be given this information sheet and be asked to sign a consent form.

5. Will my taking part in this project be kept confidential?

All information you provide to us will be kept confidential and anonymity. Only investigators will have access to it. All data collection will be stored and analysed for the research purposes only.

6. Who is organizing the research?

This research project is being undertaken at the School of Biosciences and CFF

7. Contact for further information

Name of researcher: Thai Ngoc Ro

Email address: khbx4tno@nottingham.edu.my

Phone number: 017-2430582

Name of supervisor: Dr. Susan Azam Ali

Email address: Susan.Azamali@nottingham.edu.my

Appendix 3

Consent form

Participant Consent Form

<u>Research Project:</u> Profiling of the Glycaemic Index of the underutilised fruits and vegetables

	Please initial box
1. I confirm that I have read and understand the information sheet, which explained the above research project	☐
2. I have been given an opportunity to ask questions about the project	☐
3. I understand that my participation is voluntary	☐
4. I can withdraw at any time without giving any reason.	☐
5. I understand that my responses will be kept strictly confidentiality and anonymity.	☐
6. I understand that any information which might potentially identify me will not be used in published material	☐
7. I agree for the data collected from me to be used for further research	☐

Name of Participant _____ Participant's signature _____

Date _____

A copy of this consent form and information sheet should be retained by research participant. The signed consent forms should be stored with the project's main documents which must be kept in a secure location by the researcher.

Appendix 4

Participant questionnaire

Participants Questionaire

<u>Research Project</u>: Profiling of Glycaemic Index of underutilised fruits and vegetables

Name: Gender: F/M Age:

Home address:

Email: Phone number:

Are you…?

Non-smoker past smoker current smoker cigar smoker

Have you been diagnosed with…..?

Diabetes mellitus heart disease cardiovascular disease cancer

Disease of the digestive system

Please list any food allergies:

Please circle which ethic group you belong:

Malaysian Chinese Indian Others: (specify) _____

Appendix 5

Data collection sheet

Data Collection Sheet

<u>Research Project</u>: Profiling of Glycaemic Index of underutilised fruits and vegetables

Name of researcher: Thai Ngoc Ro Supervisor: Dr.SusanAzam Ali

Date: Location:

The measurement of white rice

Name:	**Time (min)**							
Age:	**Fasting**	**0**	**30**	**60**	**90**	**120**	**150**	**180**
Gender								

The measurement of Bitter gourd

Name:	**Time (min)**							
Age:	**Fasting**	**0**	**30**	**60**	**90**	**120**	**150**	**180**
Gender								

The measurement of Ginger

Name:	**Time (min)**							
Age:	**Fasting**	**0**	**30**	**60**	**90**	**120**	**150**	**180**
Gender								

The measurement of Turmeric

Name:	**Time (min)**							
Age:	**Fasting**	**0**	**30**	**60**	**90**	**120**	**150**	**180**
Gender								

The measurement of Fenugreek

Name:	**Time (min)**

Age:	**Fasting**	**0**	**30**	**60**	**90**	**120**	**150**	**180**
Gender								

Appendix 6

ANOVA test of bitter gourd in single meal

Table 4.5: *ANOVA results of three treatments of bitter gourd in single meal*

Source of variation	d.f	s.s.	m.s.	F-ratio	P-value
Block stratum	2	0.00006146	0.00003073	2.07	
Block.*Units* stratum					
Treatment	2	0.00333375	0.00166687	112.02	<.001
Time	6	0.13405130	0.02234188	1501.45	<.001
Treatment.Time	12	0.00872470	0.00072706	48.86	<.001
Residual	40	0.00059521	0.00001488		
Total	62	0.14676641			

Table 4.6: *The Fisher's LSD test of three treatments of bitter gourd in single meal*

Fisher's protected least significant difference test

Comparison	Difference	Lower 95%	Upper 95%	t	Probability	**Significant**
Fried vs Raw	-0.00329	-0.00569	-0.000880	-2.760	0.0087	yes
Fried vs Boiled	-0.01681	-0.01922	-0.014404	-14.120	0.0000	yes
Raw vs Boiled	-0.01352	-0.01593	-0.011118	-11.360	0.0000	yes

	Mean	
Raw	0.0981	a
Fried	0.1014	b
Boiled	0.1150	c

Appendix 7

ANOVA test of ginger in single meal

Table 4.8: *ANOVA results of three treatments of ginger in single meal*

Source of variation	d.f.	s.s.	m.s.	F-ratio	P-value
Block stratum	2	0.0042859	0.0021430	2.63	
Block.*Units* stratum					
Treatment	2	0.2445049	0.1222524	149.87	<.001
Time	6	0.4278458	0.0713076	87.42	<.001
Treatment.Time	12	0.1078869	0.0089906	11.02	<.001
Residual	40	0.0326294	0.0008157		
Total	62	0.8171529			

Table 4.9: *The Fisher's LSD test of three treatments of ginger in single meal*

Fisher's protected least significant difference test

Comparison	Difference	Lower 95%	Upper 95%	t	Probability	**Significant**
Raw vs Fried	-0.0832	-0.1011	-0.06542	-9.44	0.0000	yes
Raw vs Boiled	-0.1524	-0.1702	-0.13457	-17.29	0.0000	yes
Fried vs Boiled	-0.0691	-0.0870	-0.05133	-7.84	0.0000	yes

	Mean	
Raw	0.1272	a
Fried	0.2105	b
Boiled	0.2796	c

Appendix 8

ANOVA test of turmeric in single meal

Table 4.11: *ANOVA analysis of 2 treatments of turmeric in single meal*

Source of variation	d.f.	s.s.	m.s.	F-ratio	P-value
Block stratum	2	0.0000019	0.0000010	0.00	
Block.*Units* stratum					
Treatment	2	0.598345	0.299173	112.78	<.001
Time	6	0.156635	0.026106	9.84	<.001
Treatment.Time	12	0.150179	0.012515	4.72	<.001
Residual	40	0.106110	0.002653		
Total	62	1.015871			

Table 4.12: *The Fisher's LSD test of 2 treatments of turmeric in single meal*

Fisher's protected least significant difference test

Comparison	Difference	Lower 95%	Upper 95%	t	Probability	**Significant**
Fried vs Raw	-0.0653	-0.0974	-0.0332	-4.107	0.0002	yes
Fried vs Boiled	-0.2315	-0.2636	-0.1994	-14.564	0.0000	yes
Raw vs Boiled	-0.1662	-0.1983	-0.1341	-10.457	0.0000	yes

	Mean	
Fried	0.0190	a
Raw	0.0843	b
Boiled	0.2505	c

Appendix 9

ANOVA test of fenugreek in single meal

Table 4.14: *ANOVA results of 2 treatments of fenugreek in single meal*

Source of variation	d.f.	s.s.	m.s	F-ratio	P-value
Block stratum	2	0.00032117	0.00016059	4.78	
Block.*Units* stratum					
Treatment	2	0.05029660	0.02514830	748.74	<.001
Time	6	0.03514556	0.00585759	174.40	<.001
Treatment.Time	12	0.01884473	0.00157039	46.76	<.001
Residual	40	0.00134349	0.00003359		
Total	62	0.10595156			

Table 4.15: *The Fisher's LSD test of 2 treatments of fenugreek in single meal*

Fisher's protected least significant difference test

Comparison	Difference	Lower 95%	Upper 95%	t	Probability	**Significant**
Raw vs Fried	-0.05443	-0.05804	-0.05081	-30.43	0.0000	yes
Raw vs Boiled	-0.06424	-0.06785	-0.06062	-35.92	0.0000	yes
Fried vs Boiled	-0.00981	-0.01342	-0.00619	-5.48	0.0000	yes

	Mean	
Raw	0.01900	a
Fried	0.07343	b
Boiled	0.08324	c

Appendix 10

ANOVA test of bitter gourd in mixed meal

Table 4.17: *ANOVA analysis of 3 treatments of bitter gourd with white rice in mixed meal*

Source of variation	d.f.	s.s.	m.s.	F-ratio	P-value
Block stratum	2	0.00003070	0.00001535	0.66	
Block.*Units* stratum					
Time	6	0.17364638	0.02894106	1238.60	<.001
Treatment	2	0.00173089	0.00086544	37.04	<.001
Time.Treatment	12	0.09057933	0.00754828	323.05	<.001
Residual	40	0.00093463	0.00002337		
Total	62	0.26692194			

Table 4.18: *The Fisher's LSD test of 3 treatments of bitter gourd with white rice in mixed meal*

Fisher's protected least significant difference test

Comparison	Difference	Lower 95%	Upper 95%	t	Probability	**Significant**
Raw vs Boiled	-0.004810	-0.00782	-0.001795	-3.224	0.0025	yes
Raw vs Fried	-0.012714	-0.01573	-0.009699	-8.523	0.0000	yes
Boiled vs Fried	-0.007905	-0.01092	-0.004890	-5.299	0.0000	yes

	Mean	
Raw	0.1079	a
Boiled	0.1127	b
Fried	0.1206	c

Appendix 11

ANOVA test of ginger in mixed meal

Table 4.20: *ANOVA analysis of three treatments of ginger with white rice in mixed meal*

Source of variation	d.f.	s.s.	m.s.	F-ratio	P-value
Block stratum	2	0.00002422	0.00001211	0.31	
Block.*Units* stratum					
Treatment	2	0.02635632	0.01317816	338.53	<.001
Time	6	0.33166238	0.05527706	1419.99	<.001
Treatment.Time	12	0.10409657	0.00867471	222.84	<.001
Residual	40	0.00155711	0.00003893		
Total	62	0.46369660			

Table 4.21: *The Fisher's LSD test of three treatments of ginger with white rice in mixed meal*

Fisher's protected least significant difference test

Comparison	Difference	Lower 95%	Upper 95%	t	Probability	**Significant**
Raw vs Fried	-0.02571	-0.02961	-0.02182	-13.35	0.0000	yes
Raw vs Boiled	-0.05010	-0.05399	-0.04620	-26.02	0.0000	yes
Fried vs Boiled	-0.02438	-0.02827	-0.02049	-12.66	0.0000	yes

	Mean	
Raw	0.1291	a
Fried	0.1548	b
Boiled	0.1792	c

Appendix 12

ANOVA test of turmeric in mixed meal

Table 4.23: *ANOVA analysis of two treatments of turmeric with white rice in mixed meal*

Source of variation	d.f.	s.s.	m.s.	F-ratio	P-value
Block stratum	2	0.00009432	0.00004716	0.94	
Block.*Units* stratum					
Treatment	2	0.25506022	0.12753011	2534.95	<.001
Time	6	0.13667721	0.02277953	452.79	<.001
Treatment.Time	12	0.13317422	0.01109785	220.59	<.001
Residual	40	0.00201235	0.00005031		
Total	62	0.52701832			

Table 4.24: *The Fisher's LSD test of two treatments of turmeric with white rice in mixed meal*

Fisher's protected least significant difference test

Comparison	Difference	Lower 95%	Upper 95%	t	Probability	**Significant**
Fried vs Raw	-0.0661	-0.0705	-0.06167	-30.20	0.0000	yes
Fried vs Boiled	-0.1553	-0.1597	-0.15086	-70.94	0.0000	yes
Raw vs Boiled	-0.0892	-0.0936	-0.08477	-40.75	0.0000	yes

	Mean	
Fried	0.01900	a
Raw	0.08510	b
Boiled	0.17429	c

Appendix 13

ANOVA test of fenugreek in mixed meal

Table 4.26: *ANOVA analysis of two treatments of fenugreek in mixed meal*

Source of variation	d.f.	s.s.m.s.	F-ratio	P-value	
Block stratum	2	0.00009451	0.00004725	1.53	
Block.*Units* stratum					
Treatment	2	0.05069727	0.02534863	819.80	<.001
Time	6	0.03398254	0.00566376	183.17	<.001
Treatment.Time	12	0.01848117	0.00154010	49.81	<.001
Residual	40	0.00123683	0.00003092		
Total	62	0.10449232			

Table 4.27: *The Fisher's LSD test of two treatments of fenugreek in mixed meal*

Fisher's protected least significant difference test

Comparison	Difference	Lower 95%	Upper 95%	t	Probability	**Significant**
Raw vs Fried	-0.05419	-0.05766	-0.05072	-31.58	0.0000	yes
Raw vs Boiled	-0.06476	-0.06823	-0.06129	-37.74	0.0000	yes
Fried vs Boiled	-0.01057	-0.01404	-0.00710	-6.16	0.0000	yes

	Mean	
Raw	0.01900	a
Fried	0.07319	b
Boiled	0.08376	c