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Can polyphenol-rich millets affect glycaemic response, insulinaemic response and gastric emptying in prediabetes?

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Contributions / Author's work

This thesis is the result of the author (Ameerah Almaski)'s work in terms of design, data collection/analysis, and writing the thesis. However, the only exception is the analyses of the breath samples (GE samples) which were sent to be tested in an external laboratory at Iso-Analytical Limited in London (Chapter 5/ study 4). Nevertheless, Ameerah Almaski worked on the interpretation of all findings.

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Abstract

Type-2 diabetes is a chronic metabolic condition characterised by insulin resistance and insufficient pancreatic insulin production, which results in increased blood glucose levels. Prediabetes is a common precursor to type-2 diabetes which occurs when blood sugar levels are higher than normal but not in the diabetes range. It is, therefore, essential that preventive measures, including lifestyle modifications, are developed so as to significantly reduce the risk of individuals developing the disease and suffering from its complications.

Millet is a functional grain known for its significant health benefits, which are partly attributed to its high content of polyphenols and antioxidants. Millets may exert their protective effects by reducing the glycaemic response and improving insulin sensitivity. It is possible that millet consumption may also help prevent and/or reduce the risk of developing type-2 diabetes.

The aims of this PhD were to 1) determine the levels of polyphenols and antioxidants in different species and forms of millet, 2) investigate the effect of a millet muffin on starch digestion *in vitro*, 3) conduct a sensory evaluation test to assess the overall acceptance of millet muffins, and 4) determine the effect of a millet muffin on endogenous markers of glycaemia in prediabetic individuals.

This PhD study was able to show, firstly, that the highest levels of polyphenol content and antioxidant activity were found in kodo millet grains, followed by finger millet (grain and flour). Secondly, it showed that all millet based muffins had reduced starch digestibility compared to wheat based muffins. Muffins made of finger millet grains were found to be the most acceptable in all sensory attributes when compared with wheat, finger millet flour and kodo millet grain-based muffins. In the human trial,

consumption of finger-millet muffins was shown to have no effect on glycaemic response in prediabetic and healthy adults. However, the insulin response was significantly reduced in the prediabetes group 150min and 180min after consumption of finger-millet muffins ($p<0.05$). Regarding gastric emptying, significant differences were observed in ascension times (Tasc) ($p<0.05$) (~21min after control) between wheat and finger millet grain-based muffins consumed. These differences were only observed in the prediabetes group. There was no significant difference in satiety between the two muffin types. Based on the results, regular consumption of finger millet could exert beneficial effects on the management of prediabetes through regulation of glucose and insulin.

List of abbreviations

- **ADA** American Diabetes Association
- **BMI** Body mass index
- **DNS** 3,5-Dinitrosalicylic acid
- **FCR** Folin-Ciocalteu reagent
- **FRAP** Ferric-ion reducing antioxidant power
- **GAEs** Gallic-acid equivalent
- **GE** Gastric emptying
- **GI** Glycaemic index
- **GR** Glycaemic response
- **HCl** Hydrochloric acid
- **IR** Insulinaemic response
- **OGTT** Oral glucose tolerance test
- **RDS** Rapidly digestible starch
- **RS** Resistant starch
- **SD** Standard deviation
- **SDS** Slowly digestible starch
- **SEM** Standard error of mean
- **UV** Ultraviolet radiation
- **MetS** Metabolic syndrome

Conference abstracts and publications

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General introduction

Type-2 diabetes has become a major health concern worldwide (Ren et al. 2015). Globally, it is one of the most common chronic diseases and can lead to a number of secondary complications, such as kidney disease, cardiovascular disease, limb amputation and premature death (Riaz, 2009). Prediabetes, also known as impaired glucose tolerance or impaired fasting glucose, is a condition in which the level of blood glucose is above the normal fasting level but not high enough for clinical diagnosis of type-2 diabetes (Diabetes UK, n.d.). Individuals with prediabetes have a higher risk of developing type-2 diabetes, which in turn may increase the risk of metabolic complications (Diabetes UK, n.d).

It is widely known that both the quantity and quality of dietary carbohydrates play an important role in regulating postprandial blood glucose levels. Studies have demonstrated that slowly digested and absorbed carbohydrates help reduce the risk of developing type-2 diabetes and different dietary guidelines include a recommendation for at-risk individuals to follow a glycaemic index (GI) guide when choosing meals (Ren et al. 2015).

Millet is a functional grain that has attracted interest from researchers for many years. Several lines of evidence have shown that millet has properties that may make it a beneficial dietary addition for individuals with diabetes; it is unique among other cereals due to its high nutrient and non-nutrient content. Millet contains high levels of polyphenols, antioxidants, proteins, dietary fibre and calcium (Amadou et al. 2013), as well as B vitamins, minerals and resistant starch (Talukdera and Sharmaa, 2015; Shahidi and Chandrasekara, 2013).

This PhD thesis comprises a set of *in vitro* and *in vivo* studies, which are interlinked by

their findings. A variety of millet species –finger, foxtail, barnyard, kodo, little, pearl and proso – as well as various forms of grain, flour and flakes were selected for the first study as they were postulated (from previous literature) to be rich in polyphenols and antioxidants and may have beneficial effects on the glycaemic response (GR), insulinaemic response (IR) and gastric emptying (GE). The polyphenol and antioxidant content of each species and form of millet was assessed. The three types and forms that were highest in these compounds were selected to use in muffin preparations, then measured for their effect on starch digestion *in vitro*. Sensory evaluation tests were then conducted in order to determine whether the chosen muffin was acceptable. Millet showing the best results from these studies was further used to measure its effect on the GR, IR, and GE of prediabetic individuals, after systematically evaluating existing literature on the effect of millet consumption on these parameters in healthy and type-2 diabetic individuals.

Chapter 1: Review of the literature

1.1. Introduction to millet as a functional grain

The demand for functional foods has increased recently due to improved health awareness and availability of information on the importance of a varied diet of essential nutrients and their beneficial impact on health (Hasler, 2000; Das, 2012). A functional food is defined as any food that offers health benefits beyond basic nutrition (Bech-Larsen and Grunert, 2003). There are many types of functional foods, including cereals, fruit, vegetables and dairy products; the rationale for increasing the intake of these foods is to both promote health and prevent disease (Danik, 2011). Many types of functional foods have been successfully introduced into the food market to help reduce the incidence or risk of certain dyshomeostatic conditions, e.g. high cholesterol, blood sugar, hypertension and osteoporosis (Charalampopoulos, 2002). The development of functional foods has been proven effective in improving health over time, for instance, by reducing deficiencies in A, D, and B vitamins (thiamine, riboflavin and niacin), minerals, iron and iodine (Betoret et al. 2011).

Cereal-based foods are considered to be a staple that beneficially impacts health in a variety of ways, beyond providing basic nutrition, due to a high content of carbohydrates, protein, dietary fibre, minerals, vitamins and antioxidants – all of which are vital for human health (McKevith, 2004). Moreover, there is strong epidemiological evidence that whole-grain cereals can protect from numerous diseases, including cardiovascular disease, different types of cancer and type-2 diabetes (Fayet-Moore et al. 2017; Venn and Mann, 2004). This may be attributed to the high availability of fibre and micronutrients in the outermost layer of the grain, which, once ingested, endogenously functions to combat hyperglycaemia, oxidative stress,

inflammation and carcinogenesis. Cereals also contain vitamin E, iron, zinc, sulphur, amino acids, carotenoids, manganese, folates, lignins, and phenolic acids – all of which show antioxidant function (Slavin et al. 1999). Millet, wheat, flaxseed, buckwheat, barley, and oats are the most common cereal-based functional foods and nutraceuticals (Shahidi and Chandrasekarab, 2013; Das et al, 2012).

Millet, specifically, has been a focus of scientific interest for a long time due to its significant benefits to human health (Shahidi and Chandrasekarab, 2013). Moreover, millet represents the main food source for many people in arid and semi-arid tropical regions and was domesticated over 10,000 years ago. The cereal is usually grown across Africa, Asia and also some parts of Europe (Shahidi and Chandrasekarab, 2013, Devisetti et al. 2014; Lu, 2007).

1.2. Millet

1.2.1. Definition and description of millet

Millet is a cereal crop plant that belongs to the grass family (FAO, 2011) and is named based on the French word *mille*, meaning *thousand* – indicating that the cereal produces thousands of grains (Jain, 2018). In low-income countries, millets are important crops as they can be grown in adverse weather conditions, such as drought, or where the main cereal is impossible to grow at the essential yields (Adekunle, 2012; Amadou et al. 2013).

In many low-income countries, millet grains are an untapped food source, despite their potential for consumption as food or beverages (Talukder and Sharma, 2015). Moreover, millet can be used as the main food source during famine as it can be stored for a long time and is resistant to drought and insect damage (Amadou et al. 2013). These grains are found to be more nutritious than other common cereals and can,

therefore, provide a good source of daily energy, fibre and protein (Bunkar, 2014).

1.2.2. Millet species

There are different types of millet growing around the world, the main ones being pearl (comprising 40% of total millet production), proso, finger and foxtail millet. The rarer millet types include kodo, barnyard, little, fonio, teff and guinea millet (Amadou et al. 2011).

1.2.2.1. Pearl millet (*Pennisetum glaucum*)

Pearl millet is also known as spiked millet, bulrush millet or *bajra* in India. This type of millet was first grown in tropical Western Africa and then spread to Eastern and Central parts of the continent, as well as India, as a result of the dry environment in these regions. Pearl millet can be a variety of colours, including brown, white, pale yellow, purple, slate blue and grey (Lupien, 1995).

Figure 1.1: Pearl millet (Millets, 2016)

1.2.2.2. Finger millet (*Eleusine coracana*)

Finger millet is also a common type and is also known by different names: African millet; *telebun* in the Sudan; *bulo* in Uganda; *wimbi* in Swahili; and *koracan*, or *ragi*, in India. This millet is a staple food in various parts of Africa (Central and Eastern) and India, as well as in north-eastern Zambia and Western Uganda. It can also be used

for brewing beer and can be stored for a long time without damage during famine. The most common colours of finger millet are orange-red, white, purple, brown and black (Lupien, 1995).

Figure 1.2: Finger millet (Mandua,2015)

1.2.2.3. Foxtail millet (*Setaria italica*)

Foxtail millet is known as *setaria* in Italy and Hungarian or Siberian millet in Germany. China is one of the major regions where foxtail millet is produced and it is also commonly grown in Japan and India. Seeds of foxtail millet are also found in Europe (lake dwellings). The colour of the grain ranges from pale yellow to orange red, brown and black (Lupien, 1995).

Figure1.3: Foxtail millet (Millets, 2016)

1.2.2.4. Proso millet (*Panicum miliaceum*)

Proso millet is also called *prove*, common, broom-corn, hog, brown corn or Russian millet. Historically, this type of millet was planted in Europe by lake dwellers and, due to its fast growth, it was then domesticated in Central and Eastern Asia and used by nomads. It is also grown in Arabian countries, such as Syria, Iran, Iraq and Afghanistan (Kajuna, 2001). This type of millet contains high amounts of indigestible fibre, as the seed is surrounded by a husk, and this is difficult to remove using the traditional grinding processes (Lupien, 1995).

Figure 1.4: Proso millet (Green cover seed, n.d.)

1.2.2.5. Little millet (*Panicum sumatrense*)

Little millet grains are grown everywhere in India and have seeds that are smaller than those of the common millet (Lupien, 1995). This type of millet has not received much attention from plant breeders, although it should be noted that it can thrive under conditions not suitable for other types of edible plant (Kajuna, 2001).

Figure 1.5: Little millet (Millets, 2016)

1.2.2.6. Barnyard millet (*Echinochloa colona*)

Barnyard is also known as sawa millet or Japanese barnyard. This grain is the fastest-growing of all types and can be harvested within six weeks in moist and temperate conditions (Kajuna, 2001). India, China and Japan are famous for growing this millet as an alternative to rice and it is also grown in Egypt. Furthermore, barnyard millet is known as a forage crop in the USA and can produce up to eight harvests per year (Lupien, 1995).

Figure1.6: Barnyard millet (Sherck, n.d)

1.2.2.7. Kodo millet (*Paspalum scrobiculatum*)

Kodo millet is a major grain in India, where it is limited to Karnataka, Gujarat and some parts of Tamil Nadu. Some forms of kodo millet have been found to be poisonous to humans and animals, which may be due to an infectious fungus.

However, according to Kajuna (2001), if clean and healthy, this grain poses no health issues for humans when consumed. The grains are covered by hard, corneous and persistent husks that are difficult to remove and the kodo crop is also drought-resistant. This millet can be found in a variety of colours, ranging from light red to dark grey (Lupien, 1995).

Figure 1.7: Kodo millet (Millets, 2016)

1.2.3. Traditional uses for millet

Millet grains were not seen as an important commodity in the Western world, such as North America and Europe. However, they have recently gained interest as a gluten-free grain (Saleh et al. 2013) and millet is also commonly used as a birdfood in Canada and the USA (Chandrasekara and Shahidi, 2011). In African and Asian areas, where they represent one of the main sources of traditional food, millet grains are mostly commonly used in foods, beverages and weaning foods, such as porridge, bread (fermented and unfermented) and snacks (Amadou et al. 2013; Saleh et al. 2013).

Meals prepared from millet grains differ across countries and regions and even within the same country (Amadou et al. 2013). In some African countries, millet grains are usually the main ingredient of meals and can be cooked in a variety of ways: thin porridge (*ogi*), for instance, can be prepared as a complementary meal for infants and

toddlers, while thick porridge (*to*), couscous and fermented beer are other millet-based products (Saleh et al., 2013). In Nigeria, a traditional beverage called *kunu* is made from millet and meets the nutrient requirements of humans. Analysis of *kunu* grains showed that it is a perfect source of energy, due to its high content of protein, calcium and moderate acidity (Saleh et al., 2013). In Northern China, pregnant and nursing women often consume foxtail millet as a gruel or soup or use it as a nutritional therapy (Saleh et al. 2013). In cold Japanese regions, people usually use the abundant barnyard millet to cook traditional meals – particularly in the Tohoku area, where millet is a significant grain due its capacity to keep for a long time (Watanabe, 1999). Indians commonly consume millet by preparing different foods, such as flour and malt, from the grains (Shahidi and Chandrasekarab, 2013). Meanwhile, in Jazan in Saudi Arabia, people commonly use a millet called *lohoh* to prepare bread (Osman, 2011).

1.2.4. Storage and shelf life of millet

Millet is seen as a low-cost crop that can be kept in storage for long periods (Saleh et al. 2013). Many studies have documented millet's ability to increase storage time when incorporated into other products, such as pastas and meats (Talukdera and Sharmaa, 2015). According to Devaraju (2003), making pasta containing 50% finger-millet flour increases its storage life by up to three months, with no change in the sensory attributes. In addition, Talukdera (2015) suggests that meat processors could benefit greatly from millet by including it in meat products in order to improve their shelf life or storage capacity. This is because meat contains large quantities of fatty acids (polyunsaturated) that speed up the oxidation process in the product. Many studies have found that millet contains large amounts of polyphenols, which can serve as natural antioxidants through several mechanisms, such as free-radical scavenging and attenuation of lipid oxidation. These natural antioxidant properties, when added to

meat products, increase the length of time they can be stored (Talukdera and Sharmaa, 2015).

1.3. Nutritional composition of millet

Grains and plants are widely used around the world as sources of key nutrients (Kulkarni et al. 2018). Nutritionally, millet distinguishes itself from other cereals by containing high levels of dietary fibre, calcium, protein and polyphenols (Amadou et al. 2013). Specifically, the major components of millet are carbohydrates (65–75%), dietary fibre (15-20%) and, to a lesser extent, fats (2–5%) and proteins (7–12%) (Kulkarni et al., 2018) (Table 1.1).

1.3.1. Carbohydrates

A large proportion of the carbohydrate contained in millet is made up of non-starchy polysaccharides (15% to 20%) and starch (60% to 75%) (Kulkarni et al., 2018). The non-starchy polysaccharides that are considered as dietary fibres consist of cellulose and hemicellulose (Himanshu et al., 2018). A small amount of β-glucans and lignins are present. Research has found that 90% of the total dietary fibre in millet is insoluble dietary fibre that is found in the aleurone layer and cell walls of the kernel (Himanshu et al., 2018). Finger millet, in particular, is rich in dietary fibre (around 11.5%) – more so than brown rice – and this fibre content is comparable with that found in pearl millet and wheat (Shobana et al., 2013). Kodo and little millet reportedly contain the highest amounts of dietary fibre compared with other cereals, at 37% and 38% respectively (Saleh et al. 2013).

1.3.2. Protein

According to Talukdera and Sharmaa (2015), the protein content of millet is equivalent

to that of wheat; both grains contain around 11% protein by weight. Notably, millet is a good source of essential amino acids (except for lysine and threonine) and is relatively high in methionine (Saleh et al. 2013). Protein characterisation of foxtail millet showed that its protein content makes it a potential functional food ingredient due to high levels of the amino acid lysine. Therefore, it can be used as a supplementary protein source with most cereals (Mohamed et al. 2009). Proso millet (11.6%) has also been found to contain a significantly higher amount of proteins compared to wheat, including some essential amino acids (leucine, isoleucine and methionine) (Kalinova and Moudry, 2006). Pearl millet contains a high level of niacin compared to other cereals, while finger millet is unique in its richness of sulphur amino-acid content. Pearl millet, however, displays a higher protein content compared to finger millet – 12–16% and 6–8%, respectively (Kulkarni et al. 2018). Generally, it has been shown that millet proteins contain a better essential amino acid profile than maize (Kulkarni et al. 2018).

1.3.3. Fats

Lipids are an integral ingredient of a healthy diet because they provide a source of fat and essential fatty acids. Millet is considered to be a rich source of essential fatty acids, particularly linoleic acid (Muthamilarasan et al., 2016). Among the different millet varieties, pearl millet has the highest lipid content (ranging between 3–6%) compared to foxtail, finger and kodo millet. Moreover, around 75% of the fatty acids found in pearl millet are unsaturated and are rich in linoleic acid (46.3%). Analysis of foxtail millet concluded that linoleic acid comprised about 70% of its total fatty acids (Muthamilarasan et al., 2016). It has also been shown that millet is richer in polyunsaturated fatty acids than maize, rice and sorghum (Amadou et al. 2013; Saleh et al, 2013).

1.3.4. Micronutrients

Millet is rich in vitamins that are essential to human physiology (Muthamilarasan et al. 2016); in particular, millet provides a high source of energy, B vitamins (especially niacin, B6 and folic acid) and minerals, such as iron, magnesium, phosphorus and manganese, compared to other cereals. In addition, finger millet contains the highest quantity of calcium – around 350mg/100g – compared to other cereals (Talukdera and Sharmaa, 2015; Shahidi and Chandrasekarab, 2013; Kulkarni et al. 2018), while pearl millet contains around 42 mg calcium – still more so than commonly consumed foods such as rice (10 mg), wheat (41 mg), sorghum (25 mg) and maize (9 mg) (Vanisha et al. 2011). Almost all millets also contain a high level of phosphorus, the highest being in foxtail millet (422 mg/100 g). Analysis of iron in millet showed that the barnyard species contains the highest levels (4.0 mg/100 g), followed by finger millet (3.4mg), little millet (3.2mg), kodo millet (3.2mg) and foxtail millet (2.7mg), compared to rice (1.25 mg/100 g) (Muthamilarasan et al. 2016; Kumar et al. 2018). A recent study of barnyard millet even recorded an iron content of 17.47 mg/100 g - only 10 mg lower than the total daily requirement and it consumption can meet the iron requirement of pregnant women suffering from anaemia (Kumar et al. 2018).

Table 1.1:Nutrient composition of different types of millet (per 100g)

Grains	Carbohydrates (g)	Protein (g)	Fat (g)	Energy (Kcal)	Crude fibre (g)	Minerals (g)	Ca (mg)	P (mg)	Fe (mg)
Finger millet	72	7.3	1.3	328	3.6	2.7	344	283	3.9
Kodo millet	65.9	8.3	1.4	309	9	2.6	27	188	0.5
Proso millet	70.4	12.5	1.1	341	2.2	1.9	14	206	0.8
Foxtail millet	60.9	12.3	4.3	331	8	3.3	31	290	2.8
Little millet	67	7.7	4.7	341	7.6	1.5	17	220	9.3
Barnyard millet	65.5	6.2	2.2	307	9.8	4.4	20	280	5
Pearl millet	67.5	11.6	5	361	1.2	2.3	42	296	8
Wheat	71.2	14	1.5	348	2	1.5	30	306	3.5

Source: Kulkarni et al., 2018 and Millets, 2016)
(Ca =calcium, P=phosphorus, and Fe= iron)

1.4. Polyphenols and the antioxidant capacity of millet

Polyphenols are naturally occurring compounds that are considered to be the largest group of phytochemicals found in plant parts; thus, they form an integral part of the healthy diet (Tsao, 2010). Studies show that whole grains (like millet), oilseeds, legumes, fruits and vegetables contain different bioactive phytochemicals, besides containing all essential nutrients such as carbohydrates, proteins, fats, minerals and vitamins, and therefore have numerous beneficial effects on health (Shahidi and Chandrasekara, 2013).

Polyphenols are classified into four groups, depending on the number of phenol rings and structural elements that bind these rings together. The main classes are phenolic acids, flavonoids, stilbenes and lignans (Pandey and Rizvi, 2009).

1.4.1. Polyphenols in millets

1.4.1.1. Phenolic acids

Millet mainly contains two forms of phenolic acid (free and conjugated), which are derivatives of hydroxybenzoic and hydroxycinnamic acids (Figure 1.8).

According to a review by Dykes and Rooney (2006), the hydroxybenzoic acids found in millets are gallic, protocatechuic, p-hydroxybenzoic, gentisic and syringic acids. Hydroxycinnamic acids include ferulic, caffeic, p-coumaric, cinnamic and sinapic acids. Ferulic, p-coumaric and cinnamic acids are the main phenolic acids found in millet. In finger millet, 70% of phenolic acids are found to be present in free form. Moreover, it has been shown that ferulic acid is the main bound phenolic acid in millet and protocatechuic acid was identified as the main free phenolic acid (Roe and Muralikrishna, 2002).

1.4.1.2. Flavonoids

Dietary flavonoids represent a variety of polyphenol compounds naturally occurring in plant-based foods (Hooper et al. 2008). So far, millet has been found to contain flavones; finger millet, for instance, contains the flavones vitexin, isoorientin, orientin, saponarin, violanthin, isovitexin, lucenin-1 and tricin (eight in total) (Chethan and Malleshi, 2007). Pearl millet has also been found to contain glucosylvitexin, glucosylorientin and vitexin, which may be responsible for the yellow/green colour of millet flour. Tricin has also been identified in barnyard millet (Dykes and Rooney, 2006; Hilu et al., 1978). In general, phenolic acids and flavonoids can be found in different parts of the millet grain and the concentration and structure of these compounds differ depending on the millet type (Shahidi and Chandrasekara, 2013).

1.4.1.3. Tannins

According to Dykes and Rooney (2006), the only millet that contains tannins is finger millet, with brown varieties of finger millet containing higher amounts of the compounds than white finger millet varieties (Ramachandra et al. 1977). Kodo and barnyard millet have also been found to be rich in tannins, which have known antioxidant properties (Priyankar et al. 2016). In addition, lignans are found in the bran layer of the whole grains and in the seed coat. Millets contain fairly high levels of lignans (Peterson et al., 2010).

Antioxidants are considered to be important compounds due to their major roles as lipid stabilisers and suppressors of excessive oxidation – a known contributing factor to cancer and ageing (Devi et al., 2011). For example, cooking oil containing polyunsaturated lipids that are readily oxidised when exposed to high heat may also oxidise flavours, vitamins, and pigments. It has been found that, in order to prevent

oxidation of compounds – in particular, fatty acids and oils – foods must contain antioxidants (Daker et al. 2008) that prevent the formation of toxic stable radical intermediates (Devi et al. 2011). The phenolic acids, flavonoids and tannins found in millet grain coats are multifunctional in this aspect and can act both as reducing agents and metal chelators. The antioxidant capacity of polyphenols appears to stem from their ability to donate hydrogen ions through hydroxyl groups (on benzene rings) to electron-deficient free radicals, leading to formation of a resonance-stabilised and less reactive phenoxyl radical. Studies carried out on natural antioxidants in different types of millet flour have found that finger, foxtail, little and proso millet have the highest antioxidant capacities and carotenoids level, ranging between 78 mg/100g and 366 mg/100 g (Devi et al., 2014).

1.4.2. Distribution of polyphenols in millet

Efforts have been made to determine polyphenol levels in different parts of millet grains using histochemical analyses of milling fractions. Studies have found that polyphenol compounds are not equally distributed throughout the millet grain; rather, they are mostly concentrated in the outer layers – i.e. the testa, aleurone layer and pericarp (Shahidi and Chandrasekara, 2013) – and these may be lost during the separation of the grain coat in the milling process. Around 60% of polyphenol compounds in millet grains are located in the grain coat tissue, which accounts for about 12% of the total mass of the grain (Devi et al. 2014). A study undertaken by Chethan and Malleshi (2007) found that polyphenol compounds in finger millet grains are concentrated in the grain cover, where their content comprises around 6.2% mass, and in the flour fraction (around 0.8%).

It has been reported that numerous types of millet whole grains, such as kodo, finger,

foxtail, proso, pearl and little millet, are rich natural sources of polyphenols and display high antioxidant efficacy. However, this appears to depend on the variety used (Saleh et al. 2013). Some processing technologies, such as decortications, may significantly decrease the total phenol content of grains, as well as their antioxidant capacity, fibre and mineral content, while retaining the protein and fat content (Saleh et al. 2013).

1.4.3. Variations in polyphenol content in millet

A number of studies have been conducted to estimate the levels of phenolic acids and tannins in different types of millet and have revealed there to be considerable differences that depend on the colour of the millet coat. A review by Devi et al. (2011) found that total polyphenol and tannin content varies across finger millet grain genotypes; dark-coloured finger millet contains significantly higher levels of total polyphenols and tannins than light-coloured grain, due to the grain cover being highly pigmented in the former. Lorenz (1983) confirmed that light-coloured pearl millet cultivars have much lower tannin levels than dark cultivars and are located in the tissue of millet grain.

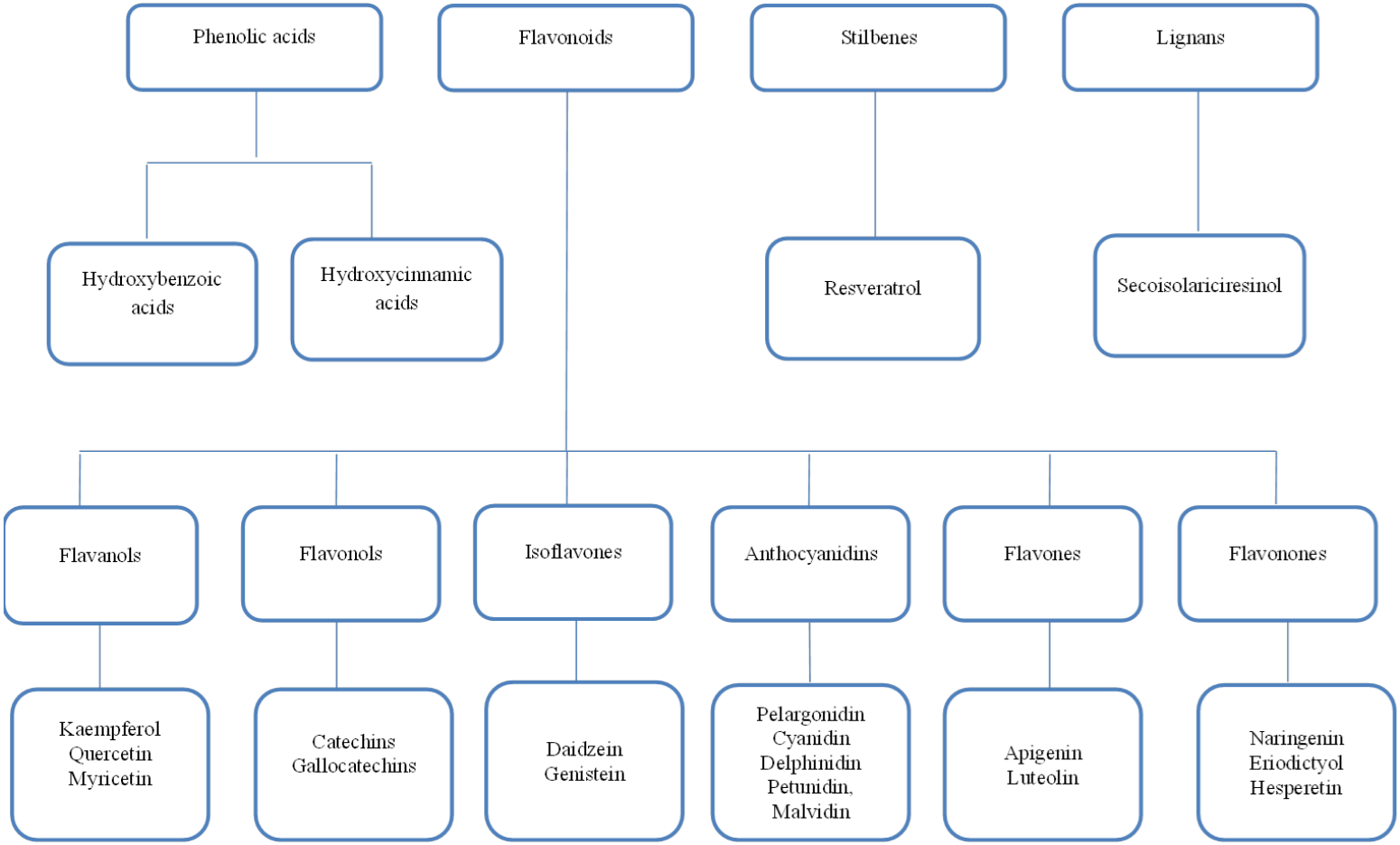
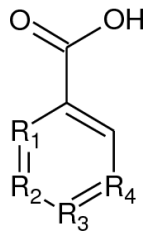
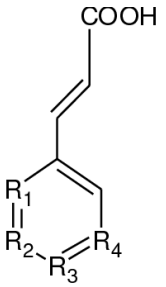


Figure 1.8: Polyphenol classification (Hardman, 2014)



Hydroxybenzoic acid

Gallic acid: R_1=H; R_2=R_3=R_4=OH
Gentisic acid: R_1=R_2=R_3=R_4=OH
p-hydroxy benzoic acid: R_1=R_2=R_4=H; R_3=OH
Salicylic acid: R_1=OH; R_2=R_3=R_4=H.
Protocatechuic acid: R_1=R_4=H; R_2=R_3=OH
Syringic acid: R_1=H; R_2=R_4=OCH_3; R_3=OH
Vanillic acid: R_1=R_4=H; R_3=OH; R_2=OCH_3



Hydroxycinnamic acid

p – coumaric acid: R_1=R_2=R_4=H; R_3=OH
Ferulic acid: R_1=R_4=H; R_2= OCH_3; R_3=OH
Sinapic acid: R_1=H; R_2=R_4= OCH_3 ; R_3=OH

Figure 1.9: Structure of some important phenolic acid derivatives in millet (Banerjee et al., 2012)

1.5. Processing factors that affect the nutritional quality of millet

Processing is used to convert inedible grain into an edible form, which improves its quality, sensory properties and the appearance of its food products. Millet grain processing involves several primary stages, namely wetting, dehulling and milling, and secondary stages, including fermentation, malting, extrusion, popping and roasting (Amadou et al. 2013). Different types of millet grain can, therefore, be consumed after being processed into flour or as popped, porridge and fermented products. Millet grains have a hard seed coat, so their processing begins with husk removal. A number of traditional methods are used to either partially or fully decorticate millet grains before further processing (Kulkarni et al. 2018).

It has been shown that decorticating millet has no effect on its protein and fat levels; however, it does cause a significant reduction in the fibre, mineral, polyphenol and antioxidant content, which may impede the applicability of millet as a functional grain for nutrition (Saleh et al. 2013). Different studies have also reported that the dehulling

of pearl millet grains decreases the levels of polyphenols, total phytic acid and tannins, which reduces the nutritional quality of millet – although, simultaneously, this process significantly increases the digestibility of protein (Kulkarni et al. 2018). This reduction in various nutrients and ant-nutrients may be explained by the fact that they mostly concentrate in the peripheral parts of the grain; thus, once the husk is removed some of these compounds may be lost. Decortication is also a process that alters the functionality of millet grains (in particular finger millet) as it becomes challenging due to their small size (Amadou et al. 2013).

Milling is a process that includes the removal of bran (the seed coat, epidermis and the aleurone layer) (Kulkarni et al. 2018) and, generally, tends to reduce the mineral and vitamin content of millet grains. Milling and heat treatment of bread made from millet decreases levels of phytic acid and polyphenols, while protein and starch digestibility are improved (Saleh et al. 2013). According to Hassan et al. (2006), the improvement in protein digestibility after undergoing processing methods, such as soaking, germination and dry heating, may be due to a reduction in anti-nutrients – such as polyphenols and tannins – that form complexes with proteins.

The processing method may also affect the content and biological activity of antioxidants in millet grains. For instance, cooking kodo or finger millet by roasting or boiling was found to reduce the antioxidant activity of the grains. Contrarily, steaming, germination and roasting of little millet increases levels of antioxidants, including total phenolic acid (21.2mg/100 g), flavonoids (25.5mg/100 g) and tannins (18.9 mg/100 g) compared to unprocessed little millet grains (Saleh et al. 2013). Hydrothermal treatments and dehulling, on the other hand, affect both polyphenol and antioxidant levels in pearl millet (Saleh et al. 2013). The reduction in antioxidant levels in different types of millet may be caused by degradation and oxidation induced by thermal

treatments such as boiling and cooking. Dehulling may also cause this through removal of the pericarp layer from grains, which is the part rich in polyphenol and antioxidant compounds. The above findings show that millet grain and millet-based food products should be processed under optimised conditions to preserve grain quality and retain the beneficial antioxidant compounds.

1.6. Carbohydrate digestibility

Starch is one of the major types of carbohydrate and is formed from two types of polymers: amylose and amylopectin. The amylopectin content of straight chains of glucose units are linked by α-1,4 glycosidic bonds and branch into small glucose chains at the α-1,6 bonds. Amylose is a straight chain of α-1,4 glucans and has limited branching units at the α-1,6 patterns. It has been found that chain length and branching pattern, as well as the ratio of amylose to amylopectin, may play important roles in the digestibility of carbohydrates (Lehmann and Robin, 2007; Kam et al. 2016).

Carbohydrates are the main energy source in the human diet. Reductions of the calorie intake, the inhibition of metabolism or the absorption of carbohydrates can help to combat clinical conditions such as obesity and type-2 diabetes, through reduction of blood glucose levels (Mahmood, 2016). Carbohydrates are broken down into monosaccharides before being absorbed by the body, by two main enzymes: amylase and glucosidase. Dietary carbohydrates that consist of monosaccharide units are found to have a high glycaemic index (GI) and are, therefore, absorbed quickly, whereas carbohydrates with a low GI are absorbed more slowly and are most beneficial to individuals with type-2 diabetes, as high GI foods cause a rapid and large increase in blood glucose levels (Mahmood, 2016). As an alternative to low GI food, a variety of products exit that are able to promote slower absorption of carbohydrates more by inhibiting the above enzymes. It has been shown that the polyphenols contained in

millet may be effective in managing type-2 diabetes by inhibiting starch-digesting enzymes (Annor et al. 2017).

1.6.1. Starch classifications

Dietary starch plays a cardinal role in supplying the metabolic energy required to execute the myriad of physiological processes enabling human health and function. Starch is generally classified into three different groups: rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS), based on the rate and extent of its digestibility. Starch fractionation is a technique that can be used to characterise the digestive property of starch *in vitro* – i.e. the Englyst method, whereby SDS is the fraction digested within 20 to 120 minutes of incubation, RDS is the fraction digested within 20 minutes of incubation and RS is the remaining undigested starch (Miao et al. 2015).

1.6.1.1. Slowly digestible starch

SDS is slowly digested in the small intestine to release glucose, which takes relatively longer to enter the bloodstream and thereby elicits a lower glycaemic response (GR). This is because the high semi-crystalline structure of SDS makes it less susceptible to metabolism by intestinal enzymes (Englyst et al. 2003). This type of starch is therefore useful for preventing diseases related to hyperglycaemia, such as diabetes. SDS is naturally found in most raw cereals, such as corn, barley and rye (Aller et al. 2011; Miao et al. 2015).

1.6.1.2. Rapidly digestible starch

RDS can be rapidly digested and absorbed in the duodenum and proximal small intestine, to result in a rapid rise of blood glucose. This increases the risk of damage to cells, tissues, and organs, which can result in diabetes and cardiovascular diseases

(Aller et al. 2011; Miao et al. 2015). RDS is found in freshly cooked starch, such as fresh white bread and potato (Miao et al. 2015).

1.6.1.3. Resistant starch

RS cannot be digested in the small intestine, although it is fermented as dietary fibre in the colon. This type of starch may improve colonic health by producing short-chain fatty acids capable of providing extra energy to the body, along with butyric acid, which is also linked to colonic health (Aller et al. 2011).

Dietary fibre is a non-digestible carbohydrate, classified into soluble and insoluble fibre. It is widely known that fibre plays a vital regulatory role in glycaemia. Food that is rich in dietary fibre (especially soluble fibre) is highly recommended for individuals with type-2 diabetes, as this type of fibre reduces enzyme access to its substrates via viscosity effect (Slavin, 2005; Kam et al. 2016).

Currently, millet is receiving increasing attention from researchers focusing on means of preventing type-2 diabetes as it is of high nutritional value and a rich source of phytochemicals and micronutrients. Millet notably has higher levels of SDS and dietary fibre compared to other cereals (Kam et al. 2016; Saleh et al, 2013).

1.7. Development of millet-based food products and sensory evaluation

1.7.1. Sensory evaluation

Sensory evaluation is a scientific method widely used to measure, analyse and interpret human responses to products through the five senses – sight, smell, touch, taste and hearing – in order to identify key attributes that may confer acceptance of the product by consumers (Beinner et al. 2010).

Sensory evaluation is an important tool used in the development of food products and their evaluation during storage. Sensory studies determine how changes in storage time affect different sensory attributes and consumers then evaluate whether these changes affect the acceptability of products (Cadena et al. 2013). Sensory evaluation is also an important factor in fortified foods with functional compounds in order to promote their acceptability by the target population (Bovell-Benjamin and Guinard, 2003). For example, food products fortified with iron help to reduce the current high prevalence of iron deficiency in the developing world. On the other hand, the fortification of food with iron has shown to cause discoloration and an unacceptable taste (Bovell-Benjamin and Guinard, 2003). In order to improve the consumer acceptance of these products, sensory evaluation programmes must be implemented to improve the sensory attributes of fortified foods (Bovell-Benjamin and Guinard, 2003).

1.7.2. Classification of sensory methods

The scientific methods involved in sensory evaluation are classified based on their primary aims. In general, scientists employ two method types: analytical and affective evaluation. The analytical method comprised of two sub-classes of tests: descriptive and discriminative (Garruti et al., 2012).

1.7.2.1. Analytical evaluation

1.7.2.1.1. Discrimination tests

Discrimination is a technique used to determine any detectable differences or similarities between two or more products at the same time. This method itself involves conducting several types of tests; for instance, the triangle test, which is used to determine the overall difference between two products (where two of the products are identical and one is different). Another test, the duo-trio test, is used to determine

any sensory difference between two products and is whether this occurs as a result of changing any of the ingredients, packaging, processing and/or storage. Products are also ranked in order of preference (Garruti et al. 2012; Zoecklein n.d; Kemp et al. 2009).

1.7.2.1.2. Descriptive tests

Descriptive tests are used to evaluate the qualitative and quantitative sensory aspects of food products (Garruti et al. 2012). This type of test offers more complicated, informative and comprehensive tools to identify the underlying ingredients and process variables, as well as other research questions, during the development stage of the food product (Garruti et al. 2012).

1.7.2.2. Affective tests

Affective tests include acceptance and preference testing. Preference tests (such as paired reference and ranking) are normally used to determine a customer's preference for one product over another. Acceptance tests, on the other hand, quantify the degree to which a product is liked or disliked and these include the hedonic scale and open-ended questions (Garruti et al. 2012).

1.7.3. Sensory attributes of millet-based products

Limited studies have been conducted on the sensory evaluation of millet-based products (McSweeney et al. 2016).

A study by Obatolu (2002) involved sensory evaluation of a product prepared by mixing pearl millet with soybeans (millet was mixed with soybean, then extruded and hot water was added to produce a gruel (15g/100ml water)). The purpose of this was to increase the nutritional value of the product. The authors reported that product was acceptable overall; however, the colour received a low rating, which made the product

ultimately unacceptable. The change in colour may have been a result of adding millet to the product, which causes an undesirable dark brown colour. However, in a recent study, varieties of germinated millet flour were reported as acceptable to consumers and the germination process actually improved the nutraceutical value of the product (Amadou and Moussa, 2018). All the germinated millet in the authors' study was produced under the same conditions; thus, the difference in results have been related to millet species and different processing methods (Amadou and Moussa, 2018).

It has been generally shown that removing gluten from baked products can impair their quality. Interestingly, however, cake baked with (gluten-free) millet displays more nutritional value and good sensory characteristics with an increased millet to wheat ratio (Emmanuel and Sackle, 2013). A study by Shukla and Srivastava (2014) found that when finger millet flour was used at two different percentages (30% and 50%) in refined wheat flour, and integrated with noodles, the 30% mixture was preferred for its sensory attributes. Moreover, data on the sensory evaluation of biryani – a famous Indian dish prepared with rice – mixed with foxtail millet showed high acceptability compared to biryani made from barnyard millet and rice. There were also no significant differences between halwa and laddu (a traditional Indian sweet) made from foxtail millet or barnyard millet compared to rice on its own (control) in terms of colour, texture, taste, appearance and overall acceptability (Verma et al. 2015).

It should be noted that the aforementioned studies involved participants from India and Africa who were familiar with millet varieties and its characteristics and, as such, were used to its consumption. Reduced or non-availability of millet-based products that are ready to consume in countries not normally producing millet unfortunately limits its wider consumption and acceptability by the wider population. Therefore, millet is limited to local consumers and people in the lower economic strata (Verma et al.,

2015). In order to popularise millet, there may be a greater requirement for nutritional education that emphasises the health benefits of including millet in the daily diet and this may encourage food companies to market millet-based products as healthy and gluten-free food alternatives.

1.8. Potential metabolic health benefits of millet: the evidence

Millet has many potential health advantages and epidemiological investigations have found that consumption of millet can also reduce the risk of diseases prevalent in the Western world, such as cardiovascular disease (Saleh et al. 2013). Millet may also play an important role in preventing type-2 diabetes, lowering blood pressure and increasing the time for gastric emptying (Chandrasekara and Shahidi, 2012; Saleh et al. 2013). The nutritional factors accounting for the overall health benefit of millet include enrichment of polyphenols, antioxidants, resistant starch and essential lipids (Chandrasekara and Shahidi, 2012).

1.8.1. Millets and cardiovascular disease

It has been demonstrated that consuming millet is beneficial for people at risk of cardiovascular disease. Proteins concentrated in foxtail and proso millet significantly improve the plasma levels of adiponectin and beneficial high-density lipoprotein (HDL) cholesterol, which in turn decrease insulin levels and restore insulin sensitivity in type-2 diabetic mice (Park et al. 2008; Rao et al. 2017). High levels of adiponectin also protect cardiovascular tissues by stimulating endothelial cell responses and inhibiting pro-inflammatory and hypertrophic mechanisms (Kumar et al. 2018). It has also been found that the starch in barnyard millet lowers blood levels of glucose and triglycerides and serum cholesterol unlike rice and other varieties of millet (Saleh et al. 2013). Finger millet and proso millet may play an important role in the prevention of cardiovascular disease, as evidenced by their reducing effect on plasma triglycerides in

hyperlipidaemic rats (Lee et al. 2010). A possible mechanism underlying the effectiveness of millet in reducing plasma triglycerides and low-density lipoprotein (LDL) may be improvement of cholesterol metabolism (Kumar et al. 2018). Pearl millet is also shown to regulate blood pressure and thereby relieve stress on the heart, due to a high magnesium content (Rao et al., 2017). Moreover, in 2012 Chandrasekara and Shahidi assessed phenolic extracts taken from different types of millets (finger, kodo, little, foxtail, pearl and proso) for their inhibitory effects on lipid peroxidation in *in vitro* copper-mediated oxidation of human LDL cholesterol and through the use of different food models such as stripped corn oil, cooked pork and emulsion of linoleic acid. It was found that millet extracts at a final concentration of 0.05mg/ml inhibited oxidation of LDL cholesterol by between 1% and 41%. All millet varieties showed effective inhibition of lipid peroxidation in food systems and kodo millet was found to produce the maximum inhibition of lipid peroxidation, similar to that observed with use of butylated hydroxyanisole at 200 ppm (Chandrasekara and Shahidi, 2012). Further research is needed to investigate the effects of millet intake on cardiovascular disease in humans, as most studies conducted so far have focused on rodents.

1.8.2. Millets and diabetes

Laboratory-synthesised inhibitors of alpha-glucosidase and pancreatic amylase have been shown to be effective in the management of postprandial hyperglycaemia. However, natural inhibitors may offer wider benefits than synthetic versions, since the intake of food rich in whole grains is found to be beneficial in prevention or/and management of type 2 diabetes. This has been shown through epidemiological studies in millet-consuming populations, which experience lower incidences of the disease (Saleh et al., 2013). For example, the consumption of millet-based food has been reported to significantly lower the level of plasma glucose, peak rise, and the area

under the curve (AUC). These results might be due to the content of fibre and polyphenols in millet-based food (Saleh et al. 2013).

Dietary management could be a successful way to monitor diabetes and its complications. According to ADA guidelines, an ideal diet for those with diabetes and prediabetes should be low in fat and high in complex carbohydrates and fibre, in an attempt to reduce postprandial hyperglycaemia and serum cholesterol levels (McMacken and Shah, 2017). It is also recommended that whole grains, legumes, fruits, and vegetables are used as a source of carbohydrates (McMacken and Shah, 2017). Many studies have shown that carbohydrates, together with high amounts of fibre, may improve both glucose tolerance and insulin sensitivity. Moreover, diets rich in complex carbohydrates are beneficial in delaying GE, which is important for type-2 diabetic individuals.

A number of studies have suggested the potential benefit of adding finger millet to the diet of type-2 diabetic individuals due to its high polyphenol content and dietary fibre. As finger millet also has a low GI, it can be consumed as a healthy snack or meal that maintains blood glucose at a steady level (Chandra et al. 2016). In a study of six type-2 diabetic subjects, consuming finger millet-based foods, such as *chapatti* (flatbread), *dosai* (rice pancake), *sevai* (rice string hoppers) and *idli* (fermented, steamed rice cake) for one month significantly reduced postprandial blood glucose levels (Chandra et al. 2016).

An interventional study by Lakshmikumari and Sumathi (2002) found that a diet rich in finger millet significantly lowered blood glucose levels due to a higher fibre content than rice and wheat. Other studies confirmed that finger millet lowers GR in type-2 diabetic individuals compared to rice; specifically, *roti* -based finger millet offers better glycaemic control compared to *idli* and *upma* made from finger millet (Thakkar

and Kapoor, 2007; Urooj et al. 2006). Regular consumption of finger millet-based foods was shown to reduce the level of fasting blood glucose by 32% and decrease insulin resistance by 43% in type-2 diabetes individuals (Chandra, 2016).

Rajasekaran et al (2004) reported studies in which skin wound-healing parameters were found to improve after feeding of finger millet for rats. Wound healing in diabetic rats is known to be impeded by increased levels of oxidative stress and a decreased presence of antioxidants. In this study, a diet of finger millet for four weeks was observed to increase levels of antioxidants, which aided in the healing process for dermal wounds, and in controlling glucose levels (Rajasekaran et al., 2004).

1.9. Progression from prediabetes to type-2 diabetes and cardiovascular disease

1.9.1. Prediabetes: A high- risk state for metabolic syndrome

In 2013, almost 3.2 million individuals in the UK alone had been diagnosed with type-2 diabetes and, if this remains unaddressed, a further five million sufferers are projected for 2025 (Diabetes UK, 2015). Diabetes mellitus is defined as a lifelong metabolic disorder characterised by hyperglycaemia, changes in the carbohydrate and protein levels and an affected lipid metabolism. It is the most common endocrine condition that either leads to reduced insulin production (type-1 diabetes) or development of resistance to insulin activity and the insulin secretory response (type-2 diabetes) (American Diabetes Association, 2009). Prediabetes is clinically diagnosed upon presentation of a higher than normal fasting blood glucose but which does not pass the threshold for diagnosis of diabetes. Prediabetes, therefore, greatly increases the risk of developing type-2 diabetes and its complications (American Diabetes Association, 2018). Annually, around 5% to 10% of people with prediabetes develop

type-2 diabetes. This is because insulin resistance begins years before the onset of type-2 diabetes, and decreased beta-cell function already exists in the prediabetes phase (Tabák et al., 2012). Different studies of both insulin sensitivity and secretion in individuals who presented with similar levels of blood glucose showed that the development from normal glucose tolerance to type-2 diabetes was an ongoing process (Tabák et al., 2012). In individuals who developed type-2 diabetes, increased amounts of blood glucose had been observed at the beginning of the follow-up stage (13 years before the diagnosis of diabetes), although glucose levels had been controlled within the normal range until two to six years before diagnosis, when a sharp increase had been observed (Tabák et al., 2012).

Weir (2004) drew up a multi-stage model of type-2 diabetes development that predicted Tabák's observations. According to Weir's model, the first stage of diabetes development consists of a long period during which insulin resistance exists and is accompanied by compensatory increased rates of insulin secretion and increased β-cell masses in order to maintain normoglycaemia. The second stage, named the "stable adaptation period", occurs when glucose levels start to increase (reaching approximately 5.0mmol/l to 6.5 mmol/l). During this stage, β-cells are unable to compensate fully for increased insulin resistance; consequently fasting and/or post-load glucose levels are not completely controlled. Much of the first and second phases occur before the pre-diabetic stage is achieved. The third stage of diabetes development is called the early unstable period of decompensation, during which glucose levels rise comparatively rapidly due to the inability of the β-cells to compensate for insulin resistance. This stage is likely to extend from prediabetes to manifest diabetes.

Prediabetes is also associated with an increased risk of vascular disease, but this matter

remains unclear if the disease risk depends on the development of diabetes. Cross-sectional studies agree that there is a high prevalence of coronary disease among prediabetic individuals who present with fasting or postprandial hyperglycaemia (Tabák et al., 2012).

Prediabetes treatment must include prevention of development into diabetes as well as reduction of the effects of some possible consequences of progression to diabetes (Tabák et al., 2012). Synthetic inhibitors of alpha glucosidase and pancreatic amylase synthesis are currently used clinically to manage postprandial hyperglycaemia; however, it has been suggested that natural inhibitors – i.e. compounds extracted from plants – may be safer for patients (Saleh et al. 2013). Regular consumption of whole grain-based foods is thought to be beneficial in helping manage or even prevent type-2 diabetes, as shown by a low incidence of type-2 diabetes in millet-consuming populations (American Diabetes Association 2010; Shobana et al. 2009; Kim et al. 2011). For instance, finger millet extracts show significant inhibitory effects on α-glucosidase and pancreatic α-amylase. The cover of the grain is rich in polyphenols, such as gentisic acid, ferulic acid, caffeic acid, vanillic acid and protocatechuic acid, which ultimately cause a reduction in postprandial blood glucose (Shobana et al. 2009).

1.9.1.1. Glycaemic response

Dietary carbohydrates normally contribute the most energy in the human diet. The glycaemic response (GR) to a food is defined as the effect that food has on blood glucose levels after consumption of a carbohydrate-rich meal. Normally, both blood glucose and insulin levels increase after ingestion of carbohydrates and then rapidly return back to fasting levels (after around 2h) (Sadler, 2011). In individuals with type-2 diabetes or prediabetes, it is important that the duration of the increase in blood

glucose after consumption of carbohydrates is prolonged (with low peak) and this is also beneficial for the general population (Sadler, 2011).

The glycaemic index (GI) was developed to classify carbohydrate-rich foods according to the effect they have on postprandial glycaemia (Sadler, 2011; Augustin et al. 2015). In the early 1980s, the GI was defined as a numeric method of demonstrating the effect of dietary carbohydrates on the concentration of blood glucose (Sadler, 2011). Since then, foods containing variable amounts of carbohydrates have been linked to different GRs in the blood. GI studies have, in fact, shown that similar quantities of carbohydrate-rich food may elicit different GRs in individuals. Although pharmacological methods have become useful for controlling glycaemia in type-2 diabetes, there is evidence of additional improvement from consuming a diet with a lower GI (Sadler, 2011; Augustin et al. 2015). Indeed, high GI diets are associated with an increased risk of type-2 diabetes (Castro-Acosta et al. 2016).

Millet is a low GI grain and is higher in nutritional content compared to other cereals (Kam et al. 2016). Thus, a number of studies have found that millet with a moderate GI is a suitable food replacement for individuals with type-2 diabetes (Shobana et al. 2007; Lakshmikumari and Sumathi, 2002).

1.9.1.2. Insulin response

Insulin is a hormone made by the beta islet cells of the pancreas that is then released into the circulation. After the consumption of carbohydrate-rich food, blood sugar levels increase as the digestive tract breaks down carbohydrates into their base molecules (sugars and starches into glucose). As glucose levels increase, insulin is released in response (NIH, 2017); however, insulin resistance occurs when the cells do not respond to insulin and, therefore, cannot absorb excess glucose from the bloodstream. As a result, the pancreas is stimulated to produce more insulin to help

clear glucose from the blood into cells. However, when the insulin still fails to transport glucose into cells, there becomes an excess of glucose in the blood and this can eventually lead to prediabetes and type-2 diabetes (NIH, 2017). It has been suggested that consuming whole grain based-foods, such as millet, may help prevent and manage type-2 diabetes (Saleh et al. 2013) and lowering dietary GI in individuals with hyperinsulinemia may reduce fasting blood glucose and insulin levels (Sadler, 2011).

1.9.1.3. Satiety

Low GI food has been shown to increase short-term (1 day or less) markers of satiety, (Bornet et al. 2007). However, it remains unknown whether this effect is directly due to changes in the GR (Sadler, 2011).

Reduction in appetite and an increase in satiety occur via two mechanisms, as explained by Lobos et al. (2017). First, low GI foods maintain blood glucose levels and other metabolic fuels for longer than high GI foods and, therefore, promote satiation signals to the brain to determine the end of a meal. Secondly, low GI foods are digested more slowly, which allows the nutrients to be kept in longer contact with the intestinal cells, potentiating the release of incretins and other endocrine mediators as a response. This effect of gut hormones leads to an increased feeling of satiety in individuals (Lobos et al. 2017). However, further research is required to determine the relationship between GI, satiety and food consumption in the long term (Sadler, 2011). The high dietary fibre and phenolic content in finger millet makes it highly beneficial for individuals with diabetic conditions. Moreover, finger millet has a low GI, which will achieve better control of the blood glucose response after a meal (Chandra et al. 2016).

1.9.1.4. Gastric emptying

GE is a measure of the time required for the stomach to be emptied of food (Markey et al. 2011). It has been found that the GE rate depends on whether solids or liquids are ingested and the macronutrient content (i.e. the amount of carbohydrates, fats or proteins). For instance, fat significantly slows GE due to its high calorie content (Phillips et al., 2015). The emptying rates of liquids and solids are markedly different, with the former emptying at an 80% faster rate (Phillips et al. 2015).

It has been shown that acute changes in the concentration of blood glucose have a major effect on gastric motor function and GE; there is an inverse relationship between the two, signifying that, in individuals with type-2 diabetes with high blood glucose, gastric emptying is slow and thus may decrease postprandial glycaemic excursions, as fast emptying is known to increase postprandial glycaemic excursions (Marathe et al. 2013). In contrast, GE is more rapid in the early stages of type-2 diabetes and then slows with solids in the later stages of the disease (Smith, 1996).

Numerous studies have shown that millet based-foods are filling and provide plenty of energy; this is supported by evidence *in vitro* and *in vivo* that starch digestion is relatively slow after consumption of millet-based food. It has been found that the satiating effects of millet-based foods may be attributed to a change in the rate of GE, which is a key determinant of macronutrient delivery to the body and positively corresponds to the GR (Wilbaux et al. 2017; Horowitz et al. 1993).

Different factors have been found to directly affect the rate of GE, such as nutrient content, macronutrient properties, bolus volume and physical properties (e.g, texture, viscosity and particle size) (Cisse et al. 2018). Cisse et al. (2018) recently found that traditional West African millet-based porridges and couscous were associated with a slower rate of GE in healthy individuals compared to non-traditional starchy foods, such as wheat pasta, rice and potato, which have only recently become available for

consumption in urban areas of Africa. It has been shown that slow GE is associated with satiety and sustained energy in West Africa and that, as a result of increasing urbanisation, the diet of the general population has drastically changed and is now associated with increasing obesity and various metabolic diseases. The authors also reported that slow GE of traditional foods prepared with millet are beneficial in increasing satiety and providing energy for longer from a single meal (Cisse et al., 2018).

1.9.2. Finger millet and diabetes: the evidence

Dietary management could be a successful way of monitoring diabetes and its complications. According to ADA guidelines, an ideal diet of those with diabetes and prediabetes should be low in fat and high in complex carbohydrates and fibre, in an attempt reduce postprandial hyperglycaemia and serum cholesterol levels (McMacken and Shah, 2017). It is also recommended that whole grains, legumes, fruits, and vegetables are used as a source of carbohydrates (McMacken and Shah, 2017). Many studies have shown that carbohydrates, together with high amounts of fibre, may improve both glucose tolerance and insulin sensitivity. Moreover, diets rich in complex carbohydrates are beneficial in delaying GE, which is important for type-2 diabetic individuals.

A number of studies have suggested the potential benefit of adding finger millet to the diet of type-2 diabetic individuals due to its high polyphenol content and dietary fibre. As finger millet also has a low GI, it can be consumed as a healthy snack or meal that maintains blood glucose at a steady level (Chandra et al. 2016). In a study of six type-2 diabetic subjects, consuming finger millet-based foods, such as *chapatti* (flatbread), *dosai* (rice pancake), *sevai* (rice string hoppers) and *idli* (fermented, steamed rice cake) for one month significantly reduced postprandial blood glucose levels (Chandra et al.

2016).

An interventional study by Lakshmikumari and Sumathi (2002) found that a diet rich in finger millet significantly lowered blood glucose levels due to a higher fibre content than rice and wheat. Other studies confirmed that finger millet lowers GR in type-2 diabetic individuals compared to rice; specifically, *roti* -based finger millet offers better glycaemic control compared to *idli* and *upma* made from finger millet (Thakkar and Kapoor, 2007; Urooj et al. 2006). Regular consumption of finger millet-based foods was shown to reduce the level of fasting blood glucose by 32% and decrease insulin resistance by 43% in type-2 diabetes individuals (Chandra, 2016).

1.10. Conclusion

In conclusion, this literature review has highlighted the importance of millet for human consumption as it has many nutritional and health benefits. Moreover, due to its high content of polyphenols, antioxidants and fibre, it can help manage and even reduce the risk of type-2 diabetes. It is, therefore, important that a commercial process be developed to increase millet consumption in large urban populations in the form of safe, ready-to-eat and ready-to-cook meals. Currently, millet consumption remains limited to rural households.

1.11. Gaps in the knowledge, novelty and aims of this PhD

Millet was known in ancient times for its uniquely high content of nutrients beneficial to human health. Although some *in vitro* and *in vivo* research is available on millet, some gaps in knowledge have been identified after critical review of the literature on this subject. Numerous studies have examined in detail the polyphenol content of different types of millet (Chandrasekara and Shadidi, 2011); however, the various millet types have as yet not been compared in their different forms in terms of

polyphenol and antioxidant content. Moreover, the majority of previous studies on millet have used traditional food products in countries such as India, Africa and Sudan, which are generally unknown in the Western world and thus unlikely to be used there. Comparing the glycaemic and gastric parameters in healthy individuals and type-2 diabetics after millet consumption is an important way to gain insight into how to manage the condition, prevent it or reduce its secondary complications. However, it is also crucial to focus on individuals who are at high risk of developing type-2 diabetes – i.e. prediabetics – as treating this group of people early may be key in preventing the development of type-2 diabetes. To the best of the author's knowledge, there are currently no available studies analysing the effect of millet consumption on the GR, IR, GE and satiety in people with prediabetes. Moreover, the majority of available studies comparing healthy individuals with type -2 diabetics used small sample sizes.

Study 1 here, therefore, aimed to determine the level of polyphenols and antioxidants in seven types of millet in different forms: grain, flour, and flakes. The aims for Study 2 were to use the form of millet that showed the highest polyphenols and antioxidant content to prepare muffins and determine their effect on *in vitro* starch digestion. Further to this, a sensory evaluation test of these millet muffins was conducted and compared to a wheat-based muffin to determine which millet muffin was most acceptable. Study 3 aimed to systematically review the intervention studies investigating the effects of consuming millet on markers of type-2 diabetes. The primary aim of the final study (Study 4) was to determine the effect of finger millet muffin consumption (which was concluded by the sensory study to be the most acceptable) on the GR, IR and GE in prediabetes individuals, in comparison to healthy controls. A secondary aim was to evaluate the potential of finger millet muffin consumption in improving satiety.

Chapter 2: Determination of the polyphenol and antioxidant activity of different types and forms of millet (Study 1)

2.1. Introduction

Millet grains are thought to have been the first cereal cultivated thousands of years ago by early humans (Shahidi and Chandrasekara, 2013). Millet is a staple food in some populations, most notably the lower income Asian and African countries, and certain Western countries, such as Canada and the USA, also use millet grain as birdseed and feed for livestock (Shahidi and Chandrasekara, 2013). At present, the popularity of these grains is rising among wheat-intolerant people due to their gluten-free composition. In addition, millet has proved unique among other cereals due to its remarkably high nutrient and non-nutrient content (Amadou et al. 2013); it contains high levels of polyphenols, antioxidants, protein, dietary fibre and calcium (Amadou et al. 2013), while also boasting high levels of B vitamins, minerals and resistant starch (Talukder and Sharma, 2015; Shahidi and Chandrasekara, 2013).

Currently, there is renewed interest in polyphenols and antioxidants due to their importance in maintaining optimal physiological function of numerous organ systems during different phases of life and reducing the risk of chronic diseases (Devi et al. 2014). Polyphenols are secondary plant metabolites that occur naturally and are largely found in cereals, vegetables (e.g. dry legumes), fruit (e.g. apples), beverages (e.g. coffee) and chocolate (Pandey and Rizvi, 2009). They are divided into four groups: phenolic acids, flavonoids, stilbenes and lignans (Pandey and Rizvi, 2009). According to Rao and Muralikrishna (2002), the major polyphenols, such as phenolic acid and tannins, are found in millet grain, among other cereals, and many of these components are shown to contribute to biological antioxidant activity (Devi et al. 2014).

Epidemiological studies have found that the daily consumption of foods high in polyphenols may be protective against many chronic diseases, such as cardiovascular disease, diabetes and cancer (Graf et al. 2005).

An antioxidant is a substance that contributes to the physiological defence system against reactive oxygen species (ROS) (Suma and Urooj, 2012) by delaying or inhibiting cellular damage via mechanisms including free radical scavenging. Some antioxidants are endogenously produced in the body as a result of normal metabolism, while others can only be obtained from certain foods. It has been shown that intake of food that is high in antioxidants might improve glycaemic control; this is because postprandial hyperglycaemia is connected with the formation of ROS as well as with oxidative stress. Moreover, antioxidants might reduce postprandial glucose response by their direct action in the gastrointestinal tract to decrease the digestion and absorption of carbohydrates and their direct reduction of postprandial oxidation (Chepulis et al., 2016). High levels of antioxidants have been found in some grains and grain-based products (Yu et al. 2002). Furthermore, much attention has been paid to the investigation of the nutraceutical and antioxidant properties of different millet varieties (Saleh, 2013). However, there is still only limited published data on antioxidant activity of different types of millets prepared in different forms (Suma and Urooj, 2012).

2.1.1 Aims and hypothesis

The polyphenol and antioxidant content of different types and forms of millet differ as a result of many factors, such as the processing (e.g. milling, soaking, germination, fermentation etc.), the shape of the grain, colours or even environmental factors during growth. The effect of these factors may vary (small or even absent), may maintain the bioactive compounds or lead to their loss (Nickel et al. 2016). Thus, it becomes

important to determine the levels of polyphenols and antioxidants in different millet varieties in common forms in order to obtain the most benefit from those foods when consumed by healthy and prediabetes adults.

It is hypothesised that, overall, millets are rich sources of polyphenols and antioxidants and the levels of these compounds may vary between types and forms. Therefore, the present study aims to determine the antioxidant and polyphenol content of seven common types of millet prepared in different forms.

2.2. Methods and materials

2.2.1. Materials

All chemicals and reagents were purchased from Sigma-Aldrich (Poole, UK). All millet grains (in different types and forms) were purchased from Madha Internationalz (Tamilnadu, India).

2.2.2. Study protocol

Seven of the most commercial varieties of millet grown in India were used in this study: finger, proso, pearl and foxtail as a major types and little, kodo and barnyard as the minor types. Three different forms of the above types were used: grain, flour and flakes – as these forms are likely to be most commonly used in cooking (Figures 2.1, 2.2 and 2.3). However, kodo and proso millets in flake form were not available from the supplier due to crops being destroyed by floods. All millet samples were delivered in pure form, free of contaminants.

Figure 2.1: Different types of millet grains

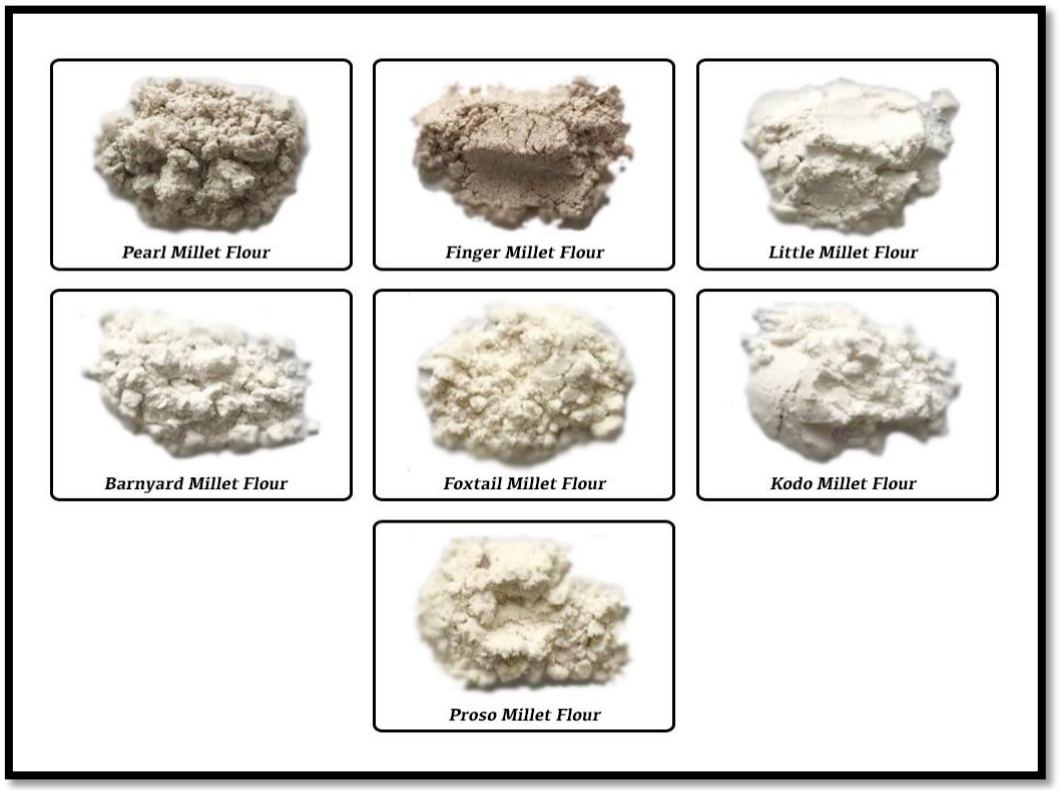


Figure 2.2: Different types of millet flour

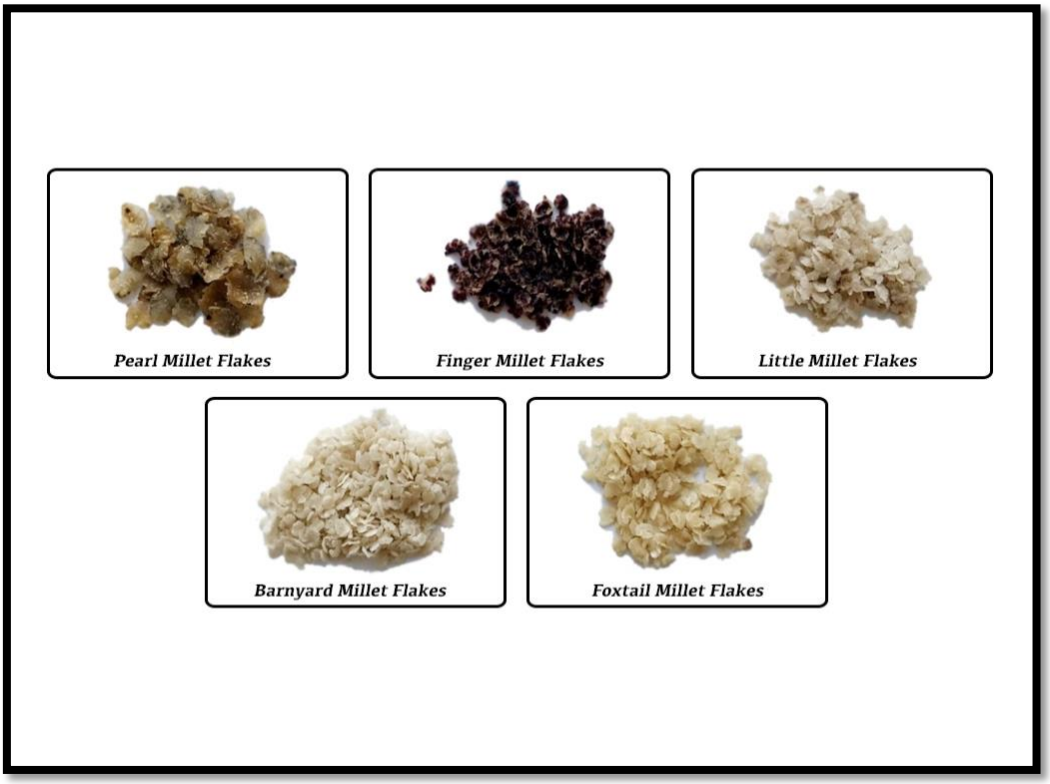


Figure 2.3: Different types of millet flakes

2.2.3. Analysis of the polyphenol and antioxidant content

2.2.3.1. Sample preparation and extraction

Each millet sample (grain and flakes) was ground immediately and in the same way, before extraction using an electric mill (Moulinex 530/380 W). A 1g sample of each type of millet in three different forms (2.2.2) was added to clean, waterproof amber bottles. The exact weight of each sample was noted and repeated in triplicate. Then, 4 ml of solvent (70% acetone) was added to each bottle, the lid securely tightened and parafilm was used around the lid to maximally prevent oxidation of the sample. All amber bottles were incubated at room temperature in a shaking water bath for 2h (Thondre et al. 2011). After incubation, all samples were transferred to glass test tubes. The millet extracts were centrifuged at 3000 × g for 10min using a bench-top centrifuge (Heraeus Instruments, Kendro Laboratory Products, D-37520 Osterode,

Germany) and the supernatant was used to determine the antioxidant activity and total phenolic content of each grain. The extracts were covered with foil to protect them from light and frozen at -20°C until analysis.

2.2.3.2. Total polyphenol content

The Folin-Ciocalteu method was used to calculate the total polyphenol content of the millets. This method is widely used for such an analysis because it is easy to perform, fast, inexpensive, and applicable in routine laboratory use (Blainski et al., 2013).

To an aliquot of millet extract (200µL), 1.5ml of freshly prepared Folin-Ciocalteu reagent (FCR) (1:10 v/v with water) was added. The mixture was allowed to equilibrate for 5min and then mixed with 1.5mL of 60 g/l sodium carbonate solution. After incubation of the mixture in the dark for 90min at room temperature, the absorbance of the mixture was measured at 760nm (using 70% acetone as a blank) using a spectrophotometer (UV-1800-Shimadzu (Figure 2.4). Gallic acid (10 mg/10 mL of 70% acetone) was used as a standard. The result was expressed as µg of gallic-acid equivalent (GAEs) per gram of millet sample (Coe et al. 2013).

2.2.3.3. Ferric-ion-reducing antioxidant power (FRAP) assay

FRAP reagent was freshly prepared from 300mM of acetate buffer (pH 3.6), 20mM of ferric chloride and 10mM of tripyridyl-s-triazine (TPTZ) made up in 40mM of HCl. All solutions were mixed together at a ratio of 10:1:1. 1ml of purified water was added to each tube and then incubated at 37°C for 5min. 25µL of millet extract or standard was added to 1.0mL of purified water and the FRAP mixture was then added to each tube. To perform the assay, the mixture was incubated at 37°C for 4min. Ferrous sulphate (1000µM) was used as a standard. Absorbance was measured at 593nm using a spectrophotometer (UV-1800-Shimadzu; Figure 2.4), using FRAP reagent as a blank (Ryan et al., 2011).

Figure 2.4: Spectrophotometer

2.2.4. Statistical analysis

Data was analysed using Microsoft Excel 2010. Statistical analysis was undertaken using the Statistical Package for Social Sciences (SPSS, version 23, USA). All experiments were carried out in triplicate and all values are expressed as mean ± SD, unless otherwise specified. Before statistical analysis, data was tested for normality using a Shapiro-Wilks test; where data was not normally distributed, non-parametric tests were used.

Based on the results of the antioxidant normality tests, a one-way between group analysis (ANOVA), an independent sample t test, a Kruskal Wallis test or a Mann-Whitney test were used to compare (inter- and intragroup) the same millet extract and different millet forms. Comparisons between polyphenol samples were carried out using ANOVA, an independent sample t test or a non-parametric Kruskal Wallis test. Statistical significance was set at $p < 0.05$. Correlation analysis was performed using Pearson's correlation. In the non-parametric test, the statistical significance was set at $p < 0.001$that based on the number of comparisons (using Bonferroni adjustment).

2.3. Results

2.3.1. Total polyphenol content of different types and forms of millet

The results for the total polyphenol content of different types and forms of millet ranged from 0.22 to 4.44 mg GAE/g (Table 2.1).

In grain form, kodo millet grain extracts contained significantly ($p < 0.001$) greater amounts of polyphenols, followed by finger millet and pearl millet. However, these three types of millets were not significantly different from each other. In flour form, finger millet extracts recorded significantly greater ($p < 0.001$) amounts of polyphenols, followed by pearl and proso millet, compared to other millet types. Total polyphenol content in pearl millet flakes, followed by finger millet, was significantly higher than in other types; however, finger millet was not statistically different from little millet in this measure ($p > 0.001$).

Table 2.1: Total polyphenol content (mg GAE/g) of each type and form of millet (mean ± SD)

	Millet form		
Millet Type	**Grain**	**Flour**	**Flakes**
Pearl millet	$2.15^{a,4,6} \pm 0.22$	$1.59^{b,2,6} \pm 0.23$	$1.76^{b,1} \pm 0.06$
Finger millet	$3.72^{a,4,6} * \pm 0.14$	$2.55^{b,2,3} * \pm 0.20$	$1.27^{c,3} \pm 0.14$
Little millet	$1.51^{a,2} \pm 0.21$	$0.52^{b,4} \pm 0.06$	$0.75^{c,2} \pm 0.03$
Barnyard millet	$1.41^{a,2} \pm 0.11$	$0.51^{b,4} \pm 0.03$	$0.69^{c,2,4} \pm 0.03$
Foxtail millet	$0.98^{a,2,3} \pm 0.06$	$0.29^{b,4,5} \pm 0.02$	$0.63^{c,2,4} \pm 0.05$
Kodo millet	$4.44^{a,1} * \pm 0.15$	$0.22^{b,1} \pm 0.04$	N/A
Proso millet	$1.14^{a,2,5} \pm 0.11$	$0.54^{b,2} \pm 0.04$	N/A

Data are expressed as mean ± SD of replicate measurements
Means followed by different lower-case letters in each row are significantly different ($p < 0.001$)
Means followed by the same lower-case letters in each row are not significantly different ($p > 0.001$)
Means in each column followed by number:
1 is significantly different with means followed by number 2
3 is significantly different with means followed by number 4
5 is significantly different with means followed by number 6
* = significantly greater than all other samples (ANOVA, $p < 0.05$)
N/A= not available

2.3.2. Total antioxidant content of different types and forms of millet

In grain form, kodo millet grain extracts contained significantly ($p < 0.001$) greater amounts of antioxidants, followed by finger millet and pearl millet. However, these three types of millet were not significantly different from each other (Table 2.2). Total antioxidant content in finger millet flour, followed by pearl millet, was significantly higher than in other types; however, finger millet was not statistically different from proso millet ($p > 0.001$). In flake form, finger millet extracts recorded significantly greater ($p < 0.001$) amounts of antioxidants, followed by pearl millet, compared to other millets types, although pearl millet was not statistically different from little millet ($p > 0.001$).

There were significant correlations between FRAP and FCR values for grain, flour and flakes (Table 2.3). There was a significant difference ($p < 0.001$) in polyphenol content and antioxidant activity between the millet types in different forms used in the present study. The results showed that millet grain extract was richer in polyphenol and antioxidant content compared to millet flour and flakes. Kodo millet grain was the highest in both polyphenols and antioxidants, followed by finger millet and pearl millet in both forms.

Table 2.2: Total antioxidant activity (FRAP/mmol/g) of each type and form of millet (mean ± SD)

	Millet form		
Millet Type	**Grain**	**Flour**	**Flakes**
Pearl millet	4583^{a,4} ± 644	4313$^{a\ ,\ B,\ 2,4,6}$ ± 322	4737$^{a,\ C,\ 3}$ ± 317
Finger millet	13979$^{a\ ,4,5}$ *± 161	9866$^{b\ 2,4,6,7}$ * ± 1016	11464$^{b,\ 1}$ * ± 1344
Little millet	3500^{a,2} ± 255	608^{b,8}± 210	1337^{c,2} ± 55
Barnyard millet	2688^{a,2,6}± 370	554^{b,5}± 370	1098^{b,2,4} ± 76
Foxtail millet	1889^{a,2,3} ± 136	352^{b,3} ± 52	1123^{c,2,4} ± 153
Kodo millet	20813^{a,1} * ± 1306	325^{b,1} ± 130	N/A
Proso millet	4351^{a,4} ± 622	922^{b,2} ± 110	N/A

Data are expressed as mean ± SD of replicate measurements
Means followed by different lower-case letters in each row are significantly different ($p < 0.001$)
Means followed by the same lower-case letters in each row are not significantly different ($p > 0.001$)
Means followed by different upper-case letters in each row are significantly different ($p < 0.001$)
Means in each column followed by number:
1 is significantly different with means followed by number 2
3 is significantly different with means followed by number 4
5 is significantly different with means followed by number 6
7 is significantly different with means followed by number 8
* = significantly greater than all other samples (ANOVA, $p < 0.05$)
N/A= not available

Table 2.3: Correlation of total polyphenol and antioxidant activity of different form extracts of millet (grain, flour and flakes)

Sample extract	FCR VS FRAP (r)
Millet grain	0.963**
Millet flour	0.968**
Millet flakes	0.602**

Ferric reducing antioxidant power assay (FRAP); Folin-Ciocalteu assay (FCR). r, Pearson's correlation coefficient.
Statistically significant ** $p < 0.01$ (2-tailed).

2.4. Discussion

The millet samples used in this study were present in different types and forms, also differing in colour and grain size. To the best of our knowledge, this is the first study to report on the total polyphenol content and antioxidant activity of different millet types and forms. All millet extracts were shown to be good sources of polyphenols and also had high antioxidant activity. Kodo millet, followed by finger millet in grain form, had the most concentrated polyphenols and antioxidant extracts out of a total of 19 different types and forms of millet extracts.

2.4.1. Polyphenols and antioxidants

Polyphenol and flavonoid compounds have been found in different parts of millet grain, with the content and structure differing depending with each type of millet (Shahidi and Chandrasekara 2013). Polyphenol compounds in grains exist as soluble conjugates (hydroxybenzoic acids) or insoluble bound forms (hydroxycinnamic acids). In a study by Chandrasekara and Shahidi, (2011), insoluble-bound polyphenols in kodo millet (81.6 μmol FAE/g defatted meal) were the predominant content with antioxidant activity compared to other types of millet. This is consistent with our results, which showed polyphenol values of 4.44 μg GAE/g and antioxidant activity of 20813.87 FRAP mmol/g for kodo millet grain. Although the methods and standards

(ferulic acid) in the previous study differ from the current one, kodo millet showed the highest values for polyphenols (FCR and DPPH radical scavenging capacity assay) and antioxidants using both methods (FRAP and β-Carotene/ linoleate model system). Antioxidants act through various mechanisms and no one assay can capture the several modes of antioxidant action. The FRAP assay was chosen in this study because it is easy to use, fast, robust, low cost and it does not require any specialist equipment. However, it has been found that FRAP is unable to detect types that act by radical quenching (H transfer) (Badarinath et al., 2010).

There was high variation in the polyphenol and antioxidant content of different grains in flour form (wheat, finger millet, pearl millet and sorghum) when extracted using different solvents. Among all flour samples, finger millet was recorded as having the greatest polyphenol content in different solvents (Siddiq and Prakash, 2015). A study by Banerjee et al. (2012) found that the total antioxidant activity of methanolic extract from millet was the highest, followed by ethanol and acetone. Sharma et al. (2017), on the other hand, reported that acetone was the most effective solvent for polyphenol extraction from kodo millet, which is in agreement with our study. This may due to the fact that both ferulic and cinnamic acid were the major free form of phenolic acid in acetone-extracted polyphenols in millet. However, methanol-extracted ferulic acid was found as the major free form phenolic acid, while cinnamic acids were the major bound form (Sharma et al. 2017). The differences in polyphenol and antioxidant activity values in the literature describing different extracts may, therefore, be attributed to variations in their phenolic content.

Dykes and Rooney (2006) found that kodo millet grain is 70% higher in antioxidant activity compared to other millet varieties. A study by Rao et al. (2011) found that kodo millet in grain form recorded the maximum phenolic content, followed by finger

millet, proso millet and foxtail millet. The results of this study concur with our results, as we found that kodo and finger millet in grain form were highest in polyphenol content compared to other millet types, and both proso (1.14 mg GAE/g) and foxtail millet (0.98 mg GAE/g) in grain form were the lowest in polyphenol content (Table 2.1). Moreover, pearl millet was the third highest millet (in grain form), after finger and kodo millets, in terms of antioxidant and polyphenol content. This may be due to a lower number of phenolic compounds being found in pearl millet grain than in finger millet – 15 and 25 compounds, respectively. In contrast, finger millet was followed by pearl millet flour values for both polyphenol and antioxidant activity and these values were higher than in kodo millet flour, and they also had higher values in flakes form compared to other millet types. Studies show that grain varieties are the highest in both polyphenols and antioxidants compared to other millet forms. According to Viswanath et al. (2009), the grain cover of finger millet is a great source of polyphenols, with significantly higher antioxidant activity compared to whole flour. The phenolic compounds present in grain are not equally distributed; rather, these compounds are primarily concentrated in the outer layers of grain (around 60% of millet polyphenols are found in the seed coat) (Devi et al. 2014). Chethan and Malleshi (2007) reported that 90% of phenolics are centred on the seed coat and the rest of the compounds are distributed throughout the endosperm cell walls. This might explain the reduction in kodo millet values in this study (polyphenols and antioxidants) in flour form compared to grain after processing and removing the seed coat.

The colour of the millet may be another reason for the observed differences in polyphenol levels; studies have shown that millets of a darker colour, such as kodo and finger millet, have higher levels of phenolic acid and tannins compared to white varieties of finger millet due to enrichment of red pigments, such as anthocyanins (Devi et al., 2014; Lorenz, 1983). Pigmented testa, located in the grain tissue, has a red

colour and contains more tannins. Moreover, finger millet and its varieties has been found to contain proanthocyanidins – also known as condensed tannins. Generally, condensed tannins have a high antioxidant capacity and this may support our finding that kodo and finger millet are higher in antioxidants compared to other millet varieties (Chandra, et al. 2016).

Millet grain is underutilised, likely due to poor availability of ready-to-eat products. Processing millet using different methods could be the answer to diversifying its use in food products to increase their nutritional value; for instance, fermentation of millet increases the polyphenol content as a result of microbial activity (Devi et al. 2014). However, Lorenz (1983) reported that tannin levels were reduced by 65–80% after dehulling of finger millet, as the tannin concentration in the hull is 5–40 times higher than in the dehulled grain. This highlights the importance of using the whole grain and not removing the grain cover, which is highly rich in polyphenol components. However, retaining the tannin content may affect the taste of the product. In contrast, Chauhan (2017) found a significant increase in total polyphenol content in germinated finger millet flour (10.6%), followed by pearl millet flour (6.49%), compared to whole raw finger and pearl millet flour. In addition, whole raw pearl millet flour was found to contain a higher level of antioxidants (2.02 FRAP μg/g) compared to whole raw finger millet flour (1.53 FRAP μg/g); however, after germination, finger millet showed a higher level of antioxidants, mainly due to its high polyphenol content in its raw form. The increased level of polyphenols compounds concentration in millet could be due to cell wall-degrading enzymes, which in turn activate other enzymes during the germination process (Sharma et al. 2015 and Pradeep and Sreerama 2015). Germination may, therefore, be a key technique for harnessing the antioxidants in finger and pearl millet. Pressure-cooking was also shown to affect the nutrient content of finger millet – it significantly decreased the total polyphenol content by 50%

(Hithamani and Srinivasan 2014). This may be due to the oxidative degradation of phenolic acids. A study by Devi and Nazn (2016) also found that roasting and pressure-cooking boosts the polyphenol content of barnyard millet grain when the cooking time is kept short. Moreover, there was an observed increase in total polyphenols in millet flour after it was roasted, which have resulted from the release of normally bound polyphenols due to the Maillard reaction, which occurs during millet roasting. This was noted to promote scavenging of ROS. It turns out that the Maillard reaction involves condensation reactions between amino acids and sugars, which are connected to polyphenols through polyphenol oxidase inhibition (Devi and Nazn, 2016). Conversely, a long cooking time may lead to a loss of polyphenols compounds. This might be due to loss or breakdown of polyphenol compound during cooking, as many bioactive polyphenols are comparatively unstable at high temperatures and easily solubilised. As discussed above, different processing methods can significantly affect the total polyphenol and antioxidant activity in different millet types (Pradeep and Sreerama, 2015).

Different environmental factors, such as cultivating location, growth season, rainfall, soil type and sunlight, are shown to affect the phenolic content of plants (Kumari et al. 2016). Our results are consistent with the findings of a previous study, where the highest phenolic content was reported for kodo millet (368mg/100g), followed by foxtail millet (106mg/100g) and finger millet (102mg/100g) (Hegde and Chandra, 2005). However, in our study, foxtail millet recorded the lowest polyphenol value (0.98mg GAE/g) amongst all the millet grains. This corroborates the findings of Chandrasekara and Shahidi (2011), who compared two types of finger millet (local and Ravi) with foxtail millet. The authors found that the lowest polyphenol content was in foxtail millet, followed by Ravi finger millet. Moreover, pearl millet showed a lower polyphenol content (2.58mg/g) than finger millet (10.2mg/g) (Hithamani and

Srinivasan, 2014) in a previous study and the current work supports these findings. In a another study, finger millet had a higher total polyphenol content compared to proso and foxtail millet; moreover, there were considerable differences in total polyphenol content among finger millet types cultivated in the same location in Sri Lanka (Kumari et al. 2016). The authors, in addition, found that millet with the highest phenolic content and antioxidant activity comes from dry zones (characterised by a distinct dry season with less rainfall) in Sri Lanka; the same millet grown in intermediate zones (with an annual rainfall of 1,750-2,500 mm and a shorter dry season) had lower phenol levels. The production of phenols is shown to be increased by low temperature, while the composition is affected positively by exposure to ultraviolet radiation from sunlight. (Kumari et al. 2016). As revealed in the aforementioned studies on millet varieties, polyphenol and antioxidant content varies significantly in the same millet species due to differences in cultivated locations and types.

It has been suggested that high antioxidant activity in foods such as millet correlate to a high polyphenol content (Mohankumar and Vaishnavi, 2012). Polyphenols may, therefore, be the prevalent antioxidants in these foods. In the current study, a significant correlation was found between the total polyphenol content (Folin–Ciocalteau method) and antioxidant activity (FRAP assays) in all millet extracts (grain, flour and flakes) (Table 2.3). Previously, a significant correlation was reported between the total polyphenol content of foxtail millet (Folin-Ciocalteu method) and its antioxidant activity, as measured by different methods (ORAC, FRAP, ABTS, DPPH) (Zhang et al., 2017). These findings are in line with those of Jubete et al. (2010), who found a significant correlation between the Folin-Ciocalteau method and antioxidant activity (DPPH and FRAP assays) in extracts of seeds, sprouts and bread (wheat, quinoa, amaranth, buckwheat). However, a study by Nsimba et al. (2008) found no correlation between polyphenols and antioxidants in extracts of amaranth and quinoa.

The conflicting evidence may be explained by different methods of measuring antioxidant activity – e.g. antioxidant solubility versus oxidation state or pH level (Jubete et al., 2010).

In the current study, kodo and finger millet in grain and flour form were found to have the highest polyphenol and antioxidant content. These two types of millet were, therefore, used to prepare muffins for digestibility and sensory evaluation in the next study.

2.5. Conclusion

There is conflicting literature on the polyphenol levels and antioxidant capacities of different millet varieties and forms, which may be expected as many different factors are shown to play important roles in the above properties of the grain – such as millet variety, part of the grain, solvent used for extraction, extraction method, processing method, agrotechnical process and environmental conditions. All the millet extracts in this study were shown to be good sources of polyphenols and demonstrated high antioxidant activity. The highest polyphenol content and antioxidant activity were recorded for kodo millet, followed by finger millet prepared in two forms (grain and flour). The results of this study may lead to improvement of the quality of millet-based meals, so as to achieve the most health benefit from the polyphenols and antioxidants contained in the grain. These results may be of interest to food manufacturers who aim to manage or reduce non-communicable diseases.

Chapter 3: *In vitro* starch digestion and sensory evaluation of millet-based muffins (Study 2)

3.1. Introduction

There has been great interest in the potential health benefits of millet, which represents a major consistent food source in the semi-arid regions of Asia and Africa. The main component of millet is starch, which accounts for 70% of the grain (the other carbohydrate in millet is sucrose) and determines the quality of millet products (Zhu, 2014). Reported differences in starch digestibility have been related to the nature and source of the starch (Aarathi et al. 2003).

Nutritionally, starches are categorised according to the extent and rate of their digestion into resistant starch (RS), rapidly digestible starch (RDS) and slowly digestible starch (SDS) (Jung Chung et al. 2008). RDS is the starch fraction that leads to a rapid increase in the blood glucose levels after ingestion, whereas SDS is the fraction that is digested more slowly in the small intestine. RS is a type of dietary fibre that is not digested in the small intestine, but ferments in the large intestine (Jung Chung et al. 2008). SDS is found in many uncooked grains, such as rice and wheat, as the polyphenols in the grains prevent digestive enzymes from breaking down the starch (Zhang and Hamaker, 2009). Compared to RDS, SDS also forms a semi-crystalline structure and is therefore less susceptible to enzymes (Englyst et al. 2003).

It has been found that the digestion, absorption and metabolism of both starch and sucrose is affected by polyphenols and their metabolites (Hanhineva et al. 2010). Furthermore, polyphenols have been shown to reduce the sugar release in foods that are rich in carbohydrates (Coe et al. 2013). The digestibility of carbohydrates in food (during the oral, gastric and intestinal phases) can be evaluated *in vivo* by measuring

blood glucose levels 2–3h after test meal consumption (Thondre et al. 2010). An alternative way is to use an *in vitro* digestion method to identify rapidly and slowly digestible starches in the test food.

The increasing demand for new and healthy baked products has given rise to a new market for bread products made from alternative ingredients. Moreover, due to recent increases in food intolerance (e.g. gluten), millet is attracting interest as an alternative gluten-free cereal (Volta and De Giorgio, 2012). Sensory evaluation of such new products plays a significant role in public acceptance of new foods. Sensory evaluation is a scientific approach to determining the sensory characteristics of food products using the five human senses: vision, smell, touch, hearing and taste (Singh-Ackbarali and Maharaj, 2014). There are different methods of sensory evaluation; the most popular are acceptance tests, discriminative tests and descriptive analysis tests (Singh-Ackbarali and Maharaj, 2014). The hedonic test is an acceptance test widely used for measuring the acceptability of baked products or for improving current products (Muresan et al., 2012).

Based on the results of the previous study (Chapter 2), kodo millet grain and finger millet prepared in two forms (grain and flour) were found to be the highest in polyphenols and antioxidant activity. These millets were therefore prepared into muffins and chosen to conduct further *in vitro* starch digestion and sensory evaluation tests. It was hypothesised that using the above millet forms and types, found to be rich in antioxidants, polyphenols and fibre, in muffins would reduce starch digestion compared to wheat-based muffins.

The aims of the current study were:

- To prepare muffins from two types of millet found in study 1 to be rich in polyphenols: kodo millet (grain) and finger millet (grain and flour), to compare

with a control (wheat) muffin;

- To evaluate the effect of kodo millet (grain) and finger millet (grain and flour)-based muffins on *in vitro* starch digestion;
- To conduct a sensory evaluation test to assess the overall acceptance of kodo millet (grain) and finger millet (grain and flour)-based muffins.

3.2. Method and materials

3.2.1. Muffin preparation

Kodo millet grain and two forms of finger millet (grain and flour) were chosen to conduct *in vitro* starch digestion and sensory evaluation tests. Muffins were prepared by replacing 50% of the wheat flour with the above millet. This was carried out in the Oxford Brookes Centre for Nutrition and Health (OxBCNH) kitchen on the day before the *in vitro* and sensory tests. The millets in grain form were washed before use and then immediately ground after drying using an electric mill (Moulinex 530/380 W).

To prepare the control muffins (Table 3.1), 5ml of vanilla extract was added to 1 medium egg and mixed for 2min, followed by addition of a liquid mixture containing 125ml of semi skimmed milk, 32g of caster sugar and 62ml of sunflower oil. This was mixed well using an electric hand-mixer (Tesco HM14). Then, 144g of wheat flour and 15g of baking powder were mixed in using the same electric hand-mixer. To prepare the test muffins, 72g of finger millet flour/crushed grain or kodo millet crushed grain was added to 72g of wheat flour and silicone muffin cups/cases were used to set the mixture. The muffins were baked at 150°C for 25min, cooled for 10min and stored until the following morning for *in vitro* digestion or sensory evaluation experiments (Figure 3.1).

Figure 3.1: Three types of millet-based muffins (kodo grain; finger millet grain or flour) and controls (wheat) were prepared for *in vitro* and sensory evaluation tests

Table 3.1: Ingredients for each type of muffin

	Muffin Type			
Ingredients (For four muffins)	**Control (wheat flour)**	**Kodo millet grain (crushed)**	**Finger millet grain (crushed)**	**Finger millet flour**
Wheat flour (g)	144	72	72	72
Millet (g)	–	72	72	72
Caster sugar (g)	32	32	32	32
Sunflower oil (ml)	62	62	62	62
Semi skimmed milk (ml)	125	125	125	125
Vanilla extract (ml)	5	5	5	5
Eggs	1 medium	1 medium	1 medium	1 medium
Baking powder (g)	15	15	15	15

Table 3.2: Nutrient and energy content by muffin type (per 100g)

Nutrient and energy content (g/100 g)	*Control muffin	*Finger millet grain muffin	**Finger millet flour muffin	**Kodo millet grain muffin
Energy (kcal)	319	300	308	304
Available carbohydrates	37.4	34.3	34	33
Total sugars	9.3	9.4	9	9
Total dietary fibre (AOAC)	1.3	4.3	1.5	2.3
Total fat	15.7	14.4	16	16
Crude protein	6.3	6.2	5.6	5.8

* Nutritional analysis was carried out by Eurofins Food Testing UK Ltd.
**Nutrition information was calculated based on the recipe, using Nutritics software.

3.2.2. *In vitro* digestibility of millet-based muffins

3.2.2.1. *In vitro* digestion

An *in vitro* digestion procedure was used to test the starch digestibility of control (wheat) and millet-based muffins. This consisted of a simulated gastric digestion phase, followed by an ileal digestion phase. The reducing sugars released were measured in the digested samples collected during the above phases: baseline, at the end of the gastric phase and during the ileal phase at 20, 60 and 120min. (Coe et al. 2013). 2.5g muffin samples were accurately weighed and placed in numbered plastic pots. Then, 30ml of distilled water was added to each muffin sample. A 0.25ml baseline sample was taken from each sample and added to a test tube containing 1ml of ethanol. Following this, 0.8 ml of 1M HCl and 1ml of 10% pepsin solution in 0.05M HCl were added to each muffin sample. All muffin samples were incubated at 37°C in a water bath for 30min to end the gastric digestion phase and 0.25ml gastric digestion samples were then transferred into test tubes containing 1ml of ethanol. In quick succession, 1ml of 2% pancreatin solution (in maleate buffer, pH 6) and 0.1ml of amyloglucosidase were added to each sample. After this step, the samples were

incubated for 120min and 0.25ml samples were taken at 20, 60 and 120min during ileal digestion. In the final step, the samples in ethanol were centrifuged at 1000 ×g for 2min in a Biofuge Primo centrifuge; the clear supernatant was used for DNS analysis of reducing sugars (Coe et al. 2013).

3.2.2.2. Analysis of reducing sugars released during digestion

Following the methodology of Englyst and Hudson (1987), sugars released from the muffins during digestion were measured using a colorimetric method (DNS) tailored to measure monosaccharides produced during the depolymerisation of starch, after amyloglucosidase secondary digestion (Thondre and Henry, 2011).

To 0.05ml of a 10mg/ml glucose standard or muffin sample from the *in vitro* digestion phase, 0.25ml of 1% amyloglucosidase (in an acetate buffer, pH 5.2) was added. Samples were incubated for 10min at room temperature, following which 0.75 ml of a 3,5-Dinitrosalicylic acid (DNS) mixture (0.5mg/mL glucose: 4M NaOH: DNS reagent; 1:1:5 ratio) was added to all samples. The samples were heated in a water bath to 95–100°C for 5min, after which they were left in a cold water bath. 4ml of water was added to stop reactions in all samples. The absorbance of each sample was read at 530nm using a Shimadzu UV-1201 spectrophotometer (Schimadzu Corporation, Rydalmere, Australia).

3.2.2.3. Rapidly digested starch and slowly digested starch

RDS values were calculated from measurement of the quantity of reducing sugars at 20min, which correlated with the amount of starch converted into glucose within 20 minutes of enzyme digestion. The SDS values were derived by the subtraction of the RDS (20min) from the level of reducing sugars measured at 120min, which allowed sufficient time to obtain extended release of glucose (Landon et al., n.d.). Nutritional analysis of the total starch present in the muffins was carried out using nutritics

software (Table 3.2).

3.2.3. Sensory evaluation: hedonic testing

3.2.3.1. Study design

The study was conducted using the 9-point hedonic method developed by David Peryam and colleagues (1955). The 9-point hedonic scale is a bipolar scale based on equal intervals, around neutral, with four positive and negative categories on each side (from 1 = "dislike extremely" to 9 = "like extremely") (Appendix 1G) (Lim, 2011). The validity, reliability and discriminative ability of the 9-point hedonic test has been proven in food acceptance analyses in the field and in the laboratory (Society of Sensory Professionals, n.d.).

The 9-point hedonic scale was used with each participant tasting four types of muffins (control, kodo millet grain, finger millet grain or flour) on two separate days (2 muffin types per day) in a random order, with at least three days between each test session. Each session lasted for up to 90min. The muffin order was randomised using a pseudo-random number generator (Research randomizer, n.d). The sessions were performed in a sensory booth in OxBCNH under standard light and temperature conditions, individually or with a maximum of 3 participants in the room, each placed in individual booths.

On the day of the test, all participants first undertook a training session on how to use the 9-point hedonic scale one hour after their last meal and drink.

Figure 3.2 shows the sensory evaluation protocol. Each participant was asked to try two different types of muffins (millet-based or control) in each session, which were randomly coded with 3-digit numbers. Cold water and crackers were provided to all participants to cleanse their palates between samples. Participants were asked to taste

the muffins and give their opinions on each product by scoring different attributes (aroma, crust, appearance, colour, flavour, taste, texture and overall acceptability) using the 9-point hedonic scale, according to their first sensory impressions. They were then asked to re-test to confirm their ratings.

Ethical approval for this study was obtained from the University Research Ethics Committee (UREC) at Oxford Brookes University (UREC Registration No: 161021). Each participant was given full details of the study protocol (Appendix 1E) and had an opportunity to ask questions. Written informed consent (Appendix 1F) was collected from each participant prior to commencing the test.

3.2.3.2. Participants

Sensory evaluation of control and millet-based muffins was conducted in 30 healthy participants. The inclusion criteria for this study was an age of 18-65 years and an absence of any known medical conditions. Participants were also eligible for the study if:

- they were not taking any medication, such as antidepressants, which may interfere with taste or olfactory sensitivity.
- they were not allergic to any of the ingredients in the study (wheat flour, vanilla, eggs, sugar, salt, baking powder, milk, oil and millet).
- they did not smoke.
- they did not experience frequent colds (including on the test day) and/or hay fever (on the test day).

Participants were recruited using posters (Appendix 1D) placed in Oxford Brookes University facilities (library, sport centre and student accommodation), via the University Research Activity Group, social media and the Oxford daily info website. The sample size of participants was based on previous recommendations in literature;

the number of panellists for a sensory test usually ranges from 3 to 50. According to Balázs (2015), 4-5 trained panellists are sufficient for such a test, whereas for semi-trained panellists, this should be between 10 and 20. In case of involvement of untrained participants, this number should be further increased for better accurate results. Gacula and Rutenbeck (2006) suggest that 5 panellists is the minimum size for a descriptive test, although, according to Lim (2011), a 9-point hedonic test can be used for a wide range of populations without training. The authors did, however, suggest using a larger sample size in order to make valid statistical inferences. In this study, 30 untrained healthy participants completed the sensory test and received a training session on the same test day to familiarise the participants with the sensory test techniques and minimise mistakes.

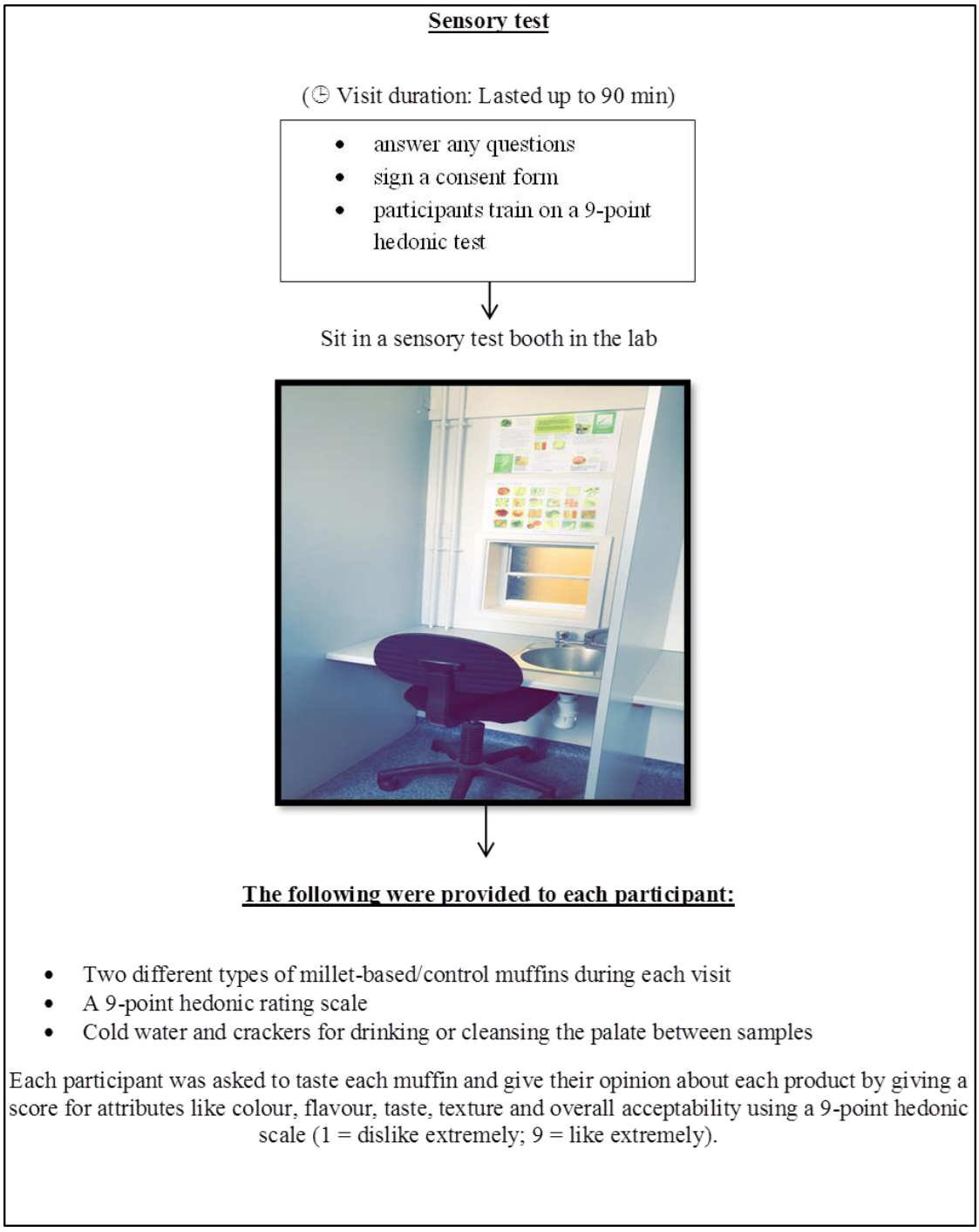


Figure 3.2: Flow diagram illustrating the design of the sensory evaluation test

3.2.4. Statistical analysis

Data were analysed using Microsoft Excel 2010. Statistical analyses were undertaken using the Statistical Package for the Social Sciences (SPSS, version 25, USA). All values are given as mean ± SD, unless otherwise specified. Before statistical analysis,

in vitro digestion data were tested for normality using a Shapiro-Wilks test. Where data were not normally distributed, non-parametric tests were used. A non-parametric Kruskal Wallis test was used to compare millet-based muffin extracts and control muffin extracts at 0min of reducing sugar released. One-way between groups ANOVA was used at all other time points.

A non-parametric Friedman test was used for sensory evaluation comparisons of each attribute between muffins, while a Wilcoxon signed rank non-parametric test was used to identify the differences between pairwise comparisons in terms of the different sensory attributes of muffins. Statistical significance was set at $p < 0.05$.

3.3. Results

3.3.1. Sugar release

The findings of reducing sugars released during *in vitro* digestion of control and millet-based muffins (kodo millet grain and finger millet as grain or flour) are shown in Figure 3.3. All three millet-based muffin samples demonstrated a reduction in sugar release, compared to wheat-based muffins (control), at each time point. At 20min, finger and kodo millet grain-based muffins were significantly lower in digestibility than finger millet flour and control muffins, although they were not statistically different from each other. The least sugar released was from finger millet grain-based muffins at 60 and 120min, as seen in Figure 3.3. However, there were no significant differences ($p > 0.05$) in starch digestibility among the finger millet grain-based muffins compared to kodo millet grain and finger millet flour-based muffins (Figure 3.3).

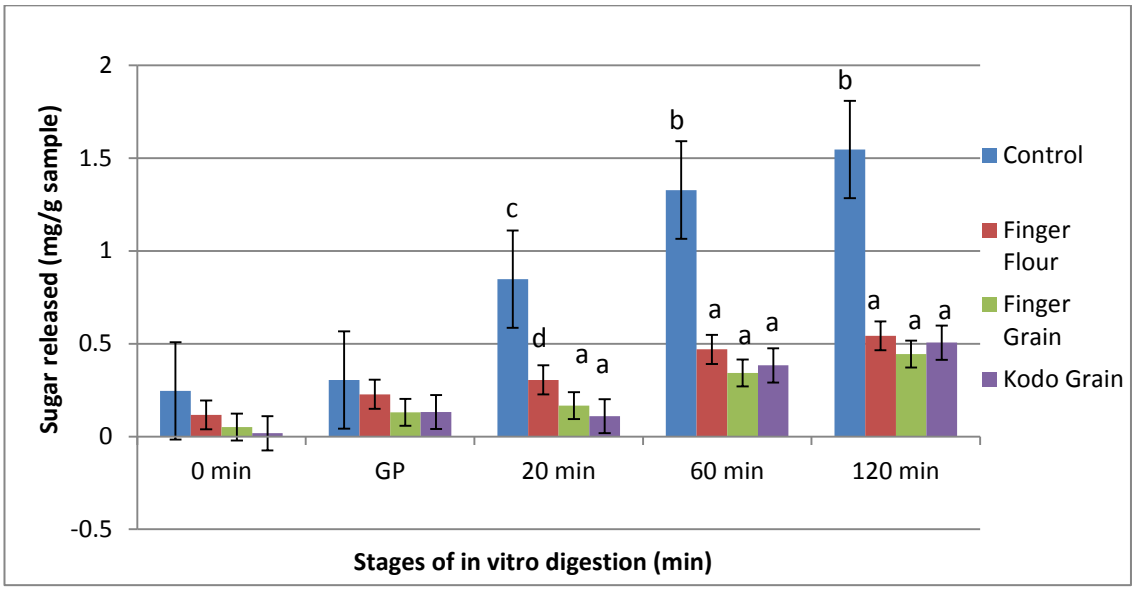


Figure 3.3: Sugar release from the control muffins (wheat) and kodo millet grain and finger millet (grain and flour) based-muffins

Values with different superscripts at each time point are significantly different from each other ($p < 0.05$)
Values with the same superscripts at each time point are not significantly different from each other ($p > 0.05$)
Values represent mean ± standard deviation (SD)
20, 60 and 12 min = stages of intestinal digestion, GP = gastric phase

The control muffins and finger-millet flour-based muffins showed a significantly greater content of RDS compared to finger and kodo-millet grain-based muffins ($p < 0.05$). The amount of SDS, on the other hand, was significantly higher in control muffins compared to all millet type muffins. There were no significant differences, however, in the amount of SDS between all types of millet-based muffins (Table 3.3).

Table 3.3: Levels of rapidly digestible starch (RDS) and slowly digestible starch (SDS) in control muffins and different types and forms of millet-based muffins

Sample	RDS (mg/g sample)	SDS (mg/g sample)	Total starch (g/100g)
Control	84.7^{b}	69.1^{b}	25.2
Finger flour	30.6^{c}	23.7^{a}	25.1
Finger grain	16.6^{a}	27.9^{a}	25
Kodo grain	11^{a}	39.6^{a}	24

Values are means of triplicates
(RDS = sugar released after 20 min, SDS = sugar released between 20 and 120min of digestion)
Values with different superscripts in each column are significantly different from each other ($p < 0.05$)
Values with the same superscripts in each column are not significantly different from each other ($p > 0.05$)

3.3.2. Sensory evaluation of millet-based muffins

Figure 3.4 shows the results of the sensory evaluation of different types and forms of millet-based muffins compared to wheat muffins (control). Analysis using a Friedman test revealed no significant difference ($p > 0.05$) between control wheat muffins and millet-based muffins in terms of aroma, colour, appearance and crust. There was also no significant difference ($p > 0.05$) in flavour between control muffins, finger millet-based muffins in both forms (grain and flour) and kodo millet grain based-muffins. In contrast, there was a significant difference between the flavours of kodo millet grain muffins and finger millet flour muffins ($p = 0.002$).

The results highlight that certain millet-based muffins were similar in texture to the control muffins, while others received markedly different scores. The control muffins were not significantly different in texture to finger millet-based muffins in either form. However, the kodo millet grain-based muffins scored as significantly less acceptable in terms of texture, compared to control ($p = 0.002$) and finger millet-based muffins in flour ($p = 0.004$) and grain form ($p = 0.001$). In terms of taste, kodo millet grain-based muffins were found to be significantly different from muffins made from finger millet grain ($p = 0.003$) and flour ($p = 0.001$) and no significant differences with wheat (control; $p = 0.009$). There was no significant difference in taste, however, between control and finger millet-based muffins in either form.

The control muffin and finger millet (flour and grain) muffins were, overall, found to be more acceptable than muffins made with kodo millet grain – with a significant difference between control and kodo millet muffins. Kodo millet based-muffins were also significantly different from both forms of finger millet muffin.

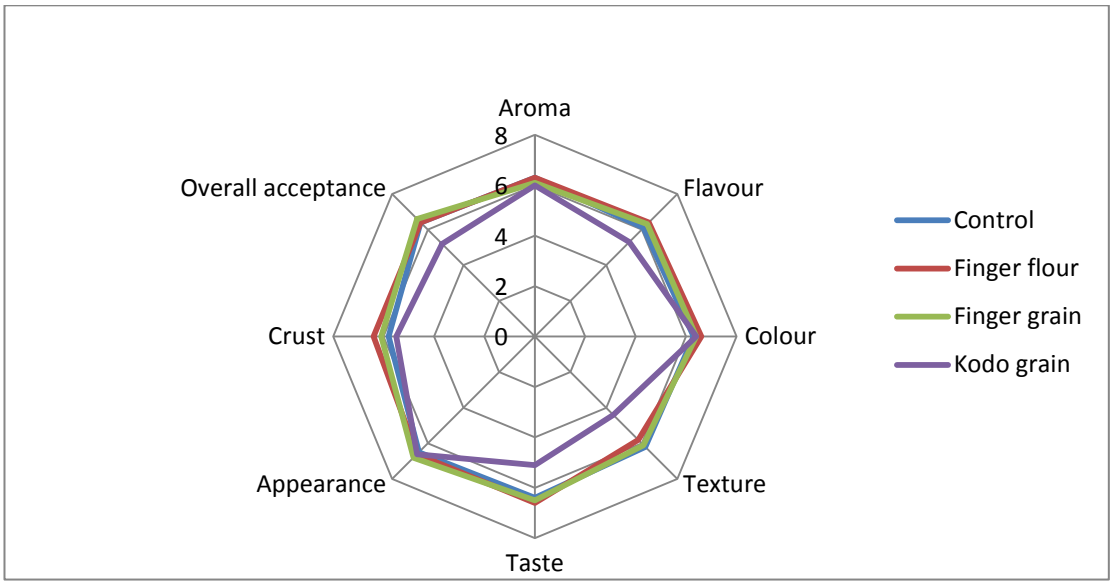


Figure 3.4: Spider diagram of sensory analysis data for wheat-based muffins (control) in comparison to kodo millet grain and finger millet (grain and flour) muffins

3.4. Discussion

3.4.1. *In vitro* starch digestion

In vitro starch digestibility is considered as one of the most important factors determining the starch profile and nutritional properties of grains (Roopa and Premavalli, 2008). The millet-based muffins, prepared from two types and in different forms (kodo millet grain and finger millet grain and flour-based) tested in this study showed significantly reduced sugar release *in vitro* compared to wheat muffins (control).

The RDS content in food accounts for the postprandial GR in the short term (Soong et al., 2014). In the current study, we found that both kodo millet grain and finger millet (grain and flour) muffins – containing 50% millet and 50% wheat –had lower RDS levels than control (100%) wheat muffins. This is in agreement with previous findings by Sharma et al. (2017), revealing that wheat flour chapattis contain more RDS compared to chapattis made with wheat and finger millet. Moreover, a study by Yousif

et al. (2012) found that flatbreads made from 100% wheat flour were higher in RDS than flatbreads containing 40% white sorghum flour and 40% red sorghum flour. It is likely that the large amounts of phenols contained in millet account for the lower RDS content in products where wheat is mixed with finger millet flour (Sharma et al. 2017).

SDS is represented by the amount of glucose that is released between 20 and 120 min of *in vitro* starch digestion and this type of starch produces a low and prolonged glycaemic response (Sharma et al. 2017). In the current study, we found that wheat-based muffins (control) were higher in both RDS and SDS. This is in agreement with previous work by Sterbova et al. (2016), which also reported higher RDS and SDS content in common white wheat flour. This may be due to the broken starch granules and smaller particles in grain compared to wholemeal wheat flour.

The variation in findings on the starch digestion differences between millet types and wheat observed here and in previous findings could be due to differences in the properties and source of starch, as well as the protein and dietary fibre content in millet; for instance, in our study, the wheat flour (control) may possess different starch digestion characteristics from kodo and finger millet muffins even under identical cooking methods. According to Bednar et al. (2001), the structure of grains, such as the pericarp and seed cover, could impede the efficiency of amylase digestion of starch in grains. Moreover, wheat-based muffins are significantly higher in SDS compared to millet-based muffins. Sharma et al. (2017) reported that storing wheat flour chapattis for 24h led to a decrease in RDS content and an increase in the amount of SDS. This may be due to starch being reorganised into an ordered crystalline state from an amorphous form during the retrogradation process. It may also be explained by the fact that most starch is digested in the first 20 min (RDS) for muffins at the expense of starch digested later or resistant starch (RS) (Sterbova et al. 2016). It has been reported

that different types of millet, such as finger and pearl millet, have high levels of dietary fibre and resistant starch (Sharma et al. 2017; Saleh et al. 2013). The resistant starch in millet escapes enzymatic digestion in the small intestine but ferments in the large intestine (Jung Chung et al. 2008).

Roopa and Premavalli (2008) compared finger millet in different varieties (hilly and base) and found that hilly varieties had higher levels of RDS and SDS than base varieties. Research has demonstrated that the carbohydrates in finger millet are digested and assimilated more slowly compared to carbohydrates in other cereals (Devi et al. 2014). The differences between millet varieties may be due to the different polyphenol compounds available, which may have different effects on sugar release (Coe, 2013). Also, differences in starch sources may affect digestion, as starches that contain high amounts of amylose tend to be lower in digestibility compared to other starches (Aarathi et al. 2003). It has been found that millet contains up to 34% of amylose and an increase in amylose starch in diets might affect the GR (Annor et al. 2017).

GI values may also play an important role in starch digestibility; it is a measurement carried out on carbohydrate-based foods to determine their physiological effect on blood glucose levels. The GI is shown to be lower in finger millet-based foods (Jayasinghe, et al. 2013); other products with a high GI could be modified by incorporating ingredients known to be rich in soluble fibre so as to delay starch digestion and the rate of glucose release (Giri et al. 2017). A study by Nidhi et al. (2014) has shown that Uthapam and Cheela dishes, made with finger and pearl millet, had lower GI values compared to the same dishes made with Semolina. It should be noted that there is a strong correlation between RDS and GI; foods with a high ratio of RDS also have a high GI (Henry, 2007).

Processing millet reduces the phenol and tannin levels, which results in an increase in the bioavailability of starch (Devi et al. 2014). Moreover, other processes, such as baking, frying and toasting, also reduce RDS levels, while others, such as puffing, increase them (Roopa and Premavalli, 2008). It has also been found that baking boosts RDS in wafers and white bread but has no significant effect on RS; notably, this could explain the observed increase in RDS levels in the control muffins here (Sterbova et al. 2016). Moreover, cooking cereal starches elicits physical and chemical changes to starch granules, including their gelatinisation (Aarathi et al. 2003). The extent to which this occurs depends on the cooking time, the amount of water present and the temperature, increasing its propensity for enzymatic digestion (Aarathi et al., 2003; Sterbova et al., 2016).

Measuring reducing sugars is a way of predicting both the rate and extent of starch digestion in the small intestine (Ahmed and Urooj, 2015). The Englyst method was used because it demonstrated a useful, inexpensive and simple way to estimate the biological response of a meal that was high in carbohydrate (Araya et al., 2002). It should be highlighted that in vitro methods may be useful tools but do not always correlate well with in vivo values because they cannot precisely imitate human processes (Argyri et al., ., 2016). Functional foods, such as millet, may be suitable for diabetics due to their ability to delay glucose release and absorption, resulting in a lower GR and consequent reduced postprandial glycemia (Lakshmi and Sumathi, 2002).

3.4.2. Sensory evaluation test

The results of the current study indicate that muffins prepared with finger millet grain were, overall, the most acceptable compared to other millet-based muffins. This contradicts the findings of Shukla and Srivastava (2014) in terms of the percentage of

finger millet in the product; the authors reported that noodles containing 30% finger millet flour scored as significantly more acceptable than noodles composed of 40% or 50% finger millet flour in all attributes, including colour, flavour, taste, texture, appearance and overall acceptance. In contrast, our study found that 50% finger millet grain-based muffins were highly acceptable for the same sensory attributes. This may be because the millet type and millet substitution level have a significant effect on the texture and colour of products prepared from finger millet (Siwela et al. 2009). Moreover, the inclusion of other ingredients in millet-based muffins in the current study, such as wheat, milk and vanilla, enhanced the flavour and texture; this combination may, therefore, have been more acceptable than control muffins prepared from the same ingredients (in the same quantities) but without millet.

Verma et al. (2015) found that biryani prepared from 100% foxtail millet grain was overall more acceptable in texture, colour, taste and appearance than control (rice) biryani and biryani prepared from other types of millet. On the other hand, there were no significant differences between *ladoo* (a sweetened food product) prepared with foxtail millet, barnyard millet and rice (control) in terms of colour, flavour, texture, appearance and overall acceptability (Verma et al. 2015). Therefore, using a high percentage of millet in meals (50-100%) may not affect the sensory characteristics of a product, showing more acceptability compared to a low percentage of millet. The type, form and quantity of millet, cooking method (e.g. soaking) and ingredients added (e.g. vegetables and spices) could be important factors that affect the taste of products and other sensory attributes. Moreover, the types of products used to incorporate millet for consumers, such as baked products, rice and sweets, may also have an effect on sensory attributes (Saleh et al. 2013; Verma et al. 2015).

Phenolic compounds are responsible for the bitter taste of many foods. In our study we

found that, in terms of sensory perception, finger millet-based muffins were more acceptable than kodo millet-based muffins; this may be due to the fact kodo millet has a higher level of polyphenols and antioxidants compared to finger millet. Moreover, in general, the degree of bitterness also depends on the strain, cultivar, ripening and storage conditions of plant foods (Drewnowski and Carneros, 2000). Kodo millet in our study was dark brown, due to tannins, and enclosed in a tough cover that was difficult to remove; this may have resulted in a crunchy, bitter sensation that scored low acceptance compared to finger millet grain, which was in smaller seeds and therefore easier to crush for preparation into muffin.

According to Lizia and John (2014), mixing different millet grains together or with other ingredients are a good way of producing good quality food products. This is because millet grain is gluten-free and possesses key functional properties that would nutritionally enrich ready-to-eat products, along with providing good texture. Conversely, making products purely from millet is likely to be sensorily unacceptable to consumers.

3.5. Conclusion

In conclusion, the results from the current study show that finger millet grain-based muffins are the most acceptable in all sensory attributes when compared to kodo millet based-muffins. There may be potential health benefits from incorporating finger millet, which is the highest in polyphenols and antioxidants than other millet species, into food products without adversely affecting consumer acceptability. The finger millet grain will reduce the RDS content of carbohydrate-rich foods, such as baked products, and this could result in lower postprandial blood glucose levels.

Chapter 4: Millet intake and risk factors for Type-2 diabetes: A systematic review (Study 3)

4.1. Introduction

Type-2 diabetes is the most common chronic metabolic condition around the world that is associated with long-term complications, such as stroke, cardiovascular disease, kidney failure, retinopathy and neuropathy (American Diabetes Association, 2009; Itagi et al. 2012). The prevalence of diabetes in the UK was 4.05 million in 2016 – an astounding 65% increase over the past decade (Diabetes UK, 2016). Preventing acute complications and reducing risk of the disease can be accomplished with medical support and education in patient self-management, as well as promoting beneficial lifestyle modifications, a healthy diet, physical activity and weight loss (American Diabetes Association, 2016).

Dietary interventions are an easy and cost-effective way to provide health benefits to people at risk and those who have been diagnosed with type-2 diabetes, in addition to improving their quality of life (Itagi et al. 2012). Current guidelines for those with type-2 diabetes are to follow a healthy, balanced diet – notably including starchy carbohydrates with a low GI and higher dietary fibre, both of which can help regulate post-prandial hyperglycaemia and reduce body weight (Diabetes UK, 2016). It has been shown that a low glycaemic carbohydrate/high-fibre diet safely reduces plasma cholesterol levels and improves blood glucose control in people with type-2 diabetes (Willett et al. 2002). For example, grains with a low GI, such as millet, are known to elicit a lower postprandial GR and increase insulin sensitivity in individuals with type-2 diabetes (Jali et al. 2012).

Millet is an important crop for populations in Africa, Asia and parts of Europe (Annor

et al, 2013; Devisetti et al. 2014). Nutritionally, millet is superior to other major cereals, such as wheat and rice, and its high content of many essential nutrients makes it an attractive ingredient for incorporation into healthy foods (Annor et al. 2013). Millet grains have been successfully used to produce adult foods, beverages and weaning foods, such as porridge, bread (fermented and unfermented) and snacks, in African and Asian areas, where they provide the main component of traditional meals (Amadou et al. 2013; Saleh et al. 2013).

Recently, millet has been found to be nutritionally superior compared to other traditional food grains due to the higher proportions of fibre, polyphenols and antioxidants (Saleh et al. 2013). This has drawn more attention to millet as a dietary option for helping manage diabetes by reducing blood glucose levels and improving insulin sensitivity (Itagi et al. 2012), which is of clinical importance for individuals with type-2 diabetes. Several reviews have addressed the beneficial effects of millet on the risk markers for type-2 diabetes (Naoyuki et al. 2009; Choi 2005; Narayanan et al. 2016). However, the results of randomised trials investigating the effects of millet on the GR have been inconsistent, with some using short intervention periods or small sample sizes that could reduce the quality of the results. Therefore, this systematic review aims to evaluate the literature on intervention studies investigating the effects of consuming different types and forms of millet on markers of type-2 diabetes and highlight the need for future adequately powered trials in this important area of research.

4.2. Methods

4.2.1. Data extraction

Search engines, including Scopus, Web of Science, Academic Research Complete,

PubMed and CAB abstract (Centre for Agriculture and Biosciences International), were used to search for studies in English, between 1990 and 2017, involving healthy, pre-diabetic and type-2 diabetic participants. The search terms used were: 'Millet AND healthy' or 'pre-diabetic' or 'type-2 diabetes AND fasting blood glucose' or 'glycaemic' or 'glycaemic response' or 'insulin response' or 'glucose tolerance' or 'insulin sensitivity'. We followed standard (PRISMA) criteria for reporting literature in this systematic review. Data extraction was conducted using a standardised table for all studies and all data was extracted for: author, year, country, study design, intervention meals and control used, millets types and forms used, and individuals characteristics (e.g. gender, age, BMI, height, weight, health condition).

4.2.2. Study selection

Data were extracted by one reviewer, as shown in the flow diagram for article selection (Figure 4.1) and all selected studies were confirmed by a second reviewer. The reference lists of all included studies were also searched to identify additional studies of interest. Studies were excluded if their titles or abstracts did not mention millet (any type or form), human participants (healthy and/or pre-diabetics and/or type-2 diabetes) or outcome (fasting blood glucose or glycaemic or glycaemic response or insulin response or glucose tolerance or insulin sensitivity). A total of 57 abstracts were included for review; from these, 30 were excluded on the basis of being duplicate studies, eight were animal studies and two were review papers. Three studies were not relevant (lacking the outcome of interest), two did not include millet as an intervention food and one was not written in English. Three more studies were identified from the reference lists of the included studies and, from these, five more studies were identified from in the reference list. In total, 19 studies met the criteria for inclusion in this review.

4.2.3. Quality assessment

The methodological quality of the 19 selected studies was independently evaluated by two reviewers using the Consolidated Standards of Reporting Trials (CONSORT) 2010 checklist for intervention studies (Consort, 2010; Visser et al. 2017). Studies were assigned a quality rating of positive, negative or neutral, based on a set of quality questions, including intervention details, outcome measures, subject criteria and statistical analysis using the checklist for primary research from the Academy of Nutrition and Dietetics (Academy of Nutrition and Dietetics, 2016; Coe and Ryan, 2016) (see appendices 2A, 2B and 2C).

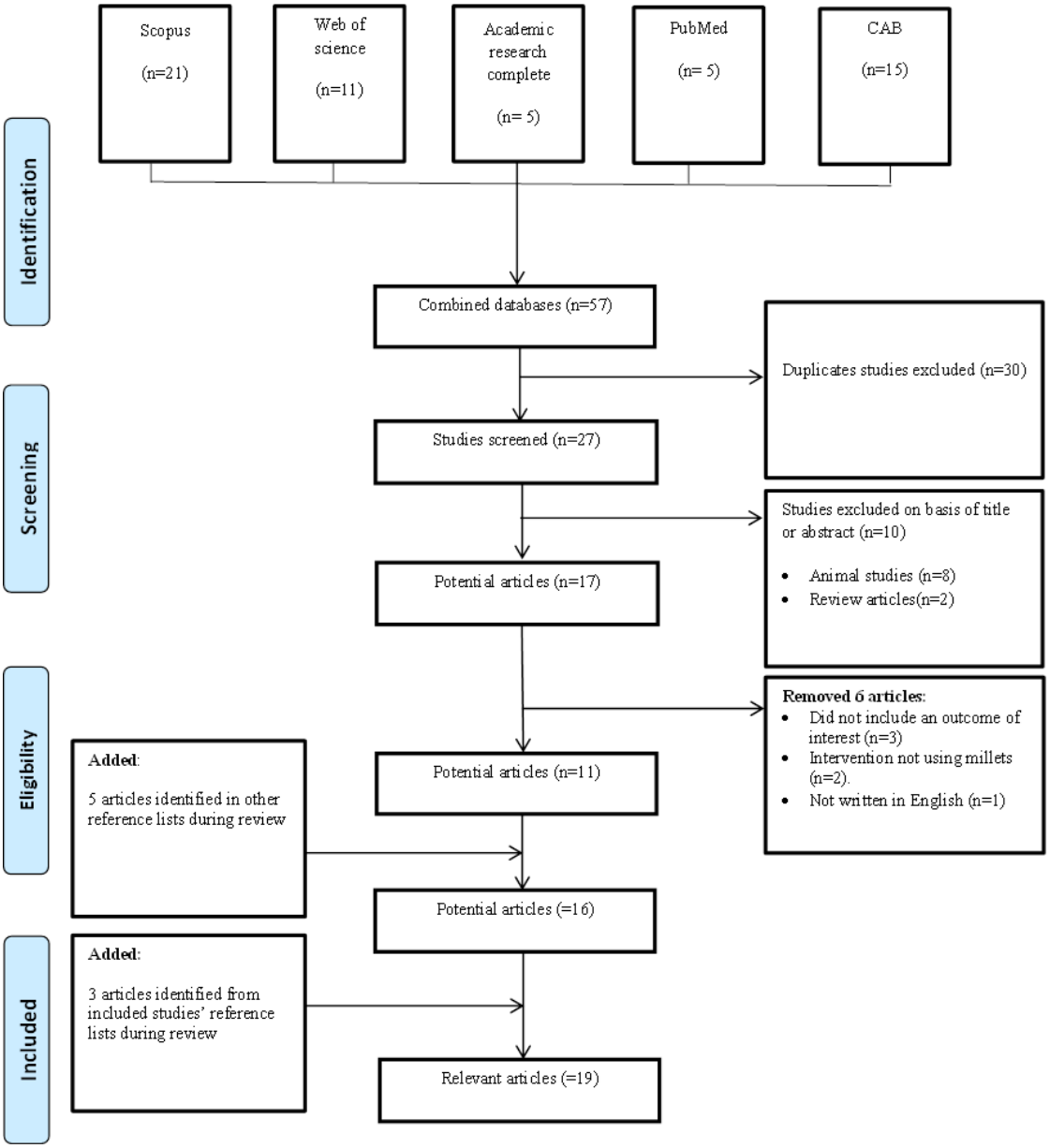


Figure 4.1: Flow diagram of the study screening and evaluation process

4.3. Results

4.3.1. Characteristics of included studies

A total of 19 studies were included in this review (Table 4.1). All studies examined an adult population, totalling 130 healthy adults (71 female and 59 male) and 482 type-2 diabetes adults (174 female and 308 male) with a mean age of 41.4 ± 12.4 years. The mean body mass index (BMI) of participants in 12 studies was 24.4 ± 2.7kg/m^2, the mean weight of participants in six studies was 59.3 ± 5.5kg and the mean height of participants in one study was 162.3 ± 8.6cm. The remaining included studies did not provide BMI, weight or height details.

The majority of trials (n=15) were carried out on participants in India, with one study based in Sudan (Abdelgadir et al. 2004), one in Kenya (Ayuo and Ettyang, 1996), one in China (Ren et al. 2015) and one in Sri Lanka (Jayasinghe et al. 2013); none were based in the UK. The methodologies of the studies were similar to some extent, in that a baseline period was generally followed by participants before being instructed to consume a millet-based test product or glucose (control). Five of the studies collected venous blood from volunteers (Ren et al. 2015; Itagi et al. 2012; Torangatti and Rama 1999; Ugare et al. 2014; Shukla et al. 1991); ten used a finger prick blood draw (Jayasinghe et al. 2013; Pathak et al, 2000; Shobana et al. 2007; Nambiar and Patwardhan, 2015; Thilakavathy and Muthuselvi, 2010; Arora and Srivastava, 2002; Thakkar and Kapoor, 2007; Ayuo and Ettyang, 1996; Shukla and Srivastava, 2014; Narayanan et al. 2016); and four studies did not stipulate on how blood was collected. In the studies that used the finger prick draw (capillary blood), blood samples were taken at the baseline and every 15 and 30 min for 2 to 3h. From those studies collecting venous blood, three (Ren et al. 2015; Torangatti and Rama, 1999; Shukla et al. 1991) took blood every 15, 30 or 60min, while others conducted long term feeding

and took blood before and after the intervention periods (Itagi et al. 2012; Ugare et al. 2014) (4 weeks and 28 days, respectively). In the studies that had no defined blood collection methods, three (Lakshmikumri and Sumathi, 2002; Urooj et al. 2006; Abdelgadir et al. 2004) had blood samples taken every 30 or 60 min and only one study (Thathola et al. 2010) had blood taken at the baseline, after a month and after two months.

4.3.2. Study quality and outcomes

All 19 studies were rated for quality using the checklist for primary research from the Academy of Nutrition and Dietetics; six studies received a positive rating (Ren et al. 2015; Urooj et al. 2006; Ugare et al. 2014; Arora and Srivastava, 2002; Abdelgadir et al. 2004; Shukla and Srivastava, 2014), 12 studies were recorded as neutral (Jayasinghe et al. 2013; Lakshmikumri and Sumathi, 2002; Pathak et al. 2000; Thathola et al. 2010; Itagi et al. 2012; Torangatti and Rama, 1999; Nambiar and Patwardhan, 2015; Thilakavathy and Muthuselvi, 2010; Thakkar and Kapoor, 2007; Ayuo and Ettyang, 1996; Shukla et al. 1991; Narayanan et al. 2016) and only one study had a negative rating (Shobana et al. 2007).

All studies investigated the impact of millet consumption on blood glucose levels as the as primary or secondary outcomes. Postprandial IR was also measured in three of the studies (Ren et al. 2015; Abdelgadir et al. 2004; Shukla et al. 1991). 12 of the studies measured the GI of meals prepared from millet (Jayasinghe et al. 2013; Pathak et al. 2000; Shobana et al. 2007; Thathola et al. 2010; Torangatti and Rama, 1999; Urooj et al. 2006; Nambiar and Patwardhan, 2015; Thilakavathy and Muthuselvi, 2010; Ugare et al. 2014; Arora and Srivastava, 2002; Shukla and Srivastava, 2014; Narayanan et al. 2016), three of the studies measured the sensory characteristics of test meals (Pathak et al. 2000; Arora and Srivastava, 2002; Shukla and Srivastava, 2014;)

and the rest reported *in vitro* and *in vivo* starch digestion, protein and lipid profiles, glycosylated haemoglobin, lipaemic response, serum cholesterol and glycaemic load as the outcomes (Ren et al. 2015; Shobana et al. 2007; Thathola et al. 2010; Itagi et al. 2012; Torangatti and Rama, 1999; Nambiar and Patwardhan, 2015; Ugare et al. 2014).

4.3.3. Millet as intervention

The most common types of millet were used in the studies (finger, foxtail, pearl, proso, kodo and barnyard millet). Eight studies used finger millet (six studies used finger millet alone and two studies used finger millet as a mixture with another millet type) (Jayasinghe et al. 2013; Lakshmikumri and Sumathi, 2002; Shobana et al. 2007; Urooj et al. 2006; Thakkar and Kapoor, 2007; Shukla and Srivastava, 2014). two studies used finger millet mixed with pearl and foxtail millet (Torangatti and Rama, 1999) or barnyard millet (Arora and Srivastava, 2002) and only one study used proso millet (Abdelgadir et al. 2004). Five used foxtail millet (Pathak et al. 2000; Ren et al. 2015; Thathola et al. 2010; Itagi et al. 2012; Narayanan et al. 2016), three used pearl millet (Nambiar and Patwardhan, 2015; Thilakavathy and Muthuselvi, 2010; Shukla et al. 1991), one used barnyard millet (Ugare et al. 2014) and one study used barnyard millet mixed with foxtail millet (Pathak et al. 2000). One study used pearl millet, kodo millet and foxtail millet (Thilakavathy and Muthuselvi, 2010) and one did not mention the type of millet used (Ayuo and Ettyang, 1996).

The majority of studies added millet to traditional meals, either alone or mixed with other grains, seeds or other ingredients. Common Indian dishes were prepared from these millets: *roti , pittu, dosa, dhokla, upma, laddu, dal, mudde, cheela, bhakri, khichdi, thalipeeth, chapati, bati* and *idli* (Abdelgadir et al. 2004; Arora and Srivastava, 2002; Ayuo and Ettyang, 1996; Itagi et al. 2012; Jayasinghe et al. 2013; Lakshmikumri and Sumathi, 2002; Nambiar and Patwardhan, 2015; Narayanan et al.

2016; Pathak et al. 2000; Shukla et al. 1991; Thakkar and Kapoor, 2007; Thilakavathy and Muthuselvi, 2010; Torangatti and Rama, 1999; Ugare et al. 2014; Urooj et al. 2006). Several studies prepared meals in the form of bread, pancake, porridge, biscuit or noodles (Ren et al. 2015; Shobana et al. 2007; Shukla and Srivastava, 2014; Thathola et al. 2010).

4.3.4. Fasting and post-prandial blood glucose

All 19 studies in this review assessed the effects of different millet types and forms on the GR. Of these, eight used finger millet; one study used 50g of available carbohydrates (from finger-millet flour) as an intervention in two different meals (*roti* and *pittu*), stoneground or industrially prepared. Meals prepared with finger-millet flour that was stoneground displayed a lower GR when compared to rubber rolling (industrial method). The GR curves indicated a lower and delayed peak (after 15min) in individuals' samples after eating both *pittu* and *roti* made with stoneground flour. However, the peak glucose concentrations in *roti* samples were relatively lower than in *pittu* samples, even when the same milling method was used (Jayasinghe et al. 2013). This finding was corroborated by a second study, where the GR to finger-millet *dosa* was compared with finger-millet *roti* (both whole and germinated); finger-millet *roti* samples were found to have a lower GR than finger-millet *dosa* (Lakshmikumri and Sumathi, 2002). Other studies also revealed a lower GR following consumption of finger-millet-based *roti* in comparison to dumplings, *idli* or *upma* made from finger millet (Urooj et al. 2006, Thakkar and Kapoor, 2007). Four studies found that blood glucose levels after ingestion of millet-based meals peaked at 30min (Shobana et al. 2007; Shukla and Srivastava, 2014) or 60 min (Torangatti and Rama, 1999; Arora and Srivastava, 2002) and then fell.

Seven studies showed reductions in the GR after consumption of foxtail-millet-based

meals, including steamed bread, pancakes, porridge, *dosa* with foxtail-millet biscuits, *burfi* made with foxtail millet and foxtail millet rice with curds and milk (Pathak et al 2000; Ren et al. 2015; Thathola et al. 2010; Itagi et al. 2012; Narayanan et al. 2016; Torangatti and Rama, 1999; Thilakavathy and Muthuselvi, 2010).

Four studies (Torangatti and Rama, 1999; Nambiar and Patwardhan, 2015; Thilakavathy and Muthuselvi, 2010; Shukla et al. 1991) showed the same positive effect of consuming traditional Indian meals including pearl millet on lowering GR. These meals included *roti*, *dhal* (Torangatti and Rama, 1999) *chapati* (Torangatti and Rama, 1999; Thilakavathy and Muthuselvi, 2010; Shukla et al. 1991) *cheela* and *bhakri* (Nambiar and Patwardhan, 2015).

Three studies tested the GR after ingestion of barnyard millet and showed a significant reduction in fasting plasma glucose level (Ugare et al. 2014). One tested a mix of barnyard millet with finger millet, which elicited a peak rise in blood glucose 60min after the meal, before returning to baseline levels after 150min (Arora and Srivastava, 2002). Another study, by Pathak et al. (2000) showed the same dampening effect of barnyard and foxtail millet (mixed) on the GR.

One study found that porridge made from proso millet significantly reduced postprandial GR – more so than wheat pancakes and maize porridge (Abdelgadir et al. 2004). Another study by Thilakavathy and Muthuselvi (2010) reported that *chappati* made from kodo millet mixed with fenugreek leaves also reduced the GR.

As mentioned, one study did not clarify the type of millet added to meals (Ayuo & Ettyang 1996). The authors showed that the mean blood glucose increments differed between all the test foods, the highest reported for millet porridge (4.0 mmol/l) and the lowest for English potatoes boiled in water with salt (1.1 mmol/l).

4.3.5. Fasting and post-prandial plasma insulin

Of all the studies in this review, only three assessed the IR after millet consumption. Plasma insulin levels changed significantly in response to millet porridge in one out of three studies ($p = 0.01$). The AUC for plasma insulin showed a significant reduction in IR after the consumption of millet porridge, compared to sorghum porridge or flat bread, wheat pancakes, maize porridge or millet flat bread (Abdelgadir et al. 2004). One of the studies (Ren et al. 2015) showed that the peak insulin concentration in blood insulin reaction curves was slightly higher after millet porridge than after a standard glucose solution, compared to other millet meals in the study. The third study in this group (Shukla et al. 1991) found that the IRs to pearl millet-based *chapati* and white bread were not significantly different between healthy and type-2 diabetic individuals.

4.4. Discussion

4.4.1. Fasting and post-prandial blood glucose

Finger millet is considered to be a suitable food for people with type-2 diabetes and other non-communicable diseases (Saleh et al., 2013). Generally, in the review by Saleh et al. (2013), all dishes made with finger millet were shown to be beneficial to reduce GR in both healthy individuals and those with type-2 diabetes. Lower GI values were measured in meals made with stoneground millet flour, in comparison with those made through the use of industrially milled millets. This was due to the large particle size of the flour produced in stoneground flour (Jayasinghe et al., 2013). *Roti* meals made from finger millet elicited a lower GR (alone or mixed with gum acacia (5g)) compared with other meal types such as *pittu, idli, upma,* and dumplings in both healthy individuals and those with type-2 diabetes (Lakshmikumri and Sumathi, 2002; Urooj et al., 2006; Thakkar and Kapoor, 2007). Many researchers have shown that

meals with low and medium GI values can be protective for people with type-2 diabetes (Potter et al., 1981). Moreover, some experiments have shown significantly lower peak levels of plasma glucose and a decreased AUC after consumption of whole-finger millet, compared with the same dishes made from germinated-finger millet (Lakshmikumri and Sumathi, 2002). Notably, however, removal of the seed coat from finger millet was shown to impede finger millet's ability to lower the GR, as reported by Shobana et al. (2007).

The variations in the GR of different products made prepared from finger millet may be due to differences in the extent of gelatinisation, which significantly increases the GR (Wolever 1991). The lower GR of whole-finger millet meals is also likely evidence of the physiological benefits of anti-nutritional factors, such as polyphenols, enzyme inhibitors, phytic acid and tannins (Lakshmikumri and Sumathi, 2002). Moreover, different processing methods, such as cooking, fermentation and the removal of seed covers, may all alter the impact of finger millet on blood glucose.

Numerous studies have shown that millet consumption elicits a reduction in blood glucose due to its high content of unavailable carbohydrates. It has been reported that dehulled and dehulled and heated barnyard millet possess low GI values of 50% and 42%, respectively, making them highly beneficial for individuals with type-2 diabetes. The average reductions in fasting plasma glucose in diabetic and non-diabetic individuals after barnyard millet intervention were 6% and 7%, respectively (Ugare et al. 2014)., which may be owed to the higher dietary fibre and low carbohydrate content (Veena et al. 2005). Although a high proportion of unavailable carbohydrates in millet may be the major contributing factor to the reduction in blood glucose, different cooking methods (pressure cooking, roasting and baking) used by Arora and Srivastava (2002) were not shown to have any significant effect on the GI of certain

traditional Indian meals (*khichdi, luddu* and *baati*).

Pearl millet (*bajra*) is one of the most popular millet types used in Indian dishes. It has been determined that the effect of *bajra* consumption on the GR depends on the cooking method used (shallow frying, roasting or steaming). It has also been shown that millet-based meals (pearl, foxtail, and finger) are associated with low GIs both in healthy individuals and those with type-2 diabetes, due to the high protein content (Torangatti and Rama, 1999; Thilakavathy and Muthuselvi, 2010). Shukla et al. (1991) showed that the GR of *chapati* made with *bajra* was significantly lower than that of white bread in healthy individuals. Moreover, the addition of 30g of fenugreek to millet *chapati* reduced the GI further, resulting in a lower GR than that observed through consumption of millet *chapati* without fenugreek. The reduction in the GR in this case may have been due to the nature and viscosity of fenugreek fibre in the leaves,which may have delayed GE (Thilakavathy and Muthuselvi, 2010).

It has been well established that there is a positive link between proso millet consumption in type-2 diabetic participants and a significant reduction in the glucose impact (Abdelgadir et al., 2004). According to Colling (1981), the method and time taken for preparation of a meal may affect both the glycaemic and insulinaemic responses. In particular, the degree of milling and duration of fermentation affected these outcomes. However, Ayuo and Ettyang (1996) found that the highest peak rise in blood glucose level was recorded after consumption of millet porridge compared with root vegetables, probably due to the higher GIs of cereals (millet, maize and rice) compared with those of root vegetables, such as cassava and English potatoes. This, along with the nature of the foods and the preparation methods, may explain the differences observed in the GRs between millet porridge and root vegetables (David et al., 1981); however, this study does not mention the type of millet used for porridge

preparation, which reduces the reliability of the findings when comparing them with findings of studies that have tested other cereals and root vegetables, as each species of millet exerts different influences on the GRs and IRs.

An intervention study (Pathak et al., 2000) reported an association between foxtail millet intake and glucose reduction. Consumption over four weeks of a ready-made mix that contained 80% foxtail millet and 10% each of wheat semolina and black gram dal with selected spices led to reductions of 16% to 19% in the plasma glucose levels of the type-2 diabetic intervention group. In addition, the carbohydrate tolerance of the intervention group improved, as shown by a reduction in the GR after four weeks. The cereal-pulse composite was concluded to be a healthy meal combination that helped to maintain normal concentrations of blood glucose. Notably, large portions of *uppuma* and *dhokla* (Indian dishes) made with foxtail millet did not induce sharp rises in blood glucose levels, indicating that they offered a healthy choice for type-2 diabetics (Pathak et al., 2000).

Foxtail-millet *dosa* caused a significant reduction ($p < 0.001$) in postprandial glucose values compared to individuals who consumed rice *dosa* (Narayanan et al. 2016). This finding corroborates that by Thathola et al. (2010), who reported that biscuits and *burfi* made from foxtail millet significantly reduced serum glucose levels from baseline by 23% after a long intervention period (30 days). According to Ren et al. (2015), cooked foxtail millet elicited the lowest post-prandial blood glucose concentration, followed by millet pancakes (without extrusion flour), foxtail millet porridge, millet pancakes (75% millet flour and 25% extrusion flour), foxtail-millet steamed bread and standard glucose solution. It has been noted that the processing methods significantly affected the GR of foxtail millet. Ren et al. (2015) found that cooked millet was found to be the best choice and preparation of millet for individuals with type-2 diabetes.

4.4.2. Fasting and post-prandial plasma insulin

It has been confirmed that there is a strong association between millet intake and reduced insulin response. A study by Abdelgadir et al., (2004), indicated that the AUC for plasma insulin after consumption of proso-millet porridge showed a significantly reduced insulin response compared with findings after consumption of sorghum (flatbread and porridge), millet (flatbread), maize (porridge) and wheat (pancake) (p = 0.0001 for all comparisons). The degree of milling and duration of fermentation was suggested to play an important role in the insulinaemic response (Colling 1981). Also, a reduction of insulin levels was observed 2h after consumption of cooked foxtail millet and foxtail-millet pancakes (which comprised 75% millet flour and 25% extrusion flour) compared with consumption of a standard glucose solution. However, Shukla et al., (1991), found no significant differences in the IRs after consumption of pearl millet compared with white bread in healthy and type-2 diabetic individuals, although the white bread produced a somewhat lower IR in type-2 diabetics 1h after intervention. In healthy individuals, pearl millet was shown to exhibit a low GI and a high insulinaemic index; however, the opposite was true for those with type-2 diabetes, in whom the GI of pearl millet was high and the insulinaemic index was low. The authors found that pearl millet evoked insulin secretion in healthy individuals, and this reduced the GR, whereas in type-2 diabetics there may have been an insufficient insulin reserve, leading to insufficient mobilisation of insulin on ingestion of pearl millet.

Millet is a functional grain known for its significant health benefits, attributed in large part to its high content of polyphenols and antioxidants, as evidenced by substantial research regarding their effects on type-2 diabetes, among other fields (Saleh et al., 2013). The present systematic review of 19 studies has demonstrated a beneficial effect

of different types and forms of millet on fasting and post-prandial blood glucose and plasma insulin levels in healthy adults and patients with type-2 diabetes. To our knowledge, this is the first systematic review to examine the effect of millet consumption on glucose and insulin sensitivity in healthy adults and those with type-2 diabetes. Overall, this review has shown that consumption of different types and forms of millet can lead to an improvement in glucose and insulin sensitivity compared with the levels of these markers after different control meals (e.g. glucose, sorghum and wheat).

4.4.3. Limitations

The quality ratings of the 19 eligible studies, assigned using CONSORT and the checklist for primary research from the Academy of Nutrition and Dietetics, indicated that, despite strong criteria that limited the number of studies that could be included (n=19), only a few were rated positively (n=6), while 12 were rated neutral and only one study received a negative rating.

There were a few limitations to the systematic review in this work. First, the 19 studies were mostly reports not of randomised clinical trials but of primary pilot trials, which did not provide reliable causality inferences or achieve sufficient statistical power to detect a significant effect of millet consumption on postprandial blood glucose and plasma insulin responses. These shortcomings were compounded by the small sample sizes and short-duration interventions in some studies. One study, as previously mentioned, did not specify the millet type (e.g. finger, pearl) or the form (e.g. grain, flour or flakes) used, which impaired the quality of the study results. Some studies mixed millet with other grains or spices, which made it hard to determine whether the millet and its components or the other ingredients were responsible for the observed reductions in the GR in participants. It should also be noted that portion size of test

meals,or the millet proportions (either added to a meal or as a replacement), were not sufficiently explained in some studies. Separate investigations across the various millet-grain foods produced from different crops may be useful to decrease heterogeneity and to obtain more robust scientific evidence of the impact of millets from specific sources on glycaemic and insulinaemic responses. This is because each millet type has a different source, type and colour, and has been shown to produce a different effect on blood glucose and insulin responses dependent on the polyphenol compound and starch content.

4.5. Conclusion

This systematic review has revealed a beneficial effect of consuming different types of millet on fasting and post-prandial blood glucose and the plasma IR in healthy and type-2 diabetic individuals. The effect of millet consumption on pre-diabetic trials with an adequate sample size should be investigated in the future. Also, it is worth exploring the potential benefits of different types of millet in managing type-2 diabetes through long-term feeding interventions.

Table 4.1: Characteristics of the 19 studies included in this review

Reference	Subject characteristics	Study design/length	Millet types & forms	Intervention	Study outcomes	Quality rating
Abdelgadir M et al. 2004	10 type 2 diabetic adults (4 females), 50.2 ± 5.3 years, BMI 27.5 ± 4.0 kg/m2.	Crossover study Study length: 6 days	Proso millet	Each participant consumed 60g of available carbohydrate id different occasions: 1-wheat gorasa (pancakes) 2-sorghum kisra (flat bread) 3-sorghum acida (porridge) 4-millet kisra 5-millet acida 6-maize acida *Control (NA).	The AUCs of the meals showed that millet acida (porridge) followed by wheat gorasa (pancakes) displayed significantly ↓ post-prandial glucose and insulin responses, whereas maize acida induced a higher post-prandial glucose and insulin response	Positive
Arora S & Srivastava S 2002	10 healthy adults (10 female), 23.5±4.95 years, BMI 21.74±3.08 kg/m2.	Not known. Study length: 6 days.	Finger millet Barnyard millet	Each participant consumed 50 g of available carbohydrate in different occasions: 1-Khichdi (millet, whole green gram and fenugreek seeds). 2-Laddu (millet, roasted soybean, malted fenugreek seeds & amaranth) 3-Baati (fenugreek seed & roasted Bengal gram flour) *Control (Standard glucose solution (50g))	Consumption of low GI millets based products (finger and barnyard millet) resulted in significantly ↓ GR (AUC)(auc for different food products ranged from 1675.23 mg min/100 ml (finger millet based khichdi) to 2377.80 mg min/100 ml (barnyard millet based laddu).	Positive

Ayuo PO & Ettyang GA 1996	15 type 2 diabetes adults, (6 female), 51 ± 3.9 years, BMI 24.2±1.2 kg/m2.	Not known. Study length: 2 months.	Not known	Each participant consumed 50 g of available carbohydrate in different occasions, 4 to 8 test meals: 1-brown bread, 2-white rice (boiled in water +salt) 3- English potatoes (boiled in water +salt) 4- maize meal (porridge) 5-millet (porridge) 6-cassava (boiled in water +salt) *Control (white bread)	The mean blood sugars at 0,60 and 120 minutes were comparable for each food, and the peak rise occurred at 60 minutes. The highest rise (4.0 mmol/I) was seen with millet porridge.	Neutral
Itagi, S et al. 2012	Group1:15 diabetic adults (5 female) & 6 diabetic adults (4 female) as control 50.78 ± 51 years, weight 69.40±74.83 kgs, BMI 27.79±29.14 kg/m2 . Group2:15 healthy adults (5 female) & 6 healthy adults (2 female) as control, 43.17±43.67 years, weight 64.33±66.00kgs,BMI 24.07±26.10 kg/m2	Not known. Study length: 4 weeks.	Foxtail millet	Each participant consumed 87 g of diabetic mix per day in breakfast/lunch and dinner. Diabetic meal mix made with 80%foxtail millet and along with 10% both wheat semolina, black gram dal & selected spices *Control(Healthy adult group)	Consumption of diabetic mix foxtail millet-based resulted in ↓ fasting plasma glucose (up to 16-19%) after 4 weeks.	Neutral

Jayasinghe, MA et al. 2013	11 healthy adults (6 female), 25 ±7.07 years, BMI 21 ±3.54 kg/m2.	Crossover study. Study length: 3 days.	Finger millet/ A bulk finger millet (half was milled using a rubber rolle &pin mill, other half by a domestic stone grinder)	Each participant consumed 50 g of available carbohydrate in different occasions: Roti and Pittu occasions made with industrially milled finger millet flour or stone ground flour. *Control (Standard glucose solution).	Foods prepared with finger millet (kurakkan) flour with a larger particle size distribution resulted in a ↓ GR.	Neutral
Lakshmikumri P & Sumathi S 2002	6 type 2 diabetes adults (male) 47.5±10.61years, weight 57±3.54 kg	Not known. Study length: 7 days.	Finger millet (Germinated)	Each participant consumed 75g of available carbohydrate: **Dosa** and **roti** made with finger millet compared with control breakfast wheat roti and rice dosa(breakfast meet one third of daily calorie requirement). *Control(wheat roti and rice dosa)	Consumption of finger millet based diets resulted in significantly ↓ plasma glucose levels, mean peak rise, and area under curve.	Neutral
Nambiar V & Patwardhan T 2015	6 healthy female, 20.5± 3.54 years, 23.0±24.9 kg/m2.	Not known. Study length:9 days.	Pearl millet	Each participant consumed 6 test meals /50g of available carbohydrate in different occasions each meal served with curd (dairy product/50 g of available carbohydrate) 1-pearl millet bhakri 2-jowar bhakri 3-Cheela 4-Khichadi 5-Thalipeeth 6-wheat roti. *Control (Standard glucose solution (50g)).	Consumption of pearl millet based meals resulted in ↓ GR (IAUC).	Neutral

Narayanan J et al. 2016	105 type 2 diabetes adults (36 female), 49.26± 9.903 years, weight 72.157±10.417, height 162.295±8.636,BMI 27.436±3.7396 kg/m2.	Not known. Study length: 2 days.	Foxtail millet	All participants are divided into two groups/each participant consumed 50 g of available carbohydrate in different occasions of: 1-millet based dosa 2-rice based dosa *Control(White bread)	Consumption of millet-based dosa resulted in significantly ↓ in the postprandial glucose level.	Neutral
Pathak, P et al,2000	5 healthy adults (female) and 5 type 2 diabetes adults (male) , 46 ± 33.94years, BMI 25.5± 4.24 kg/m2	Not known. Study length: 4 days.	Foxtail millet Barnyard millet	Each participant consumed 50 g of available carbohydrate in different occasions of: Laddu made with amaranth, foxtail millet, roasted legumes, a paste of fenugreek seeds and jaggery syrup. Uppuma made with foxtail, barnyard millet, legumes, fenugreek seeds and defatted coconut powder each Dhokla made with foxtail, barnyard millet, legumes and fenugreek seeds. *Control (NA).	Consumption of foxtail-millet-based meals resulted in a ↓ GR.	Neutral

Ren X et al. 2015	10 healthy adults (7 female), (mean) 26.0 years, (mean)BMI 20.8 kg/m2	Not known. Study length: 6 days.	Foxtail millet (flour).	Each participant consumed 50 g of available carbohydrate in different occasions of: Steamed bread, pancake or porridge made with foxtail millet flour. *Control (Standard glucose solution (50g)).	Consumption of foxtail-millet-based meals resulted in a ↓ GR.	Positive
Shobana SH et al. 2007	8 healthy adults (3 female) , 39±19.80 years	Not known. Study length: 5 days.	Finger millet	Each participant consumed 50 g of available carbohydrate in different occasions : Porridge made with wheat or millet or rice. *Control (White bread).	Consumption of finger millet-based and wheat meals resulted in a ↓ GR.	Negative
Shukla K & Srivastava S 2014	10 healthy female adults, 25.5 ± 2.12years.	Not known. Study length: 2 days.	Finger millet	Each participant consumed 50 g of available 1-carbohydrate in different occasions: 2-noodles made with finger millet flour 3-noodles made refined wheat flour *Control (Refined wheat flour noodles).	Consumption of low GI finger millet flour incorporated noodles improved blood glucose control.	Positive

Shukla,K et al. 1991	18 healthy adults, 14 type 2 diabetes adults. 49.25 years, weight 55.625±14.286 BMI 24.575 ± kg/m2.	Not known. Study length: 2 days.	Pearl millet (bajra)	Each participant consumed 50 g of available carbohydrate in different occasions: 1-Maize (chapaties) 2-bajra (chapaties) 3-barley (chapaties). *Control (White bread (50 g of available carbohydrate).	GR to pearl millet was significantly ↓ than that to white bread in healthy subjects, but the two responses were indistinguishable in T2D subjects. The IR to pearl millet and white break were not significantly different in either group of subjects.	Neutral
Thakkar R & Kapoor R 2007	42 type 2 diabetes adults, 42.5 ± 3.54 years, weight 50 to 58 kg.	Not known. Study length: 1 day.	Finger millet	All participants were randomly assigned to 7 groups (6 test groups and 1 control group). Each group consists of 6 participants. Each test meal product provided of 50 g of available carbohydrate: 1-finger millet (roti, idli, upma) 2-rice (roti, idli, upma). *Control1 (Healthy adults group).	Consumption of low GI finger millet based meals resulted in significantly ↓ GR.	Neutral
Thathola A et al. 2010	30 type 2 diabetics adults (14 female), 57±29.70 years, BMI 24.9±10.47 kg/m2	2 experiments: 1/Case control clinical trial. Study length: 30 days. 2/Crossover study (4 weeks), this trail was an extension of the case-control clinical trial. Study length: 30 days.	Foxtail millet (flour)	Each participant consumed 100g of biscuit & burfi made with foxtail millet. *Control (Type 2 diabetes adults group).	Consumption of low GI biscuits or burfi made with foxtail millet resulted in significantly ↓ serum glucose by 23% from baseline value.	Neutral

Ugare R et al. 2014	9 diabetic adults, 6 healthy adults (3 female), 37 ±40 years.	Not known. Study length: 28 days.	Barnyard millet	Each participant consumed 73 g of test meals/50g of available carbohydrate in breakfast/lunch or dinner: Upma (dehulled and roasted barnyard millet). *Control (Standard glucose solution(50g)).	Consumption of barnyard millet based meals resulted in significantly ↓GR (139.2 to 131.1 mg/dl).	Positive
Urooj A et al. 2006	7 type 2 diabetes adults (3 female) , 27±60 years, BMI 24.5 ±3.32 kg/m2 7 healthy adults (4 female), 22± 45 years , BMI 22.3±2.0 kg/m2	Not known. Study length: 4 days.	Finger millet (ragi)	Each participant consumed 4 test meals/50g of available carbohydrate in different occasions: 1-finger millet roti 2-rice roti 3-finger millet dumpling 4-jowar dumpling *Control (Standard glucose solution (50g)).	Consumption of finger millet based meals resulted in significantly ↓ GR (IAUC).	Positive
Thilakavathy S & Muthuselvi S 2010	200 type 2 diabetes adults, (100 female), 50 ±14.14 years.	Not known. Study length: 2 days.	Pearl millet (bajra) Kodo millet (varagu/paspalum scrobiculatum) Foxtail millet (Thenai/Setaria italica)	Each participant consumed test meals is different occasions: 1-chappathi made with whole wheat flour 2- 100g white bread 3- 75g of chappathi made with millet 4-75 g chappathi made with millet and 30 fenugreek leaves. *Control (50 g available carbs of white bread).	Consumption of millet based meals resulted in ↓ GR.	Neutral

Torangatti G & Rama K 1999	18 type 2 diabetes adults (2 females), 54.8±3.25years, weight 65.3±1.70 18 healthy adults(10 female) 53.2±4.10years, weight 45.45 ± 14.78	Not known. Study length: 2 days.	Pearl millet (bajra) Finger millet (ragi) Foxtail millet (Navani/Setaria italica)	Each participant consumed 50 g of available carbohydrate: the selected diabetic subjects were then randomly divided into six groups consisting of three type 2 diabetics and three normal subjects. Test meals: Group 1 - Jowar (Sorghum) roti and greengram Group 2 - Rice and redgram dhar curry Group 3 – Chapati and cabbage bhaji Group 4 – Ragi balls and redgram dhal with amaranthus curry Group 5 – Navani rice and curds with milk Group 6 – Bajra roti and brinjal bhaji *Control (Standard glucose solution (50g)).	Consumption of millets based meals (foxtail. Finger and pearl millet) resulted ↓ in GR.	Neutral

Chapter 5: The effect of muffins made with finger millet on glycaemic response, insulinaemic response and gastric emptying in prediabetic and healthy adults (Study 4)

5.1. Introduction

Reducing the prevalence of obesity, which carries with it the risk of type-2 diabetes and its complications, is a matter of urgent public attention in the Western world (Kam et al. 2016). In 2013, the NHS spent a daily average of £2.2m on drug prescriptions for managing diabetes in primary care alone – over 10% of the entire prescribing budget in the UK (Lacobucci, 2014). Prediabetes is defined as a condition where blood glucose levels are elevated above optimal levels, but remains below the threshold required for clinical diagnosis of diabetes. Each year, between 5% and 10% of individuals exhibiting a prediabetic state are shown to progress to clinical type-2 diabetes (Mainous et al. 2014). If poorly managed, this disorder is linked to adverse cardiovascular outcomes, recurrence and microvascular complications. Unfortunately, there is insufficient awareness of this among the general population with existing prediabetes (Hsueh et al. 2015). Early diagnosis and treatment of individuals with prediabetes can not only delay, but prevent the development of type-2 diabetes, as well as minimising the risk of consequent cardiovascular abnormalities (Hsueh et al. 2015). According to Hsueh et al. (2015), there are currently no approved medical treatments for prediabetes, with lifestyle changes remaining the only effective option.

Postprandial glycaemic control is important in preventing or managing diabetes, as well as the side effects of the disease, including: retinopathy, nephropathy, diabetic foot and cardiovascular diseases (Butacnum et al. 2017). The GR of a meal is the effect that consuming the meal has on blood glucose levels; both blood glucose and insulin

levels rise after food consumption and then drop back to fasting levels over a period of time, with the peak size and duration depending on the amount and type of carbohydrates ingested. Ensuring adequate regulation of the size and duration of these postprandial blood glucose increases is generally important to an individual's health and especially crucial to people with type-2 diabetes (Chandra and Bardosono, 2016). Dietary management is, therefore, a useful strategy for managing or preventing diabetes and its complications. Currently, the recommended diet for those with diabetes should be high in fibre and low in fat, as this is known to help reduce the size and the duration of the postprandial elevation in blood glucose (Itagi et al. 2012). Research has shown that diets rich in carbohydrates and fibre significantly improve glucose tolerance and insulin sensitivity, while also delaying GE and slowing down glucose absorption (Itagi et al. 2012; Thakkar and Kapoor, 2007). Decreasing the rate of GE maintains a feeling of satiety and prevents further eating and blood glucose rises (Morey et al. 2016). Blood glucose management focuses on keeping levels as close to normal as possible, without causing hypoglycaemia (Butacnum et al. 2017). A number of studies have shown that a diet including finger millet is associated with lower blood glucose levels, compared to a rice-based diet, in individuals with diabetes; this is a result of the high content of complex carbohydrate and RS in finger millet, which leads to a slower rise in blood glucose (Urooj et al. 2006).

Finger millet, known as *ragi*, is a cereal crop consumed by a large proportion of South Asian and African populations (Shobana et al. 2018). India is the main producer of finger millet and contributes around 60% of the world's production of the cereal (Shuka and Srivastava, 2014). Recently, finger millet has become of scientific interest due to its beneficial nutritional profile in regard to dietary fibre and protein. The seed cover is rich in polyphenols, dietary fibre, vitamins and minerals, particularly calcium (344mg/100g) (Chethan and Malleshi, 2007; Mamatha and Begum, 2013). In addition,

the carbohydrates in finger millet are shown to be slowly digested and assimilated, compared to other cereals (Lakshmi and Sumathi 2002). Regular consumption of finger millet has been found to significantly lower peak plasma levels and the AUC for glucose (Shukla and Srivastava, 2014; Kam et al. 2016).

There have been various studies on the effects of different millet-based meals on the GR and IR; in particular, the effectiveness of finger millet as a dietary component for type-2 diabetics has been confirmed (Kam et al. 2016). Prediabetes can be seen as an early indication that continuing an unhealthy diet and lifestyle may lead to development of type-2 diabetes. The majority of existing health guidelines and research support the notion of dietary modification as the foundation of effective management and prevention of prediabetes. The primary goal for people with higher-than-normal blood glucose levels is to regulate them during both the fasting and postprandial phases, so as to facilitate a decrease to as near normal levels as possible. This goal can be achieved by following a healthy diet rich in complex carbohydrate and fibre, which underlies the hypotheses of the current study. It was hypothesised here that consumption of finger millet-based muffins, found to be high in polyphenols and antioxidants, would reduce the GR and IR, as well as delay GE and improve satiety, in adults with prediabetes compared to healthy adults. Prediabetes can be seen as a 'grey area' between normal blood glucose and hyperglycaemia, as seen in type-2 diabetic sufferers. Thus, it is possible that finger millet consumption will have the same (or at least a similar) minimising effect on blood glucose levels here in individuals with prediabetes as shown in previous studies on those who were healthy or had type-2 diabetes.

Finger millet grain has been chosen in this study, based on the results of the previous studies (Chapters 2 and 3) confirming the high polyphenol and antioxidant content of

the grain. Moreover, this form and type of grain was found to be most accepted by a population sample subjected to sensory evaluations (Chapter 3). The primary aim of this study was to determine the effect of consumption of muffins made with finger millet on early (0 min-180 min) postprandial glycaemia and plasma insulin secretion (measured by the IAUC); and on GE in prediabetic and healthy adults. A secondary aim was to assess the effect of finger-millet muffins on satiety in these participants.

5.2. Materials and methods

5.2.1. Study design

A single-blind randomised-controlled crossover study was designed to enable participants to receive each of two muffins, one made with wheat (control) and one with finger-millet (intervention), in random order during two separate study visits with at least one day of wash-out between each visit. These study visits were undertaken after a screening session. Each visit lasted for a maximum of four hours and was conducted in the morning at the OxBCNH. The muffin order was randomised using a pseudo-random number generator (Research randomizer, n.d.). During the 24 hours prior to testing, participants were asked to avoid caffeine, alcohol, nicotine and unusual strenuous exercise. All participants fasted for 12 hours (overnight) and only consumed water.

Ethical approval for the study was obtained from the University Research Ethics Committee (UREC) at Oxford Brookes University (UREC Registration No: 161061) (Appendix 3A). Each participant was given full details of the study protocol and an opportunity to ask questions (Appendices 3E and 3D). Written informed consent was collected from each participant prior to commencing the screening and/or test (Appendix 3F).

5.2.2. Participants

Two groups of people were recruited for the study: healthy controls and those with prediabetes. The study was conducted over eight months, from May 2017 to December 2017.

Group 1: Fifteen participants were included in the (control) healthy group, under the following criteria: they had no known medical conditions, were 18-65 years of age, had a BMI $\leq 30 kg/m^2$, fasting blood glucose level <6.1mmol/l, no known diabetes and no impairment in glucose tolerance.

Group 2: Fourteen participants were included in the prediabetes group. The inclusion criteria were: 18-65 years of age, a BMI ≥ 25 kg/m^2 (based on the BMI guidelines for prediabetes (>25, NHS, 2014)), a fasting blood glucose level of 6.1–6.9 mmol/l, and/or 7.9–11.0 mmol/l 2h after an oral glucose tolerance test (OGTT) (Figure. 5.1).

In addition to the aforementioned inclusion criteria in the prediabetes group, according to the NHS (2014), certain factors may increase the risk of developing type-2 diabetes, such as being over 40 years of age, being of South Asian ethnicity, genetic factors (e.g. having a close relative diagnosed with diabetes), a history of gestational diabetes and/or polycystic ovary syndrome (PCOS). Volunteers with one or more of these factors were invited for a screening, where they were subjected to an OGTT and fasting blood glucose measurement, and their eligibility to participate was assessed via a specific protocol (Figure. 5.1). Notably, none of the participants in either group were taking any medication that would affect their glucose regulation, gastric emptying, appetite or body weight. None of the participants were smokers.

Participants for the study were recruited through advertisements (Appendix 3C) posted on University notice boards, supermarkets, the University sports centre and the

Headington and Wheatley libraries, via the University Research Activity Group. Advertisements recruiting people with prediabetes were posted in community centres, diabetes and prediabetes support groups, surgery and health centre waiting rooms in Oxford, on advertising sites (Gumtree and Daily Info) and the Diabetes UK website. On expressing interest, the volunteers were sent details of the study via email. If still interested and eligible, they were then given full details of the study protocol and were invited to attend a screening session, either before or on the morning of the test visit, where an information sheet was provided and a health questionnaire was completed, including details of smoking habits, food allergies/intolerances, metabolic diseases, physical activity, medication, eating habits and disorders (Appendices 3E, 3D and 3L).

Fasting blood glucose, anthropometric measures and blood pressure were measured to confirm the eligibility of the participants for each group. Height was recorded using a stadiometer (Seca Ltd, UK), with shoes removed. Body weight was recorded using a Tanita BC-418 MA scale (Tanita UK Ltd), with heavy garments and shoes removed. All measurements were collected in the laboratory at OxBCNH.

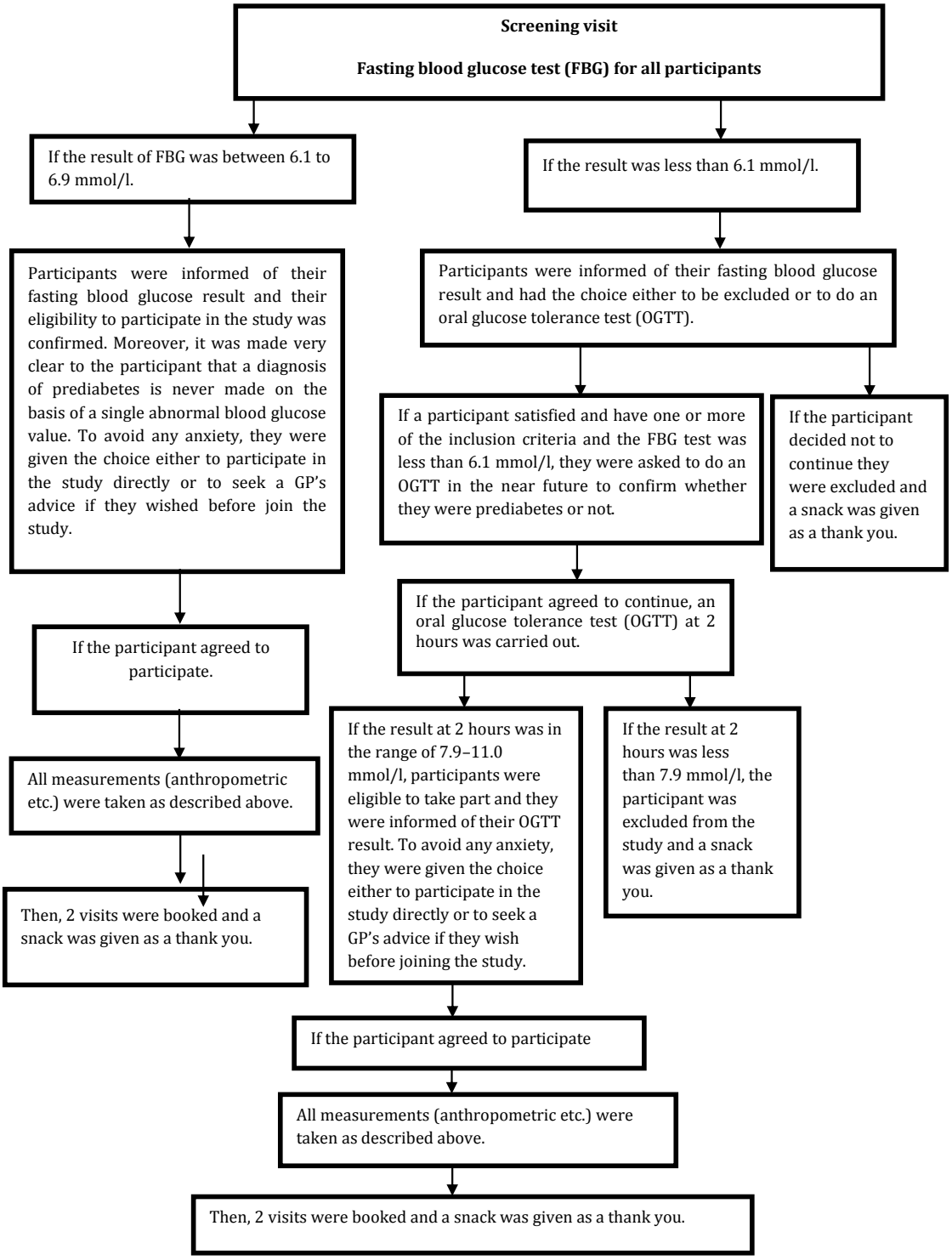


Figure 5.1: Flowchart indicates screening process used for participants in the prediabetes group

5.2.3. Test food preparation

The test meals were two types of muffins: a control muffin (100% wheat flour) and a finger millet muffin (50% finger millet grain, 50% wheat flour), which were provided on two different occasions. Finger millet was selected based on the findings in Chapters 2 and 3, which showed high polyphenol and antioxidant content, reduced RDS and best acceptability regarding all sensory attributes. Control and finger millet-based muffins were prepared in the OxBCNH kitchen using the protocol described in Section 3.2.1. The decision to replace 50% of the wheat flour in the control with 50% of millet to produce the millet-based muffin was made according to previous studies and a desire to promote utilisation of millet among the population in the UK. A study by Shukla and Srivastava (2011) found that noodles that contained 30% finger-millet flour exhibited a lower GI than wheat noodles. These noodles were more acceptable in all attributes such as taste and texture than noodles composed of 40% or 50% finger-millet flour. In our study, the percentage of millet was increased from 30% to 50% as the product was altered to muffins, which were deemed more appropriate as a breakfast food, and our previous studies had found that muffins based on 50% finger-millet grain were highly acceptable in measurements of the same sensory attributes (Chapter 2).

The finger millet muffins were prepared by the researcher one day prior to testing, using a standardised recipe modified by replacing 50% of wheat flour with crushed finger millet grain. Nutritional analysis was carried out by Eurofins Food Testing UK Ltd, which also advised on serving sizes of muffins (Table 5.1). Muffins were matched to contain 50g of available CHO per portion; control muffins (133.69g) and finger millet-based muffins (145.77g) were served with 250 mL of water.

Table 5.1: Nutrient content of all muffin types (per 100g)

Nutrition content (g/100 g)	Control muffin	Finger millet muffin
Energy (kcal)	319	300
Available carbohydrates	37.4	34.3
Total sugars	9.3	9.4
Total dietary fibre (AOAC)	1.3	4.3
Total fat	15.7	14.4
Crude protein	6.3	6.2

5.2.4. Sample collection procedures

Blood for glucose and insulin tests was obtained using the finger-prick method and a single-use lancing system (Unistick 3, Owen Mumford, Woodstock, UK) at -5min (before consumption), 0min (baseline) and 15, 30, 45, 60, 90, 120, 150 and 180min post-consumption. Before conducting the finger prick, participants were asked to warm their hand to increase the blood flow when required. The first two drops of expressed blood were discarded as they might have been contaminated and, therefore, skewed values (as advised by Hortensius et al. 2011 and WHO, 2010). The fingertips were then lightly massaged to extract blood from the base to the tip.

5.2.4.1. OGTT

An OGTT identifies issues in glucose metabolism and is, therefore, a useful method of diagnosing diabetes mellitus, insulin resistance, reactive hypoglycaemia and gestational diabetes (American Diabetes Association, 2009).

The participants were asked to fast overnight (12h) and then consume 75g of dextrose (unflavoured, Myprotein) dissolved in 250ml of water (WHO, 1999). Plasma glucose was measured while fasting and after 2h using an automatic blood glucose analyser

(Glucose 201+, Hemocue, Radiometer Ltd, Crawley, UK). A microcuvette was used to draw approximately 5µl of blood, which was immediately measured using the analyser. Hemocue is a portable analyser, commercially available and for the rapid measurement of haemoglobin and glucose from a single drop of blood taken through a finger prick. The analyser was calibrated daily using control solutions (GlucoTrol-NG, Level 2, 1.0ml) to ensure accuracy of results within a defined range. Plasma glucose was measured while fasting and after 2h. Prediabetes was diagnosed if OGTT blood glucose results after 2h were in the range of 7.9-11.0 mmol/l (Diabetes.UK, 2016).

5.2.4.2. Measurement of the glycaemic response (GR)

The method used to measure postprandial capillary blood glucose concentration was adapted from Brouns et al. (2005) and was in line with FAO/WHO (1998) recommendations. After discarding the first two drops, fasting blood samples were taken in duplicate (at -5 and 0 min) to check that the results were within a coefficient of variation (CV) of 3%.

5.2.4.3. Measurement of the insulinaemic response (IR)

For measuring insulin, blood samples were taken at the same time points and using the same finger-prick technique as for GR (5.2.4 and 5.2.4.2). At each time point, approximately 300µl of blood was collected in EDTA-coated microtainer tubes (ethylenediamine tetra-acetic acid) (Bunzl Healthcare, Enfield, UK) and stored immediately on ice. All samples were centrifuged at 4,000rpm for 10min, following which 200µl of supernatant plasma was collected and placed into 1.5ml Eppendorf tubes and frozen at -40°C, until analysis.

Plasma samples were analysed for insulin using the electrochemiluminescence immunoassay and an automated analyser (Cobas® E411; Roche Diagnostics, UK). The Cobas® system is a reliable method for determining plasma insulin concentration

(Siahanidou et al. 2011).

The changes in the GR and IR after consumption of each muffin was calculated geometrically as the incremental area under the curve (IAUC), using the trapezoidal rule (FAO, 1998), and only the area above the fasting level was included in the analysis.

5.2.4.4. Measurement of gastric emptying (GE)

GE characteristics were estimated in this study using the ^{13}C breath-test method. This method was used as it was appropriate for repeat testing. Also, the ^{13}C breath-test method was of sufficient validity and reliability compared with other GE measurement methods. GE was measured using a ^{13}C sodium acetate breath test (Morey et al. 2016); 100 mg of ^{13}C sodium acetate was added to the muffins (control and finger millet) to measure the GE rate. For this, breath samples were collected during each visit at the baseline (-5 and 0 min) prior to muffin consumption, then during the postprandial four-hour period at the same time points as for GR and IR – plus after 210 and 240min. Breath was collected using a drinking straw by blowing into a 10ml exetainer® tube (Labco, UK); after removing the straw, just prior to the end of exhalation, the cap was immediately replaced. All participants were asked to wear a nose-clip during sampling to ensure that they only exhaled through the mouth. All samples were stored at room temperature for analysis.

Breath samples were measured using GC-IRMS (gas chromatography isotope ratio mass spectrometry) and all results were expressed relative to Vienna PeeDee Belemnite (V-PDB), an international standard of known ^{13}C composition. Data was expressed as % of $^{13}CO_2$ dose recovered per hour and cumulative % of $^{13}CO_2$ recovered over time. The production of CO_2 was assumed as 300 mmol/m^2 of body surface area per hour. The body surface area was calculated using a pre-validated

weight-height formula (Haycock et al. 1978), which was then fitted into a GE model developed by Ghoos and colleagues (1993). After that, to ensure the validity of the model, the r2 co-efficient between the modelled and raw data was calculated and equalled >0.95. From this model, the lag phase and half time were then calculated.

- Lag phase (tlag): the time taken to maximal rate of $^{13}CO_2$ excretion is equivalent to the time of the inflection point.
- Half time (thalf): the time it takes for 50% of the ^{13}C dose to be excreted.
- Latency phase (tlat): the initial delay in the excretion curve, located at the point of intersection of the tangent at the inflection point of the $^{13}CO_2$ excretion curve.
- Ascension time (tasc): the latency phase and the half time, representing a period of high $^{13}CO_2$ excretion rates.

5.2.4.5. Measurement of hunger and satiety

A 7-anchor bidirectional scale (Appendix 3K) was used to assess the satiety or hunger of participants at 0, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min time points, during each visit, immediately prior to blood sampling. The AUC was calculated geometrically using baseline scores as a covariate. This practice is recommended by Blundell et al. (2010) as it corrects for baseline differences when analysing appetite scales. The rating of the scale was as follows: 6 = extremely full, 0 = extremely hungry.

5.2.5. Statistical analysis

Statistical analysis was undertaken using the Statistical Package for the Social Sciences (SPSS, v. 25, USA). All values are reported as mean ± SD unless otherwise specified. Before statistical analysis, the incremental data for the GR, IR and GE of

millet and control muffins in each group (prediabetes and healthy) were tested for normality using a Shapiro-Wilks test; where data was not normally distributed, non-parametric tests were used. Paired sample t-tests and Wilcoxon tests (for non-parametric data) were used for comparisons of the changes in the GR and IR concentrations over 3h (for GE, over 4 h).

The IAUC for the GR, IR and the GE data were analysed using a two-way repeated measures ANOVA (muffin types x participant groups) to study the differences between healthy and prediabetic participants. Pairwise comparisons were performed using a Bonferroni correction. In all analyses, p values < 0.05 were considered to be statistically significant. For the satiety AUC, a one-way ANCOVA (analysis of covariance) was carried out, using the baseline as a covariate, to determine differences between muffin types.

Previous statistical power calculations (Clegg et al. 2011) demonstrated that a total sample size of 12 was required, based on the difference in mean GR-AUC of 10 mmol/l, in order to achieve a power of 0.9 with a mean SD of 5 and an α of 0.05. In the current study, G*power (Version 3.0.10) revealed that for a medium effect size, with an α of 0.05 (two-tailed) and a power of 0.95 of power, a sample size of 15 in each group was required. Thus, 15 was the sample size for the health group; however, only 14 participants were recruited for the prediabetes group – a total of 29 subjects.

5.3. Results

5.3.1. Participants' characteristics

A total of 16 healthy and 50 prediabetic individuals who met the inclusion criteria (Figure 5.2) attended screening sessions; 16 in the healthy group (15 individuals who completed the study) and 14 in the prediabetes group who completed the study.

Demographic characteristics for the final number in each group were collected from 15 healthy adults and 14 prediabetic adults, as shown in Table 5.2. There were no significant differences in age, systolic blood pressure or height between the two groups. There were, however, significant differences ($p < 0.05$) in weight, BMI and diastolic blood pressure, as per the study protocol.

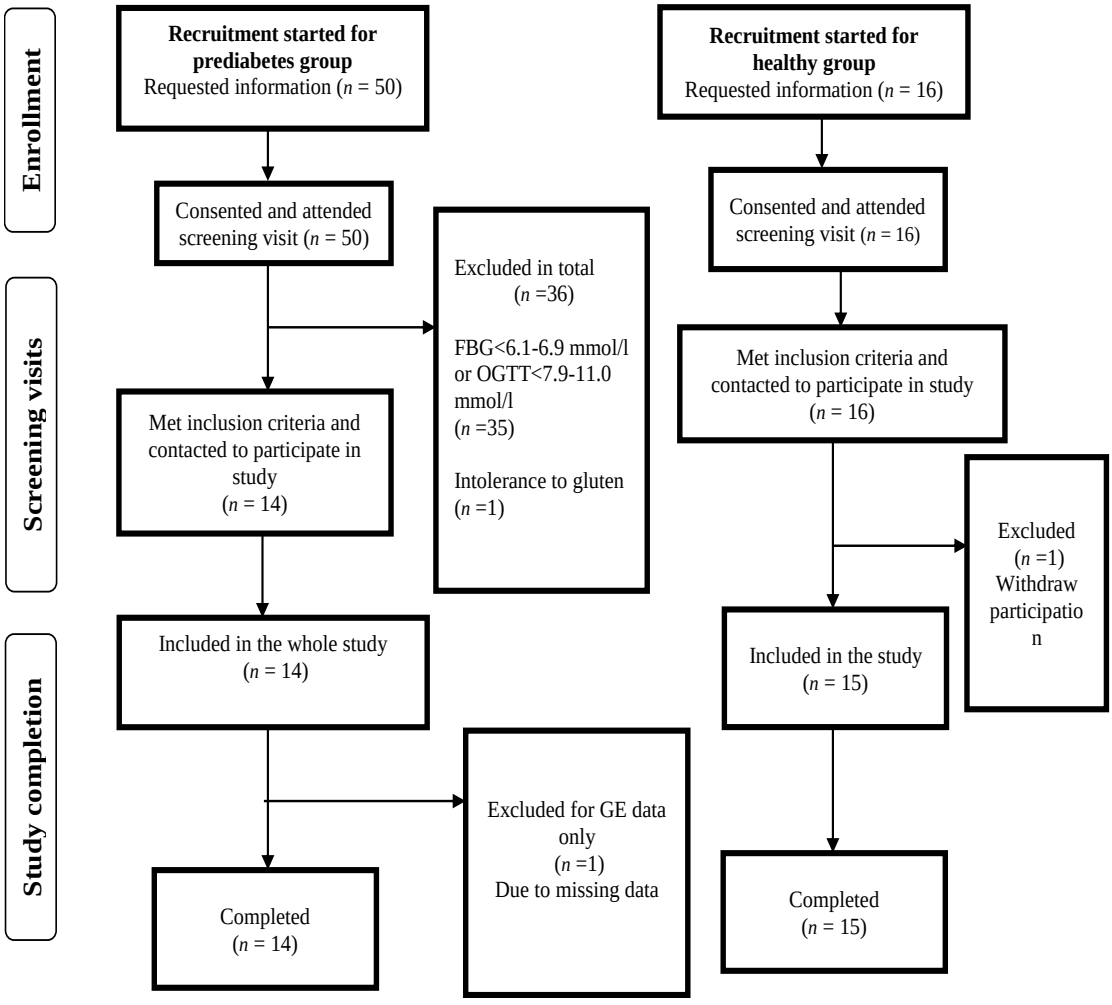


Figure 5.2: CONSORT flow diagram for the recruitment of study participants

Table 5.2: Participant characteristics for prediabetic and healthy groups

Characteristic	Prediabetes volunteers n=14	Healthy volunteers n=15	*P* value
Systolic (mm Hg)	119.9 ± 16.1	111 ± 11.8	0.063
Diastolic (mm Hg)	82.2 ± 6.4	73.1 ± 7.1	*0.001
BMI (kg/m2)	31.7 ± 3.1	24.6 ± 2.9	*<0.001
Height (cm)	167.8 ± 10.9	169.1 ± 8.5	0.054
Weight (kg)	89.7 ± 14.7	70.9 ± 14.1	*0.002
Age (years)	44.6 ± 10.7	38.6 ± 14.5	0.215
Gender	9 (female)/5 (male)	9 (female)/6 (male)	0.815

Abbreviations: BMI = body mass index. Data is displayed as mean ± SD. *Significance level $p < 0.05$ compared between both groups

5.3.2. Glycaemic response (GR)

5.3.2.1. Changes in the GR from baseline in prediabetic participants

The changes observed in postprandial capillary blood glucose concentrations from baseline in prediabetic adults are depicted in Figure 5.3. There were no significant differences in the GR between the finger millet and control muffin groups in prediabetic adults.

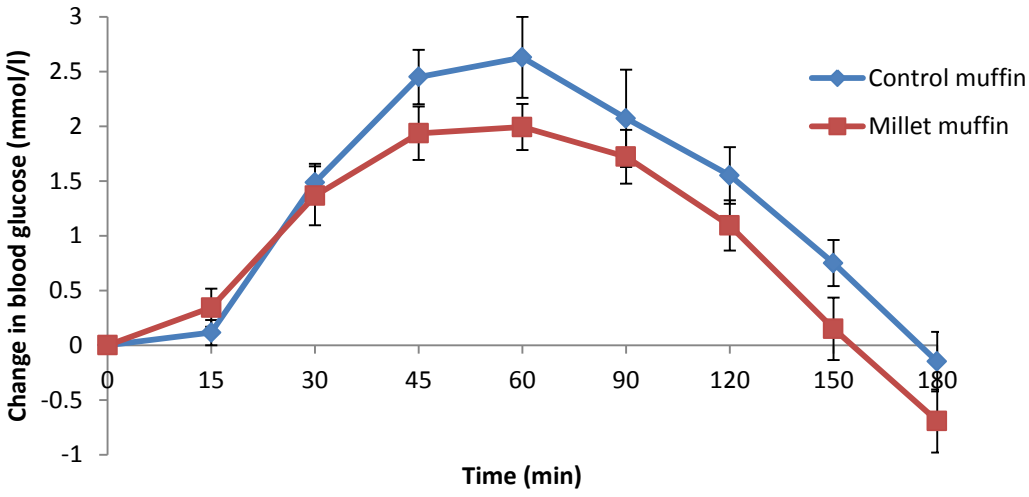


Figure 5.3: Changes in the blood-glucose response from baseline at 0-180 min (mmol/l) after muffin consumption in prediabetic participants (n=14). Data is given as means ± SD. The baseline value for control was 6.0±0.7 and for millet was 5.9±0.7(mmol/l).

5.3.2.2. Changes in the GR from baseline in healthy participants

Changes in the postprandial capillary blood glucose concentrations from the baseline in healthy adults are shown in Figure 5.4. There were no significant differences in the GR between the finger-millet and control-muffin groups in healthy adults.

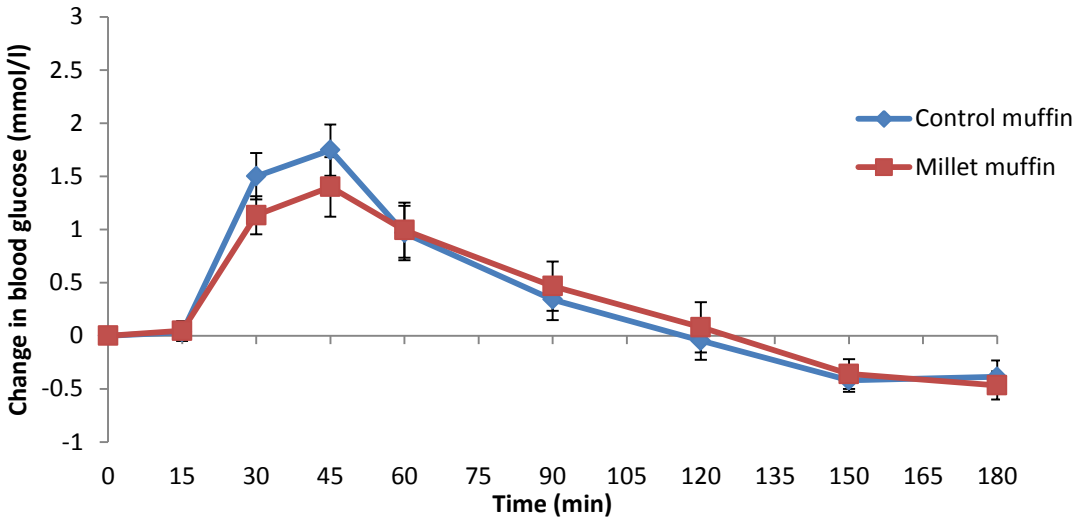


Figure 5.4: Changes in blood glucose response from baseline at 0-180 min (mmol/l) in healthy participants (n=14). Data is given as means ± SD. The baseline value for both the control and the millet groups was 5.1±0.4 (mmol/l).

5.3.2.3. GR incremental area under the curve (IAUC) for prediabetic and healthy participants after consumption of finger-millet and control muffins

A mixed between-within subjects analysis of variance was conducted to assess the impact of two different interventions (finger millet and control muffin) on the GR IAUC across different time points (60, 90, 120, 150, and 180min) in prediabetic and healthy participants. There were no significant interactions between participants and muffins at 60min (F (1.000, 27.000) = 0.026, p = 0.873), 90min (F (1.000, 27.000) = 0.004, p = 0.951), 120min (F (1.000, 27.000) = 0.031, p = 0.862), 150 min (F (1.000, 27.000) = 0.190, p = 0.666) and 180min (F (1.000, 27.000) = 3.040, p = 0.093).

Table 5.3: GR IAUC for prediabetic and healthy participants after consumption of finger-millet and control-muffins

IAUC GR (mmol/L.min)	Participant groups	*P* value	Control muffin (Mean±STD)	Millet muffin (Mean±STD)	*P* value
60	Prediabetes	*0.036	81.5±32.4	70.3±34.2	0.245
	Healthy		58.1±31.7	48.9±30.3	0.334
120	Prediabetes	*0.001	192.4±100.3	152.2±49.5	0.132
	Healthy		89.6±62.1	87.5±63.9	0.91
180	Prediabetes	*0.035	175.4±106.9	128.7±69.6	0.084
	Healthy		93.6±63.8	95.5±70.4	0.865

**P* values = significant between-subject groups (prediabetes and healthy)

5.3.3. Insulinaemic response (IR)

5.3.3.1. Changes in the IR from baseline in prediabetic participants

The changes in the IR from baseline in the prediabetes group are shown in Figure 5.5. The IR values at 150 and 180min time points after consumption of the finger millet muffin were significantly lower compared to control ($p < 0.05$).

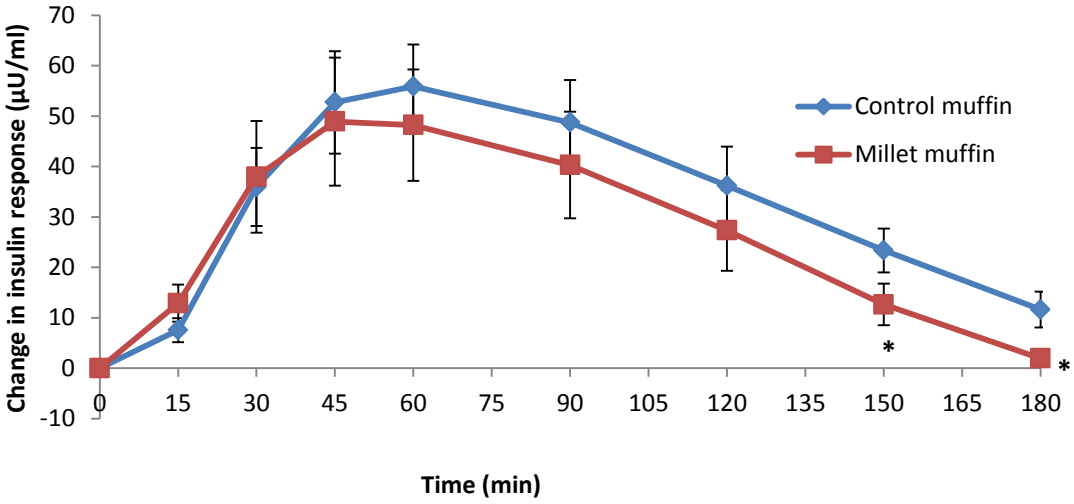


Figure 5.5: Changes in the insulin response from baseline at 0-180min in prediabetic participants (n=14). Data is given as means ± SD. *Significant difference p<0.05 compared to control muffins. The baseline value for the control group was 13.0±7.5 and for the millet group was 14.6±8.3(mmol/l).

5.3.3.2. Changes in the IR from baseline in healthy participants

The changes in the IR from baseline in healthy adults are illustrated in Figure 5.6. There were no significant differences in the IR between control and finger millet groups in healthy individuals at any time point.

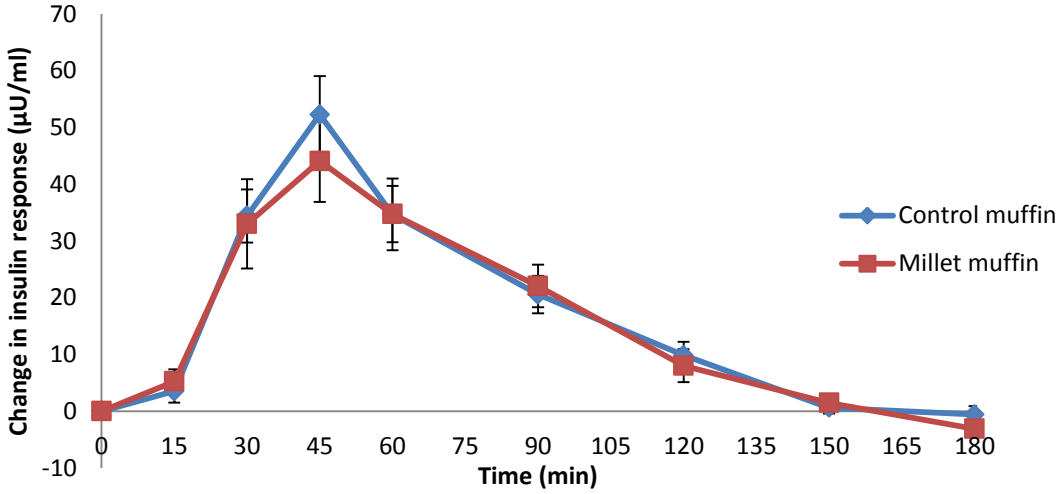


Figure 5.6: Changes in the insulin response from baseline at 0-180 min in healthy participants (n=15). Data is given as means ± SD. The baseline value for the control group was 8.4±3.9 and for the millet group was 9.2±3.9(mmol/l).

5.3.3.3. IR IAUC for prediabetic and healthy participants after consumption of finger-millet and control muffins

A mixed between-within subjects analysis of variance was conducted to assess the impact of two different interventions (finger-millet and control muffins) on prediabetic and healthy participants on the IR IAUC across different time points (60, 90, 120, 150, and 180min). There were no significant interactions between participants and muffins at 60 min (F (1.000, 27.000) = 0.002, p = 0.963), 90 min (F (1.000, 27.000) = 0.649, p = 0.428), 120min (F (1.000, 27.000) = 1.507, p = 0.230) and 150min (F (1.000, 27.000) = 2.977, p = 0.096). However, there was a significant interaction between participants and muffins for the IR (IAUC) at 180 min (F (1.000, 27.000) = 4.542, p = 0.042). Changes from baseline in glucose concentrations measured between 0 min and

–180 min differed significantly by muffin type in the prediabetes group (p = 0.041). Post-hoc analysis (using Bonferroni adjustment) revealed that at 180min the healthy group was significantly lower compared to the prediabetes group after consumption of both muffins (p <0.001; Table 5.8).

Table 5.4: IR IAUC for prediabetic and healthy participants after consumption of finger-millet and control muffins

IAUC IR (mmol/L.min)	Participant group	*p* value	Control muffin (Mean±STD)	Millet muffin (Mean±STD)	*P* value
60 min	Prediabetes	0.88	1865.9±1213.7	1860.5±1670.5	0.778
	Healthy		1623.0±750.9	1507.2±986.1	0.427
120 min	Prediabetes	0.081	4322.9±2632.3	3722.2±3141.1	0.084
	Healthy		2588.6±1163.9	2477.3±1249.6	0.91
180 min	Prediabetes	*<0.001	4264.9±2631.6	3184.5±2853.6	*0.041
	Healthy		1508.2±713.0	1510.0±726.2	0.955

**p* values = Significant between-subject groups (prediabetes and healthy)

5.3.3.6. Peak and time to peak for GR and IR in the prediabetes and healthy groups after finger millet and control muffin consumption

A mixed between-within subjects analysis of variance was conducted to assess the impact of two different interventions (finger millet and control muffin) on the peak and time to peak values for the GR and IR in prediabetic and healthy participants. There were no significant interactions between participants and muffins for the GR peak (F (1.000, 27.000) = 0.526, p = 0.475), the IR peak (F (1.000, 27.000) = 0.059, p = 0.809), the GR time to peak (F (1.000, 27.000) = 0.714, p= 0.405) and the IR time to peak (F (1.000, 27.000) = 0.794, p = 0.381).

Table 5.5: Peak and time-to-peak values for prediabetic and healthy participants after consumption of finger-millet and control muffins

	Participant group	*p* value	Control muffin (Mean±STD)	Millet muffin (Mean±STD)	*p* value
GR peak (pmol/l)	Prediabetes	*<0.001	9.1±1.5	8.5±1.0	0.69
	Healthy		7.06±1.00	6.72±0.71	0.232
GR Time to peak (min)	Prediabetes	*0.015	56.3±17.3	62.7±32.7	0.361
	Healthy		40±9.26	48±15.21	0.134
IR peak (pmol/l)	Prediabetes	0.269	80.2±40.3	78.3±51.0	0.638
	Healthy		63.6±24.9	58.62±31.12	0.14
IR Time to peak (min)	Prediabetes	*0.024	67.5±24.1	72.9±39.0	0.404
	Healthy		45±8.02	51±14.78	0.166

**p* value= Significant value between-subjects (prediabetes and healthy)

5.3.4. Gastric emptying (GE)

5.3.4.1. GE in the prediabetes group

Significant differences were observed in the ascension times (T_{asc}) ($p < 0.05$) (~21min after control) between the two muffin types for the prediabetic participants. However, there was no significant difference in the half time, latency or lag phase ($p > 0.05$).

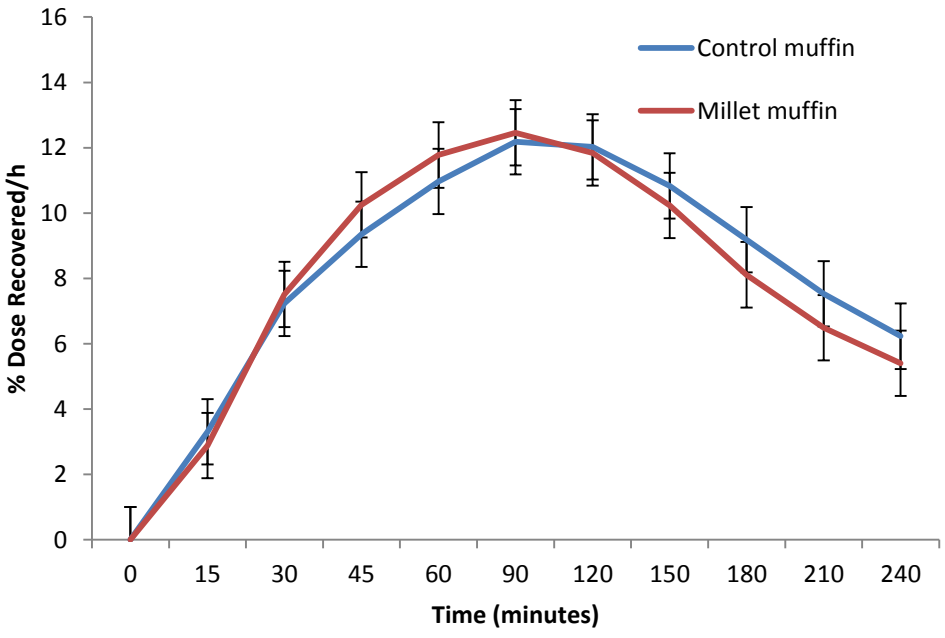


Figure 5.7: Curves show $^{13}CO_2$ levels in the breath of prediabetic participants, expressed in excess atom fraction over time following muffin consumption

Table 5.6: GE time after finger millet and control muffin consumption in prediabetic participants

Gastric emptying time (min)	Control	Millet	*P value*
T_{half}	82.02±55.06	61.40±34.81	0.133
T_{lag}	32.42±23.61	25.74±22.88	0.345
T_{lat}	37.25±20.40	35.41±16.84	0.650
T_{asc}	120.61±46.49	99.36±28.92	*0.046

Data is given as means ± SD.

5.3.4.2. GE in the healthy group

No significant differences were found in GE times between the control and finger-millet muffins in healthy individuals in terms of half time, lag, latency or ascension time ($p > 0.05$) (Table 5.11).

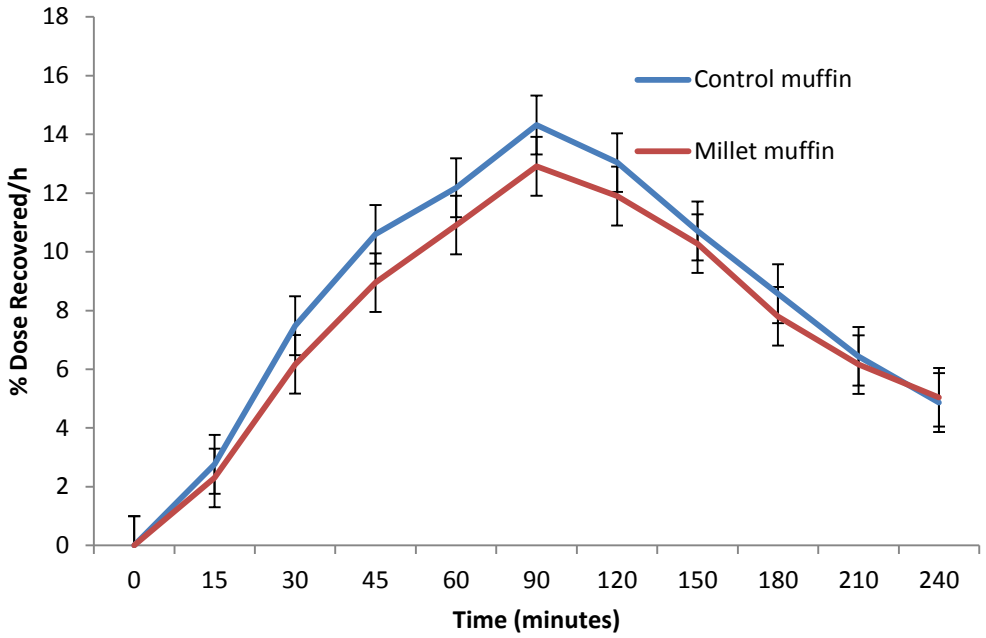


Figure 5.8: Curves show $^{13}CO_2$ levels in the breath of healthy participants, expressed in excess atom fraction over time following muffin consumption

Table 5.7: GE time after consumption of finger millet and control muffins in healthy participants

Gastric emptying time (min)	Control	Millet	*P value*
$\mathbf{T_{half}}$	55.56±17.10	62.40±21.85	0.312
$\mathbf{T_{lag}}$	26.34± 16.21	32.03±19.00	0.442
$\mathbf{T_{lat}}$	36.18±13.15	41.56±15.37	0.360
$\mathbf{T_{asc}}$	92.05±12.71	94.33±14.01	0.428

Data is given as means ± SD.

5.3.4.3. GE in prediabetic and healthy participants after finger millet or control muffin consumption

A mixed between-within subjects analysis of variance was conducted to assess the impact of two different interventions (finger millet and control muffin) on GE across different time points (T_{half}, $T_{lag,}$ $T_{lat,}$ and T_{asc}) in prediabetic and healthy participants. There were no significant interactions between participants and muffins for T_{half} (F (1.000, 26.000) = 3.634, p = 0.068), T_{lag} (F (1.000, 26.000) = 1.715, p = 0.202) and T_{lat} (F (1.000, 26.000) = 0.907, p = 0.350). However, statistically significantly interactions between participants and muffins for T_{asc} (F (1.000, 26.000) = 6.558, p = 0.017) were observed. Post-hoc analysis (using Bonferroni adjustment) revealed that the finger millet muffin was statistically significantly different compared to the control muffin in both groups for T_{asc} ($p \leq 0.05$; Table 5.10).

Table.5.8: GE time for prediabetic and healthy participants after finger millet or control muffin consumption

GE time (min)	Participant group	*p* value	Control muffin (Mean±STD)	Millet muffin (Mean±STD)
T_{half}	Prediabetes	0.689	82.02±55.06	61.40±34.81
	Healthy		55.56±17.10	62.40±21.85
T_{lag}	Prediabetes	0.986	32.42±23.61	25.74±22.88
	Healthy		26.34± 16.21	32.03±19.00
T_{lat}	Prediabetes	0.614	37.25±20.40	35.41±16.84
	Healthy		36.18±13.15	41.56±15.37
T_{asc}	Prediabetes	0.117	120.61±46.49	99.36±28.92
	Healthy		92.05±12.71	94.33±14.01

5.3.5. Satiety

Mean hunger and fullness ratings at (baseline; 0min) and after ingestion of control or finger millet muffins, in both healthy and prediabetic groups, are shown in Tables 5.13 and 5.14. Satiety was not significantly affected by either muffin type (finger millet or control) or participant group (healthy or prediabetes). However, the AUC for the 4h satiety rating in the prediabetes group after finger millet muffin consumption was tended to be higher compared to control at all time points, except at 210 and 240min (Table 5.13). Similarly, in the healthy group, the AUC for the finger millet group was tended to be higher, compared to control, at 60, 90, 120, 150, and 180min (Table 5.14).

Table 5.9: AUC for satiety following control and finger millet muffin consumption in the prediabetes group

AUC (Time in min.)	Control muffin	Millet muffin	*P* value
60	267.3±85.1	287.7±64.8	0.400
90	389.5±131.3	428.0±91.1	0.311
120	497.7±180.4	565.2±128.5	0.211
150	598.4±225.2	673.4±175.2	0.288
180	689.5±257.8	735.5±216.2	0.582
210	767.7±282.8	765.0±249.4	0.977
240	820.2±301.1	772.0±280.6	0.647

Data is given as means ± SD.

Table 5.10: AUC for satiety following control and finger millet muffin consumption in the healthy group

AUC (Time in min.)	Control muffin	Millet muffin	*P* value
60	211.5±68.9	223±71.6	0.588
90	300.5±105.1	327±111.0	0.400
120	377.5±131.5	423±148.9	0.257
150	439.5±156.9	497.5±181.7	0.193
180	490.5±180.1	524.5±197.4	0.329
210	531.5±198.5	517±203.1	0.737
240	556.0±216.6	499±210.8	0.860

Data is given as means ± SD.

5.4. Discussion

The present study demonstrates that replacing 50% of wheat flour with finger millet grain in muffins exerted an inhibitory effect on postprandial blood glucose and the IR in prediabetic individuals. Data in this study also indicates that GE was accelerated following consumption of the control muffin group compared to the millet muffin in healthy participants. Similarly, finger millet muffin consumption was found to promote

satiety in both groups.

5.4.1. The GR and IR in prediabetic and healthy individuals

A reduction in the GR from baseline after millet consumption was measured in the prediabetes group tested for this study. However, the reduction was not statistically significant. This is in contrast with a previous study by Lakshmikumari and Sumathi (2002), which showed that a larger reduction in the GR was achieved after consumption by both healthy and type-2 diabetic individuals of flatbread (*roti*) and pancake (*dosa*) prepared from whole finger millet. Addition of finger millet into the diet has been recommended for diabetic individuals, as this type of millet is high in polyphenols and dietary fibre – which accounts for the reduced GR and IR observed after consumption of different food products. The lower GR observed after whole finger millet consumption can be explained by the presence of phytic acid and tannins in whole finger millet flour (Lakshmikumari and Sumathi, 2002). The mechanisms through which polyphenols may effectively attenuate the prediabetic state (and risk of diabetes) are fractional inhibition of amylase and α-glucosidase during enzymatic hydrolysis of complex carbohydrates, as well as delayed glucose absorption, all of which ultimately lower postprandial blood glucose levels (Devi et al. 2014). Polyphenols also suppress the release of glucose from the liver and in peripheral tissues, which may improve the uptake of glucose through changes in intracellular signalling (Kim et al., 2016).

The fibre in finger millet could also play a vital role in reducing the GR and IR, as suggested by Devi et al. (2014). Fibre delays glucose absorption, increases both insulin secretion and sensitivity and also binds bile acids. Soluble fibre also reduces postprandial glucose levels by increasing the viscosity in the digestive tract, which hinders the breakdown of carbohydrates and, hence, glucose absorption (Nyambe-

Silavwe and Williamson, 2016). In Chapter 2 (*in vitro* study), it was found that millet had a high SDS content, the many health benefits of which include management of diabetes and stable glucose metabolism. This SDS content may, therefore, also be responsible for the reduction observed above.

A study conducted by Urooj et al. (2006) found that, at 30 min, the GR (AUC) was significantly lower after consumption of flatbread (*roti)* made from finger millet in comparison to finger millet-based dumplings in healthy and type-2 diabetic individuals. In the current study, the GR (IAUC) for the finger millet muffin group was lower than for the control muffin in prediabetic and healthy participants; however, this difference did not reach statistical significance. A significant difference in the GR between the prediabetic and healthy group was observed at each time point and in the IR at 150 and 180min. There are discrepancies between the results observed here and data published by other groups in relation to the general effect of millet consumption on GR reduction and, in particular, the effect of finger millet. Although both the dishes in the study by Urooj et al. (2006) and the muffins in the current work were prepared from finger millet, the exact amounts in each meal differed; 55g in *roti* and 50g in dumplings in the previous study and 36g here. The differences in fibre content may have accounted for the different sizes in GR reduction observed between the current and previous work.

Many studies have shown that numerous factors, such as cooking method and duration, heat intensity and processing method, may reduce the polyphenol content in millet and affect the ratio of the different starch fractions, which, ultimately, may differentially affect the GI and GR (Lakshmikumari and Sumathi, 2002). Cooking is known to reduce the content, and antioxidant activity, of phenolic (both free and bound) and flavonoids through thermogenic breakdown of phenolic molecules, as well as

decarboxylation and polymerisation of free phenolic acids (Kadiri, 2017). The method and duration of muffin preparation in this study were completely different from those used for preparing *roti* (toasted and processed with oil) and dumplings (boiled in paste) in the previous study; moreover, the ingredients added to each dish (vegetables, milk, and spices) could have also affected the results. A long cooking time could promote breakdown of starch granules, thereby increasing their surface area for rapid digestion and leading to an increase in the GR. In the current study, the muffins were baked at 150°C for 25min – longer than the 1.5min of cooking time required for Indian flatbread (Mir et al. 2014). As mentioned, millet processing is another factor in the GR; Chandrasekara and Shahidi (2012) pointed out that phenolic compounds contained in different types of millet grain (proso, kodo, little, foxtail, pearl) are resistant to hydrothermal processing. However, same processes have been found to significantly reduce the free phenolic content in rice cultivars (Kadiri, 2017).

Not all foods that are rich in carbohydrate induce an increase in blood glucose. Some carbohydrates, when ingested in similar amounts, have been reported to elicit different GRs in healthy and type-2 diabetic individuals. This may be due to the differences in individual carbohydrate components, such as sugars, dietary fibre and starch composition, which exert varied effects on the GR (Eleazu, 2016). Moreover, the participant's own physiology (i.e. physiological differences in healthy, prediabetic or type-2 diabetic individuals) may differentially affect the GR and IR results. Polyphenol-rich food help to prevent type-2 diabetes and the progression of metabolic syndrome (MetS) by reducing high blood sugar, blood pressure, abnormal cholesterol and body fat (which in turn increases the risk of diabetes and cardiovascular disease), which is attributed to antioxidant activity (Chiva-Blanch and Badimon, 2017). However, intake of polyphenols may have different effects on healthy people and those with MetS risk factors (Chiva-Blanch and Badimon, 2017). Moreover, foods that are

low in GI are beneficial for diabetics, in whom glucose metabolism and regulation is impaired, especially in the postprandial period, making them more liable to the various deleterious effects of excess plasma glucose (Riccardi et al. 2008). All of the above factors could, therefore, explain the differences in the results observed here in both participant groups.

Several studies have evaluated the effect of finger millet consumption on the GR in healthy and type-2 diabetic individuals; however, few have extended their focus to the IR and, to our knowledge, none have applied this rationale to the prediabetic stage. In our study, a significant reduction of the IR, and the IAUC, from baseline at 150 and 180min was seen after millet consumption in the prediabetes group. This is likely attributed to the polyphenol content of finger millet muffins. It has also been reported that an increase in insulin resistance and a decrease in insulin secretion occur as a result of β-islet dysfunction, which impairs normal glucose tolerance to result in type-2 diabetes (Paquette et al. 2017). Insulin resistance is defined as reduced tissue sensitivity to insulin, which impairs glucose uptake and usage by cells. In the early period of insulin resistance, plasma glucose is preserved at normal levels through a compensatory increase in insulin secretion from pancreatic β-cells; this is seen as the first abnormality. When this compensatory response fails, there is an increase in fasting glucose levels, which leads to the development of the prediabetes stage and, ultimately, further progression into type-2 diabetes (Paquette et al. 2017). Paquette et al. (2017) found that polyphenols prevent further increases in insulin release in the early stage of prediabetes. It has been suggested that improvement in insulin sensitivity, after consumption of polyphenol-rich (strawberry and cranberry) beverages may preclude any compensatory rise in insulin secretion. This may explain the reduction in the IR seen here in the prediabetes group who consumed polyphenol-rich finger millet, in contrast to the healthy group. Polyphenols are also suggested to

prevent oxidative damage to β-cells and activate glucose uptake receptors in insulin-sensitive tissues (Ferre et al. 2017).

It should be noted that the food matrix could also impact the IR. Our observations of IR reduction here are in agreement with a study by Abdelgadir et al. (2004), who reported a significant decrease in the AUC for insulin after millet porridge consumption in comparison to sorghum and maize porridge in type-2 diabetic individuals. The porridge (containing 292g of proso millet) was prepared by the authors using the traditional Sudanese method and served with Okra soup, minced meat, onion and spices. According to Sweeney et al. (2017), the GR of proso millet-based products depends on the product matrix – not solely on the grain type. The results from this work and that of Abdelgadir et al. (2004) showed that the reduced IR was not dependent on the type of millet consumed, as the muffins made from finger millet were mixed with wheat and the porridge was made from proso millet and served with other ingredients; both dishes lowered the IR. According to Juntunen et al (2002), the postprandial IR may also be conferred by the structure and form of food, not just the type of grain included in the product. This is because the health benefit of a food product is a combined result of the individual health benefits of each nutrient in this product (Peters, 2017).

In general, blood glucose levels reach their peak within the first 30min of meal consumption (Shobana et al. 2007). In our study, the GR peak was significantly different between the prediabetes and healthy groups and both the GR and IR peak were reduced in both groups after finger millet muffin consumption. The GR and IR time to peak were also significantly different between the prediabetes and healthy group; both occurred after 1h in the prediabetes group after consumption of finger millet muffin, while in the healthy group, they peaked at 48 and 51min, respectively.

Delaying spikes in the GR and IR after an hour may be one of the most important health effects of finger millet consumption in prediabetes. In our study, we found that the peaks in the GR and IR levels were lower after finger millet muffin consumption in both groups, although the results were not significantly different. This is in line with the findings of Jayasinghe et al. (2013), who used finger millet in *roti* and *pittu*, prepared using stoneground or industrial methods. The authors reported that the GI level was lower in the *roti* prepared with stoneground millet and both *roti* dishes displayed a lower GI than *pittu* with millet prepared using either method. Moreover, the peak glucose level was higher after consuming *pittu*, compared to *roti*, in healthy individuals, even though the same finger millet flour and the same milling method were used in both dishes. It is possible that the large particle size achieved by stone grinding finger millet results in a lower GI, as it makes starch gelatinisation comparatively difficult and hinders enzymatic attack, resulting in slower postprandial glucose release. In the current study, the electric mill was used to crush and prepare the finger millet grain, based on the methods of Jayasinghe et al. (2013), their results suggest that a smaller particular size generally increases the GI, for which reason there was no significant difference in the GR peak and time to peak after finger millet.

Finally, Coe and Ryan (2016) found that consumption of polyphenol-rich sources, in addition to carbohydrates, reduces the peaks in early-phase GR and IR in a manner that is dependent on the source of polyphenols and carbohydrates, as well as other factors (e.g. the product type). The polyphenol content may delay carbohydrate breakdown and glucose absorption (Coe and Ryan, 2016).

5.4.2. GE and satiety in prediabetic and healthy individuals

In the current study, no change or delay was measured in GE by the prediabetes or healthy groups after finger-millet consumption. A recent study by Cisse et al. (2018)

conducted a similar experimental protocol to the current study in healthy participants and observed slower GE rates after millet consumption in solid and liquid food (thick or thin porridge and couscous), compared to white rice, plain potato and wheat pasta. It is possible that the millet species or amounts play an important role in regulating the GE rate, as, in our study, we replaced 50% of wheat with ~36g of finger millet grain, while Cisse et al. (2018) used larger amounts of millet flour (750 and 1250g). Also, it is possible that the finger-millet grains became separated from the rest of the muffin mixture in the stomach and the faster emptying time represents the separation of the flour mixture from the grains. Another reason for the difference in the GE rate in our study could be participant physiology – as previously discussed (section 5.4.1.) – which would differ in the prediabetes and healthy group. It has been shown that the 'gastric motor' is accelerated in individuals with type-2 diabetes in association with hyperglycaemia (Marathe et al. 2013). The sample size may have been insufficient in this study to establish a difference in GE between the control and finger-millet-based muffins. The sample-size calculation for this study was based on the expected GR and IR. Also, results for one participant in the prediabetes group were excluded due to missing data in the GE calculation.

Other factors that may influence the time taken for food to be disintegrated and then emptied from the stomach include macronutrient properties, meal volume, calorie content and other physical properties, such as particle size (Cisse et al. 2018). Macronutrient properties (i.e. of carbohydrates fat and protein) differentially affect the GE rate; fats take the longest to empty, followed by protein and carbohydrate. Fats are strong inhibitors of GE due to their high caloric density, in comparison to foods that are less dense, such as carbohydrates (Marathe et al. 2013). Moreover, meals with a similar energy content leave the stomach at similar rates; for instance, meals that are heavy and high in calories, in both solid and liquid form, are associated with longer

GE times (Singh, 2013). This may be one of the reasons why there were no differences in the GE after finger millet and control muffin consumption in the current study; both muffins were similar in their calorie content, as well as fat and protein. The food particle size also appears to play a role in GE, as the large particles are shown to require a longer time to digest into smaller particles that can exit through the pylorus.

The reason behind the slightly delayed GE observed in healthy participants may be that the finger millet muffins had a higher fibre content compared to control muffins made from wheat. Dietary fibres delay the rate of GE (of both liquids and solids) by increasing the viscosity of the ingested food (WHO, 1998). Moreover, it has been shown that dietary fibres slow GE via food bulking – i.e. promoting gelling of food in the stomach. All the above factors delay the hydrolysis and absorption of macronutrients in the small intestine, which slows GE (Tan, et al. 2016). According to Shobana & Malleshi (2007) finger millet contains around 19.7% of insoluble dietary fibre and 2.5% of soluble dietary fibre which may have a different impact on GE.

In the current study, finger millet muffin consumption led to an increased feeling of satiety in both the prediabetes and healthy group at all time points, except at 210 and 240min, although this was not significantly different from the control muffin. This was expected; as mentioned earlier, there was no significant effect on GE, which is directly linked to satiety (Morey et al. 2016). The decrease was not significant but was observed in both groups in our study and can likely be explained by differences in muffin portion size. Benelam (2009) reported that a large portion size increases the intake of energy, thereby promoting satiety. In our study, the control muffin (50g available CHO) was smaller (133.69g) than the finger millet muffin (50g available CHO in145.77g portion) and led to lower levels of satiety, although there was no significant difference in energy content (kcal) between the muffins.

Two previous studies reported an increase in satiety scores after consumption of finger millet based-food (dumplings and couscous; Urooj et al. 2006 and Cisse et al. 2018). As discussed above in relation to GE, this may be explained by the high fibre content in finger millet muffins, which would significantly increase satiety through stomach distention, increased viscosity of stomach contents and prolonged absorption of nutrients (Skotnicka et al. 2018). In addition, the aforementioned fibre bulking effect increases chewing time and this also promotes gastric distension and satiety (Benelam, 2009). The small amount of millet included in the muffins here may account for the lower effect on GE and satiety.

Moreover, as mentioned, the participants' health is likely to play a role in in the current results. Flint et al. (2007) found that insulin acts as a satiety signal in the short term in healthy individuals, but not in those who are overweight. This may be because, in the latter group, there is insulin resistance in the central nervous system.

In our study, we did not find that consumption of finger-millet-based muffins caused a statistically significant effect on the postprandial capillary blood-glucose concentrations in either group (healthy or prediabetes). However, a larger sample size study of healthy and prediabetic populations with a higher dose of finger millet grain could provide sufficient data to detect any effect that may occur. Moreover, a study in India found that a diet which consisted of a combination of foxtail millet, split black gram and spice mix, offered for 90 days to people with type-2 diabetes, improved glycaemic control and reduced fasting levels of glucose, insulin, HbA1c, triglyceride, total cholesterol and LDL cholesterol (Jali et al., 2012). These results present indications that this diet had a positive impact on the health of type-2 diabetes patients. Further research is needed to investigate the effects of millet intake on blood glucose when combined with other dietary strategies that may provide more impact, such as the

Portfolio diet.

5.5 Conclusion

Long-term finger millet consumption could be a cheap and effective way to help reduce metabolic abnormalities in those with prediabetes, which would be instrumental in preventing conversion to type-2 diabetes, and related complications. This is the first study to look at the effects of finger millet-based muffins on the GR, IR, GE and satiety in individuals with prediabetes and compared to healthy controls. The results from this study highlight the potential of finger millet based-muffin consumption in decreasing postprandial GR, IR, GE, and satiety in those with prediabetes. However, the GR, GE and satiety findings were lower, but not significantly different, to the control results. Many studies have already shown that regular consumption of finger millet might be beneficial for managing diabetes. Future work is required to examine the effect of different percentage compositions of finger millet-based products on the GR, IR, GE and satiety; moreover, different cooking methods should undergo particular scrutiny so as to achieve optimal nutritional benefit for prediabetic individuals.

Chapter 6: General discussion and conclusion

Type-2 diabetes is a chronic disorder characterised by metabolic dysregulation and secondary complications, such as neuropathy, cardiovascular disease, nephropathy and retinopathy. It is estimated that the number of individuals diagnosed with Type-2 diabetes will reach 629 million by 2045 (Forouhi et al. 2018). In addition to medications, changes in lifestyle and diet are paramount in preventing and treating both diabetes and prediabetes. A healthy diet greatly improves the quality of life for those suffering from diabetes or at risk of developing the disease. High levels of blood glucose might stimulate free-radical production, and thus the regulation of blood glucose levels is key to the prevention of diabetes and its complications as well as reducing the risk of coronary heart disease and other cardiovascular diseases. Studies show that this can be achieved by intake of a diet rich in polyphenols and antioxidants (Kim et al., 2016). It has been found that foods high in antioxidants may improve glycaemic control by direct action in the gastrointestinal tract to decrease the digestion and absorption of carbohydrate and to reduce postprandial oxidation (Chepulis et al., 2016). Thus, it is important to consider the nutritional value of foods which could be used as potential interventions in diabetes and prediabetes (Kam et al. 2016).

Recently, millet grains have received attention as potential dietary interventions for the management of prediabetes and type-2 diabetes. There is considerable *in vitro* and *in vivo* evidence that millet has properties that make it a healthy dietary option for diabetics (Kam et al., 2016). For instance, the results of a study in India showed that levels of HbA1c, fasting blood glucose, insulin, total cholesterol, triglyceride, and LDL cholesterol were reduced in people with type-2 diabetes who consumed foxtail millet, split black gram and spice mix for 90 days (Kam et al., 2016). However, it should be noted that most of these studies were performed in healthy or type-2 diabetic

individuals after consumption of traditional millet-based foods and sample sizes were small. Indeed, prevention is seen as a more effective way of preventing diabetes than a cure; thus, focusing on prediabetes individuals who are at risk of developing type-2 diabetes and therefore reversing the condition before it is established, is ultimately a better option all round. Type-2 diabetes is preventable and may be managed much better if identified at an earlier stage (prediabetes); studies have also shown that signs of the complications associated with type-2 diabetes are also present early, prior to diagnosis. Thus, more intervention studies and clinical trials are needed to study the effect of millet consumption on the GR, IR and GE in individuals who may be at risk of developing type-2 diabetes.

In light of the above evidence on the benefits of millet consumption on controlling type-2 diabetes, this work included four novel studies. These consisted of an *in vitro* study, examining the content of polyphenols and antioxidants in millet species (Chapter 2), an *in vitro* study investigating the sugar release and a sensory evaluation of millet-based muffins (Chapter 3), a systematic review of existing literature (Chapter 4) and an *in vivo* intervention study examining the efficacy of finger millet-based muffins in adults with prediabetes (Chapter 5).

6.1. Summary of thesis aims and findings

The primary aim of this thesis was to determine the effect of a millet-based muffin, rich in polyphenols and antioxidants, on the postprandial GR, IR and GE in prediabetic individuals compared to healthy adults (Chapter 5). A secondary aim was to assess the effect of the finger millet based-muffin on satiety in the same participant groups. To achieve these aims, three studies were conducted. The first study in this thesis (Chapter 2) aimed to determine the antioxidant activity and polyphenol content of different types and forms of millet in order to determine which types and/or forms could be

most effective (high in polyphenols) for use in the subsequent human trial. The second study (Chapter 3) aimed to evaluate the effect of a polyphenol-rich millet-based muffin on *in vitro* starch digestion and to determine the best millet muffin in terms of overall sensory acceptance. Chapter 4 aimed to systematically review the published intervention studies investigating the effect of consuming different types and forms of millet on the risk factors of type-2 diabetes.

This thesis consists of a series of studies. The results of each study formed the basis for the next. To the best of our knowledge, the current study as explained in Chapter 2 is the first project to have determined the polyphenol levels and antioxidant activity of the most common types of millet around the world (pearl, foxtail, finger, kodo, little, proso and barnyard), prepared in three different forms (grain, flour and flakes). We found that millet grains were significantly higher in polyphenol content compared with other millet forms; kodo-millet grain exhibited the highest polyphenol content and antioxidant activity, followed by finger millet in two forms (grain and flour) (Chapter 2). These types of millet were each used to make muffins for the examination of *in vitro* digestion and the sensory perception of these millet types, to determine the most acceptable muffin (Chapter 3). The results showed that finger-millet grain and flour-based muffins were highly acceptable compared with kodo millet grain-based muffins. The *in vitro* digestibility studies showed that millet grain-based muffin (kodo and finger millet grain-based muffins) contained not only greater quantities of polyphenols and antioxidants, but also significantly lower levels of RDS than finger millet flour-based muffins (Chapter 3).

To the best of our knowledge, the systematic review undertaken as part of this thesis (Chapter 4) is the first to have focused on the link between millet and general health, as well as the relationship between millet consumption and risk factors for type-2

diabetes. This is an important step, as the systematic review was designed to evaluate all previous relevant studies that focused on the effect of millet intake on glycaemia in healthy and type-2 diabetic individuals. Overall, the systematic review showed that millet produced a beneficial effect on fasting and postprandial blood-glucose levels and plasma IR both in healthy individuals and in those with type-2 diabetes (Chapter 4). Consequently, Chapter 5 explains the research undertaken to evaluate the effect of millet intake on prediabetic individuals. The results highlight the potential benefits to prediabetic people of consuming finger millet, which is high in polyphenols and SDS. The results of this study show decreases in the postprandial GR and IR of prediabetic participants; however, there were no significant differences in the GR and satiety levels between those who consumed the millet muffin and those who were given the control wheat muffin. Moreover, the results of the GE test showed no significant difference between the GEs of the group given the millet muffin and those who consumed the control.

6.2. Implications, applications and future perspectives of this thesis

The findings from these studies may be instrumental in driving further research in the field. First, the beneficial effect of highly nutritious finger millet on the GR, IR, and GE in prediabetic and healthy adults was observed (Chapter 5); thus, regular consumption of millet as a main dish or a snack could help manage prediabetes, diabetes and the complications associated with these conditions through regulation of glucose, insulin, GE and satiety. A number of studies have demonstrated successful results when applying nutritional interventional strategies to prediabetes – i.e. they observed a sustained reduction in the incidence of diabetes and a general improvement in health (Bansal, 2015).

In light of the above, healthcare providers and dietitians should encourage prediabetic

individuals to engage in lifestyle changes, including healthy diet, exercise and weight reduction so as to limit the risk of developing type-2 diabetes and its complications. Including millet, which is rich in polyphenols, in diet plans could also play an important role in preventing diabetes if the grain is consumed frequently and long-term (Chiva-Blanch and Badimon, 2017).

The literature also shows that, although the potential health benefits and nutritional value of millet grains are comparable to popular cereals (such as rice and wheat), different processing methods, like soaking and fortification, were found to improve their edible and nutritional features. Millet is seldom exploited for its qualities in diets in the Western world; most commonly, it is a staple food in rural households in developing countries. This may be due to the lack of available processing technologies required to transform millet grain into ready-to-eat or ready-to-cook products that are safe and easy to prepare (Saleh et al. 2013). Therefore, further research could encourage food manufacturers and bakeries to provide a range of millet-based meals and snacks that are ready to eat, as well as beverages with limited calories. Moreover, it is important to minimise dust and other contaminants when producing or processing millet grains to be ready to cook in the UK. Millet grain is usually used as animal feed and birdseed around the world. In the UK, to our knowledge, there are virtually no ready-to-cook or -eat products made with millet available. Our efforts to find millet grain in the UK were unsuccessful and, where available (e.g. in organic food shops), the type of millet was not specified. This was an important consideration, as numerous studies cited in this thesis, have shown that the different millet types and forms are not equal in their content of polyphenols and antioxidants and, thus, may not offer the same health benefits. Due to its nutritional characteristics and low cost, this might encourage the bakeries in the UK to start importing the millet types in different forms from the countries provide it like India, then making local fresh baked healthy (as well

as gluten-free) products like bread, cake, and biscuit.

Third, healthcare providers and dietitians should be made aware of the best cooking methods needed to maintain the nutritional value of millet, as well as the types and forms of millet that yield the maximum benefits in terms of fibre, polyphenol and antioxidant content – important in controlling glycaemia. This could be done by providing short videos, brochures, seminars, workshops and cooking booklet guidelines for prediabetics, encouraging them to include millet in their diet (the benefits of which are outlined in the systematic review in Chapter 4).

These studies encourage the diversification of food products at the national and household levels. Replacing refined carbohydrates with whole grain may be an important factor in preventing a number of diseases, such as type-2 diabetes (Dixit et al. 2012). Millets are high in fibre and protein and can be used to make healthy dishes that are appetising, culturally appropriate, and nutritionally balanced; they could, therefore, prevent, delay and reduce the global burden of chronic diseases such as diabetes (Dixit et al. 2012).

As a final point, millet is fast-growing and may be grown under adverse weather conditions, such as drought, or during low harvest of more popular cereals. These advantages of millet may encourage farmers to cultivate millet in different countries around the world. Nowadays, the demands for healthy gluten-free products are increasing; thus, this may further promote the utility of millet grains in Western diets. Gluten-free food has been prescribed to support the treatment for patients with coeliac disease. The specialist gluten-free foods such as bread and pasta or snacks like biscuits are often three to four times more expensive than normal gluten-containing varieties. Importantly, millet is gluten-free and adding millet in the diet make it is possible to follow a healthy gluten-free diet on a budget.

6.3. General limitations

The main limitations of this thesis are:

- In the first study (Chapter 2), we could not test two types of millet in flake form (kodo and proso) as the crops were destroyed by floods in India and our supplier was only able to provide grain and flour forms of these millet types. In addition, we could not test these in the subsequent study as they would have given different values for polyphenol and antioxidant content.
- In the second study (Chapter 3), due to financial and time constraints, we only tested sensory evaluation on one baked product (muffin) – which was at times judged as unacceptable by potential consumers (kodo millet in grain form). A bigger choice of dishes, like bread, porridge and drinks should be used to give participants a variety of foods, which should increase acceptability (e.g. of kodo millet, which showed the highest content of polyphenol compounds). Another limitation was the sample size in the sensory study, although all participants were semi-trained just before the sessions. More trained participants are required in order to provide more statistical confidence in the data. A large sample size would be required in further tests in order to obtain a perfect understanding of consumer opinions, a minimum of 50 participants is needed.
- In the third study (Chapter 4), the systematic review was not registered with PROSPERO (international prospective register of systematic reviews), which is a protocol for systematic reviews relevant to health and social care – required in order to avoid duplication. Another limitation is the quality of the studies included in the systematic review, as some used small sample sizes and, in

others, millet grain was mixed with other grain, making it difficult to ascertain which was predominantly responsible for the observed reductions in glycaemia.

- In the last study (Chapter 5), variation in fasting blood glucose levels in the prediabetes group may have affected the GR and IR. To diagnose prediabetic people in this study, we used FBG and OGTT. For some participants, the FBG was in the prediabetes range (6.1 to 6.9 mmol/l); however, some participants displayed normal blood glucose levels (<6.1 mmol/L) but reported one or more risk factors for diabetes; this made them eligible for the OGTT – the gold standard test for diagnosing prediabetes. In this case, we observed a large variation in values at 0min – i.e. not all values were ≥6.1 mmol/l although the OGTT determined prediabetes. Another limitation is the dose/amount of finger millet used in our study. Although we replaced 50% of wheat with finger millet, however, might be not enough to conduct effect in GR, IR, GE and satiety in prediabetes.

6.4. Recommendations for future research

- This PhD thesis highlights the importance of consuming finger millet as a functional grain in prediabetic individuals. However, it is important to carry out further adequately powered full-scale trials to assess the true effect of long-term millet consumption on preventing diabetes diagnosis in prediabetic individuals. It is also worthwhile to test the effects of products made from pure millet on glycaemia in prediabetic individuals. This would help raise the profile of millet in the food industry and popularise millet-based food products, especially in countries where millet is not commonly consumed.

- A further study using a larger sample size to investigate the effect of finger millet consumption on the GR, IR and GE on prediabetic individuals would be useful, as well as standardising the fasting blood glucose range. This will provide more reliable results and lead to more informed recommendations for millet consumption in the prevention of type-2 diabetes.
- Further studies linking millet and glycaemia are required, with a particular focus on the different processing and cooking methods – which are shown to significantly impact blood sugar.
- More sensory evaluation tests on different varieties of millet, in different food and beverage products, are required to promote development of a millet industry in Western countries and commercialise ready-to-eat products containing millet.

6.5. General conclusion

This PhD thesis provides novel knowledge for type-2 diabetes prevention research and contributes to our understanding of functional grains, such as millet, in how they impact on glycaemia, insulin, GE and satiety. The results of this thesis should inform future research into novel functional products that include millet as a dietary means of improving glucose regulation in prediabetes and preventing the development of type-2 diabetes .

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Appendices

Appendix 1A Certificate Of finger millet muffin Analysis

eurofins

Helen Lightowler
Oxford Brookes University
Functional Food Centre
Headington Campus
Gipsy Lane
Oxford
OX3 0BP

PO Number IC 433138

AR-17-UD-134781-01

Reported on 23/03/2017
Reported by Nicholas Cockburn, Analytical Services Manager

Certificate Of Analysis

Sample number	400-2017-20031882	Received on	14/03/2017
Your sample reference	Finger Millet	Your sample code	Sample B

Test Code	Analyte	Result		SOP No.
Nutrition				
UD006	Moisture	37.9	g/100 g	Q/005 H/007
UD001	Crude Protein (Nx6.25) (Dumas)	6.2	g/100 g	Z/001
UD007	Ash	2.9	g/100 g	Q/001
UD017	Carbohydrates (available)	34.30	g/100 g	Q/035
UD08W	Fructose	<0.1	g/100 g	CHROM/344
UD08W	Galactose	<0.1	g/100 g	CHROM/344
UD08W	Glucose	<0.1	g/100 g	CHROM/344
UD08W	Lactose	1.2	g/100 g	CHROM/344
UD08W	Maltose	0.1	g/100 g	CHROM/344
UD08W	Sucrose	8.0	g/100 g	CHROM/344
UD08W	Total sugars	9.4	g/100 g	CHROM/344
UD003	Total fat	14.4	g/100 g	Q/002
B7039	Total dietary fibre (AOAC)	4.3	g/100 g	H/085
UD771	Energy value (kcal)	300	kcal/100 g	Q/035
UD771	Energy value (kJ)	1256	kJ/100 g	Q/035
		Energy has been calculated to include the contribution from fibre, as required by Regulation (EU) No 1169/2011		
UD815	Salt (via sodium x 2.5)	1.71	g/100 g	
Fatty Acids				
UDFB1	Monounsaturated fatty acids	4.18	g/100 g	CHROM/215
UDFB1	Polyunsaturated fatty acids	7.49	g/100 g	CHROM/215
UDFB1	Saturated fatty acids	2.05	g/100 g	CHROM/215
UDFB1	Trans Fatty Acids	< 0.1	g/100 g	CHROM/215
Elements				
UD015	Sodium	0.685	g/100 g	ICP/003

Unless stated, all results are expressed on a sample as received basis.
† Indicates that this test was subcontracted

Key: cfu colony forming units
< denotes less than
> denotes greater than
~ estimated value

* Indicates that this parameter is not included in the UKAS accreditation schedule for the laboratory.
Opinions and/or interpretations within this report are outside our accreditation scope.

UKAS TESTING 0342

Eurofins Food Testing UK Ltd
i54 Business Park
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Regd Office: i54 Business Park
Valiant Way
Wolverhampton WV9 5GB
Regd in England No: 5009315

Appendix1B Certificate Of control muffin Analysis

eurofins

Helen Lightowler
Oxford Brookes University
Functional Food Centre
Headington Campus
Gipsy Lane
Oxford
OX3 0BP

PO Number IC 433138

AR-17-UD-134780-01

Reported on 23/03/2017
Reported by Nicholas Cookburn, Analytical Services Manager

Certificate Of Analysis

Sample number	400-2017-20031881	Received on	14/03/2017
Your sample reference	Control Muffin	Your sample code	Sample A

Test Code	Analyte	Result		SOP No.
Nutrition				
UD006	Moisture	36.6	g/100 g	Q/005 H/007
UD001	Crude Protein (Nx6.25) (Dumas)	6.3	g/100 g	Z/001
UD007	Ash	2.7	g/100 g	Q/001
UD017	Carbohydrates (available)	37.40	g/100 g	Q/035
UD08W	Fructose	<0.1	g/100 g	CHROM/344
UD08W	Galactose	<0.1	g/100 g	CHROM/344
UD08W	Glucose	<0.1	g/100 g	CHROM/344
UD08W	Lactose	1.1	g/100 g	CHROM/344
UD08W	Maltose	0.2	g/100 g	CHROM/344
UD08W	Sucrose	7.8	g/100 g	CHROM/344
UD08W	Total sugars	9.3	g/100 g	CHROM/344
UD003	Total fat	15.7	g/100 g	Q/002
B7039	Total dietary fibre (AOAC)	1.3	g/100 g	H/085
UD771	Energy value (kcal)	319	kcal/100 g	Q/035
UD771	Energy value (kJ)	1334	kJ/100 g	Q/035
	Energy has been calculated to include the contribution from fibre, as required by Regulation (EU) No 1169/2011			
UD815	Salt (via sodium x 2.5)	1.81	g/100 g	
Fatty Acids				
UDFB1	Monounsaturated fatty acids	4.44	g/100 g	CHROM/215
UDFB1	Polyunsaturated fatty acids	8.32	g/100 g	CHROM/215
UDFB1	Saturated fatty acids	2.18	g/100 g	CHROM/215
UDFB1	Trans Fatty Acids	< 0.1	g/100 g	CHROM/215
Elements				
UD015	Sodium	0.724	g/100 g	ICP/003

Unless stated, all results are expressed on a sample as received basis.
† Indicates that this test was subcontracted

Key: cfu colony forming units
< denotes less than

Appendix1C UREC Ethical approvals for sensory study

OXFORD BROOKES UNIVERSITY

Dr Helen Lightowler
Director of Studies
Department of Sport and Health Sciences
Faculty of Health and Life Sciences
Oxford Brookes University
Headington Campus

6 July 2016

Dear Dr Lightowler

UREC Registration No: 161021
Evaluation of millet based baked products: Phase I Sensory Evaluation in Healthy Adults

Thank you for the email of 4 July outlining the response to the points raised in my previous letter about the PhD study of your research student Ameerah Almaski and attaching the revised documents. I am pleased to inform you that, on this basis, I have given Chair's Approval for Phase 1 of the study to begin.

The UREC approval period for phase 1 of the study is two years from the date of this letter, so 6 July 2018. If you need the approval to be extended please do contact me nearer the time of expiry.

Should the recruitment, methodology or data storage change from your original plans, or should any study participants experience adverse physical, psychological, social, legal or economic effects from the research, please inform me with full details as soon as possible.

Once Phase 1 has been completed and the researcher is able to move to Phase 2 of the study, please submit an application to UREC, paying particular attention to the recruitment of pre-diabetic participants.

Yours sincerely

Dr Sarah Quinton
Chair of the University Research Ethics Committee

cc Sangeetha Thondre and Shelly Coe, Supervisory Team
Ameerah Almaski, Research Student
Dido Green, Research Ethics Officer
Jill Organ, Research Degrees Team
Louise Wood, UREC Administrator

Appendix1D Sensory evaluation Study advertisement

Ameerah Almaski

Email: 14107967@brookes.ac.uk

PhD Research Study - September 2016 to December 2016

Supervisors:

Dr Helen Lightowler Dr Sangeetha Thondre Dr Shelly Coe

OXFORD BROOKES UNIVERSITY

Evaluation of millet-based baked products

Evaluation of millet based baked products: Phase I Sensory Evaluation in Healthy Adults

- We are looking for healthy adult volunteers aged between 18 and 65 years
- You will be asked to consume millet-based products
- You will be asked to express you opinion about each product by giving a score to attributes like colour, flavour, taste, texture and overall acceptability
- The study will involve two visits to the Functional Food Centre, Oxford Brookes University, and each visit lasting up to 90 minutes.
- You will be trained on the sensory test in your first visit before testing the product

If you would like to get involved and need more information, please contact the researcher

You will receive a £10 Amazon voucher after you finish the study

Contact Ameerah Almaski 14107967@brookes.ac.uk

Appendix1E Participant information sheet for sensory evaluation study

Information sheet

Evaluation of millet-based baked products

Evaluation of millet based baked products: Phase I Sensory Evaluation in Healthy Adults

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully:

The purpose of the study:

Millet is a functional grain that has attracted the attention of scientists for some years due to its significant benefits to human health. Millet is the main food source for many people and is usually grown in different countries such as African, Asian and different sections of Europe and consumed as a main food in semi-arid and tropical regions of the world. Research has shown that millets have a high antioxidant capacity and polyphenol content which can contribute to a reduced risk of some chronic diseases such as type 2 diabetes and its complications. Acceptance of a food product can be tested with the help of a sensory evaluation test.

This project is based on using millet grain in baked products and studying the degree of consumer acceptance and satisfaction regarding those products in terms of taste, texture, flavour and appearance.

Why have I been invited to participate?

You will have seen our advertisements inviting healthy adults
The inclusion criteria:

- Healthy adult aged 18-65 years

The exclusion criteria:

- Health aspects or use of medication such as antidepressants that interfere with taste and/or olfactory sensitivity such as loss of smell and/or taste
- Allergies to the foods presented in the study (flour, vanilla, egg sugar, salt, baking powder, milk, oil and millet)
- Diabetes (fasting blood sugar 7.0 mmol/l or more).
- Frequent cold (this will include cold and blocked nose on test day)
- Hay fever (on test day).
- Smokers.

What will happen to me if I take part?

If you are interested in taking part you will be sent the health questionnaire to make sure that you are eligible to participate in the study via email or by hand (from the Functional Food Centre).

Then, if you are eligible to participate in the study, you need to sign the consent form and you will be asked to come on two separate days for the test in Functional Food Centre in Oxford Brookes University, one hour after your last meal.

Visit 1:

- Visit one will last up to 90 minutes
- You will be trained on the test procedure
- You will taste two different types of millet-based baked products
- You will be asked to express your opinion about each product by giving a score to attributes like colour, flavour, taste, texture and overall acceptability using 9-point hedonic score system (9 = like extremely; 1 = dislike extremely).

Visit 2:

- Visit two will last up to 90 minutes.
- Will be the same as visit 1 using two types of millet-based baked products

In each session:

- Cold water and crackers will be supplied to you for drinking or cleansing your palate between the samples.

There will be at least 3 days between the tests.

The diagram illustrates the test methods in each visit.

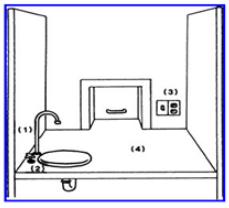
First visit

(🕒 Visit duration: Last up to 90 minutes)

1. Answer any question
2. Sign consent form
3. Train the participant on 9-hedonic test

↓

Sitting in sensory evaluation booths in lab (room S404)



↓

Providing the following to participant:

4. Two different types of millet-based baked products
5. A hedonic rating scale
6. Cold water and crackers for drinking or cleansing the palate between samples

↓

Ask the participant to taste the millet-based baked products and express their opinion about each product by giving a score to attributes like colour, flavour, taste, texture and overall acceptability using 9-point hedonic score system (9 = like extremely; 1 = dislike extremely).

At least 3 days

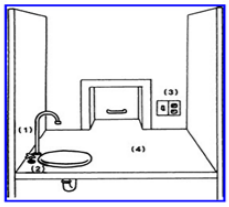
Second visit

(🕒 Visit duration: Last up to 90 minutes)

Answer any question

↓

Sitting in sensory evaluation booths in lab (room S404)



↓

Providing the following to participant:

1. Another two different types of millet-based baked products
2. A hedonic rating scale
3. Cold water and crackers for drinking or cleansing the palate between samples

↓

Ask the participant to taste the millet-based baked products and express their opinion about each product by giving a score to attributes like colour, flavour, taste, texture and overall acceptability using 9-point hedonic score system (9 = like extremely; 1 = dislike extremely).

Benefits of the study

- You will receive a £10 Amazon voucher on completion of the study.

Health and safety issues

There are no major risks.

Data protection and withdrawal

- Confidentiality of any information provided can only be protected within the limitations of the law
- All records will be coded and will only be available to the researchers involved in the study; your name will never appear in any published work
- All data from the study will be owned by Oxford Brookes University and will be will be retained and kept securely in accordance with the University's policy of Academic Integrity for a period of ten years.
- You are free to withdraw from the study at any time, without giving a reason, and to withdraw any unprocessed data previously supplied.

What happens if I do want to take part?

If you would like to take part in this research study you can do so by contacting the researcher at the address, phone number or email address given below. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form.

Participation is completely voluntary. If you decide to take part, you are still free to withdraw at any time and without giving a reason. If you are a student, by choosing to either take part or not take part in the study or to withdraw at any time will have no impact on your marks, assessments or future studies. If you are a member of staff by choosing to take part, or not to take part, in the study or to withdraw at any time will have no impact on your employment status at the university, no details will be recorded for monitoring purposes.

Who is organising and funding the research?

I (Ameerah Almaski) am conducting the research as a PhD researcher at Oxford Brookes University under the supervision of Dr Helen Lightowler, Dr Sangeetha Thondre and Dr Shelly Coe in the Department of Sport & Health Sciences. Ameerah Almaski is fully funded by the Embassy of Saudi Arabia.

Will this study be kept confidential?

All information collected about you will be kept strictly confidential. Your personal data will be in a locked drawer at Oxford Brookes University, and if saved on a computer, will be securely encrypted with a password. Data in paper or electronic form will be de-identified by a code and kept securely for a period of 10 years after the completion of this research project. Only researchers directly involved in the study will have access to personal data.

The data will be statistically analysed in the UK under supervision of Dr Helen Lightowler, Dr Sangeetha Thondre and Dr Shelly Coe at Oxford Brookes University.

What will happen to the results of this research study?

Ultimately, the results of the study will be written up as part of a PhD thesis. In addition, the results will be published in peer-reviewed journals and presented at meeting and conferences. Participants will not be identified in any publications.

Who has reviewed the study?

This study has been approved by Oxford Brookes University Research Ethics Committee (UREC). Any concerns about the conduct of the study should be referred to the Chair of UREC on ethics@brookes.ac.uk

Contact for further information

You can contact the researcher Ameerah Almaski at any time if you have any questions or concerns regarding this study:
EMAIL: 14107967@brookes.ac.uk
PHONE: 01865 483283

Appendix 1F Consent form

Consent form

Study tittle: To conduct a sensory principles test to assess the acceptance of meal prepared from millet

Contacts:

1. Ameerah Almaski, PhD researcher in human nutrition.
2. Dr Helen Lightowler, Operations Director of the Functional Food Centre, senior lecturer in human nutrition.
3. Dr Sangeetha Thondre, senior lecturer in nutrition.
4. Dr Shelly Coe, lecturer in nutrition.

Tel: 01865 483245 - 1865 483988-1865 483839
Email: 14107967@brookes.ac.uk/ hlightowler@brookes.ac.uk / Pthondre@brookes.ac.uk / scoe@brookes.ac.uk

Please **INITIAL** the appropriate box

	Yes	No
1. I confirm that I have read and understand the information sheet for the above research project.	☐	☐
2. I confirm that I have had the opportunity to ask questions and have received satisfactory answers to all my questions.	☐	☐
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, or to withdraw any unprocessed data previously supplied.	☐	☐
4. I understand that confidentiality of information provided can only be protected within the limits of the law.	☐	☐
5. I agree to take part in the above research.	☐	☐

Name of Participant .....Date .....
(block capitals)

Signature .....

Contact number: ..... email: .....

Name of Researcher .....Date .....
(block capitals)

Signature

Appendix 1G 9-point hedonic rating scale (sensory evaluation study)

Hedonic Rating Scale

Tray number:

Name:

In front of you is one sample. Taste the sample and tick ✕ how much you like or dislike each of the characteristics. You can taste the sample more than once.

1. Aroma:

Like extremely	Like very much	Like moderately	Like slightly	Neither like Or dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

2. Flavour:

Like extremely	Like very much	Like moderately	Like slightly	Neither like Or dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

3. Colour:

Like extremely	Like very much	Like moderately	Like slightly	Neither like Or dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

4. Texture:

Like extremely	Like very much	Like moderately	Like slightly	Neither like Or dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

5. Taste:

Like extremely	Like very much	Like moderately	Like slightly	Neither like Or dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

6. Appearance:

Like extremely	Like very much	Like moderately	Like slightly	Neither like Or dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

7. Crust:

Like extremely	Like very much	Like moderately	Like slightly	Neither like Or dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

8. Overall acceptance:

Like extremely	Like very much	Like moderately	Like slightly	Neither like Or dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

Thank you

Appendix 2A Consort checklist form

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	
	2b	Specific objectives or hypotheses	
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	
	13b	For each group, losses and exclusions after randomisation, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Appendix 2B Prisma checklist form

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: www.prisma-statement.org.

Appendix 2C Quality criteria checklist primary research

Quality Criteria Checklist Primary Research — Handout A

Symbols Used

+ **Positive:** Indicates that the report has clearly addressed issues of inclusion / exclusion, bias, generalizability, and data collection and analysis.

-- **Negative:** Indicates that these issues have not been adequately addressed.

Ø **Neutral:** Indicates that the report is neither exceptionally strong nor exceptionally weak.

Quality Criteria Checklist: Primary Research

RELEVANCE QUESTIONS		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for patients / clients / population group? (NA for some Epi studies)	Yes No Unclear N/A
2.	Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about?	Yes No Unclear N/A
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	Yes No Unclear N/A
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes No Unclear N/A
If the answers to all of the above relevance questions are "Yes", the report is eligible for designation with a plus(+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.		
VALIDITY QUESTIONS		
1.	**Was the research question clearly stated?** 1.1 Was the specific intervention(s) or procedure [independent variable(s)] identified? 1.2 Was the outcome(s) [dependent variable(s)] clearly indicated? 1.3 Were the target population and setting specified?	
2.	**Was the selection of study subjects / patients free from bias?** 2.1 Were inclusion / exclusion criteria specified (e.g. risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study? 2.2 Were criteria applied equally to all study groups? 2.3 Were health, demographics, and other characteristics of subjects described? 2.4 Were the subjects / patients a representative sample of the relevant population?	Yes No Unclear N/A
3.	**Were study groups comparable?** 3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT) 3.2 Were distribution of disease status, prognostic factors, and other factors (e.g. demographics) similar across study groups at baseline? 3.3 Were concurrent controls used? (Concurrent preferred over historical controls.) 3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and / or were pre-existing differences accounted for by using appropriate adjustments in statistical analysis? 3.5 If case control study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.) 3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")?	Yes No Unclear N/A
4.	**Was method of handling withdrawals described?** Were follow up methods described and the same for all groups? Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and / or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%) Were all enrolled subjects / patients (in the original sample) accounted for? Were reasons for withdrawals similar across groups? If diagnostic test, was decision to perform reference text not dependent on results of test under study?	Yes No Unclear N/A

5.	**Was blinding used to prevent introduction of bias?**	Yes	No	Unclear	N/A
	5.1 In intervention study, were subjects, clinicians / practitioners, and investigators blinded to treatment group, as appropriate?				
	5.2 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)				
	5.3 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?				
	5.4 In case control study, was case definition explicit and case ascertainment not influenced by exposure status?				
	5.5 In diagnostic study, were test results blinded to patient history and other test results?				
6.	**Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?**	Yes	No	Unclear	N/A
	6.1 In RCT or other intervention trial, were protocols described for all regimens studied?				
	6.2 In observational study, were interventions, study settings, and clinicians / provider described?				
	6.3 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?				
	6.4 Was the amount of exposure and, if relevant, subject / patient compliance measured?				
	6.5 Were co-interventions (e.g. ancillary treatments, other therapies) described?				
	6.6 Were extra or unplanned treatments described?				
	6.7 Was the information for 6.4, 6.5 and 6.6 assessed the same way for all groups?				
	6.8 In diagnostic study, were details of test administration and replication sufficient?				
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes	No	Unclear	N/A
	7.1 Were primary and secondary endpoints described and relevant to the question?				
	7.2 Were nutrition measures appropriate to question and outcomes of concern?				
	7.3 Was the period of follow-up long enough for important outcome(s) to occur?				
	7.4 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures?				
	7.5 Was the measurement of effect at an appropriate level of precision?				
	7.6 Were other factors accounted for (measured) that could affect outcomes?				
	7.7 Were the measurements conducted consistently across groups?				
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes	No	Unclear	N/A
	8.1 Were statistical analyses adequately described the results reported appropriately?				
	8.2 Were correct statistical tests used and assumptions of test not violated?				
	8.3 Were statistics reported with levels of significance and / or confidence intervals?				
	8.4 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?				
	8.5 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)?				
	8.6 Was clinical significance as well as statistical significance reported?				
	8.7 If negative findings, was a power calculation reported to address type 2 error?				
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes	No	Unclear	N/A
	9.1 Is there a discussion of findings?				
	9.2 Are biases and study limitations identified and discussed?				
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes	No	Unclear	N/A
	10.1 Were sources of funding and investigators' affiliations described?				
	10.2 Was there no apparent conflict of interest?				

MINUS / NEGATIVE (-)
If most (six or more) of the answers to the above validity questions are "No", the report should be designated with a minus (-) symbol on the Evidence Worksheet.

NEUTRAL (Ø)
If the answers to validity criteria questions 2, 3, 6 and 7 do not indicate that the study is exceptionally strong, the report should be designated with a neutral (∅) symbol on the Evidence Worksheet.

PLUS / POSITIVE (+)
If most of the answers to the above validity questions are "Yes" (including criteria 2, 3, 6, 7 and at least one additional "Yes"), the report should be designated with a plus symbol (+) on the Evidence Worksheet.

OXFORD BROOKES UNIVERSITY

Dr Sangeetha Thondre
Director of Studies
Department of Sport and Health Sciences
Faculty of Health and Life Sciences
Oxford Brookes University
Headington Campus

6 March 2017

Dear Dr Thondre

UREC Registration No: 161061
The effect of millet-based muffins on glycaemic, insulinemic response and gastric emptying in pre-diabetic adults

Thank you for your email of 7 February 2017 outlining your response to the points raised in my previous letter about the PhD study of your research student Ameerah Almaski and attaching the revised documents. I am pleased to inform you that, on this basis, I have given Chair's Approval for the study to begin.

The UREC approval period for this study is two years from the date of this letter, so 6 March 2019. If you need the approval to be extended please do contact me nearer the time of expiry.

Should the recruitment, methodology or data storage change from your original plans, or should any study participants experience adverse physical, psychological, social, legal or economic effects from the research, please inform me with full details as soon as possible.

Yours sincerely

Dr Sarah Quinton
Chair of the University Research Ethics Committee

cc Helen Lightowler and Shelly Coe, Supervisory Team
Ameerah Almaski, Research Student
Anne Delextrat, Research Ethics Officer Jill Organ, Research Degrees Team Louise Wood, UREC Administrator

Appendix 3B GR, IR & GE study advertisement (healthy group)

The effect of millet based muffins on glycaemic response (GR), insulinemic response (IR) and gastric emptying in healthy adults volunteers (without any known medical condition)

OXFORD BROOKES UNIVERSITY

Who can participate?

- We are looking for healthy volunteers aged between 45 and 65 years.
- Body mass index (BMI) ≤ 30kg/m2
- Fasting blood glucose < 6.1 mmol/l
- Non-pregnant and non-lactating
- No known diabetes or impaired glucose tolerance
- No medical condition(s) or medication(s) known to affect glucose regulation or appetite and/or which influence digestion and absorption of nutrients
- No major medical or surgical event requiring hospitalisation within the preceding three months
- Not taking steroids, protease inhibitors or antipsychotics

Ameerah Almaski
PhD Research Study
Sep 2017
to
Dec 2017
Email:
ameerah.almaski-2015@brookes.ac.uk

What would be expected of participants?

The study will involve 2 visits, each visit lasting up to 4 hours.

In each visit you will need to :

- ❖ fast overnight (10-12 hr).
- ❖ be prepared to have a minimum of 10 small finger pricks on each occasion
- ❖ consume a muffin made with wheat and a muffin made with millet
- ❖ collect breath samples every 15 minutes for 4 hours

You will receive (£30) Amazon voucher after you finish the study

Ameerah Almaski
Email: ameerah.almaski-2015@brookes.ac.uk

Appendix 3C GR, IR & GE Study advertisement (pre-diabetic group)

Effect of millet based muffins consumption on glycaemic, insulinemic response and gastric emptying in pre-diabetic adults

OXFORD BROOKES UNIVERSITY

We are looking for pre-diabetic volunteers
aged between 18 to 65 years

According to NHS in the UK if you have one or more of the following risk factors you might be at risk for developing type 2 diabetes and you should be tested for prediabetes:

- Adults who have been diagnosed with prediabetes.
- Fasting blood glucose 6.1-6.9 mmol/l or/and an Oral glucose tolerance test (OGTT) at 2 hours 7.8 to 11.0 mmol/l.
- Women with polycystic ovarian syndrome
- Age – being over the age of 40
- Genetics – having a close relative with the condition, such as a parent, brother or sister
- Weight – being overweight or obese (BMI ≥ 25 kg/m2)
- Ethnicity – being of south Asian, Chinese, African-Caribbean or black African origin, even if you were born in the UK

You will need to come for a screening visit to measure your body weight and height, blood pressure and fasting blood glucose in the Functional Food Centre.

Note: Oral glucose tolerance test is required if your fasting blood glucose level is normal.

The study will involve 2 visits (or 3 visits for oral glucose tolerance test for 2h) , each visit lasting up to 4 hours.

In each visit:

- You will need to fast overnight.
- You will need to be prepared to have a minimum of 10 small finger pricks on each occasion
- You will need to consume control muffin (wheat) and one test muffin (millet).
- We will need to collect breath samples every 15 minutes for 4 hours for measurement of gastric emptying to know about digestion process.

You will receive (£50) Amazon voucher after you finish the study

Ameerah Almaski - PhD Research Study - April 2017 to May 2018

Ameerah Almaski 1410796?@brookes.ac.uk

Appendix 3D Participant information sheet GR, IR and GE study (healthy)

Information sheet

The effect of millet based muffins on glycaemic response (GR), insulinemic response (IR) and gastric emptying in healthy adults volunteers (without any known medical condition)

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully:

The purpose of the study:

Millet is a functional grain that has attracted the attention of scientists for some years due to its significant benefits to human health. Millet is the main food source for many people, is usually grown in Africa, Asia and different regions of Europe and consumed as a main food in semi-arid and tropical regions of the world. Research has shown that millets have a high antioxidant capacity and polyphenol content which can contribute to a reduced risk of some chronic diseases such as type 2 diabetes and its complications.

The study aims to assess the GR, IR and gastric emptying time after the consumption of a millet-based muffin in healthy participants in order to determine the effect of millet consumption on GR and IR compared to the consumption of a non-millet-based, control muffin.

Why have I been invited to participate?

We are inviting volunteers without any known medical condition to participate in the study.
The inclusion and exclusion criteria will be as follows:

Inclusion criteria and exclusion criteria

- Aged 18-65 years
- Body mass index (BMI) ≤ 30kg/m2
- Fasting blood glucose < 6.1 mmol/l
- Non-pregnant and non-lactating
- No known diabetes or impaired glucose tolerance
- No medical condition(s) or medication(s) known to affect glucose regulation or appetite and/or which influence digestion and absorption of nutrients
- No major medical or surgical event requiring hospitalisation within the preceding three months
- No use of steroids, protease inhibitors or antipsychotics
- No food allergy to:
    - millet
    - wheat
    - egg
    - milk

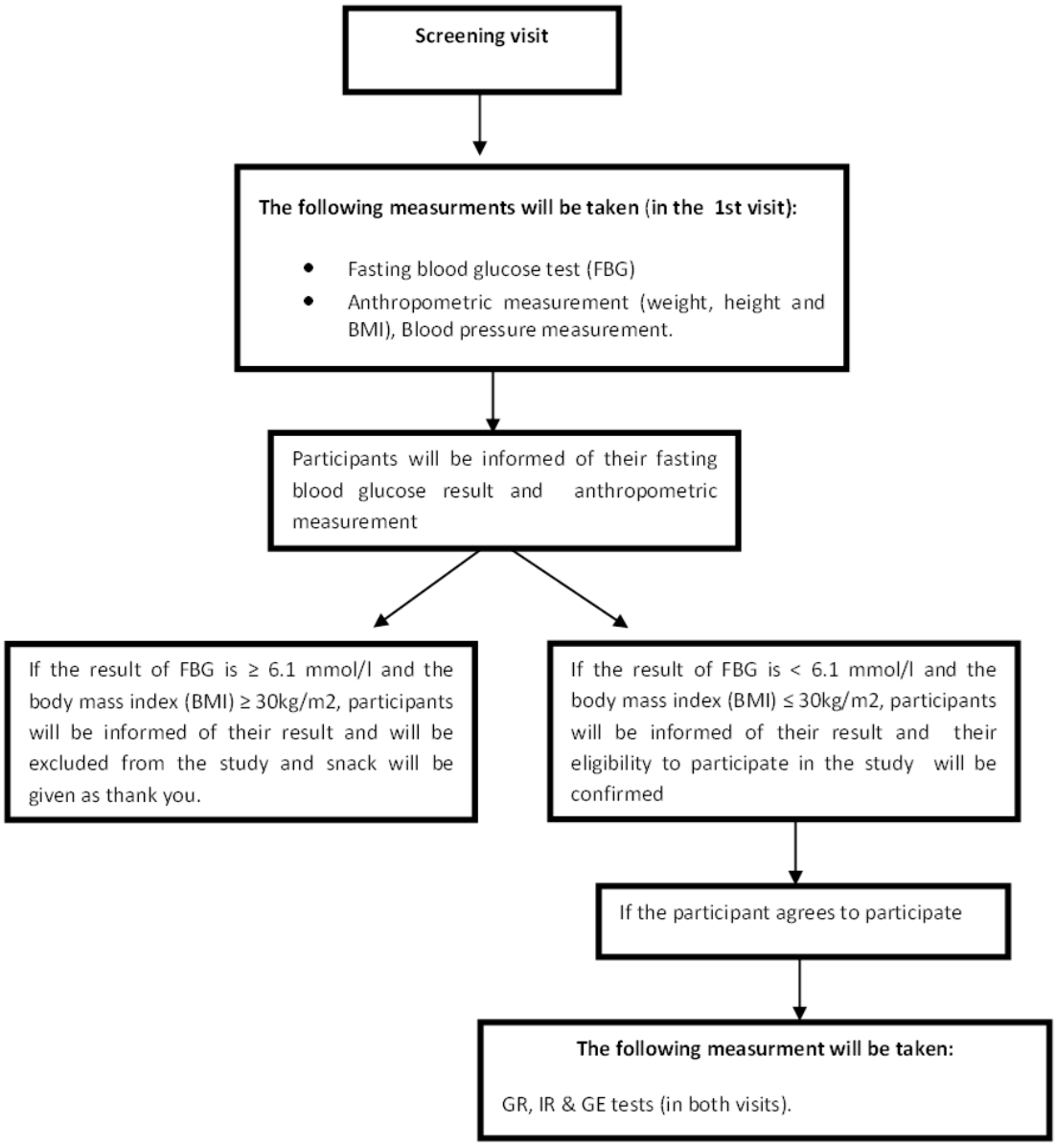
Should you agree to participate, and after signing the consent form, you will be asked to complete a health questionnaire and screening to make sure that you are eligible to participate in the study.

What the study involves

- You will be required to attend the Oxford Brookes Centre for Nutrition and Health (OxBCNH)for two test sessions, with at least one-day between each test. Each test session will last for approximately 4 hours (3 hours for glycaemic, insulinaemic response test and an additional 1 hour to complete gastric emptying).

Part 1:

Interested participants will be invited for a screening test (on the same day of the test) to check their fasting blood glucose level and will go through the following protocol. Testing is completely voluntary to the participant:



Part 2:

If you are eligible to take part in the study,you will be asked to sign a consent form and complete the first session, which will involve the following:

- Two fasting blood glucose measurements (at -5 minutes and 0 minutes before the meal) – this involves two small finger-pricks.
- Further finger-prick blood samples for glucose and insulin at 15, 30, 45, 60, 90,120, 150 and 180 minutes after the start of consumption of a control (wheat) or millet muffin.
- At the same time intervals, you will be asked to rate how hungry you feel.
- Gastric emptying: You will need to give breath samples for measurement of gastric emptying which will be taken by blowing into a small glass tube through a straw. They will be collected every 15 minutes for 4 hours after consumption of control and millet-based baked products.

The second session will be the same as part 2 in session one.

How to prepare for the study

- You need to fast overnight (approximately 10-12 hours) – this means **no food or drink**, apart from drinking water in moderation
- For example, if you start your test session at 8.30 am, you need to stop eating/drinking (apart from water) at 8.30 pm the previous evening

On the day before a test:

- limit your caffeine intake (maximum of 2-3 cups of coffee/tea)
- limit your alcohol intake (maximum of 1 small glass of wine/1 pint of beer)
- restrict participation in intense physical activity (e.g. long periods at the gym, excessive swimming, running, aerobics)

- Do not smoke on the morning of the test

Benefits of the study

- The study will provide valuable information on the effect of millet-based muffins on glycaemic, insulinemic response and gastric emptying in pre-diabetic adults.
- You will receive a "health check" profile, including anthropometric and body composition measurements.
- You will receive **£30 of Amazon voucher** when you have completed all the sessions.

Health and safety issues

- Each measurement requires a small amount of blood; therefore the finger-prick will be small, with minimal discomfort. A light bruising may also occur in some people, but this should disappear within a couple of days and will not affect your ability to work.
- The researcher (Ameerah Almaski) will take finger-prick blood samples using standard procedures. There will be a designated clean area where finger-prick blood samples will be taken.
- Clinical waste procedures will be followed at all times. No cellular material will be stored after 24 hr; only plasma will be frozen for insulin assay.
- If you have a high blood glucose level and blood pressure, this does not always mean diabetes/hypertension. The diagnosis of diabetes/hypertension is never made on the basis of a single abnormal blood glucose value/blood pressure reading. You will be advised to contact your GP for further tests if necessary.

Data protection and withdrawal

- Confidentiality of any information provided can only be protected within the limitations of the law
- All samples and records will be coded and will only be available to the researchers involved in the study; your name will never appear in any published work

You are free to withdraw from the study at any time, without giving a reason, and to withdraw any unprocessed data previously supplied.

What happens if I do want to take part?

If you would like to take part in this research study you can do so by contacting the researcher at the address, phone number or email address given at the end of this information sheet. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form.

Participation is completely voluntary. If you decide to take part, you are still free to withdraw at any time and without giving a reason. If you are a student, by choosing to either take part or not take part in the study or to withdraw at any time will have no impact on your marks, assessments or future studies.

Who is organising and funding the research?

I (Ameerah Almaski) am conducting the research as a PhD researcher at Oxford Brookes University under the supervision of Dr Sangeetha Thondre, Dr Helen Lightowler, and Dr Shelly Coe in the Department of Sport, Health Sciences and Social Work. Ameerah Almaski is fully funded by the Embassy of Saudi Arabia.

Will this study be kept confidential?

All information collected about you will be kept strictly confidential. Your personal data will be in a locked drawer at Oxford Brookes University, and if saved on a computer, will be securely encrypted with a password. Data in paper or electronic form will be de-identified by a code and kept securely for a period of 10 years after the completion of this research project. Only researchers directly involved in the study will have access to personal data.

What will happen to the results of this research study?

Ultimately, the results of the study will be written up as part of a PhD thesis. In addition, the results will be published in peer-reviewed journals and presented at meeting and conferences. Participants will not be identified in any publications.

Who has reviewed the study?

This study has been approved by Oxford Brookes University Research Ethics Committee (UREC). Any concerns about the conduct of the study should be referred to the Chair of UREC on ethics@brookes.ac.uk.

Contact for further information

You can contact the researcher Ameerah Almaski at any time if you have any questions or concerns:

Ameerah Almaski PhD student 14107967@brookes.ac.uk

Thank you so much

Appendix 3E Participant information sheet GR, IR and GE study (prediabetes)

Information sheet

The effect of millet based muffins on glycaemic, insulinemic response and gastric emptying in pre-diabetic (impaired glucose tolerance) adults(18-65 years old)

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully:

The purpose of the study:

Millet is a functional grain that has attracted the attention of scientists for some years due to its significant benefits to human health. Millet is the main food source for many people, is usually grown in Africa, Asia and different regions of Europe and consumed as a main food in semi-arid and tropical regions of the world. Research has shown that millets have a high antioxidant capacity and polyphenol content which can contribute to a reduced risk of some chronic diseases such as type 2 diabetes and its complications.

Pre-diabetes (impaired glucose tolerance) is a condition in which blood glucose levels are higher than normal, but not high enough to be classified as diabetes.
The study aims to assess the GR, IR and gastric emptying time after the consumption of a millet-based muffin in pre-diabetic participants in order to establish whether this lowers GR and IR compared to the consumption of a non-millet-based, control muffin.

Why have I been invited to participate?

We are inviting pre-diabetic volunteers to participate in the study and people with pre-diabetes could be included in the study without screening.
The inclusion and exclusion criteria will be as follows:

Inclusion criteria

The inclusion criteria for screening people without prediabetes are one of the following:

- BMI ≥ 25 kg/m^2
- Aged 40+ years
- Women with polycystic ovarian syndrome.
- Genetics – having a close relative with diabetes, such as a parent, brother or sister
- Ethnicity – being of south Asian, Chinese, African-Caribbean or black African origin, even if born in the UK

After screening, people will be recruited for the study if they have one of the following:

- Fasting blood glucose 6.1-6.9 mmol/l (108-125 mg/ dl) - (WHO, 2006) **or/and** an Oral glucose tolerance test (OGTT) at 2 hours 7.9 to 11.0 mmol/l

Exclusion criteria

- Pregnant and lactating
- Diabetes
- Medical condition(s) or medication(s) known to affect glucose regulation or appetite and/or which influence digestion and absorption of nutrients
- Medical or surgical event requiring hospitalisation within the preceding three months
- Use of steroids, protease inhibitors or antipsychotics (because of their effects on glucose regulation of appetite or influence digestion and absorption of nutrients).
- Food allergy to study products (millet. wheat, egg, milk, butter, sugar).

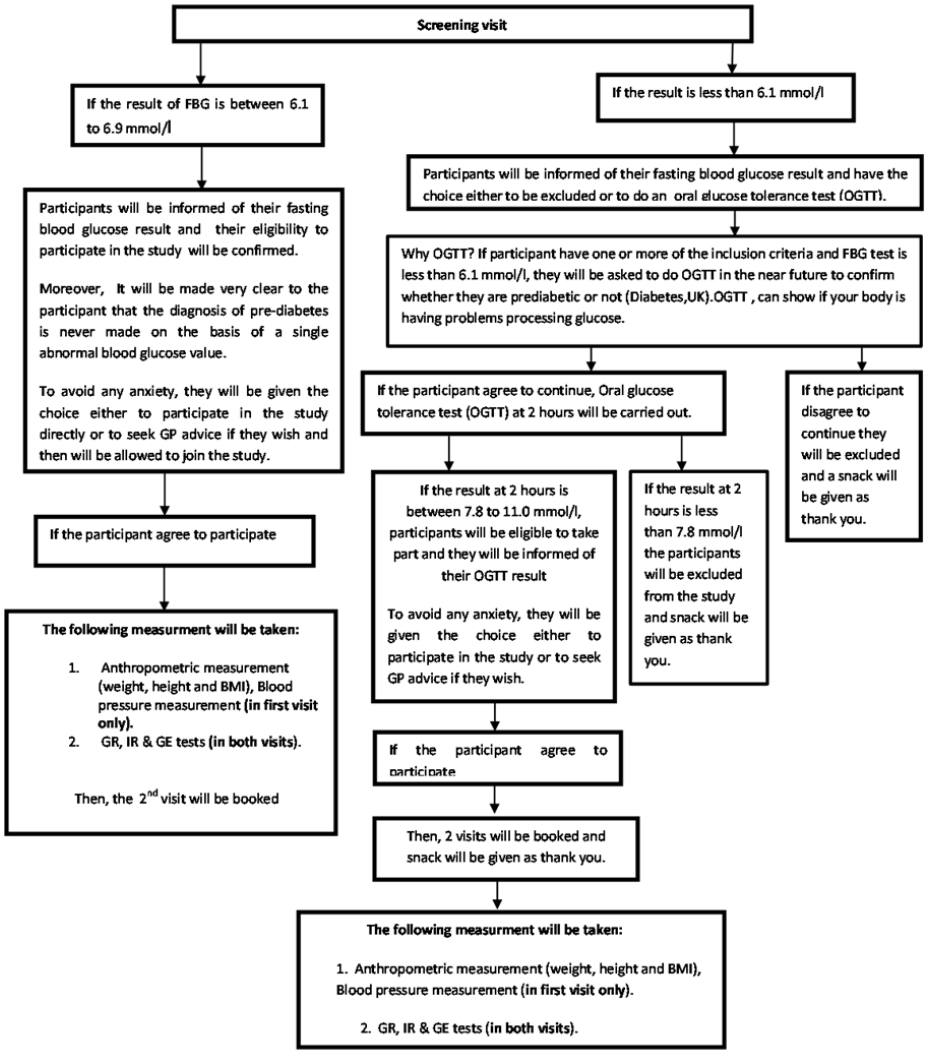
Should you agree to participate, and after signing the consent form, you will be asked to complete a health questionnaire and screening to make sure that you are eligible to participate in the study.

What the study involves

- You will be required to attend the Functional Food Centre for two test sessions, with at least one-day between each test. However, if you need to complete an oral glucose tolerance test (OGTT) in the screening visit, you will be asked to attend another two sessions for testing (three sessions in total).
- Each test session will last for approximately 4 hours (3 hours for glycaemic, insulinaemic response test and an additional 1 hour to complete gastric emptying).

Part 1:

Interested participants will be invited for screening test to check their fasting blood glucose level and will go through the following protocol. Testing is completely voluntary to the participant:



Part 2:

If you are eligible to take part in the study,you will be asked to sign a consent form and complete the 2nd part of the first session, which will involve the following:

- Two fasting blood glucose measurements (at -5 minutes and 0 minutes before the meal) – this involves two small finger-pricks.
- Further finger-prick blood samples for glucose and insulin at 15, 30, 45, 60, 90,120, 150 and 180 minutes after the start of consumption of a control (wheat) or millet muffin.
- At the same time intervals, you will be asked to rate how hungry you feel.
- Gastric emptying: You will need to give breath samples for measurement of gastric emptying which will be taken by blowing into a small glass tube through a straw. They will be collected every 15 minutes for 4 hours after consumption of control and millet-based baked products.

The second session will be the same as part 2 in session one.

How to prepare for the study

- You need to fast overnight (approximately 10-12 hours) – this means **no food or drink**, apart from drinking water in moderation
- For example, if you start your test session at 8.30 am, you need to stop eating/drinking (apart from water) at 8.30 pm the previous evening

On the day before a test:

- limit your caffeine intake (maximum of 2-3 cups of coffee/tea)
- limit your alcohol intake (maximum of 1 glass of wine/1 pint of beer)
- restrict participation in intense physical activity (e.g. long periods at the gym, excessive swimming, running, aerobics)

- Do not smoke on the morning of the test

Benefits of the study

- The study will provide valuable information on the effect of millet-based muffins on glycaemic, insulinemic response and gastric emptying in pre-diabetic adults.
- You will receive a "health check" profile, including anthropometric and body composition measurements.
- You will receive **£50 of Amazon voucher** when you have completed all the sessions.

Health and safety issues

- Each measurement requires a small amount of blood; therefore the finger-prick will be small, with minimal discomfort. A light bruising may also occur in some people, but this should disappear within a couple of days and will not affect your ability to work.
- The researcher (Ameerah Almaski)will take finger-prick blood samples using standard procedures.There will be a designated clean area where finger-prick blood samples will be taken.
- Clinical waste procedures will be followed at all times. No cellular material will be stored after 24 hr.; only plasma will be frozen for insulin assay.
- If you have a high blood glucose level and blood pressure, this does not always mean diabetes/hypertension. The diagnosis of diabetes/hypertension is never made on the basis of a single abnormal blood glucose value/blood pressure reading. You will be advised to contact your GP for further tests if necessary.

Data protection and withdrawal

- Confidentiality of any information provided can only be protected within the limitations of the law
- All samples and records will be coded and will only be available to the researchers involved in the study; your name will never appear in any published work

You are free to withdraw from the study at any time, without giving a reason, and to withdraw any unprocessed data previously supplied.

What happens if I do want to take part?

If you would like to take part in this research study you can do so by contacting the researcher at the address, phone number or email address given at the end of this information sheet. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form.

Participation is completely voluntary. If you decide to take part, you are still free to withdraw at any time and without giving a reason. If you are a student, by choosing to either take part or not take part in the study or to withdraw at any time will have no impact on your marks, assessments or future studies.

Who is organising and funding the research?

I (Ameerah Almaski) am conducting the research as a PhD researcher at Oxford Brookes University under the supervision of Dr Sangeetha Thondre, Dr Helen Lightowler, and Dr Shelly Coe in the Department of Sport & Health Sciences. Ameerah Almaski is fully funded by the Embassy of Saudi Arabia.

Will this study be kept confidential?

All information collected about you will be kept strictly confidential. Your personal data will be in a locked drawer at Oxford Brookes University, and if saved on a computer, will be securely encrypted with a password. Data in paper or electronic form will be de-identified by a code and kept securely for a period of 10 years after the completion of this research project. Only researchers directly involved in the study will have access to personal data.

What will happen to the results of this research study?

Ultimately, the results of the study will be written up as part of a PhD thesis. In addition, the results will be published in peer-reviewed journals and presented at meeting and conferences. Participants will not be identified in any publications.

Who has reviewed the study?

This study has been approved by Oxford Brookes University Research Ethics Committee (UREC). Any concerns about the conduct of the study should be referred to the Chair of UREC on ethics@brookes.ac.uk.

Contact for further information

You can contact the researcher **Ameerah Almaski** at any time if you have any questions or concerns:

Email: 14107967@brookes.ac.uk

Thank you so much

Appendix 3F Consent form for healthy and pre-diabetic group

Consent form

Study tittle: Effect of millet based muffins consumption on glycaemic, insulinemic response and gastric emptying in healthy and pre-diabetic adults

***Contacts*:**

1. Ameerah Almaski, PhD researcher in human nutrition.
2. Dr Helen Lightowler, Operations Director of the Functional Food Centre, senior lecturer in human nutrition.
3. Dr Sangeetha Thondre, senior lecturer in nutrition.
4. Dr Shelly Coe, lecturer in nutrition.

Tel: 01865 483245 - 1865 483988-1865 483839
Email: 14107967@brookes.ac.uk/ hlightowler@brookes.ac.uk / Pthondre@brookes.ac.uk / scoe@brookes.ac.uk

Please **INITIAL** the appropriate box

	Yes	No
1. I confirm that I have read and understand the information sheet for the above research project.	☐	☐
2. I confirm that I have had the opportunity to ask questions and have received satisfactory answers to all my questions.	☐	☐
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, or to withdraw any unprocessed data previously supplied.	☐	☐
4. I understand that confidentiality of information provided can only be protected within the limits of the law.	☐	☐
5. I agree to take part in the above research.	☐	☐

Name of Participant .....Date .....
(block capitals)

Signature .....

Contact number: ..... email: .....

Name of Researcher .....Date .....
(block capitals)

Signature

Appendix 3G Sample gatekeeper email for the health centres or doctor's surgeries

Dear XXX

I am a PhD student from Oxford Brookes University and I am interested in looking at the assessment of glycaemic,insulinemic response and gastric emptying in pre-diabetic participants following consumption of millet-based muffins.

I am emailing you because I am interested in recruiting adult pre-diabetic volunteers (male and female) aged between 18 and 65 years for the above-mentioned study. The participants will have a minimum of 10 small finger pricks each occasion to measure their glycaemic and insulinaemic response. Also, I will need to collect breath samples from them every 15 minutes for 4 hours for measurement of gastric emptying to know about digestion process.

Would you consider allowing me to post the study advertisement on the notice board in your clinic for people who are interested in waiting room. I attach a copy of the study advertisement and participant information sheet to provide further information about what is expected from potential volunteers.

This study has been fully approved by the university ethics committee under Registration No: 161061

I would be most grateful for your support in our efforts to recruit participants for this study. Please feel free to contact me should you have any questions. Alternatively, you may wish to contact my project supervisor, Dr Sangeetha Thondre email: pthondre@brookes.ac.uk if you would like any further information or to arrange a phone call.

Many thanks for taking the time to read this and I hope to hear from you soon
Ameerah Almaski, MSc,

PhD Research Student
Functional Food Centre
Oxford Brookes University
Telephone number: 01865 483283
Email address: ameerah.almaski-2015@brookes.ac.uk
Supervisors: Dr. Sangeetha Thondre Senior Lecturer in Nutrition
Dr. Helen Lightowler Senior Lecturer in Human Nutrition
Dr. Shelly Coe Lecturer in Nutrition

Appendix 3H Data Collection Sheet

Data Collection Sheet

Product number: ☐ 1 ☐ 2

Subject: Date: / /

Yesterday evening

What did you eat yesterday evening?

What was the quantity of your meal?

☐ Small ☐ medium ☐ large

Did you do any exercise yesterday?

☐ Yes ☐ No

If yes what was the intensity of it?

☐ light ☐ medium ☐ strenuous

What was the duration of the exercise?

☐ 20min ☐ 30 min ☐ 1 hour ☐ other .....

Did you drink coffee yesterday evening?

☐ Yes ☐ No

Did you drink alcohol yesterday evening?

☐ Yes ☐ No

If yes, please fill the table below:

Types of alcohol drinks	**How much alcohol did you drink last night?** **(cups, shots, pints, glass…etc)**
Wine	
Beer	
Spirits	
Cider	
Liqueurs	

Thank you

Appendix 3J Glycaemic response data collection

Glycemic response (glucose level)

Fasting blood glucose level (screening)	

Blood pressure: _____

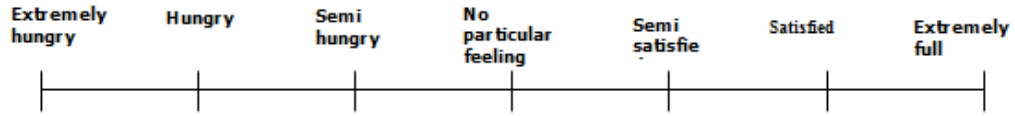
Glycemic response (glucose level):

Time of sampling (Minutes)	Glucose level (mmol/l)	
-5		Average
0		
15		
30		
45		
60		
90		
120		
150		
180		

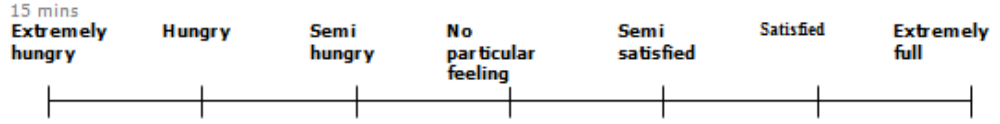
Appendix 3K Satiety/hunger scales

Satiety/hunger scales

0 mins



15 mins



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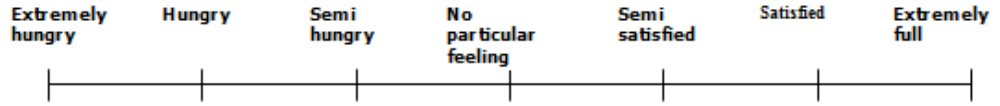

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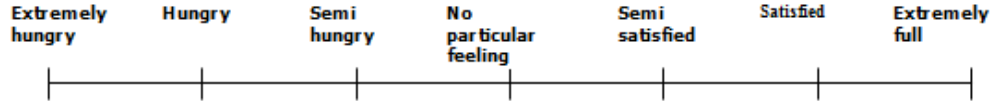

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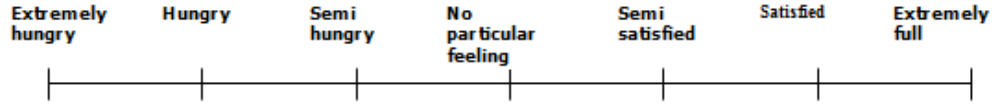

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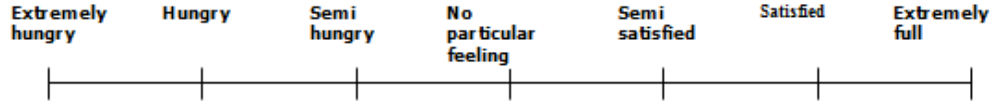

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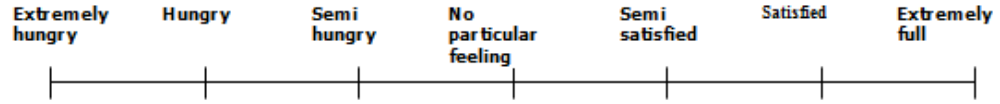

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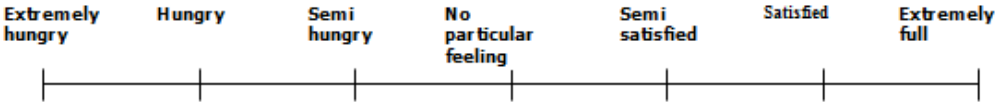

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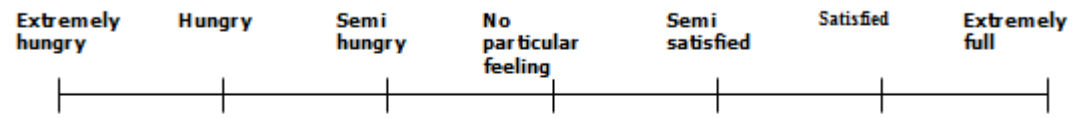
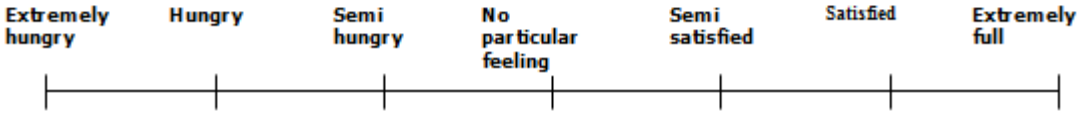
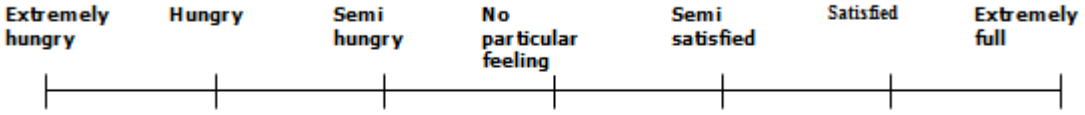


210 mins



240 mins



Thank you

Appendix 3L Health Questionnaire

Health Questionnaire

(Please circle as appropriate)

- Contact details:

Email address: _____

Telephone number: _____

The preferred method to contact: _____

Gender:

Male ☐ Female ☐

Are you allergic to any foods? Yes or No

If yes, which one(s)? _____

Do you have a genetic or metabolic disease? Yes or No

Are you taking any medication? Yes or No

If yes, which one(s)? _____

Have you undergone any major medical/ surgical event in the last 3 months? Yes or No

Are you a smoker? Yes or No

If yes, cigarettes/day:_____

Are you following a special diet? Yes or No

If yes, which one(s)? _____

Do you tend to restrain your food intake? Yes or No

Do you exercise or participate in any sports? Yes or No

How often a week?_____ Duration:_____ Intensity:_____

Are there any foods you dislike? _____

If you are female:

- Are you on the contraceptive pill Yes No
- How long is your usual menstrual cycle(if applicable) .....
- How many days has it been since the start of your last period (if applicable)?

.....

Thank you

Appendix 3M OGTT screening

Participant code:

Fasting blood glucose level
After 2 h

Blood pressure: _____

Test results: _____

Is it eligible to participate? Yes ☐ ☐

Appendix 3N Absolute GR results for the prediabetes and healthy group

Absolute GR results for the prediabetes

Absolute glucose values (mmol/l) at baseline and after the consumption of control and millet muffins in prediabetic participants.

Time (min.)	Control muffin	Millet muffin
0	6.0±0.7	5.9±0.7
15	6.1±0.9	6.3±0.9
30	7.5±0.9	7.3±1.0
45	8.5±1.0	7.9±1.2
60	8.6±1.4	7.9±1.2
90	8.1±1.9	7.7±1.2
120	7.6±1.2	7.0±1.2
150	6.8±1.1	6.1±1.5
180	5.9±1.5	5.4±1.4

Data is given as means ± SD.

Absolute GR results for the healthy group

Absolute glucose values (mmol/l) at baseline and after consumption of control and millet mufins in healthy participants.

Time (min.)	Control muffin	Millet muffin
0	5.1±0.4	5.1±0.4
15	5.2±0.5	5.1±0.5
30	6.6±0.9	6.2±0.6
45	6.9±1.1	6.5±1.0
60	6.1±1.0	6.1±1.0
90	5.5±0.7	5.5±0.8
120	5.1±0.5	5.1±0.8
150	4.7±0.4	4.7±0.5
180	4.8±0.6	4.6±0.5

Data is given as means ± SD.

Appendix 30 Absolute IR results for the prediabetes and healthy group

Absolute IR results for the prediabetes group

Absolute insulin values (mmol/l) at baseline and after consumption of finger millet and control muffins in prediabetic participants.

Time (min.)	Control muffin	Millet muffin
0	13.0±7.5	14.6±8.3
15	20.6±12.1	27.5±18.6
30	49.0±33.1	52.6±46.2
45	65.8±41.6	63.5±52.8
60	68.9±35.4	62.8±46.7
90	61.8±35.1	54.8±43.1
120	49.2±32.6	41.9±33.9
150	36.4±20.0	27.2±18.7
180	24.7±15.5	16.5±10.6

Data is given as means ± SD.

Absolute IR results for the healthy group

Absolute insulin values (mmol/l) at baseline and after consumption of finger millet and control muffins in healthy participants.

Time (min.)	Control muffin	Millet muffin
0	8.4±3.9	9.2±3.9
15	11.9±6.4	14.4±8.7
30	42.8±19.0	42.2±31.2
45	60.6±27.9	53.3±29.5
60	43.1±26.7	43.9±21.1
90	28.9±13.1	31.3±14.8
120	18.2±9.6	17.2±11.6
150	9.0±4.1	10.7±5.7
180	7.8±6.7	6.1±2.7

Data is given as means ± SD.