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Starch Digestibility of Porridges from Unrefined and Refined Maize, Pearl Millet and Sorghum

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Starch Digestibility of Porridges from Unrefined and Refined Maize, Pearl Millet and Sorghum

By

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Submitted in partial fulfilment of the requirements for the degree

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I declare that the dissertation herewith submitted for the degree MSc Food Science at the University of Pretoria, has not previously been submitted by me for a degree at any other university or institution of higher education.

ABSTRACT

STARCH DIGESTIBILITY OF PORRIDGES FROM UNREFINED AND REFINED MAIZE, PEARL MILLET AND SORGHUM

by

Salvatory Theobald Kundi

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The incidence of diabetes mellitus in Tanzania, as in many developing countries, appears to be increasing among people living in urban areas, as compared to rural areas. The major carbohydrate staple of most of the people living in the rural areas of Tanzania is stiff porridge prepared from unrefined maize, sorghum or pearl millet, while in urban areas it is stiff porridge prepared from refined maize. This change from unrefined to refined porridge and from sorghum and pearl millet to maize could have contributed to the apparent increasing incidence of diabetes among urban people.

An *in vitro* assay involving pre-chewing of the porridge, followed by digestion with pepsin and α-amylase in dialysis tubing was used to determine the rate of starch digestibility. The rates of starch digestibility of porridges prepared from unrefined and refined maize, sorghum and pearl millet using white wheat bread as a standard were determined. Hydrolysis Indices (HIs) were calculated and used to predict the Glycaemic Indices (GIs). The effects of species, variety and refinement on the rates of *in vitro* starch digestibility of the porridges from the three cereals were determined.

All the porridges prepared from the three cereals, except that from refined sorghum variety NK 283, had a lower rate and extent ($p < 0.05$) of *in vitro* starch digestibility than that of bread.

Cereal species did not affect the rates of *in vitro* starch digestibility of the stiff porridges. The probable reasons are that all three cereals are C4 crops and the proximate compositions, endosperm structures, gelatinisation temperatures and the shape of their starch granules are similar.

Apparently, due to the higher proportion of amylopectin in the starch, porridge from refined sorghum NK 283 was more digestible than the porridges from other varieties. However, the stiff porridge made from the unrefined flour did not show this effect.

Refinement of cereal grain flours did not in general improve the rates of *in vitro* starch digestibility of the stiff porridges prepared from non-tannin low polyphenol grains. However, it did increase the rate of digestibility of sorghum variety NK 283 and pearl millet variety SDMV 91018, both of which contained relatively high levels of non-tannin polyphenols in the grain and much lower levels in the refined flour. It is possible that high levels of non-tannin polyphenols inhibit starch digestibility.

Since porridges from maize, sorghum and pearl millet in unrefined or refined forms did not in general differ significantly in-terms of GI, the three cereals in unrefined or refined forms can probably be used without discrimination by diabetic people. None of the three cereals can be claimed as more suitable than the others in diabetes management. However, if there are varieties known to have a high amylopectin/amylose ratio in their starches, like sorghum variety NK 283, they should be avoided as a diet for diabetic people, because this type of starch is associated with higher rate of starch digestibility and hence higher GI which is unsuitable for diabetics. On the other hand, varieties known to contain high levels of non-tannin polyphenols may be useful for diabetics in the unrefined form, as these grains have shown both lower starch digestibilities and GIs.

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My parents, my wife, my children, my brothers and sisters, my in-laws and my friends. Thanks for your prayers.

Above all, glory to God Almighty, for his grace and this life that has given us to accomplish such an achievement.

DEDICATION

This dissertation is dedicated to the following people:

MY DEAR PARENTS: Mr. Theobald Peter Kundi and Mrs. Prisca Theobald Kundi
For their great vision in education and their relentless efforts in educating their children. It is through this vision and efforts that has made me what I am today.

MY BELOVED WIFE: Mrs. Grace-Anna Salvatory Kundi
For her willingness to remain behind during my studies, encouragement, prayers and her magnificent devotion to her children and the whole family.

MY DEAR CHILDREN: Witness and Gloria
For missing their Daddy's company throughout the duration of the studies.

The fear of the Lord is the beginning of wisdom.
(Psalm 111 : 10a)

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CHAPTER 1

INTRODUCTION

Cereal grains constitute the least expensive high carbohydrate diets, which are the most important source of energy for much of the world's population (FAO, 1997). Cereals are important in the diet for preventing undernutrition and to help prevent and treat diseases of overnutrition (Vorster and Venter, 1993). Whole-grain cereals prevent undernutrition by being an inexpensive, nutrient-dense source of energy, plant protein, starch, dietary fibre, polyunsaturated fat, a variety of vitamins, minerals, trace elements and electrolytes (Vorster and Venter, 1993). The role of cereals in preventing diseases of overnutrition, is more complex. In unrefined foods, the presence of fibre is likely to slow carbohydrate absorption by interfering with the digestion of starch or other saccharides. Also, plant cell walls or bran layers in cereal grains can serve as a barrier to the penetration of digestive enzymes (Schneeman and Tietyen, 1994).

Dietary carbohydrates are digested and absorbed at different rates and to different extents in the human small intestine, depending on their botanical source and physical form of the food (Englyst, Englyst, Hudson, Cole and Cummings, 1999). It has been suggested that diets that contain large amounts of rapidly digested carbohydrates, which elevate blood glucose and insulin responses, may be detrimental to health. Carbohydrates may directly influence human diseases by affecting physiological and metabolic processes (FAO, 1997). One of the diseases of metabolic disorder that is associated with dietary risk factors is non-insulin dependent diabetes mellitus (National Research Council, 1989; Reviewed by Feskens, 1992; FAO, 1997).

According to Cahill, Arky and Perlman (1991), Anderson and Geil (1994), Anderson and Deskins (1995), diabetes mellitus (DM) is a chronic metabolic condition characterized by major derangements in metabolism of glucose which results in inappropriate hyperglycaemia, and abnormalities in metabolism of fat, protein, and other substances. Non-insulin dependent diabetes mellitus (NIDDM or Type II) is the most dominant type

of diabetes mellitus, occurring in the mid-adulthood and constituting to about 85 to 90% of all the diabetic cases, of which 90 to 95 percent are normally overweight. According to FAO (1997) high rates of NIDDM in all population groups are associated with cultural changes in populations previously consuming traditional diets which were unrefined, and also with increasing obesity. Foods rich in dietary fibre and carbohydrate-containing foods with slower starch digestibility or low Glycaemic Index (GI) appear to protect against diabetes, the effect being independent of body mass index.

During recent years, Tanzania has been experiencing an increase in the incidence of diabetes. The mostly affected people are among the employees of the middle and high incomes and prosperous business people who had moved from rural to urban and peri-urban areas (personal experience).

In the rural areas, maize, sorghum and pearl millet are ground to whole-grain flours, sometimes maize is mixed with sorghum to improve food palatability (Kundi, 1999). Mechanical dehuller/decorticator for rice and maize are located in urban and peri-urban areas (personal experience). Sorghum and millet are mainly consumed in the rural areas whereas urban dwellers are said to prefer maize (Hammond and Shayo, 1999; Shayo, Laswai and Kundi, 1999). In many parts of Tanzania, when people move from rural to urban areas they change from eating stiff porridges prepared from unrefined flours of maize, sorghum and pearl millet to stiff porridge prepared from refined flour of maize.

This study will try to find out if changes in eating patterns which follow after migrating from rural to urban areas implies changing from slowly digested stiff porridge to faster digested stiff porridge. A method of determining *in vitro* starch digestibility will be employed and will examine the effect of species (maize, sorghum and pearl millet), variety and refinement on the rate of starch digestibility. The outcomes of this study will provide more understanding on the rates of *in vitro* starch digestibility of the stiff porridges made from the three cereals, because there are significant number of people who believe that sorghum and pearl millet are slowly digested starchy foods, and therefore, can be used as therapeutic diet in diabetes management (personal experience).

CHAPTER 2

LITERATURE REVIEW

In this review, refinement of cereal grains, changing of food habits and their implication in general will be discussed. Composition, structure and effect of refinement on maize, sorghum and pearl millet will be reviewed. Starch, and factors affecting starch digestibility in general, and its *in vitro* determination will also be discussed. At this juncture, an attempt will be made to find out if there are any effects of species, variety or refinement on starch digestibility between maize, sorghum or pearl millet. Finally, it will be attempted to judge if the influence of species, variety or refinement on starch digestibility can be related to the increasing incidence of diseases such as diabetes mellitus.

2.1 Refinement of the cereal grains

Refinement of cereal grains includes processes such as milling, that separate anatomical parts of the grain to produce a palatable foodstuff (Kent and Evers, 1994; Hoseney, 1994; Anon, 1998). Milling generally involves removal of the material the miller calls bran, i.e., the pericarp, the seed coat, the nucellar epidermis, and the aleurone layer. In addition, the germ is usually removed because it is relatively high in oil, which makes the product become rancid faster, thereby decreasing its palatability (Hoseney, 1994). The most palatable (lowest fibre), and most stable (lowest fat) parts of the grains are not necessarily the most nutritious, and if only these are consumed, much of the potential benefit can be lost (Kent and Evers, 1994). This results from the fact that many nutrients such as vitamins and minerals reside in the embryo and outer parts of the grains (mainly the aleurone tissue) (Kent and Evers, 1994). However, while milling may reduce the mineral and vitamin content of cereal grains, a related concern is that whole cereal grains may contain biologically unavailable forms of these nutrients (Roderuck and Fox, 1987). Traditionally maize grain (Rooney and Serna-Saldivar, 1987), and sorghum and millet grains (Murty and Kumar, 1995) are decorticated partially or completely by traditional

methods before further processing and consumption. Whole grains of sorghum and millet are also directly dry-milled to fine flour (Hoseney, Andrews and Clark 1987; Murty and Kumar, 1995).

Foods rich in fibre and other factors such as enzymes inhibitors, tannins, starch-protein and starch-lipid interactions which reduce the rates of both digestion and glycaemic responses, have been consumed in relatively large amounts in the diets of more primitive cultures. However, these types of foods have been reduced in concentration both by processing and by food preferences in the Western diet (Jenkins, Taylor and Wolever, 1982a). Groups consuming high-fibre diets in Africa were found to have lower prevalence of diabetes than groups consuming diets with lower levels of fibre (Walker, 1961; Walker, Walker and Richardson, 1970). The dietary fibre and the resistant starch from cereals have several health benefits; firstly is the fermentation by microbial enzyme in the large gut, providing *inter alia* acetate, propionate and butyrate which are believed to protect against colon cancer by inhibiting the growth and proliferation of tumour cells; secondly is the absorption of the short chain fatty acids formed as energy; thirdly is to increase the stool bulk and decrease intestinal transit time which contribute to the lowering of risk for colon cancer; fourthly is the protection from glucose intolerance (National Research Council, 1989; Vorster and Venter, 1993; Baghurst, Baghurst and Record, 1996).

2.2 Changing of the food consumption patterns and preparation and its implications

Changes in nutrition habits are apparent all across Africa, even in rural populations; the influence of "urban culture" in the country, resulting from increasing migration to urban areas, is unmistakeable. People who move from the country to the city adopt city-eating habits (Steller, 1993). As a result of this situation consumption of sorghum and millet is decreasing in many African countries while consumption of maize and importation of wheat and rice is increasing (Steller, 1993). Sorghum and millet food products are not commonly found in urban and semi urban markets, probably because of the drudgery

involved in their domestic processing, as the mechanical processing is not in place, and also the low prestige attached to them (Murty and Kumar, 1995).

According to Popkin (1998) rapid changes in diet, activity level and body composition in the developing world have resulted into positive and negative aspects: Positive- the decreased incidence of infant mortality related to undernutrition, wasting and stunting; Negative- increased incidence of obesity, NIDDM and cardiovascular diseases which are related to NIDDM. This type of nutrition transition normally occurs after the major shifts in population growth, age structure and urbanization. Unlike, in the United States (National Research Council, 1989) where the incidence of diabetes mellitus is highest among the poor, in developing countries the incidence is positively associated with the socioeconomic status. South Africans who have changed from their traditional foods of low-fat and high-fibre (unrefined foods) to the Western diet of high-fat, low-fibre (refined foods) and high-simple carbohydrate foods, have amongst the highest incidence of diseases of affluence such as coronary heart disease and diabetes mellitus, in the world (Vorster and Venter, 1993). Specific patterns of food consumption and preparation are associated with diabetes and obesity in a native Canadian community (Gittelsohn, Wolever, Harris, Harris-Giraldo, Hanley & Zinman, 1997).

2.3 Role of maize, sorghum and pearl millet in human nutrition

Maize (*Zea mays* L.) (Rooney and Serna-Saldivar, 1987) and sorghum (*Sorghum bicolor* (L.) moench.) and pearl millet (*Pennisetum glaucum* (L.) R. Br.) (Murty and Kumar, 1995) play a key role as the staple food for large groups of people in Asia and Africa.
Maize is the dominant cereal food grain produced in Africa. Other crops produced at a significant level are grain sorghum and millet in the drier areas and wheat in the more temperate regions (Cownie, 1993). Sorghum and millets are mainly considered as subsistence crops because of their unique tolerance to draught and adaptation to dry tropical and subtropical ecosystems throughout the world (Serna-Saldivar and Rooney, 1995).

Porridges made from sorghum and maize are the staple diet of the black people of southern Africa (Taylor, Dewar, Taylor and Von Ascheraden, 1997). Bello, Rooney and Wanisika (1990) reported that porridges prepared from sorghum, pearl millet, and maize are popular in many African and Asian countries. Traditional African thick porridges are generally prepared by cooking a slurry of unfermented or fermented flour in boiling water (acidic, neutral or alkaline) with continuous stirring; the resulting thick porridge after cooling is known by different names such as tô, tuwo, aseda, ugali and mudde depending on geographical region. In many parts of Tanzania, food and stiff porridge are synonymous terms. Though stiff porridges from composite flours are available, stiff porridges are mainly prepared from single flours. Stiff porridges prepared from fermented flours are not common in Tanzania. Due to high preference and availability, maize in the form of unrefined and refined flours is the mostly used. Following maize are sorghum and pearl millet mainly in the form of unrefined flours. Composite flours used are from sorghum/maize, sorghum/cassava, maize/pearl millet and maize/cassava (personal experience).

2.3.1 Structure and chemical composition of maize, sorghum and pearl millet grains.

Maize, sorghum and pearl millet have more similarities than differences in terms of their kernel structures. Dent maize has a large flattened seed. Figure 1 (Hoseney 1994), shows the various parts of the maize kernel. It is by far the largest of the common cereal grains, weighing in average 350 mg. White and yellow are the most common colours of maize kernel. The hull or pericarp constitutes about 5 – 6% of the kernel; the germ is relatively large, constituting 10 – 14 % of the kernel with the remainder 80 – 85 % being endosperm (Hoseney, 1994; Watson, 1987). Like maize, the sorghum endosperm is divided into horny and floury parts. Sorghum kernels are generally spherical, range in weight from 20 to 30 mg, and may be white, red, yellow, or brown. Figure 2 (Hoseney, 1994), shows the various parts of the sorghum kernel. The composition of the kernel is approximately 7.9% pericarp, 9.8% germ, and 82.3% endosperm (Hoseney, 1994). Like maize and sorghum, the endosperm of pearl millet is divided into horny and floury parts. Pearl millet consists of small (average about 8.9 mg) tear shaped kernels. They vary in

colour, with slate grey being most common, although yellow, white, and brown varieties are also known. The germ in pearl millet is large (17%) in proportion to the rest of the kernel (Hoseney, 1994). Figure 3 (Hoseney, 1994), shows the size of the germ relative to the endosperm. Other components of the pearl millet kernel are 7.2 – 10.6% pericarp and 71 – 76% endosperm (Serna-Saldivar and Rooney, 1995).

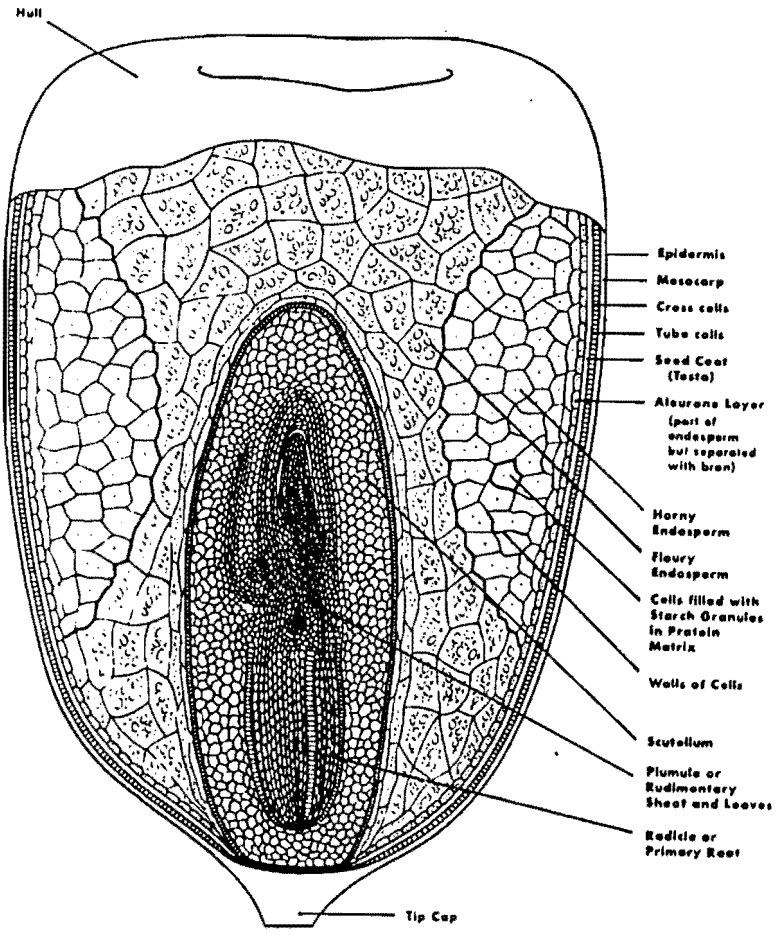

Figure 1. Longitudinal section of maize kernel (Hoseney,1994)

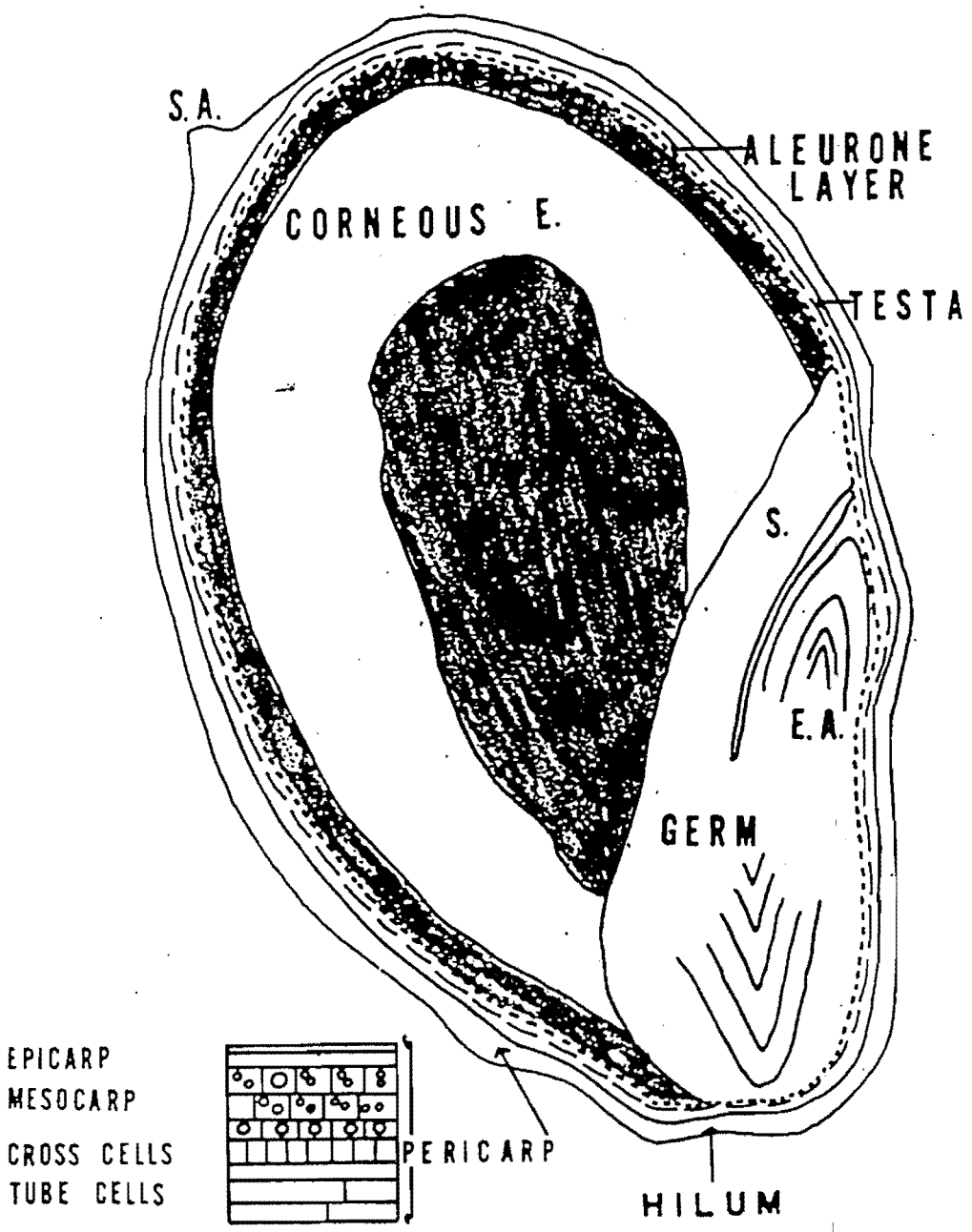

Figure 2. The structure of sorghum kernel (Hoseney, 1994)

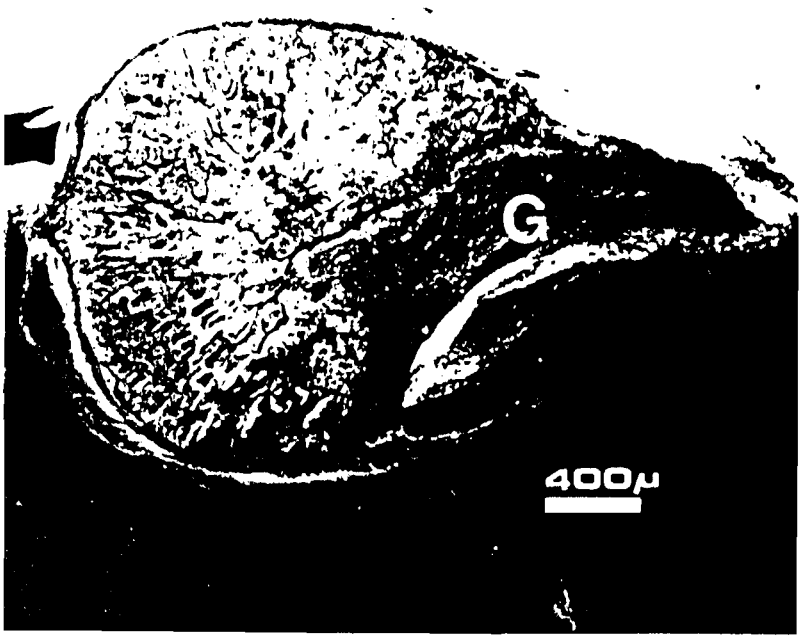

Figure 3. Low magnification of a longitudinal section of a pearl millet kernel (Hoseney, 1994).

2.3.1.1 Germ

The germs of maize, sorghum and pearl millet grains are composed of two parts, the embryonic axis (rudimentary root and shoot) and the scutellum which functions as a storage organ. The germ is relatively high in protein, sugar, oil and minerals. It also contains vitamins especially B vitamins and vitamin E (Hoseney, 1994; Anderson and Deskins, 1995; Anon, 1998). Cereal lipids are rich in essential fatty acid, linoleic acid (18 : 2; 30 – 60% of total fat acids) and practically devoid of saturated fatty acids (Serna-Saldivar, 1993). Cereals contain trace quantities of phytosterols; they do not have any cholesterol (Serna-Saldivar, 1993). Pearl millet contains high protein and oil due to the large proportion of germ to endosperm (Serna-Saldivar and Rooney, 1995).

2.3.1.2 Bran

Bran is the fibre-rich part of grains and seeds (Anderson and Deskins, 1995). For many years, the term "bran" referred only to wheat bran. With the growth of interest in dietary fibre since 1970, the term is now used to describe the outer layer of any cereal grain, external to the starchy endosperm, but beneath the hull, if the grain is encased in a hull (Stephens 1993). The bran is the term used to denote a mixture of seed coat, pericarp and the aleurone layer of the cereal grains removed during milling (Hoseney, 1994). For the wheat kernel, the bran layer is easily distinguishable and is separated from the endosperm by the aleurone layer. Unlike in wheat, the oat bran layer is not as clearly separated from the underlying kernel. Maize bran resembles wheat bran in relation to its separation from the rest of the grain (Stephens 1993). Oat bran differs from the other brans in being largely soluble non-starch polysaccharide (NSP), while the others are mainly insoluble. The major polysaccharides in the other brans of the cereal grains are cellulose and arabinoxylans, both of which contribute to their insolubility and resistant nature (Stephens 1993). Only small fraction of the bran is made up of soluble dietary fibre, the major fraction is insoluble fibre (Serna-Saldivar and Rooney, 1995). The bran of maize, sorghum and pearl millet grains consists of cellulose, hemicellulose, minerals, protein, total phosphorus, phytate phosphorus, fat and niacin. It also contains thiamine and riboflavin (Hoseney, 1994; Kent and Evers 1994; Serna-Saldivar and Rooney, 1995).

2.3.1.3 Endosperm

The endosperm of the three cereals contains mostly starch and protein with small amounts of fat and fibre (Hoseney 1994). About 98% of the starch in maize is located in the endosperm. The endosperm also contains about 75% of the kernel protein, of which the majority is storage proteins (Reviewed by Pedersen, Knudsen and Eggum, 1989). The maize endosperm contains about 86 – 89% of starch and about 8% of protein. (Reviewed by Pedersen, *et al.*, 1989). In general, yellow dent maize and yellow sorghum are nearly equivalent in feeding values, with maize slightly higher in gross and metabolizable energy (Serna-Saldivar and Rooney, 1995). The carbohydrates of sorghum and millets are composed of starch, soluble sugar, pentosans, cellulose and hemicellulose. Starch is the most abundant chemical component, while soluble sugar and crude fibre are low (Serna-

Saldivar and Rooney, 1995). From one-half to three fourths of grain weight is starch (Serna-Saldivar and Rooney, 1995). In the native form, they are considered as pseudo crystals that have crystalline and amorphous areas (Serna-Saldivar and Rooney, 1995). Regular endosperm sorghum types contain from 23 to 30% amylose (Serna-Saldivar and Rooney, 1995). Pearl millet starches have an amylose content ranging from 20 – 21.5% and their starches appear to have a higher swelling power and solubility than other starches (Serna-Saldivar and Rooney, 1995). The amylose content of normal maize starch ranges from 25 – 30% (Boyer and Shannon, 1987). According to Hoseney (1994) and Kent and Evers (1994) the amylose content of cereal grains vary from 20 to 35% of the total starch. The summary of the major components in average percentages of the normal maize, sorghum and pearl millet is shown in Table 1.

Table 1. Summary of major components in average percentages of normal maize, sorghum and pearl millet (Boyer and Shannon, 1989; Hoseney, 1994; Serna-Saldivar and Rooney, 1995).

Cereal	Pericarp	Germ	Endosperm	Amylose[1]
Maize	5.5	12.0	82.5	27.5
Sorghum	7.9	9.8	82.3	26.5
Pearl millet	8.9	17.0	73.5	20.8

1 Of the total starch

2.4 Effect of refining or milling on the chemical composition of maize, sorghum and pearl millet grains.

Changes in nutritional properties of cereals result from several types of processing, such as: refinement, cooking and supplementation (Kent and Evers, 1994). Since milling generally involves removal of the bran and germ which are relatively rich in protein, B vitamins, minerals, and fat, it implies therefore, the milled product is lower in these entities than was the original grain (Hoseney, 1994). Thus, as a result of milling, the palatability is increased but the nutritional value of the product is decreased (Hoseney, 1994; Kent and Evers, 1994; Anon, 1998). The degree of change depends upon the

degree of separation that occurs or on the length of the extraction process (Kent and Evers, 1994; Anon, 1998). The composition of milling products of maize and sorghum are shown on Table 2. From the Table, it is clear that, the milled or rather refined products of maize and sorghum have lower levels of protein, fat, ash, and crude fibre as opposed to the levels of starch which are higher than those of the unrefined products.

Table 2. Composition of milling products of maize and sorghum grains (in percentage dry basis) (Kent and Evers, 1994; Reviewed by Pedersen *et al.*, 1989).

Cereal and product	Protein	Fat	Ash	Crude fibre	Carbohydrate
Maize[1]					
Maize grain	11.2	4.8	1.7	1.9	80.4
Flour	8.1	1.5	0.7	1.0	88.7
Maize[2]					
Whole maize	9.9	5.2	1.4	NA[3]	76
Degermed maize	8.7	1.4	0.4	NA	89.2
Sorghum[1]					
Whole sorghum	9.6	3.4	1.5	2.2	NA
Pearled sorghum	9.5	3.0	1.2	1.3	NA
Flour, crude	9.5	2.5	1.0	1.2	NA
Flour, refined	9.5	1.0	0.8	1.0	NA

1 From Kent and Evers (1994)

2 From Review of Pedersen *et al.* (1989)

3 Not available

Data for the composition of milling products of pearl millet is not available, but according to Hoseney *et al.* (1987) the composition of the pearl millet (whole grain) in percentage dry basis is given in Table 3 below.

Table 3. Chemical composition of the pearl millet whole grain (in percentage dry basis) (Hoseney *et al.*, 1987).

Protein	Fat	Ash	Crude fibre	Starch
9 – 19	3 – 7	1.5 – 3.9	1.96 – 3.88	56 – 65

2.5 Starch

Next to water, starch in one or more forms is the most abundant constituent in the human diet. Starch occurs naturally in most plant tissues, including roots and tubers, cereal grains, legumes, green vegetables, and fruits. It is also added to prepared foods, often in modified forms (BeMiller, 1992). The majority of starch in cereal grains is located in the endosperm (Jackson, 1993a). Many grains can be referred to as having 'soft or floury endosperm' or 'hard or vitreous endosperm'. There is little evidence that the molecular components of their starches are any different. The packing of the starch granules within a kernel, however is different (Jackson, 1993a). Floury maize kernels have less protein binding the starch granules together, and starch granules within more flinty maize kernels are encased more tightly within a protein matrix (Jackson, 1993a). The starch granules of maize and sorghum are very similar to each other in size, shape, and gelatinisation properties (Hoseney, 1994). They average about 20 μm in diameter and their shape varies from polygonal, to almost spherical. Starch granules in cells near the outside of the kernel (in the vitreous endosperm) tend to be polygonal, whereas those in cells from the center of the kernel (in the opaque endosperm) tend to be spherical (Hoseney, 1994). As far as is known, the properties such as gelatinisation and pasting of the differently shaped granules are the same. Pearl millet starch is also similar to that of maize and sorghum, except that its granules are smaller, averaging about 12 μm in diameter (Hoseney, 1994).

Starch is a mixture of two glucose polymers amylose and amylopectin. These polymers are initially enclosed within a semicrystalline granule formed inside starch-synthesizing plant organelles (Jackson, 1993a). In cereals, the starch granules are formed in plastids called amyloplasts. In the case of maize, wheat, rye, barley, sorghum and millets, each

amyloplast contains only one starch granule (Hoseney, 1994). As the starch molecules form in an amyloplast, they combine with one another to form a compact, ordered mass that is semicrystalline (Whistler and BeMiller, 1997). Amylopectin, the larger of the polymers, is an α-1,4-linked, α-1,6-branched (4 – 6% branching) polymer with an average molecular weight near 10^8 Daltons. Amylose, a smaller mostly linear polymer, is also composed of α-1,4-linked glucose units; long chains are sometimes connected with α-1,6 branches, although branching probably accounts for < 1% of the glucose unit connections. The molecular weight of amylose is approximately 10^5 Daltons. The molecular weight of starch polymers varies depending upon its plant source. Amylopectins have been reported to vary from 1600 to 1 x 10^6 Daltons. The degree of branching also varies (Jackson, 1993a). The structures of amylose and amylopectin are shown in Fig. 4 (Alais and Linden, 1991) below.

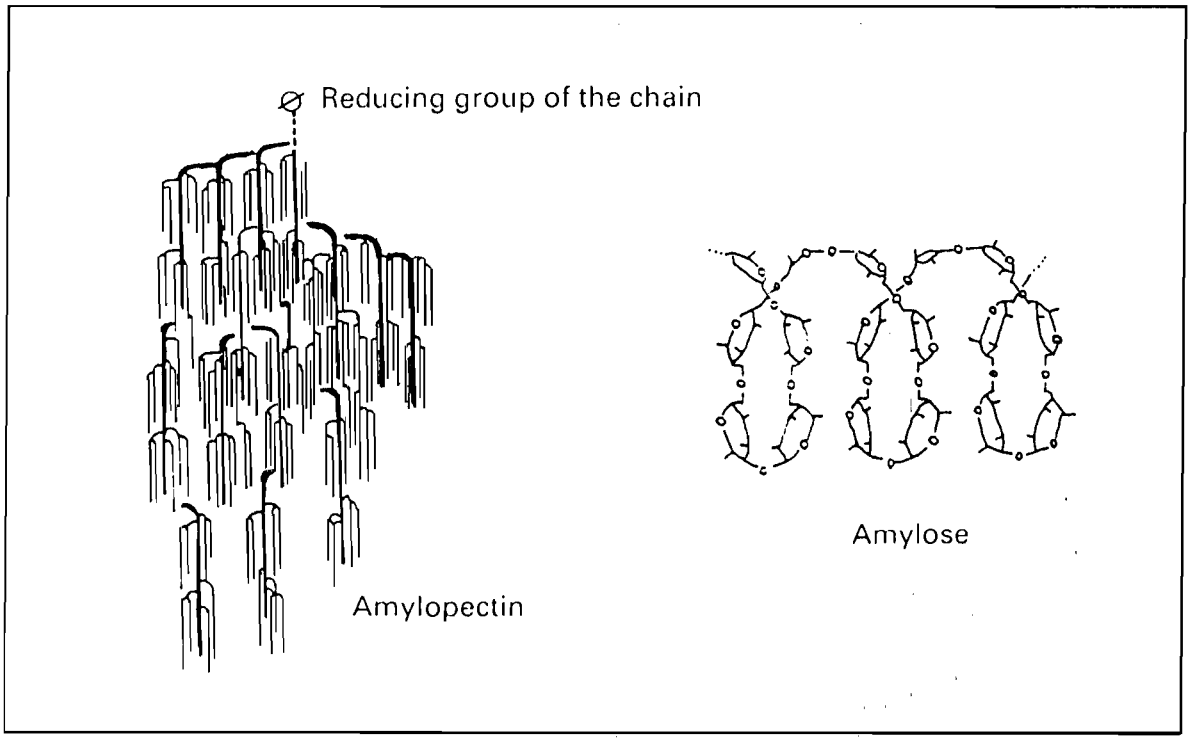

Figure 4. The structures of amylose and amylopectin (Alais and Linden, 1991)

2.5.1 Starch gelatinisation

When heated in water, starch granules gelatinise. Gelatinisation is the collapse (disruption) of molecular order within starch granules, manifested in irreversible changes in properties such as granule swelling, native crystallite melting, loss of birefringence, and leaching of soluble components (primarily amylose). Some amylose leaching can occur at temperatures below the gelatinisation temperature. (BeMiller, 1992; Thomas and Atwell, 1999). The temperature of initial gelatinisation and the range over which the gelatinisation occurs depends on the method used to determine it and is governed by the starch concentration method of observation, granule type, and heterogeneities within the granule population under observation (BeMiller, 1992; Thomas and Atwell, 1999). When starch granules are exposed to liquid water below 40 – 50°C, the amorphous gel-like portions of the starch granule absorb water causing the granule to swell. As the temperature is raised from 40 – 50°C, and water is in excess, granules begin to undergo reversible swelling and, around 60 – 80°C lose birefringence and irreversible swelling occurs (Jackson, 1993a). During the transition from reversible swelling to loss of birefringence, the amorphous regions have a rubber-like structure and the specific volume of the amorphous areas increases as does the mobility of molecular segments which are thermally softened and plasticized by water (Jackson, 1993a). At the gelatinisation temperature, the mobility of molecules increases rapidly and the granule components are in a more flowable state.

Generally, gelatinisation temperature of tuber and root starches such as potato and tapioca is slightly lower than that of cereal starches such as maize and wheat (Thomas and Atwell, 1999). All three starches of maize, sorghum and pearl millet have a 50% gelatinisation temperature of about 67°C, somewhat higher than that of wheat, barley, and rye (Hoseney, 1994).

Pasting follows gelatinisation when a starch slurry containing excess water is heated. Although there is no clear separating line at which gelatinisation ends and pasting begins, pasting is usually associated with the development of the viscosity. Paste viscosity is

highest when a majority of fully swollen, intact granules are present in the cooking medium (Thomas and Atwell, 1999). Pasting involves further granule swelling, additional leaching of soluble components, and eventually, total disruption of granules, when shear is applied, resulting in molecules and aggregates of molecules in dispersion or solution, although in most, if not all, cases granule remnant remain (BeMiller, 1992; Thomas and Atwell, 1999). The property of forming thick pastes or gels is the basis of most starch uses. The extent of starch gelatinisation and pasting is the principal factor controlling texture and other product properties such as storageability and digestibility (BeMiller, 1992). In some baked goods, many starch granules remain ungelatinised (as much as 90% in pie crust and some cookies that are high in fat and low in water content) (BeMiller, 1992).

2.5.2 Starch retrogradation

Starch molecules in an unordered state (in solution, in a dispersion, or in gelatinised granules) will undergo a process termed retrogradation. Retrogradation (setback) occurs when molecules that have become disordered during cooking begin to reassociate in an ordered structure (BeMiller, 1992). In the initial phases of retrogradation linear segments of two or more starch chains may form a simple juncture point that then may develop into more extensively ordered regions. Ultimately, under favourable conditions, a crystalline order appears. The result is gelation or precipitation. Generally, extensive retrogradation is undesirable (BeMiller, 1992). Retrogradation is especially evident when amylose-containing starches are cooled (Baghurst *et al.*, 1996; Thomas and Atwell, 1999). Upon cooling, less energy is available to keep the solubilised starch molecules apart. Amylopectin can also slowly retrograde upon cooling, but linear amylose molecules have a greater tendency to re-associate and form hydrogen bonds than the larger amylopectin molecules which are in the form of tumbleweed like structure (Thomas and Atwell, 1999). Amylose retrogradation may be largely complete by the time the product has cooled to room temperature but retrogradation of amylopectin requires much longer time (Whistler and BeMiller, 1997).

2.5.3 Effect of amylopectin, amylose, and lipids on swelling and gelatinisation of cereal starches

In cereal starches amylose (AM) content is often correlated with lipid content and it is difficult to distinguish the effects of each on granule swelling and gelatinisation (Tester and Morrison, 1990). Furthermore, selecting starches from unrelated varieties of the same cereal or from different species of cereals is likely to introduce considerable variation attributable to differences in amylopectin (AP), which makes it impossible to interpret the results satisfactorily (Tester and Morrison, 1990). Inclusion complexes similar to those involving iodine also form between starch and lipid components. This effect was first noticed when it was discovered that lipids interfere with amylose-iodine complex formation (Jackson, 1993b). The carbon-chain segment of a lipid is located in the α-helical structure of amylose molecules and in long, linear segments of amylopectin (or amylopectin-like 'intermediate material') molecules. Hence the binding of lipid is somewhat dependent on the molecular availability or solubility of starch polymers, especially amylose. If starch is in the process of gelatinisation or fully gelatinised (amylose solubilised), as the chain-length of saturated fatty acids increases the amount of starch-lipid binding also increases. Since starch-lipid binding is not a surface effect, amylose molecules are not readily available for binding with lipid if they are not gelatinised. Therefore as the starch granule swells during gelatinisation, more amylose is molecularly available for binding with lipid (Jackson, 1993b). Swelling is evidently a property of AP, and AM is thus a diluent. However, AM and lipids in the normal starches also inhibit swelling under conditions when AM-lipid complexes are likely to be formed (Tester and Morrison, 1990; Jackson, 1993b). Polysaccharide (AM, AP or both, depending on the starch) leached from the granules is generally highly correlated with the extent of swelling for each starch (Tester and Morrison, 1990; Jackson, 1993b).

2.5.4 Starch digestibility

Essentially, only cooked starch can be digested effectively by humans. Amylases are the enzymes that catalyze the hydrolysis of the glycosidic bonds of the polysaccharides of

starch (BeMiller, 1992). α-Amylases are endo-enzymes, i.e., enzymes that catalyze the hydrolysis of internal bonds of starch polysaccharides. Although saliva contains an α-amylase, very little starch hydrolysis occurs in the mouth (BeMiller, 1992).

Almost all starch digestion and absorption takes place in the small intestine. The pancreatic juice secreted into the small intestine contains another α-amylase. This enzyme effects a rapid reduction in molecular weight of the starch polysaccharides producing starch oligosaccharides (maltooligosaccharides), primarily of six and seven α-D-glucopyranosyl units. The α-amylase then acts more slowly on these oligosaccharides to reduce them to smaller fragments (maltose and maltotriose). Finally maltase catalyses the hydrolysis of maltose, 4-O-(α-D-glucopyranosyl)-D-glucose to D-glucose which is absorbed in the body (BeMiller, 1992).

For nutritional purposes, starch in foods may be classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst, Kingman and Cummings,1992). It has been suggested also that diets rich in slowly digested carbohydrates may protect against chronic diseases (Englyst *et al.*,1992). Clinical studies of persons with diabetes have found improved glycaemic control with such diets (Englyst *et al.*,1992). There is much evidence to suggest that prolonged digestion and absorption of carbohydrates is preferable not only in individuals with diabetes but also in healthy individuals as it affects positively a number of physiological factors (Bjorck and Asp, 1994).

2.5.5 Relationship between starch digestibility, Glycaemic Index (GI) and the Hydrolysis Index (HI).

In a simple definition, dietary GI is defined as an indicator of carbohydrate's ability to raise blood glucose levels within two or three hours after eating (Brand-Miller, 1994; Mendosa, 1999). GI is an *in vivo* measurement based on the glycaemic response to carbohydrate-containing foods, and allows foods to be ranked on the basis of the rate of digestion and absorption of the carbohydrates that they contain. GI values are normalized

to a reference amount of available carbohydrate and do not reflect the amounts of carbohydrate normally present in the foods (Truswell, 1992; Bjorck and Asp, 1994; Englyst, Veenstra and Hudson, 1996). The simple carbohydrate exchange has been challenged by the demonstration that not all starchy foods produce the same glycaemic response (Jenkins *et al.*, 1982a). For example, a food with a low content of carbohydrates will have a high GI value if that carbohydrate is digested and absorbed rapidly in the human small intestine (Englyst *et al.*, 1996). Comparing five starchy foods, including bread, rice, potato and maize, it has been demonstrated that significant differences in both the character of the glycaemic response and the total amount of insulin secreted exist. It was concluded that this was related to differences not in the fibre but in the digestibility of the different starches. It was reasoned that the more rapidly digested bread and potato caused higher rises in blood glucose and insulin levels (Jenkins *et al.*, 1982a).

The difference between GI and HI indices is that GI is an *in vivo* while HI is an *in vitro* measurement of starch digestibility. Mathematically they are expressed as follows:

HI of food is calculated from the area under the digestibility curve of the test product divided by the area under the digestibility curve of the reference material (white wheat bread) normally for 3 hours of starch digestion and multiply by 100 (Granfeldt, Bjorck, Drews and Tovar, 1992).

$$HI = \frac{\text{Area under digestibility curve of sample (0 – 180 min) x 100}}{\text{Area under digestibility curve of white bread reference (0 –180 min)}}$$

GI of a food is calculated from the area under the curve plotted to show the change in blood glucose level that arises over a fixed time (marked on the diagram by a vertical dashed line) after consumption as shown in Fig. 5 (Bjorck and Asp, 1994). The area under this curve (shaded is compared to the area obtained for a reference substance (either glucose or, white bread) of equivalent carbohydrate content (usually 50 g) which is defined as having a GI of 100:

$$\text{GI (test product)} = \frac{\text{Area (test product)}}{\text{Area (reference)}} \times 100\%$$

The cut-off measurement time is usually 120 min, but in a healthy subjects a shorter cut-off of 95 min is recommended. A product that releases glucose more rapidly than the reference substance has a higher or earlier peak glucose level, and thus the GI value greater than 100; products that release glucose less rapidly have GI values less than 100 (Bjorck and Asp, 1994).

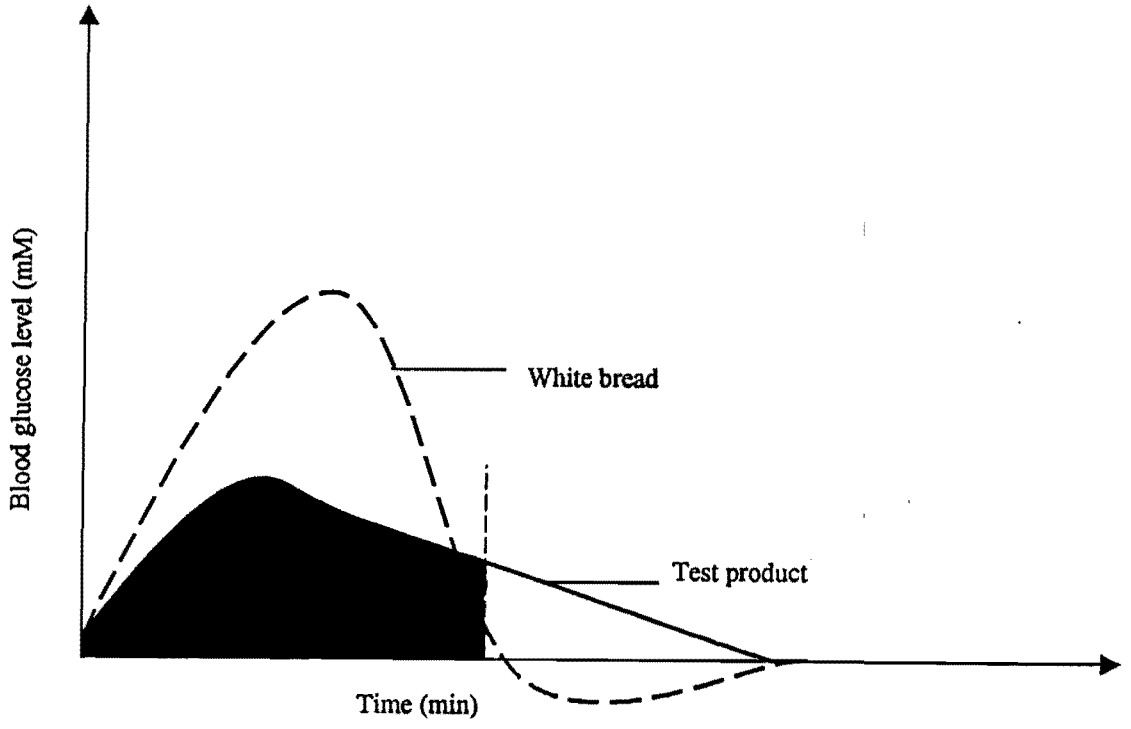

Figure 5: Calculation of the glycaemic index (GI) of a food product (Bjorck and Asp, 1994; adapted).

The ranges in GI for some important Western starchy foods is shown in Fig. 6 (Bjorck and Asp, 1994). Most legume products display a low GI, some even below 15 (using white bread as reference). In contrast, breakfast cereals, for example corn-flakes or puffed cereals, have GIs of approx. 125. The GIs of rice vary considerably depending on the amylose : amylopectin ratio and/or the type of processing it has undergone. Pasta

products, on the other hand, have GIs in the lower range, whereas most flour-based breads are characterized by high indices. GIs on glucose reference for unspecified maize porridges in the international tables by Foster-Powell and Brand-Miller (1995) ranges from 42 to 75. In the same list there is South African maize meal porridges grouped into unrefined with GI of 71 and refined with GI of 74. Venter, Vorster, Van Rooyen, Kruger-Locke and Silvis (1990) with *in vivo* studies observed a GI of 50 to 66 for maize porridge, which showed that maize porridge is a slow to intermediate starchy digested food. The range of the GI given by Venter *et al.* (1990), covered different temperatures of the maize stiff porridge. Hot maize porridge had a GI of 66 while reheated maize had a GI of 56 and cooled maize porridge had a GI of 50.

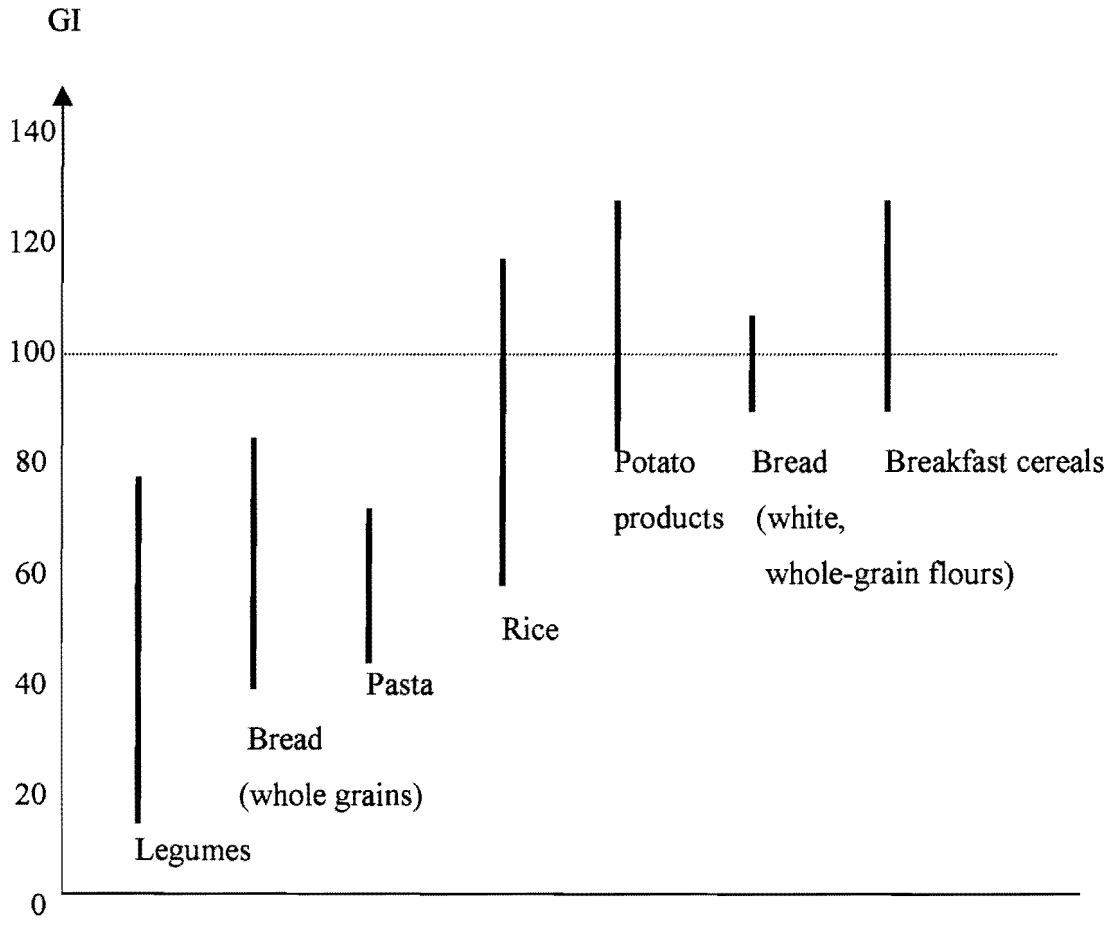

Fig. 6. Approximate ranges in GI for some of the main sources of starch in a Western-type diet (Bjorck and Asp, 1994).

Not only the fibre and the nature of the starch (discussed later in this Chapter) but also the form in which the food is eaten has been suggested as an important determinant of the glycaemic response. Thus higher rises were seen for pureed as opposed to whole apples, ground compared with whole rice and cooked versus uncooked starch (Jenkins *et al.*, 1982a). Wolever and Bolognesi (1996) observed that both the amount and source of carbohydrate consumed are important determinants of postprandial glucose and insulin responses of mixed meals in non-diabetic subjects. Regarding the amount of carbohydrate eaten (National Research Council, 1989) it can disturb the metabolic pathways, including those concerned with glucose metabolism, if taken in excess. The deleterious effects of overeating are more felt in obese people.

One of the assumptions latent in the fibre hypothesis is that the rate of digestion and hence of absorption is a major determinant of the glycaemic response. If this is so it will be influenced by many factors, which alter digestibility, other than fibre. The possible closeness of this relationship has only recently been demonstrated. A wide range of food factors which might be responsible for such differences in digestibility and hence GI, include: enzyme inhibitors, lectins, phytates, tannins, starch-protein and starch-lipid interactions (discussed later in this Chapter) (Jenkins *et al.*, 1982a).

2.5.5.1 Factors affecting the measured glycaemic index values

- Methodological variability

According to Perlstein, Willcox, Hines and Milosavljevic (1997) the method used to assess the glycaemic response to foods and presentation of the results may differ. The variables that can affect the GI are as follows:

- The choice of the standard food (glucose or white bread).
- The size of the portion (For low concentration carbohydrate foods, a 25 g available carbohydrate portion may be used instead of 50 g, because the volume of the food needed to supply 50 g of carbohydrate would be too large to consume.
- The method, frequency and length of time that blood is sampled.
- The method of calculating the area under the glycaemic response curve.
- The degree of control of the individuals involved in the test.

- The blood glucose levels before the test.

- Properties of the sample
- Many factors such as the nature of the carbohydrate, the physical form, the levels of fibre, antinutrients, fats and protein have been proposed to affect the glycaemic response to food (Perlstein *et al.*, 1997). These factors affect glycaemic response by first affecting the rate of absorption or digestion or both. Detailed discussion of these factors is on Section 2.6.

2.5.6 The role of slowly digested starch or low GI foods in the treatment and control of diabetes mellitus.

Diet plays an important role in the management of diabetes mellitus (National Research Council, 1989; Osman, 1995; Anderson and Geil, 1994; ADSA, 1997). Importance of diet in the treatment of diabetes has been recognized for centuries, but fashions have changed considerably even in recent years (Wright, 1993). Ancient civilizations in Egypt, Greece, Rome, and India recognized diabetes and recommended dietary modifications (Anderson and Geil, 1994). One of the major aims of diabetes therapy is to normalize the blood glucose profile, including the fasting and postprandial blood glucose concentrations (National Research Council, 1989; Brand-Miller, 1994; Anderson and Geil, 1994). Clinical studies of persons with diabetes have found improved glycaemic control with low digested carbohydrate diets (Englyst, *et al.*, 1999).

Bornet, Billaux and Messing (1997) have reported on nine studies which have compared the long-term nutritional impact of diets based on foods having high or low GIs in non-insulin-dependent (NIDDM) or insulin-dependent patients (IDM). The results of eight studies out of nine showed that by using low GI foods it was possible to reduce the GI of the patients between 14% and 28%. The remaining study which was considered as negative could only achieve an overall GI reduction of 6%. The reduction in the majority of the studies was achieved through consumption of lower GI foods such as pasta and rice instead of high GI foods such as bread and potatoes. The subject's metabolic control was

seen to improve significantly during the low-index diet period. This was confirmed by the corresponding reduction of plasmatic fructosamine and daily doses of insulin. Also reduction in the plasmatic rate of triglycerides and phospholipids was observed.

Jenkins, Wolever, Collier, Ocan, Venketshwer, Buckley, Lam, Mayer and Thompson (1987) did a study on health subjects to find out the impact of low GI diet. They observed that following a low GI diet for two weeks affected significant changes in the parameters of carbohydrate metabolism (reduction of serum fructosamine), lipid metabolism (reduced total cholesterol) and renal function (reduced creatinine clearance) in a group of six healthy subjects with reference to a high GI diet period.

2.6 Factors affecting the rate of starch digestibility

Starch digestibility is limited by the degree of gelatinisation, granule size, amylose content, starch-protein interactions, starch-lipid complexes and, perhaps most importantly, the degree of crystallinity, including that formed by retrogradation during processing (Whistler and BeMiller, 1997). Factors affecting digestibility of starch can be divided into intrinsic factors (i.e. properties of the food) and extrinsic factors (processing, chewing, transit through the bowel, concentration of the enzyme, amount of the starch and the presence of other interfering ingredients) (Englyst *et al.*, 1992; Annison and Topping, 1994).

2.6.1 Intrinsic factors affecting starch digestibility

2.6.1.1 Starch structure

Examination of raw starch granules of different sizes from cassava and maize suggests that the smaller the granule, the greater the extent of *in vitro* digestion by bacterial α-amylases and fungal amyloglucosidase (Annison and Topping, 1994). The digestibility of starches from some plant species is much less than that of others. Raw potato and field bean starches are poorly digested *in vitro* compared with most cereals (Annison and Topping, 1994). The X-ray diffraction analysis of native starches yields two types of

spectral patterns, A type and B type, which points to two types of crystalline structures. Cereal starches yield the A-type pattern, whereas the tuber starches and amylose-rich starches yield the B-type pattern. Legume starches yield C type pattern which is the combination of the A and B patterns (Englyst *et al.*, 1992; Annison and Topping, 1994). In general, starch granule showing X-ray diffraction patterns of the B or C type tend to be more resistant to digestion by pancreatic amylase and the degree of resistance is dependent on the plant source (Englyst *et al.*, 1992; Annison and Topping, 1994). This type of resistance to hydrolysis with pancreatic amylase affects the digestibility of starchy foods normally eaten raw such as banana, and processed foods, such as biscuits, where the starch has been incompletely gelatinised (Englyst *et al.*, 1992).

Though potato and cassava are both tubers, but cassava is more susceptible to α-amylase hydrolysis than potato, probably due to differences in their starch granules' surface areas. Potato starch granules (B-type) are very large and therefore have a low surface area relative to volume compared with tapioca starch granules (B-type spherulites), which are rapidly fragmented by amylases, a process that increases the area exposed to attacking enzymes (Annison and Topping, 1994).

2.6.1.2 Amylose : amylopectin ratio

According to the studies made on rice and maize, which are available in a variety of genotypes with different amylose : amylopectin ratios, a high amylose content resulted in both reduced rate of digestion and absorption, and also an increased yield of resistant starch in the finished product (Bjorck and Asp, 1994; Sagum and Arcot, 2000). The higher resistant starch content is probably mainly related to retrogradation of the amylose component, although enzyme resistance due to limited swelling of starch granules cannot be excluded in the case of certain maize varieties and other cereals with exceptionally high amylose contents (Bjorck and Asp, 1994). Sagum and Arcot (2000) observed a negative correlation between amylose content and the rates of starch digestibility of three varieties of rice (Japonica, Inga and Doongara). The amounts of amylose for the three varieties of rice from the lowest to the highest were 11% (Japonica), 20% (Inga) and 31% (Doongara). The *in vitro* starch digestibility of Doongara rice was significantly lower ($p < 0.05$) than those of Inga rice and Japonica rice in both boiling and pressure cooking

processes. Sagum and Arcot (2000) attributed the significantly lower rate ($p < 0.05$) of *in vitro* starch digestibility of Doongara rice to its higher amylose content and the higher *in vitro* starch digestibilities of Inga rice and Japonica rice to their lower amylose content.

2.6.1.3 Formation of amylose-lipid complex superstructures

Complexation between amylose and lipids, which readily takes place during heat processing of starch, also appears to influence the susceptibility of starch to enzymic degradation (Seneviratne and Biliaderis, 1991). According to Urooj and Puttraj (1999) in food systems, starch forms molecular complexes with lipids and protein which render it less susceptible to enzymatic digestion. Although complexed amylose was highly resistant to α-amylase *in vitro*, compared to free amylose in solution, complete digestion of the complex was obtained when a large excess of enzyme was added (Holm, Bjorck, Ostrowska, Eliasson, Asp, Larsson and Lundquist, 1983). Complexes of amylose with lysolecithin were also found to be completely absorbed in the rat small intestine. Nevertheless, the plasma glucose and insulin levels, following ingestion of complexed amylose, were significantly lower than those after ingestion of free solubilised amylose (Seneviratne and Biliaderis, 1991). These results, therefore, imply a slower degradation of amylose-lipid complexes for both *in vitro* and *in vivo* situations (Seneviratne and Biliaderis, 1991).

The presence of complexing lipids, appears to pose competitive mechanism between amylose chain association and amylose-lipid complex formation during cooling of high amylose starchy food (Czuchajowska, Sievert and Pomeranz, 1991). Lower yields of resistant starch in the sample in which lipids were added than in the control sample, it confirms that the added lipids interacted with amylose chains that were involved in the re-crystallization process in the control sample. In other words, the presence of complexing lipids affect the re-association behaviour of amylose upon retrogradation of starch and thus formation of resistant starch type 3 (RS_3) (Czuchajowska *et al.*, 1991).

2.6.1.4 Starch-protein interaction

The effect of protein on the starch digestibility of cooked sorghum flours was studied by Zhang and Hamaker (1998) and observed the following:

- Cooked sorghum flours had lower starch digestibility (15 – 25%) than normal maize flour, regardless of whether the endosperm type was floury, dense floury, or vitreous.
- The increase in starch digestibility when sorghum flour was pepsin-pretreated before cooking, or by cooking with a reducing agent, suggests that protein plays a large role in its low starch digestibility.

The probable role of sorghum protein in creating less digestible starch was explained in two folds: First, according to Chandrashekar and Kirleis (1988) endosperm protein may restrict the starch granules from fully gelatinising, thereby resulting in lower digestibility. The authors did not find ungelatinised starch granules in any of the cooked flours. However, gelatinised sorghum had lower soluble starch than the maize counterpart. Second, a starch-protein interaction may occur during cooking or cooling that causes gelatinised sorghum starch to be in a less digestible state. The rigidity of the protein body and matrix in hard grain sorghums that restrict the starch granules from fully gelatinising has been attributed to disulphide bonds (Chandrashekar and Kirleis 1988)

Urooj and Puttraj (1999) observed both lower rate of starch digestion and digestibility index in chapati, poori, semolina idli and idli and suggested that such results might have been caused by the presence of protein matrix around the starch granules, which restricts the amylase penetration into the granules. In some processed foods, protein may encapsulate the starch granules, e.g., encapsulation by gluten in wheat products such as pasta (Colonna *et al.*, 1990; Annison and Topping, 1994). According to Guerrieri, Eynard, Lavelli and Cerletti (1997) various proteins were observed to have different effects on the following gelatinised polysaccharide substrates; starch, amylopectin, amylose and β-dextrins when these were digested with amyloglucosidase enzyme indicating different interactions of the molecules on each polysaccharide.

2.6.1.5 Effect of dietary fibre on starch digestibility

In unrefined foods, the presence of fibre is likely to slow carbohydrate absorption by interfering with the digestion of starch or other saccharides. In unrefined foods, plant cell walls or bran layers in cereal grains can serve as a barrier to the penetration of digestive enzymes (Schneeman and Tietyen, 1994). For example, in rice kernels with the bran layer intact (whole brown rice) and rice kernels with the cell walls intact (whole white rice), amylase digestion of starch is relatively low, whereas grinding the whole rice kernels to a fine powder increases starch digestion substantially (Snow and O'Dea 1981). The potential benefit of fibre in slowing carbohydrate utilization may be achieved by consuming foods with intact cell walls, not isolated fibre supplements (Schneeman and Tietyen, 1994). Viscous dietary fibres reduce the rate of absorption of glucose or other carbohydrates, resulting in a reduction of the blood glucose and insulin responses (Wolever, 1993; Schneeman and Tietyen, 1994; Baghurst *et al.*, 1996).

The effects of fibre naturally present in foods are not necessarily the same as those of purified fibres added to foods (Wolever, 1993). There is only a weak relationship between the total fibre content of foods and their blood glucose responses, with no relationship between soluble fibre and the blood glucose response (Wolever, 1993). This is most likely because the chemical measurement of fibre does not indicate its physical properties, and because many other food-related factors (e.g. type of starch, particle size, processing and antinutrients) affect the glycaemic responses. Normally, 2 – 10% of the available carbohydrate in refined food enters the colon, increasing to 15 – 20% for high-fibre foods (Wolever, 1993).

Regarding the relationship between dietary fibre and prevention of chronic diseases such as diabetes mellitus, the National Research Council (1989) has concluded that, although the evidence generally suggests that the risk of diabetes mellitus is inversely associated with diets high in fibre-containing foods, but the nature of the association has not been established as causal.

2.6.1.6 Anti-nutrients

Anti-nutrients are also other food components which might affect the rate of starch digestion and absorption. These include phytic acid, lectins and tannins (Jenkins *et al.*, 1982a; Bjorck and Asp, 1994). The mechanism is not clear but might involve the inhibition of starch-degrading enzymes in the gastrointestinal tract (Bjorck and Asp, 1994). Tannins, phenolic acids and derivatives and flavanoids form a group of compounds known as polyphenols. These compounds are some of the most numerous and widely distributed groups of natural products in the plant kingdom (Reviewed by Salunkhe, Jadhav, Kadam and Chavan 1982; Reviewed by Bravo, 1998). For example, tannins isolated from green carob beans significantly inhibited the activities of digestive enzymes like trypsin, lipase, and amylase (Reviewed by Salunkhe *et al.*, 1982). Alonso, Aguirre and Marzo (2000) observed that among other methods such as soaking and germination, decortication was the most effective in reducing the levels of condensed tannins and polyphenols, and as a result, starch digestibility was highly increased in faba and kidney beans.

Sorghum is unique among major cereals like maize, rice and wheat because some cultivars produce polymeric polyphenols known as tannins (Waniska and Rooney, 2000). Phenols are present in all sorghums but very few sorghums contain a pigmented testa with B1 and B2 genes that produce condensed tannins or proanthocyanidins. Most sorghums only contains flavonoids (Waniska and Rooney, 2000). In sorghum grain, polyphenols are mainly located in the pericarp and testa (Rooney, Blakely, Miller and Rosenow, 1980). According to Reichert, Youngs and Christensen (1980) polyphenols in millet grain are not as nutritionally adverse as the tannins present in the testa layer of some cultivars of sorghum. Despite being nutritionally harmless, these polyphenols pose an aesthetic problem due to a grey pigmentation in the peripheral areas of the seed. The polyphenols present in pearl millet are non-tannin polyphenols and are concentrated in the hull/pericarp and testa which is about 3 times that is in the whole grain. Decorticated millet seed would be expected to contain approximately 40% less than the whole seed. Pearl millet and low tannin sorghums have no measurable level of tannin (Reichert *et al.*, 1980).

2.6.1.7 Amylase inhibitor

Stone-ground wheat wholemeal flour has been found to be hydrolyzed more slowly than the other flours *in vitro*. This may have been due to the presence of an amylase inhibitor, which has been isolated from the germ fraction of wheat (Snow and O'Dea, 1981).

2.6.2 Extrinsic factors affecting starch digestibility

2.6.2.1 Degree of gelatinisation (DG)

Raw starch is only slowly digested by enzymes *in vitro,* whereas cooking increases the susceptibility considerably because of the rupture and disintegration of the compact crystalline granular structure (Snow and O'Dea 1981; Holm, Lundquist, Bjorck, Eliasson and Asp, 1988; Annison and Topping, 1994). Furthermore, the glucose and insulin responses *in vivo* are significantly greater after ingestion of cooked compared with raw starches. Consequently DG is an extremely important factor in the rate of starch hydrolysis and metabolic response (Holm *et al.*, 1988).

The extent of gelatinisation is dependent on moisture availability, time, temperature and pressure and generally can be explained by the method of processing to which the foods have been subjected to (Urooj and Puttraj, 1999). The degree of gelatinisation varies depending on the cereal used and method of cooking. It is reported that wheat starch swells in a mode which differs from other starches (Urooj and Puttraj, 1999).

2.6.2.2 Physical form

Starch digestion is slowed in the small intestine if the physical form of the food hinders access of pancreatic amylase. This occurs if starch is contained within whole or partly disrupted plant structures such as grains or seeds; if rigid cell walls inhibit swelling and dispersion of starch, as in legumes (Wursch,1989); or if starch is very densely packed in a food such as spaghetti (Englyst *et al.*, 1992). When the rate of starch digestion is decreased, postprandial glucose and insulin responses are reduced or delayed (Englyst *et al.*, 1992). Snow and O'Dea (1981) made observations on rolled cereals and whole kernels on one side and cereal flours on the other side and found that the finely milled cooked flours were hydrolyzed much faster compared to the rolled and whole kernel

cooked foods. Granfeldt *et al.* (1992) also demonstrated that kernel and coarse breads gave significantly lower blood glucose responses than bread produced from the corresponding wholemeal flour. Kernels or coarse grain, contain starch granules entrapped in cell walls which do not swell completely due to the limited amount of water (Colonna, Leloup and Buleon, 1992). As a result of the limited swelling disruption does not take place and limited solubilisation was observed on thermal treatment by (Colonna, Barry, Cloarec, Bornet, Guilloud and Galmiche, 1990). Intact structures provide starch that is physically inaccessible to hydrolytic enzymes due to the barrier created by the cell walls and hence causing lower digestibility (Snow and O'Dea 1981; Colonna *et al.*, 1992; Bravo, Englyst and Hudson, 1998).

Processing such as milling, grinding, puffing, canning, flaking and dry heating of grains has been associated with increasing glycaemic responses (Perlestein *et al.*, 1997). Decreasing the particle size by grinding greatly increases the surface area and results in much more rapid digestion and absorption of the rice with resultant increased insulin secretion (Snow and O'Dea, 1981; Annison and Topping, 1994). Finely grinding red kidney beans or lentils prior cooking resulted in 7-fold increase in a starch hydrolysis rates (Wong, Traianedes and O'Dea, 1985). Particle size and surface area to starch ratio are important factors in determining the availability of starch to the hydrolytic enzymes. This was clearly demonstrated for both raw and cooked cereals. For example, starch in cooked flour (bread) was still hydrolyzed faster than that in cooked rolled wheat (porridge) (Snow and O'Dea, 1981). Processing may elevate GI by 40 to 50 units. It has been shown that canning increased the GI of dried beans by 17 units and hypothesized that the high pressure used in the canning process could alter the physical nature of the starch and antinutrient content (Perlstein *et al.*, 1997).

2.6.2.3 Formation of retrograded starch

When starch granules are fully gelatinised and dispersed, the starch becomes easily digestible (Annison and Topping, 1994). However, as the gel cools and ages, the polymers once more form a partially crystalline structure. Re-crystallization or retrogradation depends on the formation of inter-chain hydrogen bonds and occurs most

rapidly for the linear amylose. Retrogradation of amylopectin is limited by its branched structure and the polymers of retrograded amylopectin are less firmly bound than those of retrograded amylose. Retrograded starch characteristically forms the B-type pattern of starch granules which tend to be more resistant to digestion by pancreatic amylase (Annison and Topping, 1994). Retrogradation leads to the formation of resistant starch in the diet and slows down the rate of starch digestion (Bjorck and Asp, 1994).

2.6.2.4 The presence of other ingredients

Added fat and protein in foods do not normally slow digestion by blocking the enzyme accessibility to the starch granules but rather by reducing the gastric emptying, and for this to happen they should be present in large quantities (Perlstein *et al.*, 1997). Studies have shown that fat and protein generally alter the GI of foods containing carbohydrate if present in quantities greater than 25 g in a food containing 50 g of carbohydrate (Perlstein *et al.*, 1997).

2.7 *In vitro* determination of starch digestibility

In vivo methods which involve studies of postprandial glucose and insulin responses to starchy foods are in many aspects laborious, and demand several motivated subjects during a long period of time (Granfeldt *et al.*, 1992). Strict regulations are required when using human subjects; this makes the situation difficult as some people may decide not to adhere to the regulations such as diet and medication.

There is also a common problem of lack of facilities for most of the laboratories involved in food research especially in the developing world (Granfeldt *et al.*, 1992).

In vivo digestibility is automatically modified by numerous stimuli e.g. food itself. This means that enzymes which are involved with protein, carbohydrate and lipid digestion adapt to any changes in substrate intake (Corring, Juste, and Lhoste, 1989). Also, antinutritional factors and dietary fibre affect enzyme secretions. It is on these grounds that, several scientists have concluded that, the *in vivo* conditions can never be

completely simulated under *in vitro* conditions (Boisen and Eggum, 1991; Granfeldt *et al.*, 1992; Urooj and Puttraj, 1999).

Advantages of *in vitro* techniques are; they are simple techniques, can be designed to use specific enzymes either to give maximal digestibility values or to measure the initial rate of hydrolysis. In both cases the enzymes used should have specificities similar to those which are present in the digestive tract (Boisen and Eggum, 1991). The applicability of the results depends on a high degree of simulation or correlation with *in vivo* values obtained under standardized conditions using identical material (Boisen and Eggum, 1991; Asp and Bjorck, 1992). There are various methods for determining *in vitro* starch digestibility and they vary in the way the sample is prepared, the enzymes that are used the conditions of incubation (time and temperature) and other requirements. Below in Table 4 are some few examples of different methods.

Table 4: Some few examples of methods used during determination of starch digestibility *in vitro*

Sample prep.	Enzyme used	Time Incub.	Temp. incub.	Restriction	Agitation	Measure of digest. Prod.
Chewing sample containing 1 g starch	Salivary α-amylase, pepsin & pancreatic α-amylase	210 minutes	37°C	Dialysis tubing	Stirred water bath/ constant stirring	3,5-dinitrosalicylic acid method
The method above was used by Granfeldt and Bjorck (1991); Granfeldt *et al.* (1992); Liljeberg, Granfeldt, and Bjorck (1992); Liljeberg and Bjorck (1994); Van der Merwe, Erasmus and Taylor (2001).						
No prep. Sample containing 0.2g starch	α-amylase and amyloglucosidase	Whole night	50°C	None	Shaking water bath	Para-hydroxy benzoic acid hydrazide (Pahbah)
The method above was used by Snow and O'Dea (1981); Wong *et al.* (1985).						
No prep. Sample containing 2 g starch	Salivary α-amylase, pepsin & pancreatic α-amylase	365 minutes	37°C	Dialysis tubing	No	3,5-dinitrosalicylic acid method
The method above was used by Casiraghi, Brighenti, Pellegrini, Leopardi and Testolin (1993).						
Ground sample containing 1 g starch	Salivary α-amylase & pancreatic α-amylase	180 minutes	37°C	Dialysis tubing	No	Standard glucose-oxidase method
The method above was used by Urooj and Puttraj (1999).						
Ground sample containing 2 g starch	Salivary α-amylase & pancreatic α-amylase	300 minutes	37°C	Dialysis tubing	Stirred water bath	Standard glucose-oxidase method
The method above was used by Jenkins, Ghafari, Wolever, Taylor, Jenkins, Barker, Fielden and Bowling (1982b).						
Isolated starch 200 mg	pancreatic α-amylase	240 minutes	37°C	None	Constant stirring	HPLC
The method above was used by Faulks and Bailey (1990).						

2.7.1 Sample preparation

Different pretreatment methods of the sample have been shown to influence the rate of *in vitro* starch digestibility. Holm and Bjorck (1992) managed to get significantly different results of the *in vitro* starch digestibility between white bread and coarse bread when the samples were chewed but failed when the samples were milled. Before Granfeldt *et al.* (1992) invented the chewing method that enables *in vitro* studies of the food with the structure 'as eaten', the food structure was more or less destroyed mechanically by methods like grinding, sieving or shaking. If the *in vitro* techniques are to mimick the *in vivo* situation then it is very important to make sure that the botanical structure is not destroyed beyond the level of ordinary chewing during the critical sample preparation (Granfeldt *et al.*, 1992). Physical characteristics of starchy foods influences the outcome of the starch they contain. In other words, starch that is contained within whole plant cells or within a dense food matrix may escape digestion in the human small intestine (Englyst and Hudson, 1996).

Englyst *et al.* (1992) concurred with Granfeldt *et al.* (1992) on the chewing method which will enable food to be analysed as eaten but with caution. For both *in vitro* and *in vivo*, the rate and extent of starch digestion of foods with particular shape and form, are critically dependent on the way food sample is divided (Englyst *et al.*, 1992). Chewing is highly unique and variable method of dividing food. The extent to which a food is chewed will depend on its texture or form and on such factors as dental health, the degree of hunger of the consumer, the presence of other foods and individual chewing habits (Englyst *et al.*, 1992). Due to a number of shortcomings on chewing method, scientists should be careful not to imitate chewing to closely when preparing food analysis *in vitro*. Englyst *et al.* (1992) suggested that, the method chosen should reflect the average division of the food achieved by chewing, at the same time exhibiting greater degree of reproducibility.

In line with Englyst *et al.* (1992), Granfeldt *et al.* (1992) standardized chewing procedure by incorporating the following conditions: - Subjects were told not to eat for 1.5 h to 2 h

prior to the experiment, rinse their mouths with tap water and chew the food 15 times within approximately 15 s. With this standardization, better results than those obtained without preparation or by disruption of particulate structures mechanically without considering food analysis 'as eaten' were obtained (Granfeldt, Liljeberg, Drews, Newman and Bjorck, 1994; Liljeberg and Bjorck, 1994; Akerberg, Liljeberg and Bjorck, 1998).

2.7.2 Enzyme used to digest the starchy food samples

Some researchers like Urooj and Puttraj (1999) and Jenkins *et al.* (1982b) had used α-amylases only (from saliva and pancrease) while others like Granfeldt *et al.* (1992) and Van der Merwe *et al.* (2001) used a combination of proteolytic and α-amylases enzymes. The proteolytic enzyme used in these cases was pepsin, and the purpose was to simulate the situation which takes place in the stomach when one consumes starchy food. Starch-protein interactions, restricting the susceptibility to α-amylolysis *in vitro*, are present in foods such as boiled and durum wheat flour (Holm and Bjorck, 1988). Tovar, Bjorck and Asp (1990) observed an increase on the rate of *in vitro* hydrolysis of starch in red kidney beans when a pre-treatment with proteolytic enzymes was carried out. There are also some scientists like Snow and O'Dea (1981), and Wong *et al.* (1985) who had used amyloglucosidase together with α-amylase. Since amyloglucosidase is not a mammalian enzyme, it is therefore difficult to compare the results obtained to those of *in vivo*.

2.7.3 Incubation conditions

Time of incubation depends on the purpose of experiment and the quantities of the sample and the reagents used. Most of the *in vitro* digestibility experiments, which were done to mimick *in vivo* conditions were carried out for at least 3 h; this is roughly the time taken by the food to pass out of the small intestine. Some examples are Faulks and Bailey (1990) for 240 min; Granfeldt *et al.* (1992) and Van der Merwe *et al.* (2001) for 210 min; Casiraghi *et al.* (1993) for 365 min and Urooj and Puttraj (1999) for 180 min.

In most cases the temperature used for incubation was 37°C which is the temperature of the human body. The major reason for using this temperature during *in vitro* experiments is to make sure that the results obtained are as close as possible to those of *in vivo*. Other temperatures have been used with non-mammalian enzymes such as amyloglucosidase at 50°C by Snow and O'Dea (1981) and Wong *et al.* (1985).

Most of the *in vitro* experiments make use of mechanical agitation such as shaking water bath or magnetic stirrer, only in few cases whereby you will find manual agitation or no agitation at all.

Dialysis tubing has been used to simulate the gastrointestinal motility and absorption (Jenkins *et al.*, 1982b). After splitting up of the starch by the α–amylases the products obtained are hydrolyzed by the brush border enzymes in the small intestine and are absorbed as glucose. In the dialysis tube, sugars (maltose and maltotriose) and oligosaccharides smaller than the mean pore diameter (2.4μm) will diffuse through the dialysis membrane to be measured as the products of carbohydrate digestion (Jenkins *et al.*, 1982b). Another advantage of the dialysis tubing is the creation of the viscous environment like the one found in the intestine (Granfeldt *et al.*, 1992). The viscosity of the gastrointestinal content is known to affect the glycaemic response, a situation which can be compared to the rate of appearance of maltodextrins in the dialysate as it is also being affected by the viscosity inside the dialysis tubing (Granfeldt *et al.*, 1992). Dialysis tubing offers prevention of the end-product inhibition on the enzyme by diffusing the end products out (Boisen and Eggum 1991).

2.7.4 Measurement of digestion end products

Amylases from both salivary and pancreatic juice are the enzymes that catalyze the hydrolysis of the glycosidic bonds of the polysaccharides of starch. (Jenkins *et al.*, 1982b; BeMiller, 1992). Although saliva contains an α-amylase, almost all starch digestion and absorption takes place in the small intestine (BeMiller, 1992). The α-amylase in the small intestine hydrolyses the starch polysaccharides to oligosaccharides, maltose, maltotriose

and very small amount of glucose (Jenkins *et al.*, 1982b; BeMiller, 1992). Concerning the measurement of digestion end products, some researchers like Granfeldt *et al.* (1992) and Van der Merwe *et al.* (2001) used the method of Bernfeld (1955) of determining the reducing power of the products of digestion by 3,5 dinitrosalcylic (DNS) acid. Maltose which is in the highest proportion among the digested products, reacts with 3,5 dinitrosalcylic acid to form a complex with a colour intensity that is proportional to its concentration, hence making use of absorbance measurements at 540 nm. Other researchers like Jenkins *et al.* (1982b) and Urooj and Puttraj (1999) analysed maltose and oligosaccharides together as glucose, after acid hydrolysis with HCl and neutralization with NaOH. The glucose formed was analysed by a standard glucose oxidase method. Snow and O'Dea (1981) and Wong *et al.* (1985) reacted the products of digestion with parahydroxy benzoic acid hydrazide to form a coloured aromatic hyrazide which had an absorbance at 415 nm proportional to the monosaccharide concentration. Faulks and Bailey (1990) ran the products of digestion through HPLC and analysed for glucose and its oligomers (G2 – G5) by comparison of peak areas with G2 – G5 standards.

From the discussion above regarding *in vitro* starch digestibility, it is evident that, the methods and conditions are quite variable. But despite the variability a high correlation with *in vivo* glycaemic response has been obtained by a good number of researchers (Jenkins *et al.*, 1982b; Granfeldt *et al.*, 1992; Granfeldt *et al.*, 1994; Akerberg *et al.*, 1998). Dialysis tubing can to some extent be compared to small intestine by its capability to allow smaller molecules of the products of digestion to pass through and the creation of viscosity inside the tube. Reliable and simple *in vitro* methods for the determination of the rate and extent of starch digestibility can be very useful in studying the behaviour of different starchy foods before embarking into metabolic studies.

2.8 Conclusions

Refinement of cereal grains such as maize, sorghum and pearl millet through milling produces the most palatable (lowest fibre), and most stable (lowest fat) flours. In the process of reducing the fibre, which is mainly made up by the bran, other components

like protein and antinutrients are also removed. Likewise the removal of the germ does not only reduce the fat content but also some protein. It is clear that the refinement process of the cereal grain flours reduces some of the intrinsic factors which are known to slow down the rate of starch digestibility. Intrinsic factors which are mostly reduced during grain flour refinement process, are; lipids, dietary fibre and anti-nutrients. Others, such as protein are only minimally affected. Sometimes amylase inhibitor found in the germs of some cereal grains, such as wheat, is also reduced. Since porridges from refined flours contain lower quantities of intrinsic factors, which slow down the rate of starch digestibility, their rate of *in vitro* starch digestibility might be higher than those from unrefined flours.

Different cereal species and varieties would have different rates and extents of starch digestibility if they have different intrinsic factors resulting from different chemical compositions and endosperm properties. Unlike maize, sorghum is considered to have a rigid protein body and matrix cover, which prevents the starch granules undergoing full gelatinisation, and hence has lower starch digestibility. On the other hand, pearl millet has a higher proportion of fat which interacts with amylose to form complexes, leading to the lowering of the susceptibility of the starch to α-amylase.

A high rate of starch digestibility translates into high glycaemic response, which is associated with the risk factor for chronic diseases such as diabetes mellitus. This might be one of the reasons for the increased incidence of diabetes mellitus among people who are changing from unrefined to refined flours and also changing from sorghum and pearl millet to maize.

CHAPTER 3

RESEARCH OBJECTIVES

Objective 1

To determine whether stiff porridges made from flours of different cereals; maize, sorghum and pearl millet have different rates of *in vitro* starch digestibility.

Objective 2

To determine whether stiff porridges made from flours of different varieties of the same species have different rates of *in vitro* starch digestibility.

Objective 3

To determine the rates of *in vitro* starch digestibility of stiff porridges prepared from unrefined and refined flours of maize, sorghum and pearl millet, and find out whether refinement improves digestibility.

Hypothesis 1

Digestibility of the starch from stiff porridges prepared from sorghum flours would be lower than those from maize and pearl millet, due to the presence of rigid protein body and matrix cover in sorghum, which probably restrict the starch granules from fully gelatinising and hence resulting to lower starch digestibility.

Hypothesis 2

Digestibility of the starch from stiff porridges prepared from unrefined pearl millet flours would be lower compared to the other cereals, due to its high fat content from the large germ in the grain. The large amount of fat might block some parts of the starch surface area and limit accessibility of the starch to enzyme action; also, the formation of amylose-lipid complex superstructures is known to lower starch susceptibility to α-amylase.

Hypothesis 3

The rate and extent of starch digestibility of stiff porridges prepared from unrefined flours would be lower than those from refined flours. The presence of bran (pericarp, testa and aleurone layer), fat and antinutritional substances which are present in higher amounts in unrefined than in the refined flours may directly or indirectly interfere with the enzymic digestion. This could be through restriction or inhibition of the enzyme activity, or possibly by blocking or binding of the starch granules.

CHAPTER 4

EXPERIMENTAL

4.1 Materials

4.1.1 Maize

Two varieties of maize; PAN 6335 grown in 1997 in Mpumalanga, South Africa and PAN 6043 grown in the same year in Free State also in South Africa were used.

4.1.2 Sorghum

Two varieties of sorghum; white sorghum KAT 369 (grown in 1997 in Cheplambus, Baringo, Kenya) and NK 283 red hybrid (grown in 1997 in Nola, Randfontein, in South Africa) were used.

4.1.3 Pearl millet

The two varieties of pearl millet used were: SDMV 89004 and SDMV 91018 grown in 1999. These two varieties were supplied by the SADC/ICRISAT research station for sorghum and millet at Bulawayo, Zimbabwe.

4.1.4 White wheat bread

The bread used in this study was white wheat pan bread, it was bought in a fresh state and from one batch a few days before starting of the experiment on August 2000 at Pick 'n Pay Supermarket at Hatfield, Pretoria, South Africa. It was cut into slices of about 15 mm thick, each slice was vacuum sealed in a plastic bag and stored at – 20°C before defrosted for use. Only the bread crumb was used.

All the grain and grain flours were store under dry conditions at 10°C.

The proximate composition of maize, sorghum and pearl millet flours is shown in Table 5, and that of white wheat bread in Table 6.

Table 5. Proximate composition of unrefined and refined flours of maize, sorghum and pearl millet

Sample	Moisture (%)	Protein[1] (% dwb)[4]	Fat (% dwb)	Ash (% dwb)	Starch (% dwb)
Maize PAN 6043 (unrefined)	11.31^{c2} (0.36)[3]	10.76^{ef} (0.05)	4.26^{d} (0.02)	1.31^{c} (0.03)	69.45^{de} (1.59)
Maize PAN 6043 (refined)	10.06^{def} (0.34)	9.69^{g} (0.02)	0.66^{h} (0.01)	0.36^{f} (0.01)	83.74^{a} (1.00)
Maize PAN 6335 (unrefined)	11.70^{bc} (0.03)	9.69^{g} (0.12)	4.37^{d} (0.0.4)	1.15^{de} (0.02)	75.62^{bc} (2.62)
Maize PAN 6335 (refined)	10.01^{def} (0.15)	8.58^{h} (0.02)	0.70^{h} (0.01)	0.27^{f} (0.01)	87.19^{a} (3.13)
Sorghum KAT 369 (unrefined)	11.70^{bc} (0.07)	10.49^{f} (0.05)	3.35^{e} (0.04)	1.81^{a} (0.03)	68.76^{de} (1.58)
Sorghum KAT 369 (refined)	12.37^{a} (0.07)	9.47^{g} (0.08)	1.66^{g} (0.07)	1.04^{e} (0.14)	78.85^{b} (2.70)
Sorghum NK 283 (unrefined)	12.19^{ab} (0.22)	11.76^{d} (0.14)	3.54^{e} (0.12)	1.53^{b} (0.06)	72.63^{cd} (1.79
Sorghum NK 283 (refined)	12.21^{ab} (0.22)	10.92^{e} (0.31)	2.30^{f} (0.03)	1.20^{cd} (0.04)	79.26^{b} (2.52)
Pearl millet SDMV 89004 (unrefined)	10.57^{bc} (0.27)	12.64^{c} (0.11)	6.60^{a} (0.03)	1.74^{a} (0.04)	68.23e (3.67)
Pearl millet SDMV 89004 (refined)	10.27^{de} (0.02)	10.67^{ef} (0.04)	4.88^{c} (0.08)	1.24^{cd} (0.03)	77.15^{b} (0.58)
Pearl millet SDMV 91018 (unrefined)	9.89^{ef} (0.09)	15.32^{a} (0.08)	6.33^{b} (0.02)	1.70^{a} (0.03)	66.95^{e} (2.02)
Pearl millet SDMV 91018 (refined)	9.51^{f} (0.04)	13.22^{b} (0.06)	3.54^{e} (0.22)	1.03^{e} (0.03)	78.83^{b} (0.96)

1 N x 6.25

2 Values with different letters in the superscript are statistically significantly different ($p < 0.05$)

3 Values in brackets are the standard deviations

4 Percentage dry weight basis

Table 6. General proximate composition of bread

Sample	Moisture (%)	Protein[1] (% dwb)[5]	Fat (% dwb)	Ash (% dwb)	Starch (% dwb)
White wheat bread	45.3[6] (0.2)[2]	13.1[3]	2.77[3]	2.94[4]	69.7[6] (1.2)

1 N x 5.70 for wheat

2 Values in brackets are the standard deviations

3 According to South African food composition tables (Langenhoven, Kruger, Gouws and Faber, 1991)

4 According to Van Heerden, Anderson, Van Niekerk and Wight (1990)

5 Percentage dry weight basis

6 According to the analyses carried out in this study.

4.2 Methods

Unrefined and refined flours of two varieties of each cereal grain (maize, sorghum and pearl millet) were prepared. The steps involved in the preparation are discussed below.

4.2.1 Removal of the bran and germ

4.2.1.1 Degerming maize

Degerming was done to separate the germ and the bran from the maize kernel so that the resulting endosperm could produce highly starch-concentrated refined flour after grinding. Before degerming, the moisture content of the maize kernels was increased to toughen the bran, which makes it easier to remove together with the germ from the endosperm (Uhlig & Bhat, 1979). The conditioning was done in two steps: the maize grains were conditioned to 16% moisture for overnight, and then 30 min before milling the grains were conditioned to a moisture content of 18%.

Degerming was done with a small-scale maize degermer designed by the Council for Scientific and Industrial Research (CSIR) in collaboration with the South African Maize Board. This small-scale maize degermer, works in the same principles as the industrial-scale Robinson or Beall degermer.

4.2.1.2 Decortication of sorghum and pearl millet

Sorghum and pearl millet were decorticated at CSIR using a rice polisher machine, unlike maize there was no conditioning of the grains. According to Rooney and Miller (1982) and Serna-Saldivar and Rooney (1995), the germ in sorghum and pearl millet is deeply interred in the grain and therefore making it difficult to remove through decortication/dehulling. High milling losses, up to 40%, may be incurred if the fat content of the meal is to be reduced to about 1%, a fat content that would ensure reasonable shelf stability of the meal. To make sure that no excessive endosperm was removed, an attempt was made to limit the amount of bran and germ removed to about 20% of the grain weight, which is figure recommended by the FAO according Kent and Evers (1994). Due

to the lack of suitable decorticating machine to remove exactly the outer surface material equivalent to 20% of the grain weight, it was found eventually that, the amounts of the bran and germ together removed from sorghum and pearl millet grains ranged from 13.96% to 29.94%. Sorghum and pearl millet remained with a high proportion of the germ after decorticating as per the above explanation.

4.2.2 Milling maize, sorghum and pearl millet to flour

- Unrefined flour was prepared by grinding the whole grain of the two varieties of each cereal.
- The refined flour was prepared by grinding degermed and decorticated maize grain and decorticated sorghum and pearl millet grains from the two varieties from each cereal.
- Grinding was done by a laboratory hammer mill using 800 μm sieve.

4.2.3 Maize, sorghum and pearl millet stiff porridge cooking procedure

The recipe used in this experiment is the one which is commonly used in most parts of East and Southern Africa. Since the quantities of water and flour had to be reduced, the suitable method was that of adding the whole amount of flour into the boiling water at once. Unlike the alternative method in which a thin porridge is made first before adding the rest of the remaining flour, this method had the advantage of reducing excessive evaporation and hence minimizing the amount of variation. Every day two samples of stiff porridge were prepared by picking a pair of flour samples in a complete randomized table. Every sample was cooked in triplicate. The steps involved were as follows:

Flour (60 g) for each sample was weighed into a 250 ml plastic beaker.

Tap water (150 ml) for each sample was measured by using a measuring cylinder and poured into a saucepan. The saucepan was covered and put on a hotplate (double solid hot plate with settings 0, 1, 2, 3, 4, 5, and 6) and temperature was set at 4 mark. The stop watch set at 20 min was started counting down when the power was switched on from the socket and after approximately 6 min and 10 s the water was brought to boiling point.

The flour was poured in and the stirring was done gently for 30 s and then covered for 40 s before performing another stirring which unlike the first one, this was vigorous for 30 s and then covered for 40 s. While the first saucepan was still covered the second saucepan which was started 1 min and 40 s later was coming to the boiling point and the same procedure was followed. After 5 min and 10 s, for each saucepan the temperature settings was turned down to 2 while stirring and covering continued for another 4 min and 5 s when the temperature settings was turned to 0. Quickly the saucepan was removed from the hot plate and the porridge was pulled together and covered to avoid drying on the surface while cooling at room temperature. The same was done to the porridge on the second saucepan.

4.3 Analyses

4.3.1 Hardness of the grains

Before milling, the hardness of the grains was visually characterized. This method involved cutting the whole grain into longitudinal section and making visual comparison of the ratios of the vitreous to floury areas with standard sections developed from sorghum grains (Rooney and Miller, 1982). The standard sections are grouped into three categories; namely floury, intermediate and corneous and use a scale from 5 to 1. The category of floury uses a scale of 5, category of intermediate to corneous uses a scale from 4 to 2 while the category of corneous uses a scale from 1 to 2.

4.3.2 Weight of the grains (1000 kernel weight)

The grains were counted by seed counter (Numigral seed counter, Tripette & Renaud). Only the whole grains and decorticated grains of sorghum and pearl millet varieties were counted. It was impossible to count decorticated and degermed maize grains because it was converted to maize samp which consisted mainly of broken maize kernels.

4.3.3 Proximate analysis

The proximate composition of the samples was expressed on dry weight basis. Quantities calculated from the experiments were converted to dry weight basis by the equation below:-

$$\text{Quantity (\% dry weight basis)} = \frac{\text{Quantity (\% as is)} \times 100}{(100 - \text{moisture content (\%)})}$$

4.3.3.1 Moisture

AACC Method 44 – 15A (American Association of Cereal Chemists, 1983b) was used. For samples that with moisture less than 13% (maize, sorghum and pearl millet flours), the one-stage air oven method was used. Approximately 2 –3 grams of well-mixed sample was placed into a moisture dish that had previously been dried, cooled in a dessicator and weighed. The dish was covered with its lid and weighed. The sample was put in an air oven at 103 ± 1°C while the lid was put under the dish. The sample was heated for 60 min counting from when the oven regained the temperature of 103 ± 1°C after introducing the samples. The dish was covered with its lid and the sample was placed in a dessicator to cool to room temperature. The moisture content was determined as a loss in moisture using the following equation:

$$\% \text{ Moisture} = \frac{A \times 100}{B}$$

In which A = moisture loss in grams, B = original weight of sample

In the case of the bread and the maize, sorghum, and pearl millet stiff porridges, the two-stage air oven procedure of the same method was followed. The pre-weighed moisture dish was filled nearly full with a representative portion of the sample. The sample weight was recorded. The sample was placed in a ventilated air oven at 30°C over night to reduce the moisture content to about 10%. The sample was taken out of the oven and left outside for 2 hours to equilibrate to atmospheric moisture content. The air-dried sample was

weighed and the percentage loss due to air-drying recorded. The particle size of the air-dried sample was then reduced using a clean, dry food liquidiser. The one-stage procedure described above was then followed. The total moisture content was calculated with the following equation:

$$\% \text{ Total moisture} = X + \frac{(100 - X)\,Y}{100}$$

Where X = percent moisture loss on air drying, Y = percent moisture loss as determined by oven drying.

4.3.3.2 Ash

AACC Method 08-01 (American Association of Cereal Chemists, 1983a) was used to determine the ash content of the maize, sorghum and pearl millet flours. Approximately 4 grams of sample was weighed accurately into a silica ashing crucible which had previously been ignited, cooled in a dessicator and weighed. The samples were incinerated in a muffle furnace until a light grey ash was obtained, cooled in a dessicator and weighed. Ash content was calculated as follows:

$$\% \text{ Ash} = \frac{(\text{weight of crucible} + \text{ash}) - \text{weight of an empty crucible} \times 100}{\text{weight of sample}}$$

4.3.3.3 Protein content

Samples were analysed for crude protein using a Kjeldahl method (modified AACC Method 46 – 12, American Association of Cereal Chemists, 1983c). Approximately 0.5 g sample was weighed accurately into a digestion tube. One Kjeltab (Thompson & Capper, Cheshire, UK), a 5 g tablet consisting of 100 parts K_2SO_4, 6 parts $CuSO_4.5H_2O$ and parts selenium was added. To that, 20 ml of concentrated H_2SO_4 was added. Samples were digested for approximately 1.5 h using a Buchi 430 Digestor (Buchi, Flavil, Switzerland). Distillation of ammonia, reaction with boric acid and titration with standard HCl (0.1 M)

were done with a Buchi 322 Distillation unit (Buchi, Flavil, Switzerland). The crude protein content was calculated using the following equation:

$$\% \text{ Protein} = \frac{(\text{ml std NaOH} \times \text{M of NaOH}) \times 1.4007 \times \text{factor}}{\text{sample weight (g)}}$$

The same factor of 6.25, was used for maize, sorghum and pearl millet.

4.3.3.4 Crude fat

The crude fat contents of the flours of maize, sorghum and pearl millet were determined by the modification of AACC Method 30 – 25 (American Association of Cereal Chemists, 1983d). Approximately 20g of well-mixed sample was weighed accurately onto a thimble. A piece of fat-free absorbent cotton wool was placed on top to prevent escape of the flour from the thimble and distribute solvent as it dropped on the sample. The sample was extracted with petroleum ether which was put into a flask previously dried in an oven, cooled in dessicator and weighed. The extraction was done for 5 h in a Soxhlet extractor and maintained at condensation rate of 5 – 6 drops per second. The solvent was evaporated on a water bath in a fume cupboard. The flask with fat was then dried completely in an oven at 103°C for approximately 1h. The flask was then cooled in a dessicator and weighed. The crude fat content was calculated as follows:

$$\% \text{ Crude fat} = \frac{((\text{weight of flask} + \text{fat}) - (\text{weight of flask})) \times 100}{\text{weight of sample}}$$

4.3.3.5 Total starch

Total starch assay kit, α-amylase/amyloglucosidase method (AA/AMG 9/97, Megazyme International Ireland Limited, Wicklow, Ireland, www. Megazyme.com). This method has been adopted first action by the AACC (Method 76.13).

The analysis included solubilisation of the starch with 80% ethanol and then dimethyl sulphoxide, digestion with thermostable α-amylase and digestion with amyloglucosidase. The formed glucose was then determined with a glucose oxidase/peroxidase reagent

(GOPOD) and the absorbance read at 510 nm. The total starch was calculated by the following equation:

$$\% \text{ Starch} = \frac{\Delta E \times F \times 90}{W}$$

Where ΔE is the absorbance read against the reagent blank, F is the conversion from absorbance to μg glucose, 90 is the adjustment from free glucose to anhydrous glucose (as occurs in the starch) and W is the weight of the sample.

4.3.4 Amylose content

A modification of the method of Knutson (1986) was used. Flour sample (50 mg) was dissolved in 10 ml 90% dimethyl sulphoxide containing 6 x 10^{-2} M iodine and heated at 50°C overnight in a shaking water-bath. The aliquots of this solution was diluted x 200 with distilled water and the absorbance was measured at 620 nm. The amylose content was determined by comparison with a standard curve. Mixtures of pure amylose and amylopectin from Sigma in different ratios between 0 and 100% was used to plot the standard curve. The purity of the amylose and amylopectin used for calibration was not checked. Thus, the amylose/amylopectin ratios can be considered as precise rather than accurate. The values of the amylose content obtained were percentages of dry weight flour. These values were converted to percentage weight of total starch by multiplying them with the following factor: $\frac{100}{TS_f}$

Where 100 is a constant and TS_f is the amount of the total starch contained in the dry flour sample.

4.3.5 Total polyphenols

Total polyphenols were determined by a modification of the method of Daiber (1975). 250 mg of the flour sample was mixed with 5.0 ml of the dimethyl formamide extractant and stirred vigorously in a vortex mixer after every 10 min for 1 h at room temperature. The polymeric phenols extracted were reacted with ferric ions (green ammonium ferric citrate) in an alkaline solution and the green/yellow dye produced was measured in a spectrophotometer at 525 nm.

4.3.6 Texture

Porridge was analysed by using TA – XT2 Texture analyser (Stable Micro Systems, Godalming: England) which consists of a computer with a texture expert programme and up and down movable vertical device mounted with a compressing probe. All the samples were subjected to the same conditions. Each porridge sample was prepared from the same amount of flour (60 g) and water (150 ml) and packed in strong and rigid small cylindrical plastic containers of 32 mm internal diameter and height of 40 mm. Thin steel wire for cutting cheese was used to cut the porridge bulging at the top of the porridge cylinders to make a smooth flat surface and then cooled at room temperature for 30 min. The texture of the porridges were measured by penetrating a 20 mm cylindrical probe with a flat end, 4 mm deep in the porridge at a speed of 0.2mm/s. The units of the porridge's texture was expressed as compression force in Newtons exerted during penetration.

4.3.7 In vitro starch digestibility

The Granfeldt *et al.* (1992) method involving pre-chewing of the food was used. The flow diagram in Figure 7 summarises the procedure. The whole *in vitro* starch digestibility experiment consisted of 12 treatments or samples of stiff porridges which were done in triplicate, and each replicate was divided into three portions. All the stiff porridges were cooked on a hot plate under the same conditions.

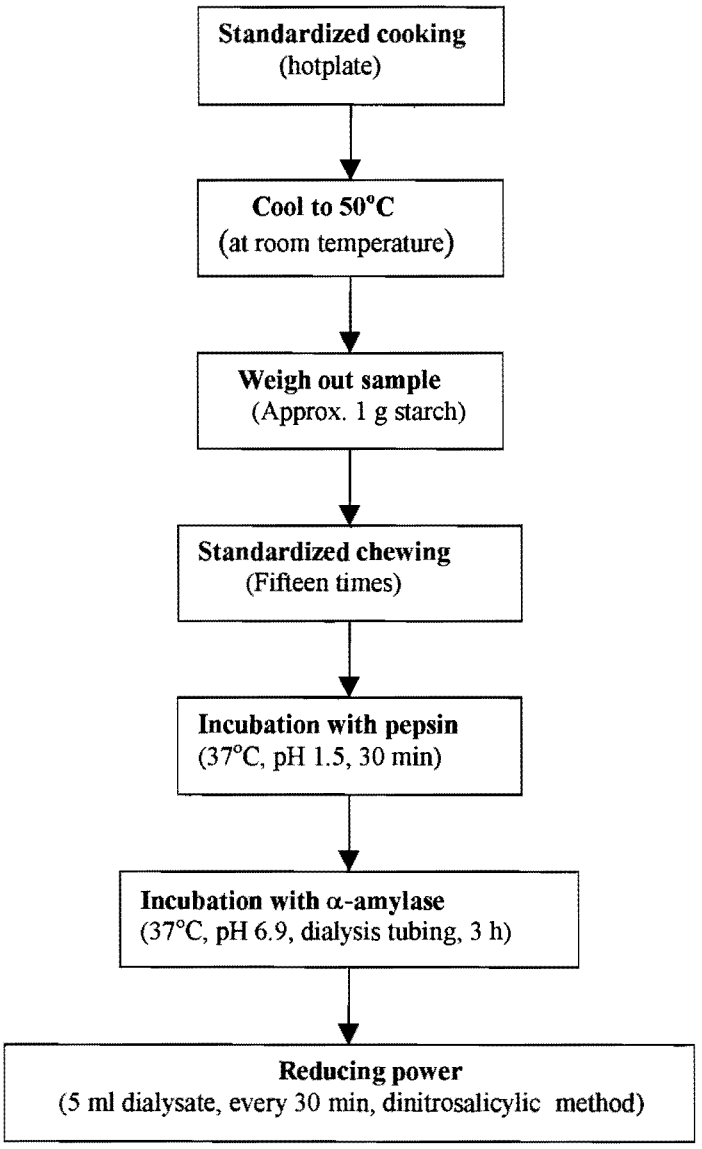

Figure 7: Flow diagram of the procedure used to determine the *in vitro* starch digestibility of stiff porridges from maize, sorghum and pearl millet.

4.3.7.1 Preparation of dialysis tubing

Dialysis tubing (Visking ex Labretoria, Pretoria) with a dry flat width of 42 mm and a molecular weight cut-off of 12 – 14 kDa was cut into 15 cm strips. The tubing was soaked in distilled water at 15°C overnight. One end of the tube was closed by tying it with a piece of string. The tubing as well as the extra pieces of string later used to close the other end of the tube were boiled in distilled water for 5 min to remove the sulphur used as preservative by the manufacturers. The tubing was then covered with fresh distilled water and used the same day. There was no tubing that was stored for more than a day, and therefore the preservative sodium benzoate acid (0.2 % m/v) that is commonly used to inhibit cellulolytic micro-organisms was not used.

4.3.7.2 Sample preparation

Two samples were cooked at a time on a saucepan, but with a difference of 2 min between the two in-terms of starting and finishing. After finishing cooking, porridge of each sample was pulled together in the form of a ball and covered the saucepan with a lid. The porridge was allowed to stand for about 18 min, the time by which it cooled to about 50°C at room temperature. Three portions from each sample containing about 1 g starch were weighed into the weighing boats and put inside a warm container with lid. The inside of the container was kept warm by a hot water sealed in a plastic bag. Weighing always started with the first sample that had been cooked.

Along with the two samples, there was also a bread which was used as a standard. Cubes of about 20 x 20 x 15 mm were cut from defrosted crumb of white wheat bread slice which had been stored in vacuum plastic bags at – 20°C. Two portions containing about 1 g starch were again cut from the cubes and weighed into the weighing boats and put together with the other portions from the two samples inside the warm container.

4.3.7.3 Chewing

Throughout the experiment no food was consumed in the 2 h before chewing took place. The mouth was rinsed with tap water before and after chewing any sample. After chewing, the sample was carefully expectorated into a 50 ml glass beaker containing 50

mg pepsin (2000 FIB-U/g, Merck, Darmstadt, Germany) and 6 ml of 0.05 M Na,K-phosphate buffer (containing 0.4 g/l NaCl) adjusted to pH 1.5 with HCl. The mouth was rinsed with 5 ml phosphate buffer (pH 6.9) for 30 s and the rinsing solution was also expectorated into the beaker. Twelve samples were handled per day.

4.3.7.4 Enzyme incubation

Before incubating with pepsin, the sample pH was adjusted to 1.5 with 2 M HCl and the beaker was covered with aluminium foil. It was incubated in a 37°C water bath for 30 min to simulate digestion in the stomach. The sample was mixed three times during incubation.

Following the incubation with pepsin, the pH was adjusted to 6.9 with 2 M NaOH. Porcine pancreatic α-amylase (A 6255 Sigma) was then added. The enzyme (35 µl) was dissolved in 9 ml of 0.05 M phosphate buffer and pH 6.9, and 1 ml of this solution was added to the sample. This represented approximately 147 Sigma units per gram of starch.

The sample was transferred to dialysis tubing. The beaker was rinsed with an amount of phosphate buffer that would bring the final volume in the dialysis tube to 30 ml (this volume varied between 7 ml to 8 ml according to sample). The dialysis tube was then tied and suspended in a 1 l beaker with 800 ml of 0.05 M phosphate buffer and pH 6.9. The beaker with the dialysis tube was then covered with aluminium foil to reduce evaporation and incubated in a water bath at 37°C for 3 h.

4.3.7.5 Measurement of products of digestion

Every 30 min a 5 ml aliquot of the dialysate was removed after thorough stirring of the contents in the beaker with a table spoon. It was analysed for reducing power by the 3,5 – dinitrosalicylic (DNS) acid method (Bernfeld, 1955). The aliquot was added to 5 ml DNS reagent (1% DNS in 0.4 M NaOH containing 30% w/w sodium potassium tartrate) in a 25 ml volumetric flask. The flask was immersed in a boiling water bath for 5 min to develop the colour, cooled and the sample was made up to volume. Absorbance was measured at 540 nm. A maltose standard curve was also constructed and used to convert

the absorbancy readings to maltose concentration (mg/ml). From maltose concentration the starch digestibility was calculated as follows:

Maltose liberated (mg) = maltose concentration (mg/ml) x 830

Where 830 ml is the total volume of the contents of the dialysis tube plus the buffer in the beaker.

Starch in porridge sample (mg) = mass of sample (g) x solids of porridge x starch content of sample x 1000

$$\text{Starch digestibility (\%)} = \frac{\text{mg maltose liberated x 100.}}{\text{mg starch in porridge sample}}$$

4.3.7.6 Blanks and reference sample

With every set of samples, a blank porridge sample was run in duplicate. The blank sample was not chewed, instead it was transferred to the beaker containing 50 mg pepsin in 6 ml of 0.05 M phosphate buffer and pH 1.5. Addition of 5 ml of 0.05 M and pH 6.9 buffer as it was done for the samples, was also done for the blank and it was broken up slightly with a glass rod. With the exception of chewing and addition of 1 ml of enzyme solution of which 1 ml of phosphate buffer (pH 6.9) was added, the blanks were treated in the same way as the samples.

White wheat bread was used as a reference, because it is often used as a reference when GI is determined (Granfeldt *et al.*, 1992; Perlstein *et al.*, 1997)

4.3.7.7 Calculation of Hydrolysis Index (HI) and predicted GI

A Hydrolysis Index (HI) was calculated according to Granfeldt *et al.* (1992):

$$\text{HI} = \frac{\text{Area under digestibility curve of sample (0 – 180 min) x 100}}{\text{Area under digestibility curve of white bread reference (0 –180 min)}}$$

Granfeldt (in her Ph.D. thesis, 1994) found a significant correlation (r = 0.862) between HI and GI which was also used by Akerberg *et al.* (1998) to predict GI by using the following equation:

$$GI = 0.862HI + 8.198$$

The result was converted to a glucose reference basis by multiplying by 0.7, as was done by Foster-Powell & Brand-Miller (1995).

4.3.8 Statistical analysis

Statistical analysis was done by using Statistica for Windows Release 5.0 (StaSoft Inc. 1984 – 1995, Tulsa, USA) and Microsoft Excel 97 (Microsoft Corporation, 1985 – 1997). Mrs. L Swart (Department of Statistics, University of Pretoria) and Prof. Van der Linde (Department of Information Technology, University of Pretoria) were consulted regarding the experimental design and statistical analysis.

Significant differences between means were obtained with both Tukey's honest significant difference and least significant difference tests. Significant differences between the regression coefficients (slopes) of straight lines after linear regression were determined by using the following equation:

$$t\ (student) = \frac{b_1 - b_2}{(S^2b_1 + S^2b_2)^{1/2}}$$

Where b_1 and b_2 were the regression coefficients and S_1 and S_2 were the standard errors of b_1 and b_2. The degrees of freedom of the t-distribution were calculated as $n_1 + n_2 - 4$, where n_1 and n_2 were the number of measurements taken in 1 and 2 respectively and 4 is for the two regression coefficients and intercepts that had to be estimated. The p-value of the test was 1 minus the p obtained from a t-table. In all the significance tests, a p-value of smaller than 0.05 was considered to be statistically significant.

CHAPTER 5

RESULTS

5.1 Grain hardness (by visual characterization)

Table 7 shows the mean scores for grain hardness of maize, sorghum and pearl millet varieties by visual characterization.

Table 7: Grain hardness of maize, sorghum and pearl millet varieties

Varieties	Mean scores for grain hardness
Maize PAN 6043	3.5[1]
Maize PAN 6335	3.3
Sorghum KAT 369	3.1
Sorghum NK 283	3.1
Pearl millet SDMV 89004	2.7
Pearl millet SDMV 91018	2.5

1 Scale is from 1 to 5. The categories are; from1 to 2 hard grain; from 2 to 4 hard to intermediate grain and from 4 to 5 soft grain (Rooney and Miller, 1982).

All the varieties of the three cereal grains fell in the category of intermediate grain.

5.2 Total polyphenols in the refined and unrefined flours of maize, sorghum and pearl millet

Table 8 shows the total polyphenol content of the flours of the three cereals (maize, sorghum and pearl millet).

Table 8: Total polyphenol content of the refined and unrefined flours of maize, sorghum and pearl millet

Samples	Mean total polyphenol content (mg/100g) dry weight flour
Maize PAN 6043 Unrefined	60 (60)[1]
Maize PAN 6043 Refined	51 (37)
Maize PAN 6335 Unrefined	52 (71)
Maize PAN 6335 Refined	47 (64)
Sorghum KAT 369 Unrefined	72 (54)
Sorghum KAT 369 Refined	40 (43)
Sorghum NK 283 Unrefined	169 (31)
Sorghum NK 283 Refined	100 (49)
Pearl millet SDMV 89004 Unrefined	91 (48)
Pearl millet SDMV 89004 Refined	47 (31)
Pearl millet SDMV 91018 Unrefined	149 (20)
Pearl millet SDMV 91018 Refined	23 (32)

1 Values in the brackets are standard deviations.

The amounts of polyphenols in these cereal grains were very low. Polyphenols were highest in the unrefined flours of sorghum NK 283 and pearl millet SDMV 91018, and lowest in the refined flour of pearl millet 91018. Analysis of variance could not assign significant differences as a result of large standard deviations.

5.3 Amylose content

Table 9 shows the amylose content of the refined and unrefined maize, sorghum and pearl millet flours.

Table 9: Amylose content of refined and unrefined maize, sorghum and pearl millet flours

Samples	Amylose content (% of total starch)
Maize PAN 6043 Unrefined	31.0[a1] (1.3)[2]
Maize PAN 6043 Refined	30.6[ab] (0.9)
Maize PAN 6335 Unrefined	28.5[bc] (1.2)
Maize PAN 6335 Refined	29.5[abc] (1.0)
Sorghum KAT 369 Unrefined	29.2[abc] (0.8)
Sorghum KAT 369 Refined	31.4[a] (0.2)
Sorghum NK 283 Unrefined	21.7[d] (0.3)
Sorghum NK 283 Refined	23.2[d] (0.6)
Pearl millet SDMV 89004 Unrefined	29.9[abc] (0.8)
Pearl millet SDMV 89004 Refined	31.2[a] (0.7)
Pearl millet SDMV 91018 Unrefined	28.0[c] (0.7)
Pearl millet SDMV 91018 Refined	29.9[abc] (0.8)

1. Values with different letters in superscript are statistically significantly different ($p < 0.05$).
2. Values in the brackets are standard deviations.

There was no significant difference ($p < 0.05$) in amylose content between refined and unrefined flours of the same variety. The results for varieties and cereals were; all the varieties of maize, pearl millet and sorghum variety KAT 369 essentially had no significant differences in amylose content, but, they were significantly higher ($p < 0.05$) in amylose than unrefined and refined sorghum NK 283.

5.4 Texture

Table 10 shows the texture of stiff porridges prepared from refined and unrefined flours of maize, sorghum and pearl millet in terms of compression force (N) exerted by the probe by penetrating 4 mm deep.

Table 10: Texture in terms of compression force (N) for stiff porridges prepared from refined and unrefined flours of maize, sorghum and pearl millet

Samples	Compression Force (N)
Maize PAN 6043 Unrefined	19.3^{c} (1.2)[2]
Maize PAN 6043 Refined	33.2^{a} (1.8)
Maize PAN 6335 Unrefined	20.8^{c} (1.9)
Maize PAN 6335 Refined	29.0^{b} (1.6)
Sorghum KAT 369 Unrefined	11.6^{d} (0.9)
Sorghum KAT 369 Refined	11.6^{d} (0.4)
Sorghum NK 283 Unrefined	6.6^{e} (0.3)
Sorghum NK 283 Refined	4.6^{e} (0.1)
Pearl millet SDMV 89004 Unrefined	12.0^{d} (0.4)
Pearl millet SDMV 89004 Refined	13.1^{d} (0.6)
Pearl millet SDMV 91018 Unrefined	12.7^{d} (1.2)
Pearl millet SDMV 91018 Refined	13.5^{d} (0.2)

1. Values with different letters in superscript are statistically significantly different ($p < 0.05$).
2. Values in the brackets are the standard deviations.

Refined flours of maize PAN 6043 and maize PAN 6335 produced the stiffest ($p < 0.05$) porridges while refined and unrefined flours of sorghum NK 283 produced the softest porridges. Refinement increased stiffness in maize porridge but not in sorghum and pearl millet.

5.5 *In vitro* starch digestibility

5.5.1 Starch digestibility of white wheat bread and porridges prepared from refined and unrefined flours of maize, sorghum and pearl millet.

After chewing, the bread and the maize stiff porridges formed thicker mashes than sorghum and pearl millet stiff porridges. Comparing sorghum and pearl millet, sorghum formed softer mash, especially that from sorghum NK 283. Unlike bread, sorghum and pearl millet, maize stiff porridges formed lumps in their mashes. In the process of incubation with pepsin, adjusting the pH and finally incubation with α-amylase, the mashes became thinner because these processes involved additional of solutions and gentle stirring. However, the thin mashes of maize stiff porridges were still containing lumps, but, not as larger as those in the thick mashes. On the other hand, there were no lumps present in either thick or thin mashes of the bread and those of sorghum and pearl millet stiff porridges.

Figure 8 compares the rate of *in vitro* starch digestibility of stiff porridges prepared from unrefined flours of maize, sorghum and pearl millet to that of white wheat bread.

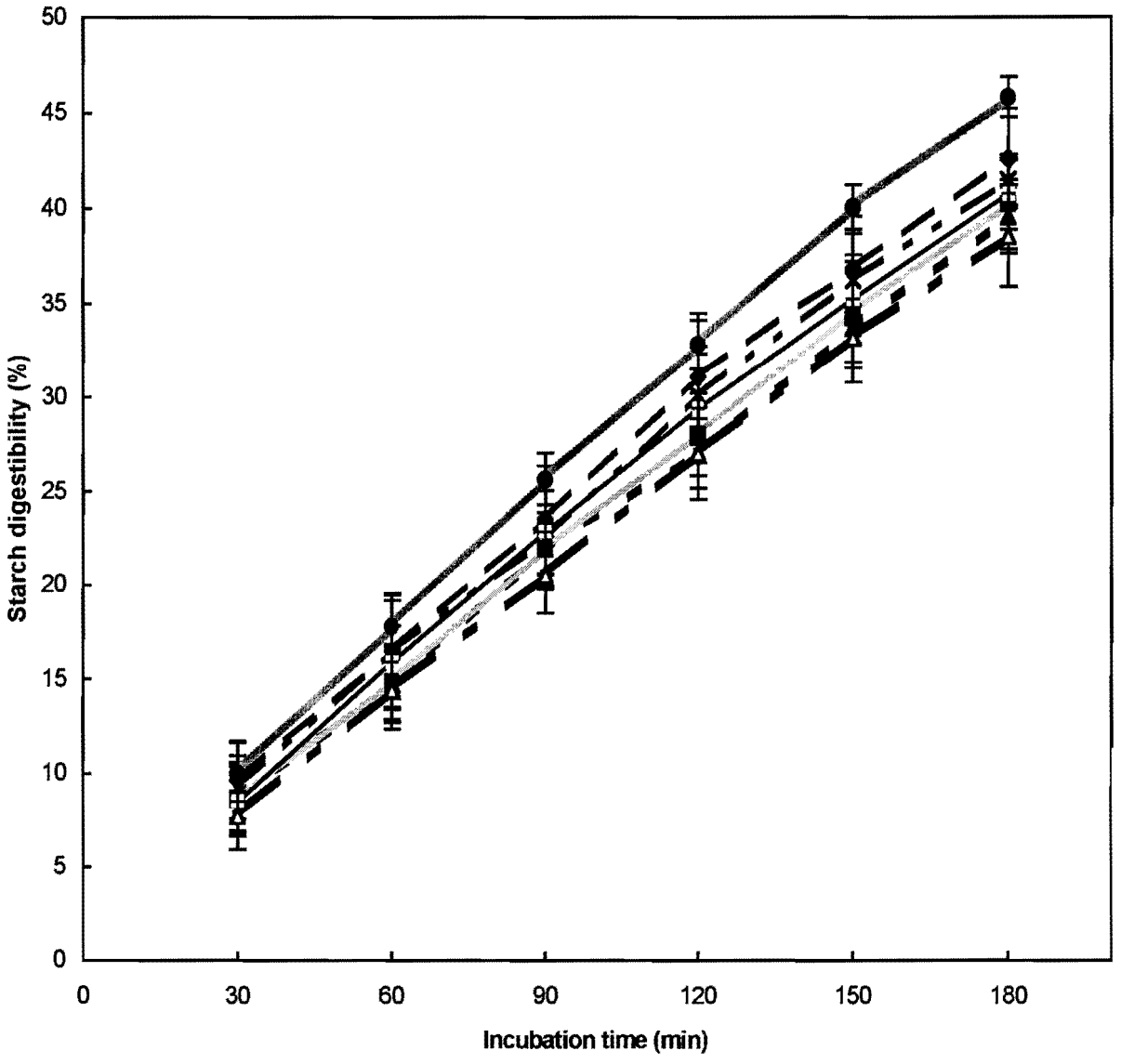

Figure 8: ***In vitro*** **starch digestibility of stiff porridges prepared from unrefined cereal grain flours compared to white bread. (– – ▲– –) maize PAN 6043; (..... ■ .....) maize PAN 6335; (— —◆— —) sorghum KAT 369; (— . ×—) sorghum NK 283; (——+——) pearl millet SDMV 89004; (— . Δ— –) pearl millet SDMV 91018 and (..... ● .....) white wheat bread.**

As seen in Figure 8, there were some differences in the rates of *in vitro* starch digestibility between white bread and the porridges and also between the porridge samples themselves. To make these differences more clear, linear models were fitted to the data. The model is expressed in the equation $y = mx + c$, where y is starch digested (%), x is incubation time (min), m is the slope of the line and c the intercept.

Figure 9 shows the fitted lines of starch digested against incubation time of stiff porridges prepared from unrefined flours of maize, sorghum and pearl millet compared to that of white wheat bread.

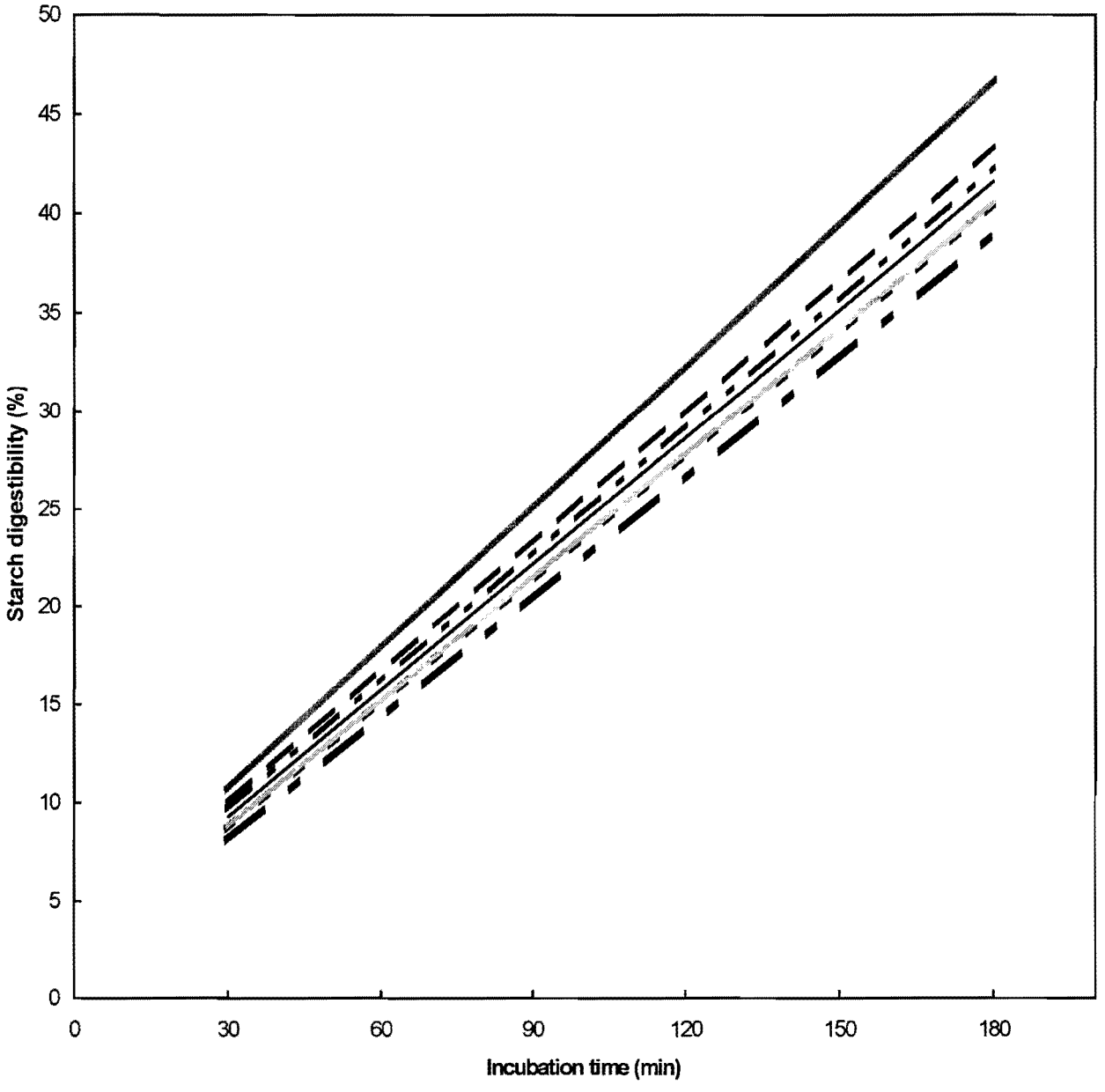

Figure 9: Fitted linear models of percentages starch digested against incubation time of stiff porridges prepared from unrefined cereal flours compared to white bread. (_ _ _) maize PAN 6043; (.....) maize PAN 6335; (___ __) sorghum KAT 369; (___ . .) Sorghum NK 283; (_____) pearl millet SDMV 89004; (___ . __) pearl millet SDMV 91018 and (.....) white wheat bread.

Table 11 gives the regression statistics of the fitted models.

Table 11: Regression statistics of the linear models fitted to the data of digestibility against incubation time for white wheat bread and porridges prepared from unrefined flours of maize, sorghum and pearl millet

Sample	Coefficient of Determination (R^2)	Slope	Intercept
Maize PAN 6043	0.964	$0.206^{c,1}$	2.58
Maize PAN 6335	0.964	0.213^{bc}	2.36
Sorghum KAT 369	0.946	0.223^{b}	3.32
Sorghum NK 283	0.957	0.218^{bc}	3.13
Pearl millet SDMV 89004	0.962	0.215^{bc}	2.81
Pearl millet SDMV 91018	0.967	0.207^{c}	1.88
White wheat bread	0.999	0.241^{a}	3.35

1 Slopes with different letters in the superscript are statistically significantly different ($p < 0.05$).

White wheat bread had a significantly higher rate ($p < 0.05$) of *in vitro* starch digestibility than the stiff porridges prepared from unrefined flours of maize, sorghum and pearl millet. Among the stiff porridges, only the stiff porridge from sorghum KAT 369 that had a significantly higher rate ($p < 0.05$) of *in vitro* starch digestibility than maize PAN 6043 and pearl millet SDMV 91018.

Figure 10 compares the rate of *in vitro* starch digestibility of stiff porridges prepared from refined flours of maize, sorghum and pearl millet to that of white wheat bread.

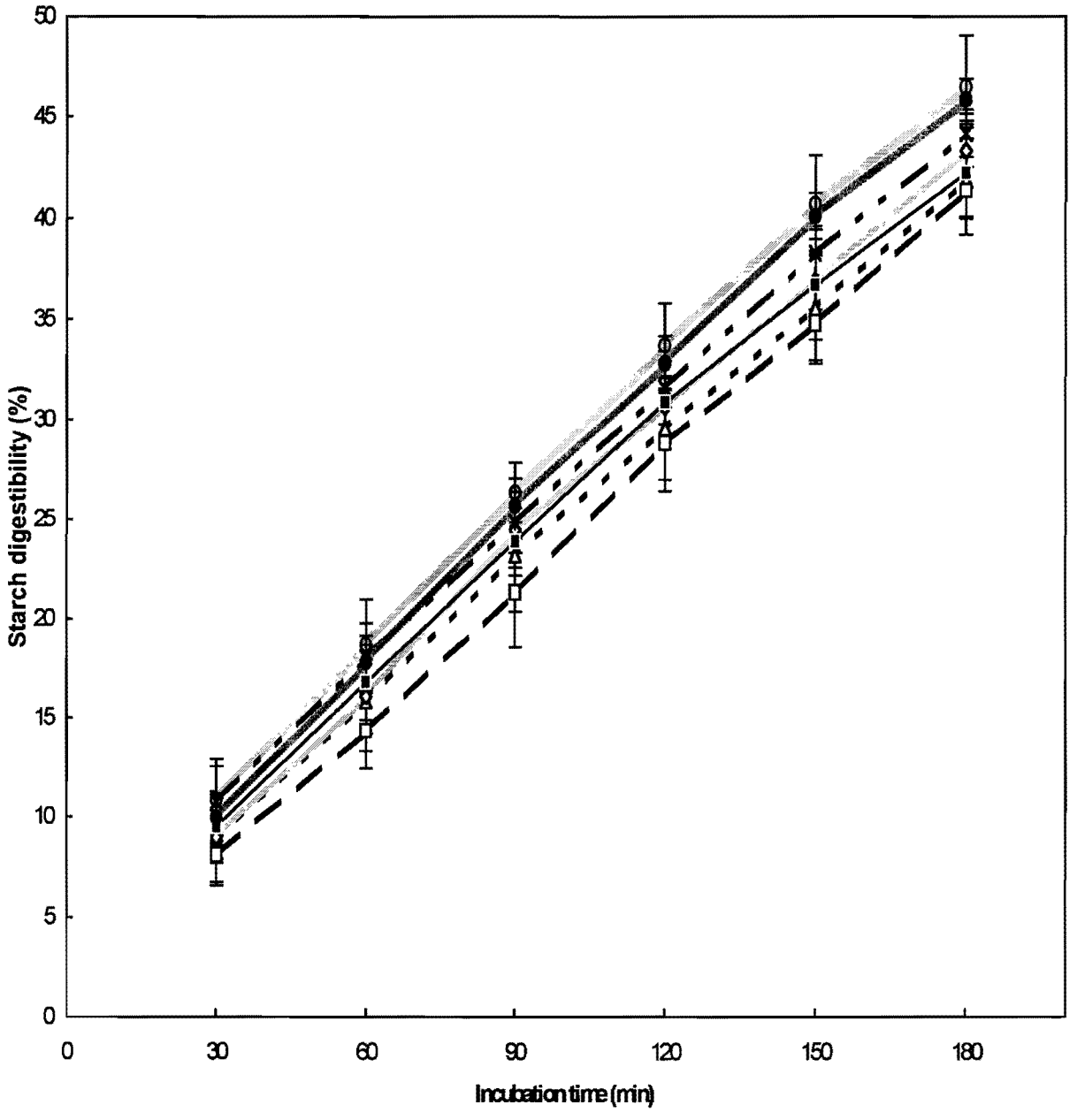

Figure 10: *In vitro* starch digestibility of stiff porridges prepared from refined cereal grain flours compared to white bread. (– – Δ – –) maize PAN 6043; (—— —□ ——) maize PAN 6335; (····· ◊ ·····) sorghum KAT 369; (····· O ·····) sorghum NK 283; (—— · · ✻ ——) pearl millet SDMV 89004; (—— ■ ——) pearl millet SDMV 91018 and (····· ● ·····) white wheat bread.

As seen in Figure 10, there were some differences in the rates of *in vitro* starch digestibility between white bread and the porridges and also between the porridge samples themselves. To make these differences more clear, linear models were fitted to the data. The model is expressed in the equation $y = mx + c$, where y is starch digested (%), x is incubation time (min), m is the slope of the line and c the intercept.

Figure 11 shows the fitted lines of starch digested against incubation time of stiff porridges prepared from refined flours of maize, sorghum and pearl millet compared to that of white wheat bread.

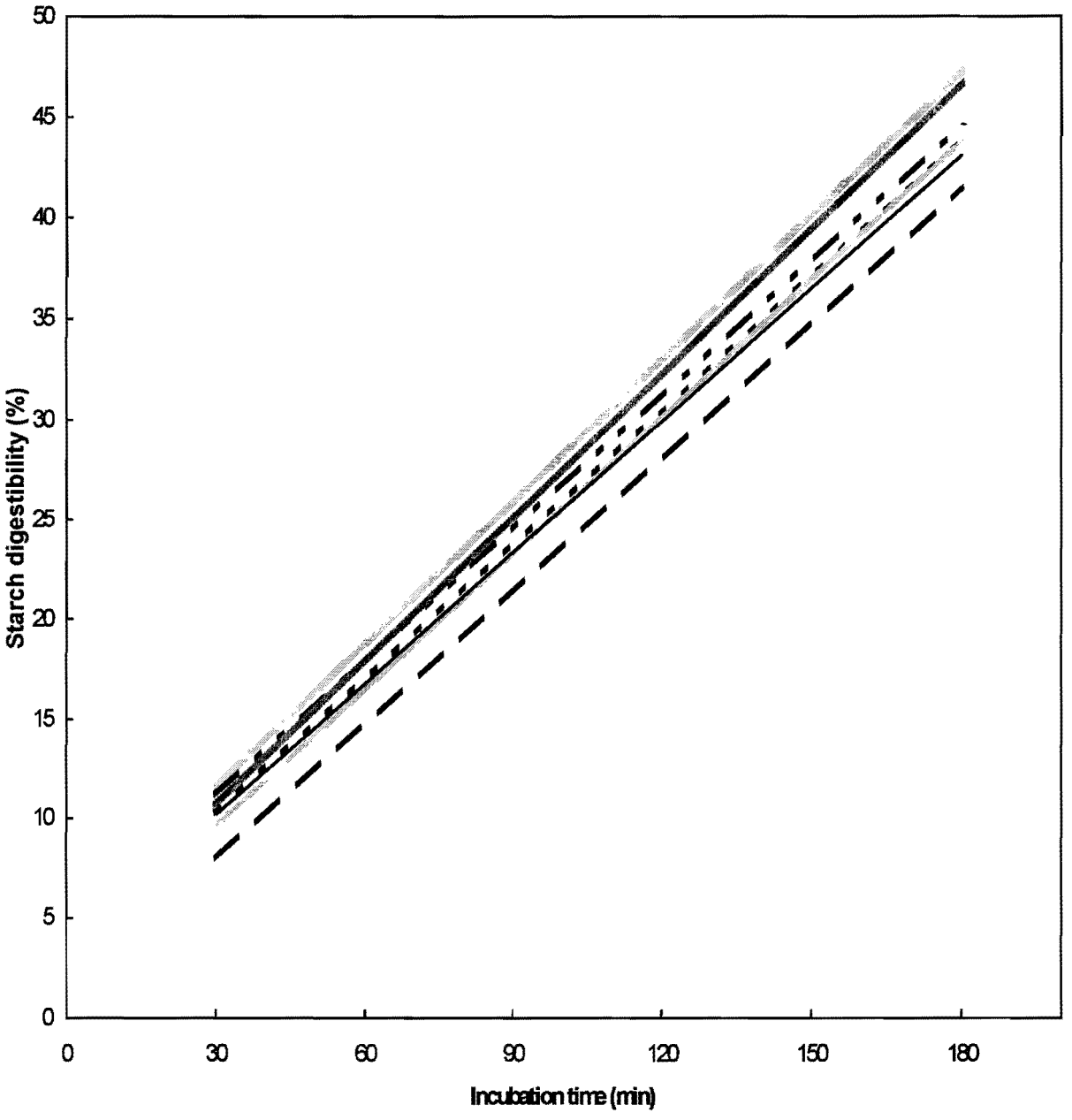

Figure 11: Fitted linear models of percentages starch digested against incubation time of stiff porridges prepared from refined cereal grain flours compared to white bread. (– – –) maize PAN 6043; (—— ——) maize PAN 6335; (.....) sorghum KAT 369; (.....) sorghum NK 283; (—— . .) pearl millet SDMV 89004; (——) pearl millet SDMV 91018 and (.....) white wheat bread.

Table 12 gives the regression statistics of the fitted models.

Table 12: Regression statistics of the linear models fitted to the data of digestibility against incubation time for white bread and porridges prepared from refined flours of maize, sorghum and pearl millet

Sample	Coefficient of Determination (R^2)	Slope	Intercept
Maize PAN 6043	0.953	$0.219^{c,1}$	2.82
Maize PAN 6335	0.972	0.224^{c}	1.28
Sorghum KAT 369	0.980	0.229^{bc}	2.70
Sorghum NK 283	0.969	0.240^{ab}	4.31
Pearl millet SDMV 89004	0.983	0.223^{c}	4.53
Pearl millet SDMV 91018	0.968	0.219^{c}	3.64
White wheat bread	0.999	0.241^{a}	3.35

1 Slopes with different letters in the superscript are statistically significantly ($p < 0.05$) different.

With the exceptional of stiff porridge prepared from sorghum NK 283, white bread had a significantly higher rate ($p < 0.05$) of *in vitro* starch digestibility than all the stiff porridges prepared from refined flours of maize, sorghum and pearl millet. Porridges prepared from refined flours of maize and pearl millet had in general the slowest rate of *in vitro* starch digestibility. There was no significant difference in the rate of *in vitro* starch digestibility between the two sorghum varieties.

Figure 12 compares the rate of *in vitro* starch digestibility of stiff porridges prepared from refined and unrefined flours of maize PAN 6043 to that of white wheat bread.

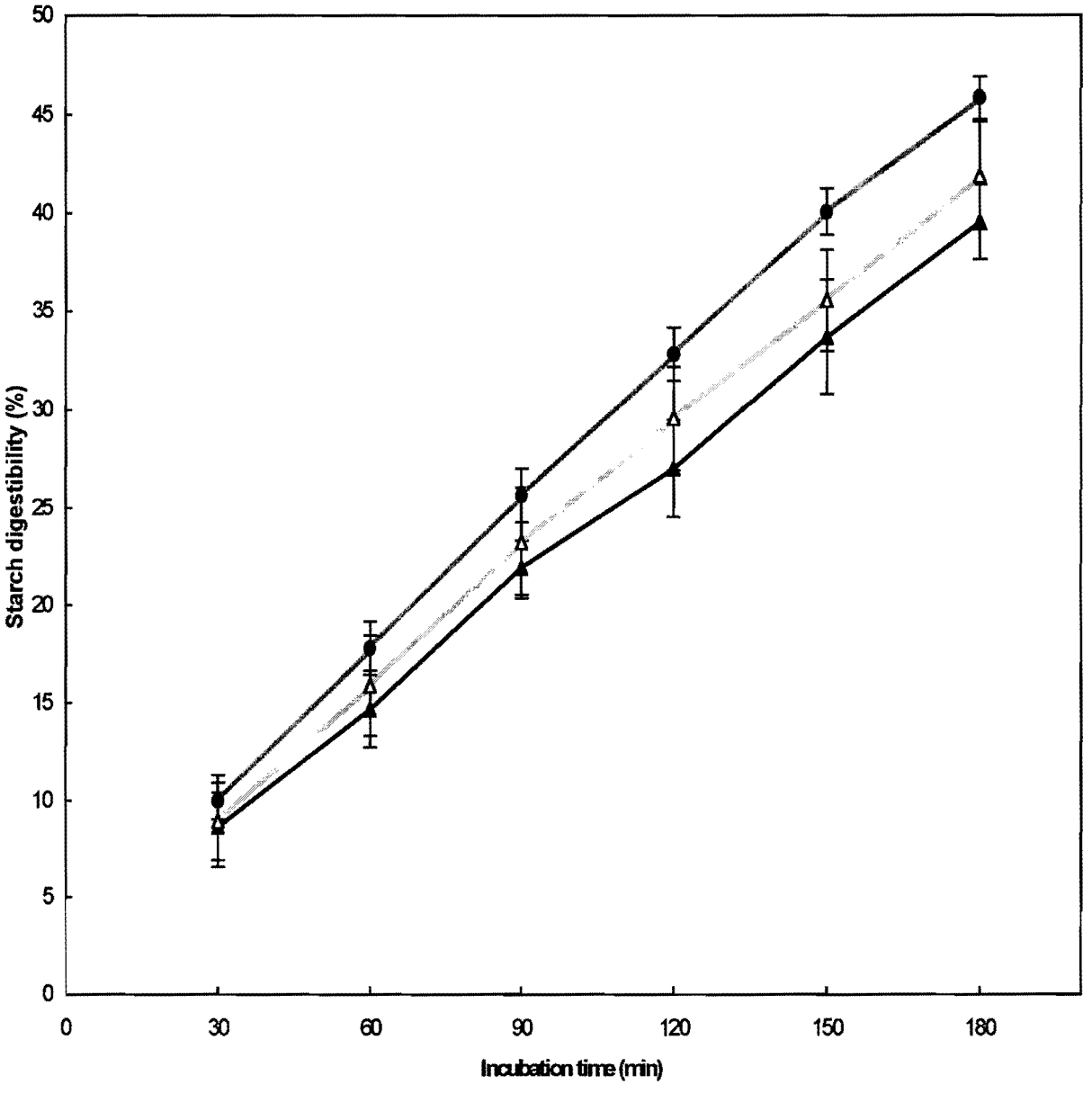

Figure 12: *In vitro* starch digestibility of stiff porridges prepared from refined (Δ) and unrefined (▲) flours of maize PAN 6043 compared to that of white wheat bread (●)

As seen in Figure 12, there were differences in the rates of *in vitro* starch digestibility between white wheat bread and stiff porridges prepared from both refined and unrefined flours of maize PAN 6043. To make these differences more clear, linear models were fitted to the data. The model is expressed in the equation $y = mx + c$, where y is starch digested (%), x is incubation time (min), m is the slope of the line and c the intercept.

Figure 13 shows the fitted lines of starch digested against incubation time of stiff porridges prepared from refined and unrefined flours of maize PAN 6043 compared to that of white wheat bread.

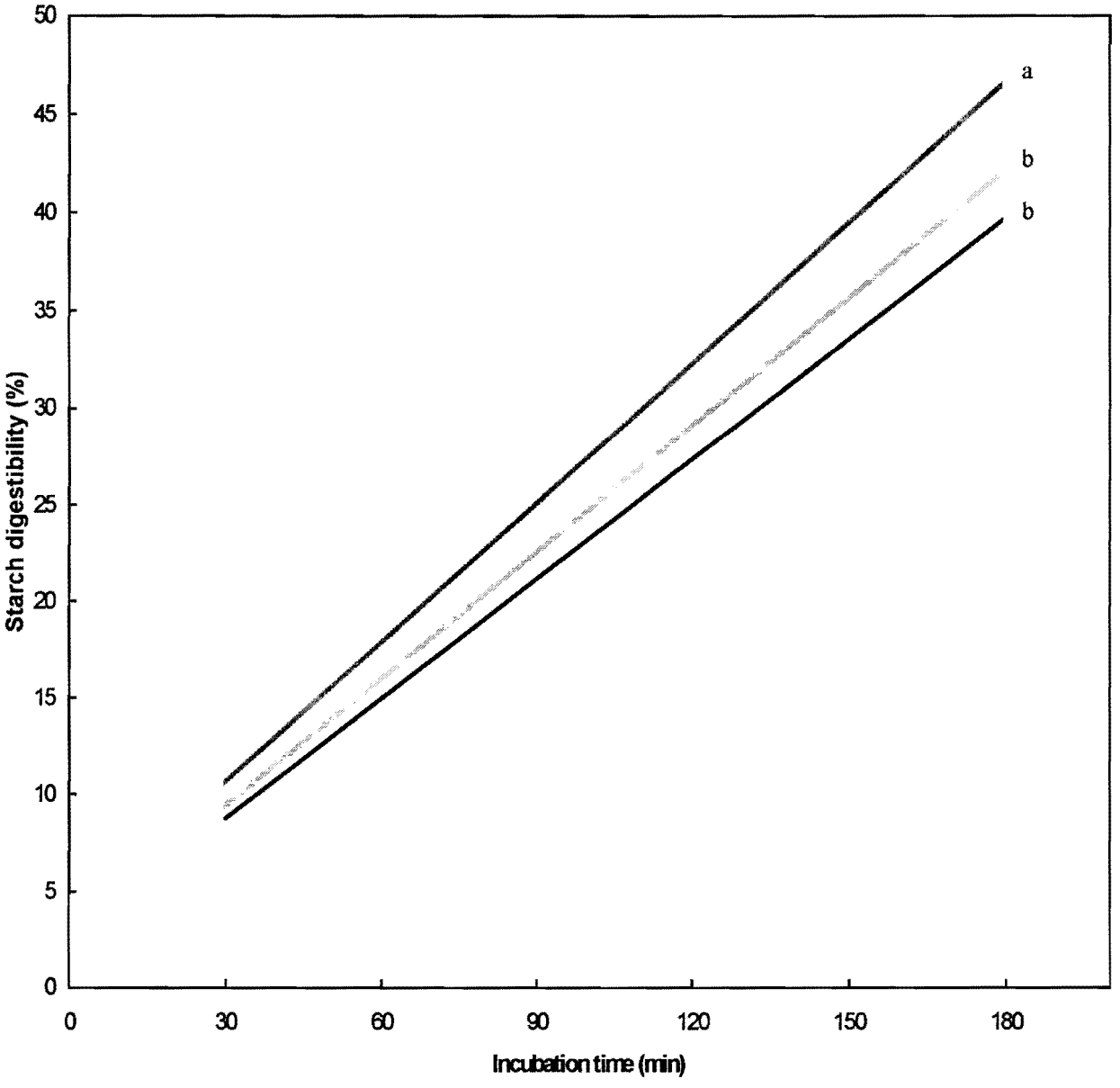

Figure 13: Fitted linear models of percentages starch digested against incubation time of stiff porridges prepared from refined (.....) and unrefined (——) fours of maize PAN 6043 compared to that of white wheat bread (.....)

The fitted lines with different letters show the samples that were significantly different.

The fitted lines in Figure 13 show that white wheat bread had a significantly higher rate ($p < 0.05$) of *in vitro* starch digestibility than those from the stiff porridges prepared from refined and unrefined flours of maize PAN 6043. There was no significant difference ($p < 0.05$) in the rate of *in vitro* starch digestibility between the stiff porridge prepared from refined and that prepared from unrefined flour of maize PAN 6043.

Figure 14 compares the rate of *in vitro* starch digestibility of stiff porridges prepared from refined and unrefined flours of maize PAN 6335 to that of white wheat bread.

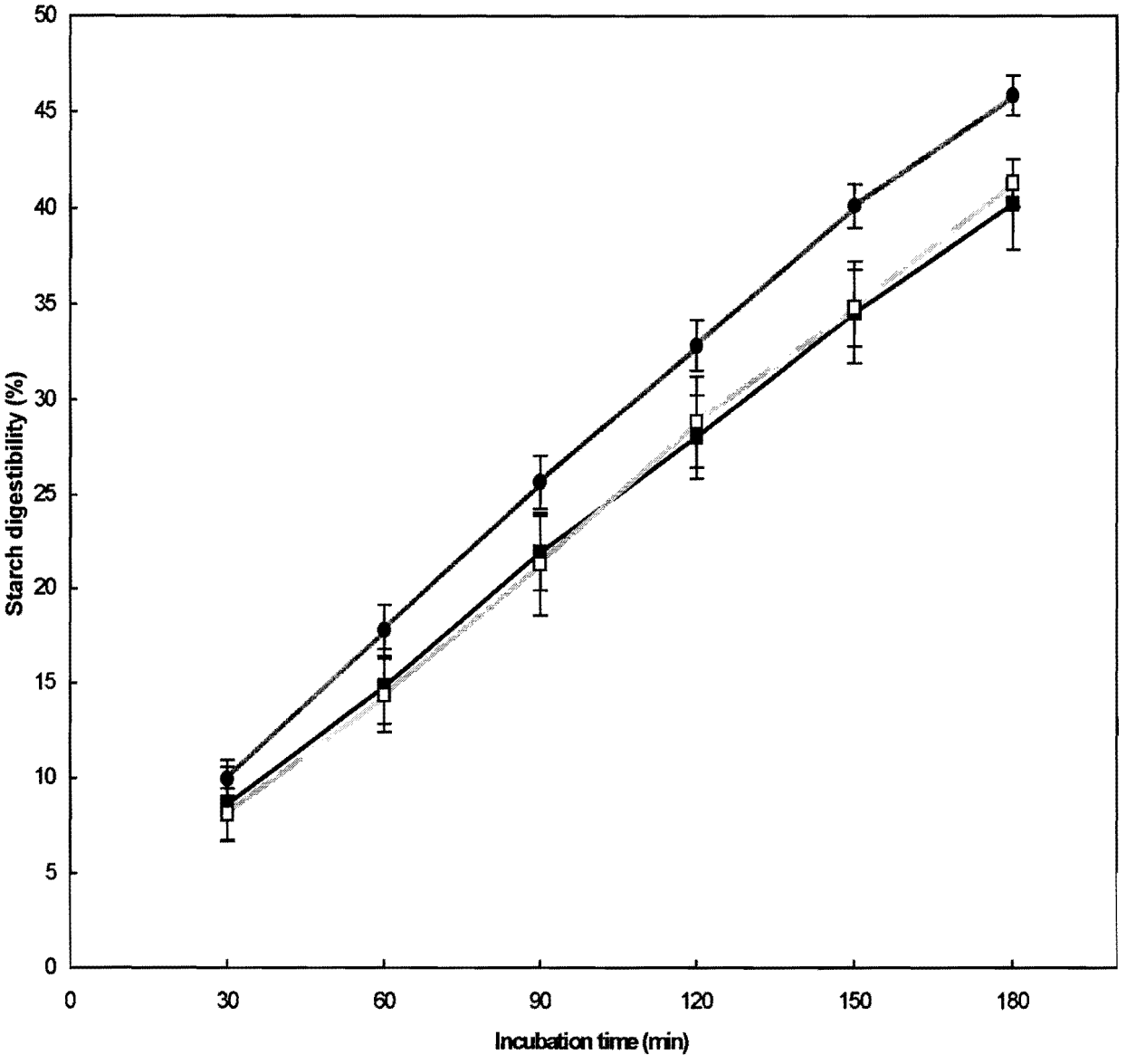

Figure 14: *In vitro* starch digestibility of stiff porridges prepared from refined (□) and unrefined (■) flours of maize PAN 6335 compared to that of white wheat bread (●)

As seen in Figure 14, there were differences in the rates of *in vitro* starch digestibility between white wheat bread and both of the stiff porridges prepared from refined and unrefined flours of maize PAN 6335. To make these differences more clear, linear models were fitted to the data. The model is expressed in the equation $y = mx + c$, where y is starch digested (%), x is incubation time (min), m is the slope of the line and c the intercept.

Figure 15 shows the fitted lines of starch digested against incubation time of stiff porridges prepared from refined and unrefined flours of maize PAN 6335 compared to that of white wheat bread.

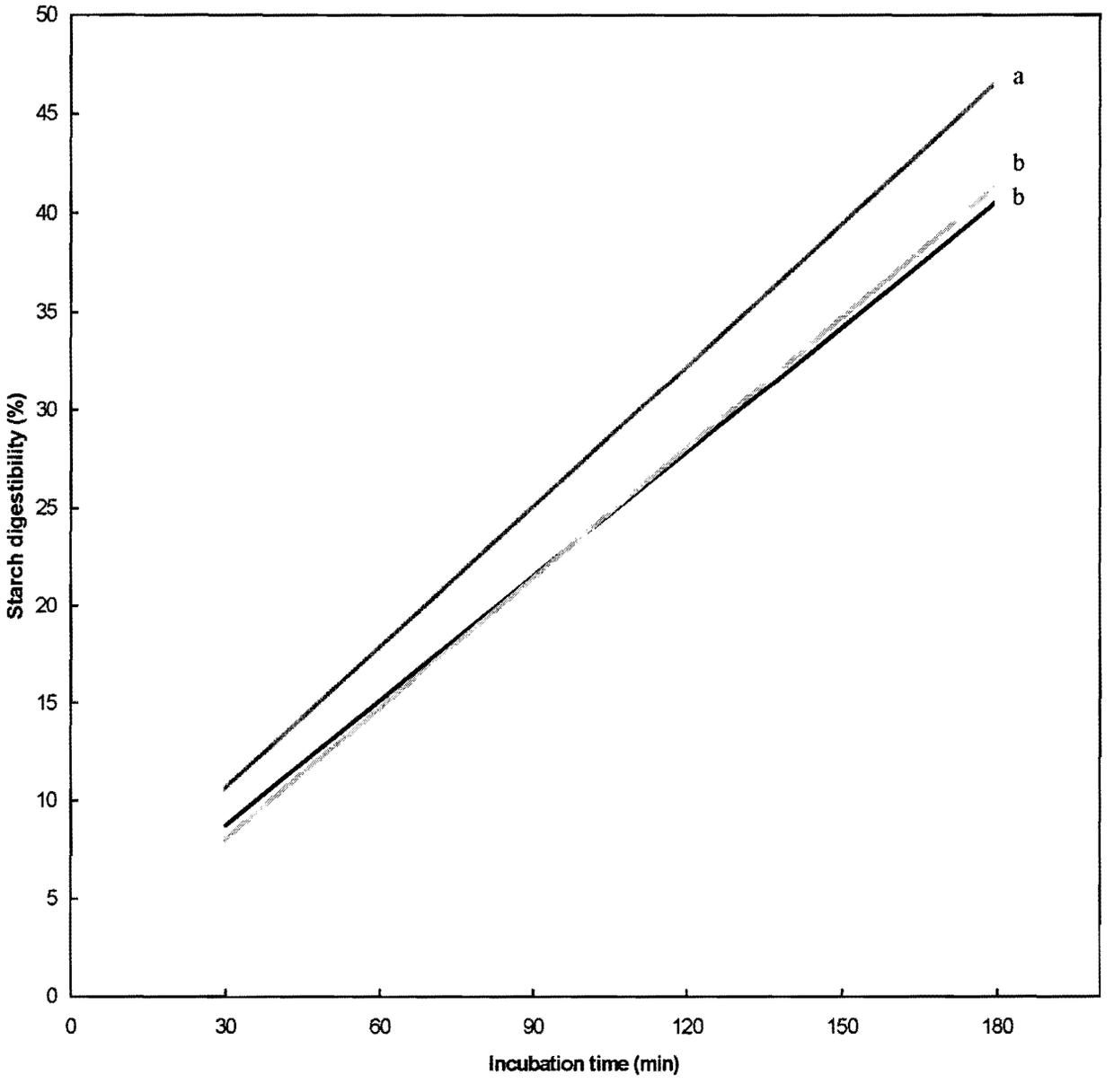

Figure 15: Fitted linear models of percentages starch digested against incubation time of stiff porridges made from refined (.....) and unrefined (——) flours of maize PAN 6335 compared to that of white wheat bread (.....)

The fitted lines with different letters show the samples that were significantly different.

The fitted lines in Figure 15 show that white wheat bread had a significantly higher rate ($p < 0.05$) of *in vitro* starch digestibility than those from the stiff porridges prepared from refined and unrefined flours of maize PAN 6335. There was no significant difference in the rate of *in vitro* starch digestibility between the stiff porridge prepared from refined and that prepared from unrefined flour of maize PAN 6335.

Figure 16 compares the rate of *in vitro* starch digestibility of stiff porridges prepared from refined and unrefined flours of sorghum KAT 369 to that of white wheat bread.

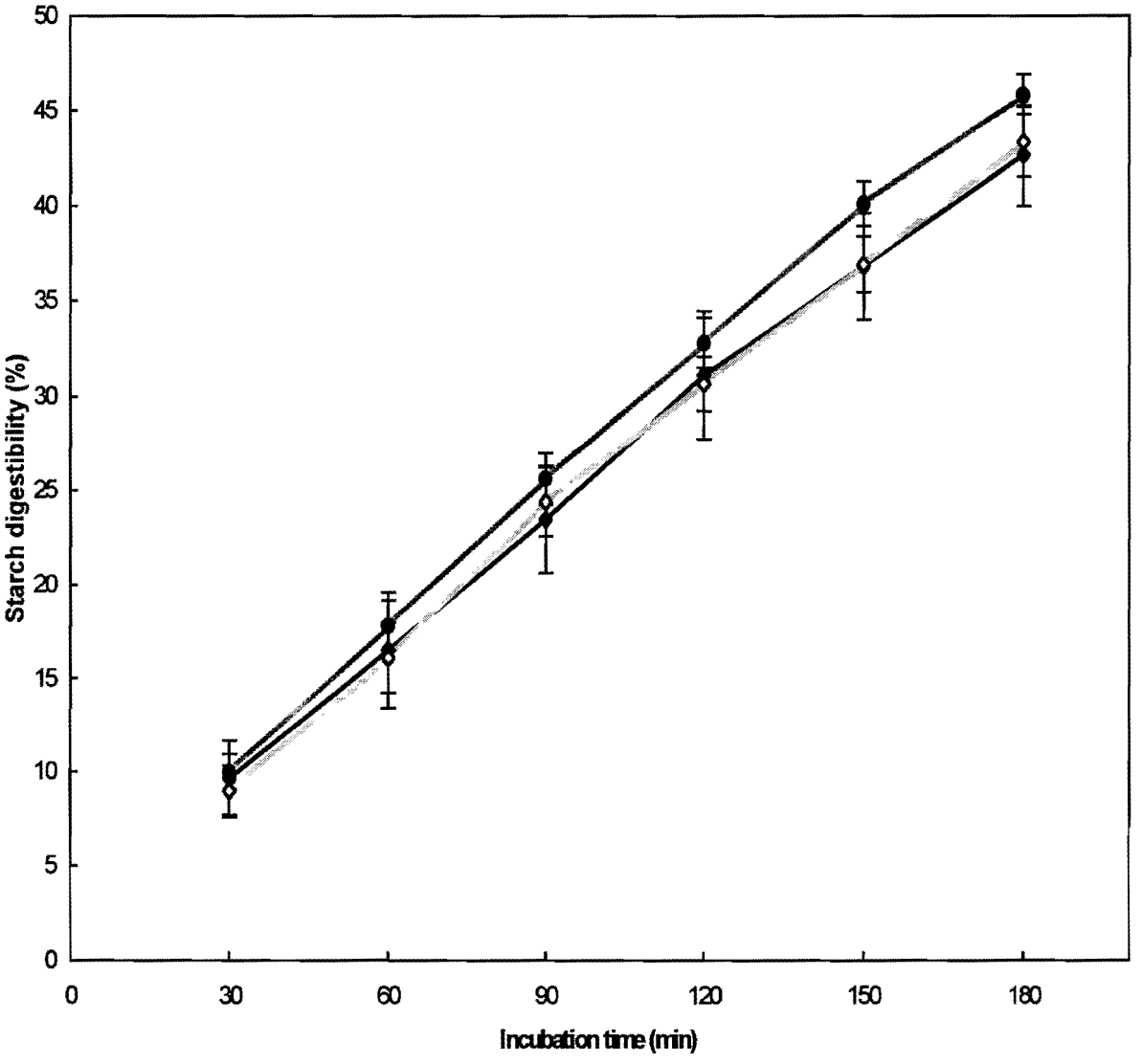

Figure 16: *In vitro* starch digestibility of stiff porridges prepared from refined (◊) and unrefined (◆) flours of sorghum KAT 369 compared to that of white wheat bread (●)

As seen in Figure 16, there were some differences in the rates of *in vitro* starch digestibility between white wheat bread and both of the stiff porridges prepared from refined and unrefined flours of sorghum KAT 369. To make these differences more clear, linear models were fitted to the data. The model is expressed in the equation $y = mx + c$, where y is starch digested (%), x is incubation time (min), m is the slope of the line and c the intercept.

Figure 17 shows the fitted lines of starch digested against incubation time of stiff porridges prepared from refined and unrefined flours of sorghum KAT 369 compared to that of white wheat bread.

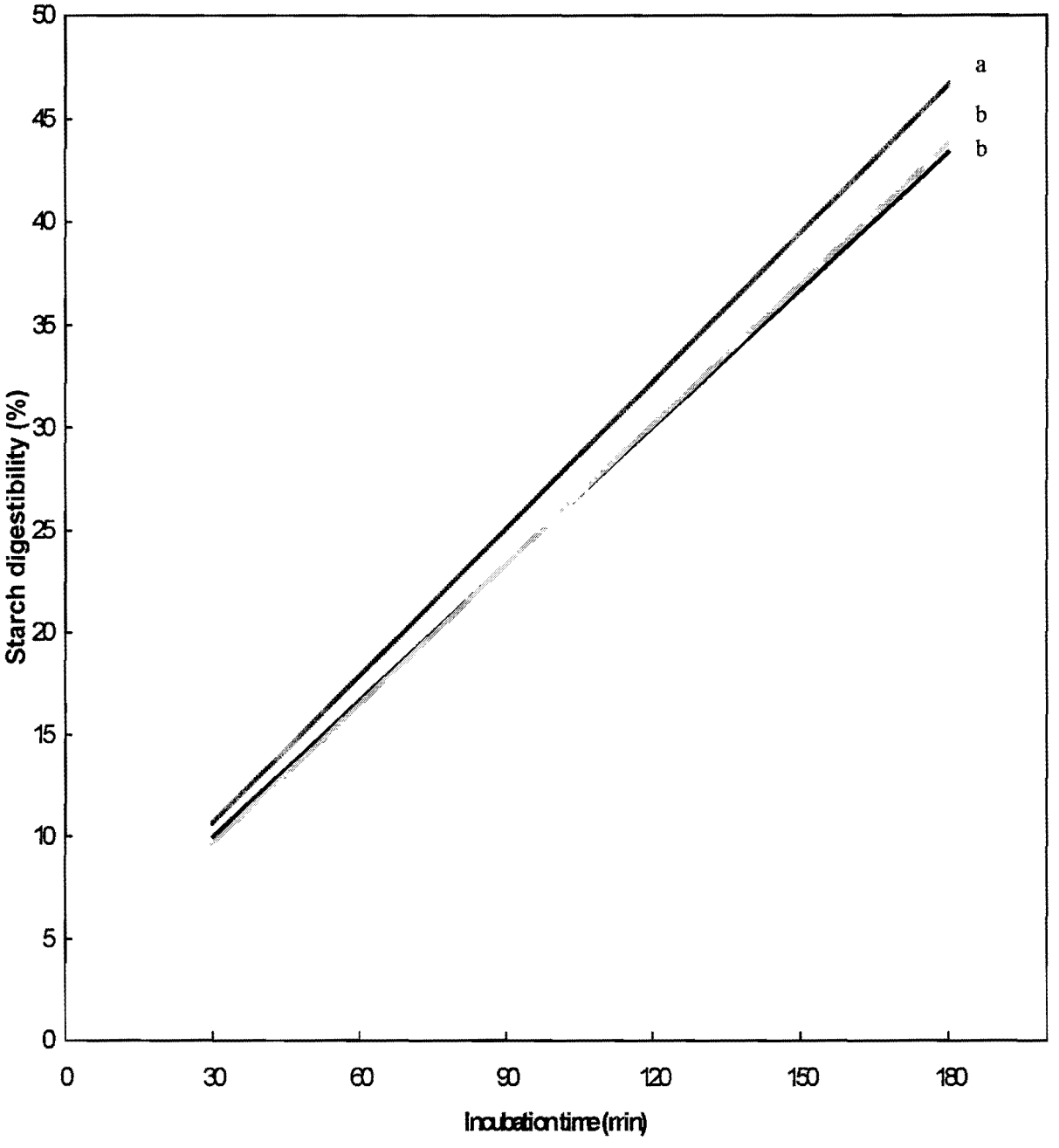

Figure 17: Fitted linear models of percentages starch digested against incubation time of stiff porridges prepared from refined (.....) and unrefined (——) flours of sorghum KAT 369 compared to that of white wheat bread (.....)

The fitted lines with different letters show the samples that were significantly different.

The fitted lines in Figure 17 show that white wheat bread had a significantly higher rate ($p < 0.05$) of *in vitro* starch digestibility than those from the stiff porridges prepared from refined and unrefined flours of sorghum KAT 369. There was no significant difference ($p < 0.05$) in the rate of *in vitro* starch digestibility between the stiff porridge prepared from refined and unrefined flours of sorghum KAT 369.

Figure 18 compares the rate of *in vitro* starch digestibility of stiff porridges prepared from refined and unrefined flours of sorghum NK 283 to that of white wheat bread.

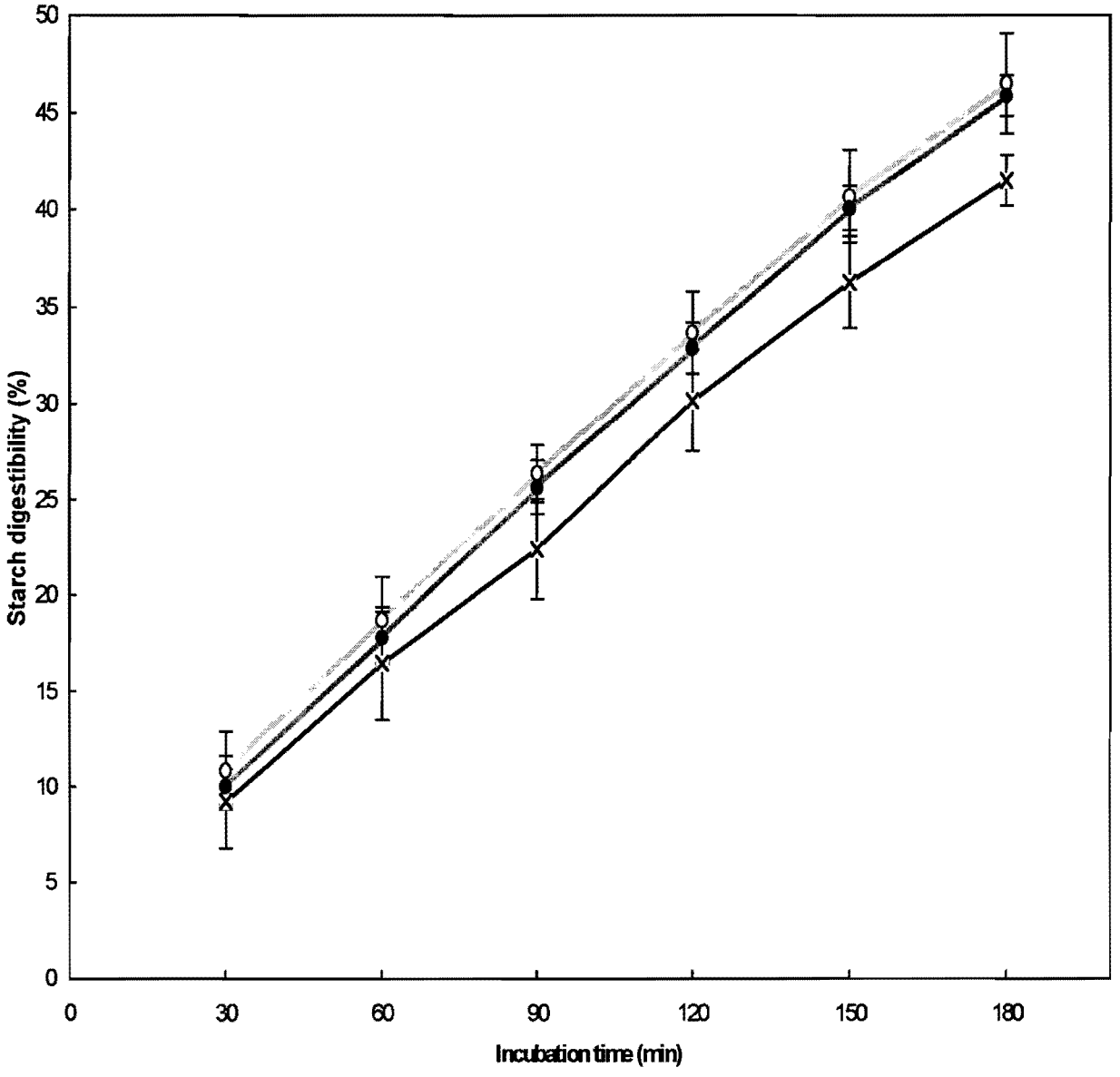

Figure 18: *In vitro* starch digestibility of stiff porridges prepared from refined (O) and unrefined (×) flours of sorghum NK 283 compared to that of white wheat bread (●)

As seen in Figure 18, there was a difference in the rates of *in vitro* starch digestibility between white wheat bread and the stiff porridge prepared from unrefined flour of sorghum NK 283. To make these differences more clear, linear models were fitted to the data. The model is expressed in the equation $y = mx + c$, where y is starch digested (%), x is incubation time (min), m is the slope of the line and c the intercept.

Figure 19 shows the fitted lines of starch digested against incubation time of stiff porridges made from refined and unrefined flours of sorghum NK 283 compared to that of white wheat bread.

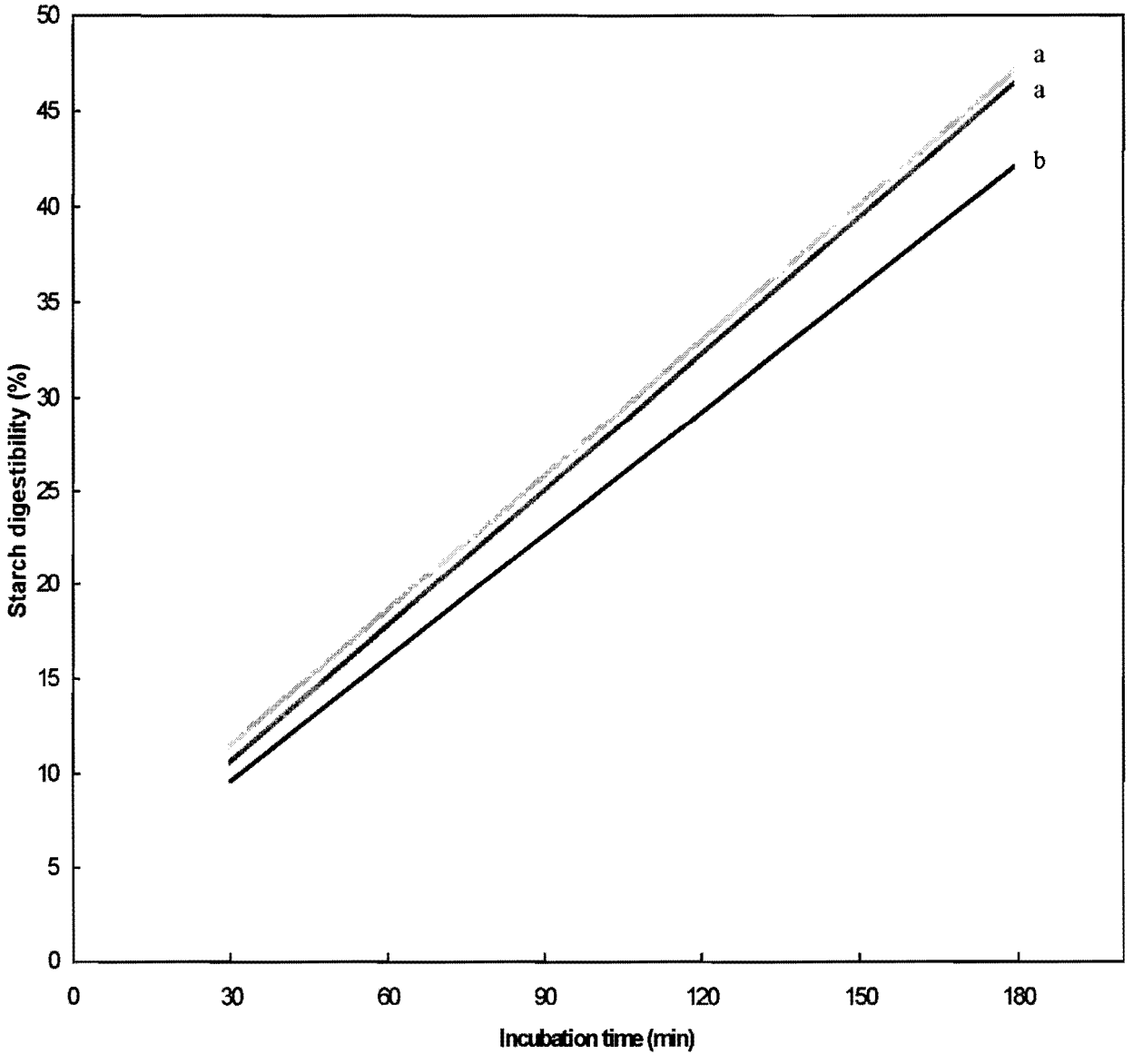

Figure 19: Fitted linear models of percentages starch digested against incubation time of stiff porridges prepared from refined (.....) and unrefined (——) flours of sorghum NK 283 compared to that of white wheat bread (.....)

The fitted lines with different letters show the samples that were significantly different.

The fitted lines in Figure 19 show that white wheat bread had a significantly higher rate ($p < 0.05$) of *in vitro* starch digestibility than that of the stiff porridge prepared from unrefined flour of sorghum NK 283. On the other hand stiff porridge prepared from refined flour of sorghum NK 283 did not differ significantly ($p < 0.05$) from the white wheat bread.

Figure 20 compares the rate of *in vitro* starch digestibility of stiff porridges made from refined and unrefined flours of pearl millet SDMV 89004 to that of white wheat bread.

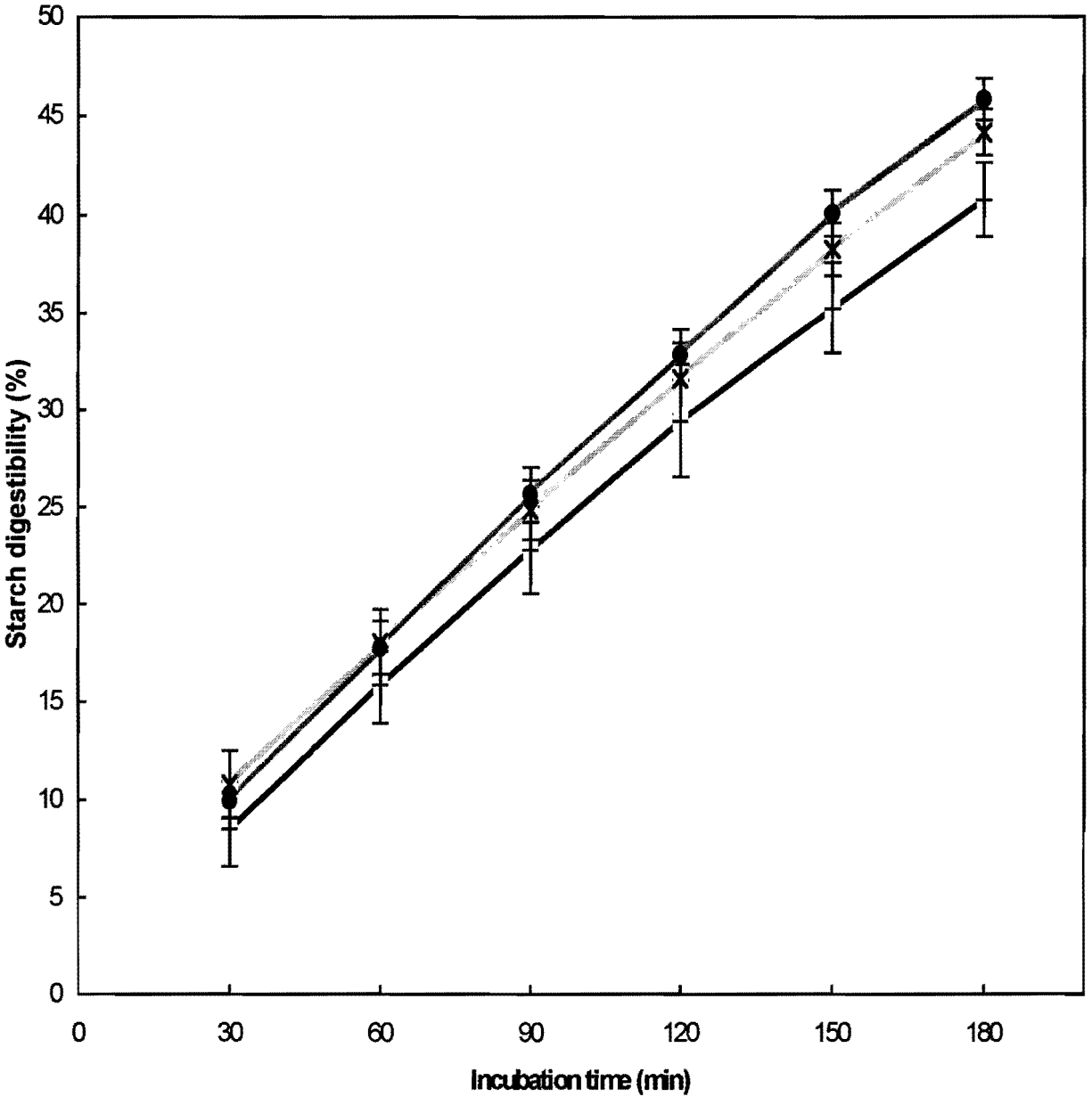

Figure 20: *In vitro* starch digestibility of stiff porridges made from refined (✻) and unrefined (+) flours of pearl millet SDMV 89004 compared to that of white wheat bread (●)

As seen in Figure 20, there were differences in the rates of *in vitro* starch digestibility between white wheat bread and the stiff porridges prepared from refined and unrefined flours of pearl millet SDMV 89004. To make these differences more clear, linear models were fitted to the data. The model is expressed in the equation $y = mx + c$, where y is starch digested (%), x is incubation time (min), m is the slope of the line and c the intercept.

Figure 21 shows the fitted lines of starch digested against incubation time of stiff porridges made from refined and unrefined flours of pearl millet SDMV 89004 compared to that of white wheat bread.

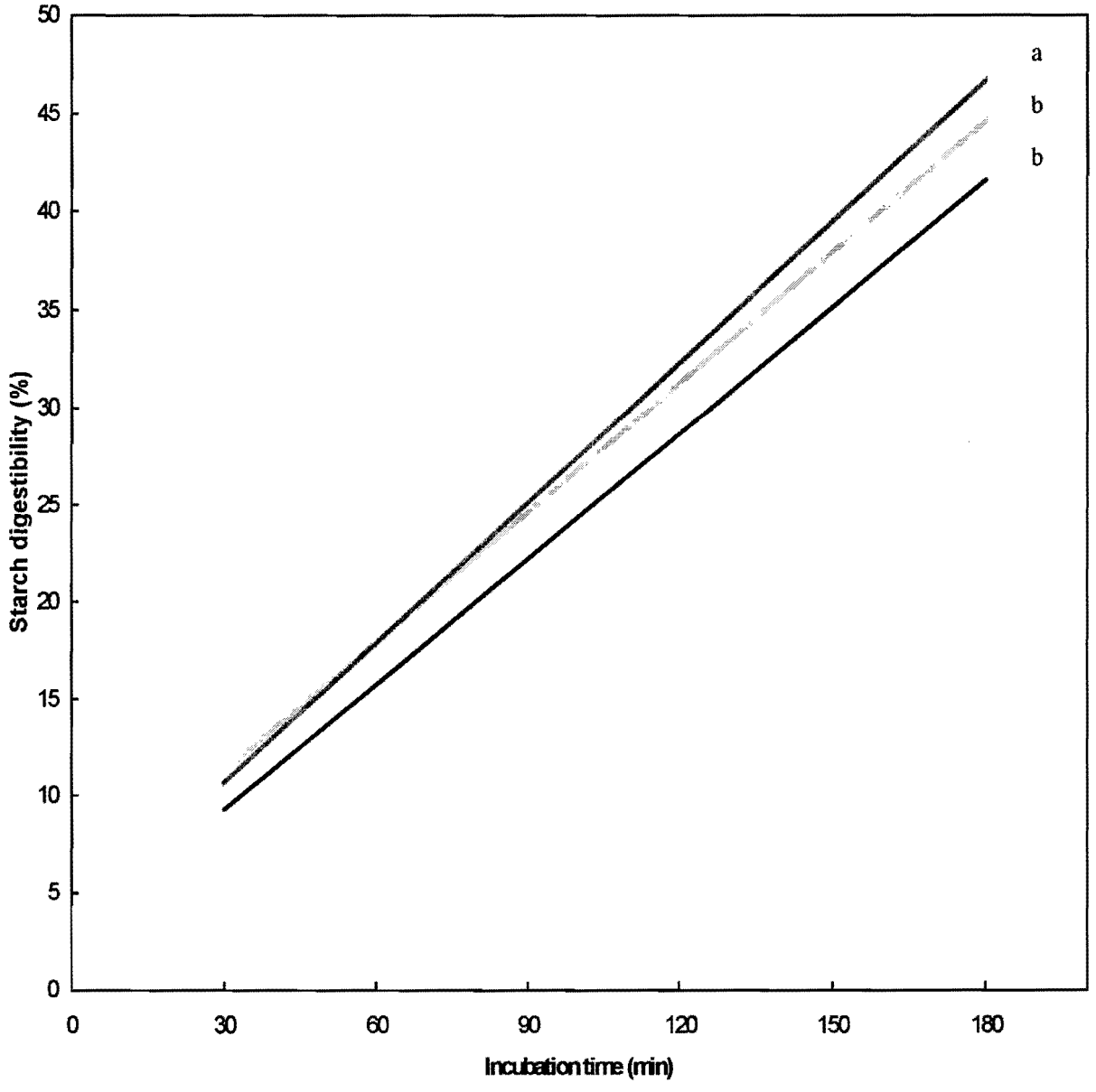

Figure 21: Fitted linear models of percentages starch digested over time of stiff porridges made from refined (.....) and unrefined (——) flours of pearl millet SDMV 89004 compared to that of white bread (.....)

The fitted lines with different letters show the samples that were significantly different.

The fitted lines in Figure 21 show that bread had a significantly higher rate ($p < 0.05$) of *in vitro* starch digestibility than those from the stiff porridges prepared from refined and unrefined flours of pearl millet SDMV 98004. There was no significant difference ($p < 0.05$) in the rate of *in vitro* starch digestibility between the stiff porridge prepared from refined and that prepared from unrefined flour of pearl millet SDMV 89004.

Figure 22 compares the rate of *in vitro* starch digestibility of stiff porridges made from refined and unrefined flours of pearl millet SDMV 91018 to that of white wheat bread.

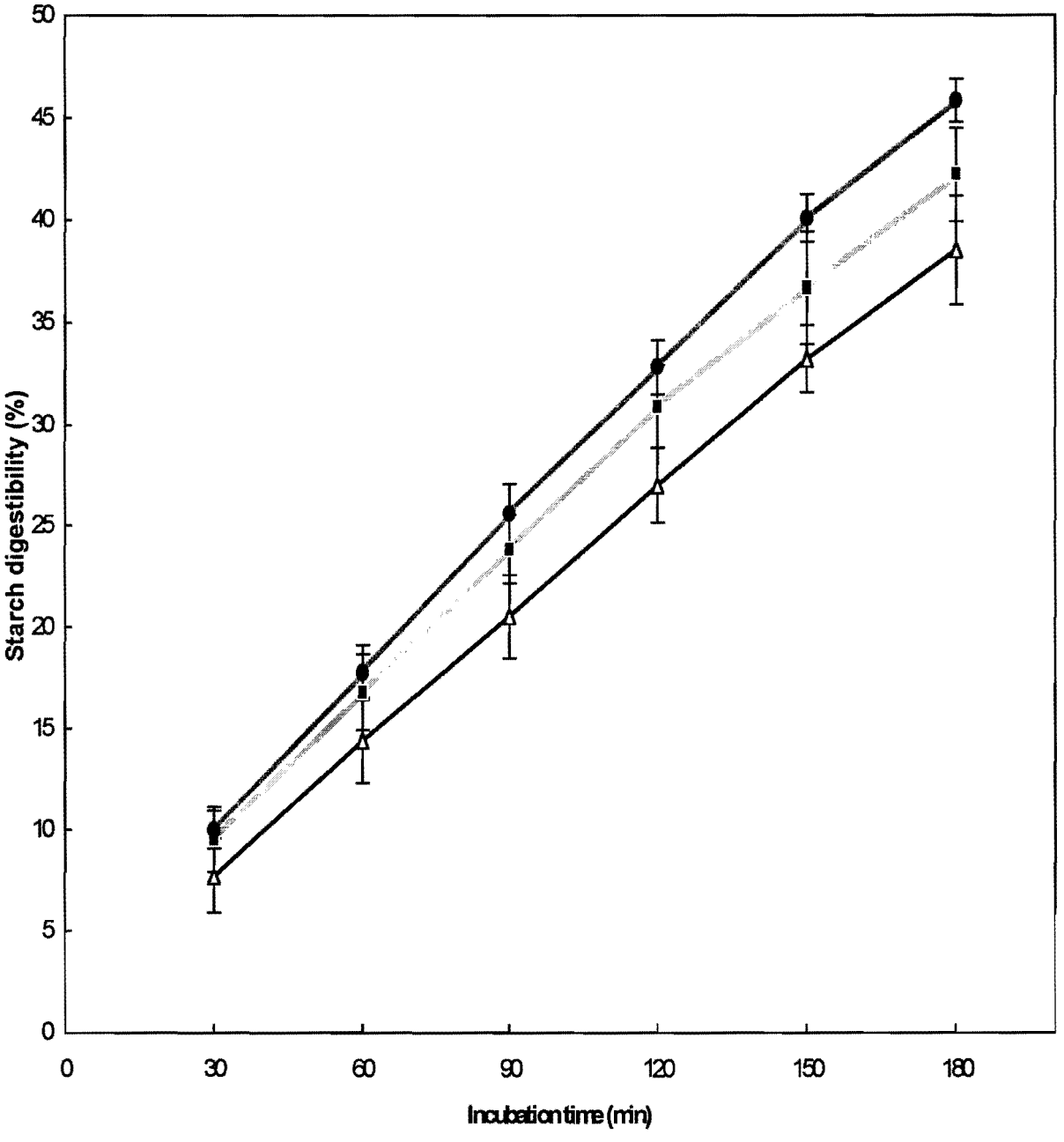

Figure 22: *In vitro* starch digestibility of stiff porridges made from refined (■) and unrefined (Δ) flours of pearl millet SDMV 91018 compared to that of white wheat bread (●)

As seen in Figure 22, there were differences in the rates of *in vitro* starch digestibility between white wheat bread and both of the stiff porridges prepared from refined and unrefined flours of pearl millet SDMV 91018. To make these differences more clear, linear models were fitted to the data. The model is expressed in the equation $y = mx + c$, where y is starch digested (%), x is incubation time (min), m is the slope of the line and c the intercept.

Figure 23 shows the fitted lines of starch digested against incubation time of stiff porridges made from refined and unrefined flours of pearl millet SDMV 91018 compared to that of white wheat bread.

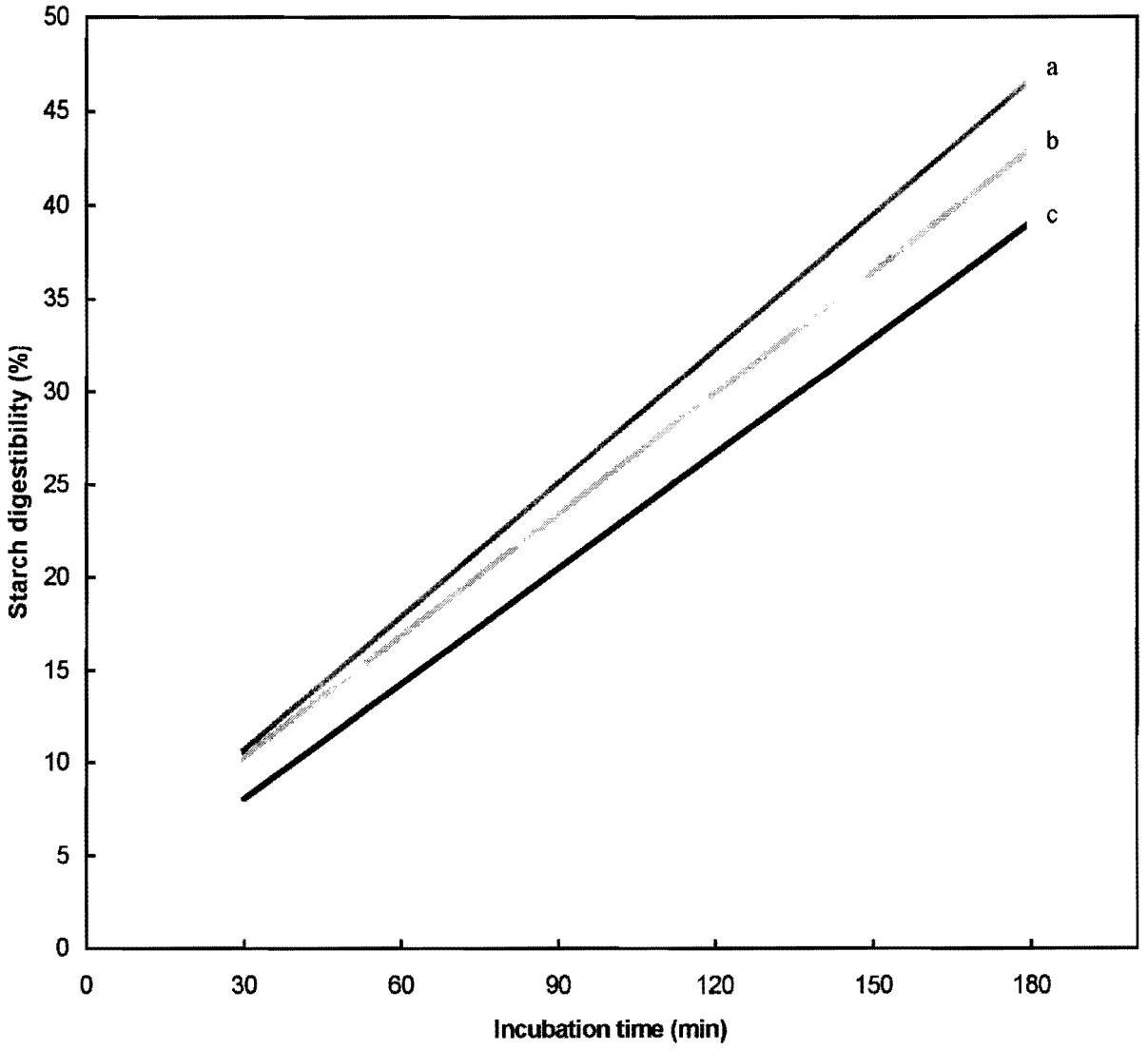

Figure 23: Fitted linear models of percentages starch digested over time of stiff porridges made from refined (.....) and unrefined (——) flours of pearl millet SDMV 91018 compared to that of white wheat bread (.....)

The fitted lines with different letters show the samples that were significantly different.

The fitted lines in Figure 23 show that white wheat bread had a significantly higher rate ($p < 0.05$) of *in vitro* starch digestibility than those from the stiff porridges prepared from refined and unrefined flours of pearl millet SDMV 91018. Also the stiff porridge prepared from refined flour of pearl millet SDMV 91018 had a significantly higher rate ($p < 0.05$) of *in vitro* starch digestibility than that prepared from unrefined flour.

5.6 ANOVA between the unrefined and refined treatments on maize, sorghum and pearl millet

Table 13 below shows the mean percentage starch digestibility of the stiff porridges and the 'F' value from the analysis of variance between the unrefined and refined treatments on the three cereals.

Table 13: Mean percentage starch digestibility and ANOVA between the unrefined and refined treatments on maize, sorghum and pearl millet

Treatments	Mean percentage starch digestibility of the stiff porridges
Unrefined	25.6 (11.4)[1]
Refined	27.4 (12.1)
F = 0.4601 (Not significant)	

1 Values in the brackets are standard deviations.

The results of the analysis of variance between the unrefined and refined treatments showed that, overall, there was no significant difference between the two treatments.

5.7 Hydrolysis index (HI) and predicted glycaemic index (GI)

Granfeldt (1994), according to Akerberg *et al.* (1998) found a significant correlation (r = 0.862) between HI and GI which was used to predict GI by using the equation GI = 0.862HI + 8.198. Table 14 below shows the calculated HI and predicted GI for the bread and stiff porridges by using bread and glucose references.

Table 14: Hydrolysis index (HI) and predicted glycaemic index (GI) of maize, sorghum, pearl millet and bread

Samples	HI	GI (Bread ref.)	GI (Glucose ref.)
Bread	100^{a1} (0)[2]	94^{a} (0)	66^{a} (0)
Maize PAN 6043 Unrefined	92^{bc} (1)	87^{bc} (1)	61^{bc} (1)
Maize PAN 6043 Refined	96^{ab} (1)	90^{ab} (1)	63^{ab} (1)
Maize PAN 6335 Unrefined	87^{c} (6)	83^{c} (5)	58^{c} (4)
Maize PAN 6335 Refined	88^{c} (2)	84^{c} (1)	59^{c} (1)
Sorghum KAT 369 Unrefined	91^{bc} (5)	87^{bc} (4)	61^{bc} (3)
Sorghum KAT 369 Refined	90^{bc} (6)	86^{bc} (5)	60^{bc} (3)
Sorghum NK283 Unrefined	92^{bc} (1)	88^{bc} (1)	61^{bc} (1)
Sorghum NK 283 Refined	102^{a} (0)	96^{a} (0)	67^{a} (0)
Pearl millet SDMV 89004 Unrefined	86^{c} (1)	83^{c} (1)	58^{c} (1)
Pearl millet SDMV 89004 Refined	92^{bc} (6)	88^{bc} (5)	61^{bc} (3)
Pearl millet SDMV 91018 Unrefined	85^{c} (0)	81^{c} (0)	57^{c} (0)
Pearl millet SDMV 91018 Refined	92^{bc} (4)	88^{bc} (4)	61^{bc} (3)

1 Values with different letters in superscript in the same column are statistically significantly different ($p < 0.05$).

2 Values in the brackets are standard deviations.

Bread and the stiff porridges prepared from refined flours of sorghum NK 283 and maize PAN 6043 had a significantly higher ($p < 0.05$) hydrolysis and glycaemic indices than all the other stiff porridges prepared from unrefined and refined flours of maize, sorghum and pearl millet.

Porridges prepared from the unrefined flours of pearl millet SDMV 89004 and SDMV 91018 and unrefined and refined flours of maize PAN 6335 had the lowest HI and GI.

CHAPTER 6

DISCUSSION

In this discussion, the term refinement refers to the milling process that separates wholly or partially, pericarp and germ from the cereal grain before processing to flour. In this study refined cereal grain flour from maize was produced by grinding decorticated and degermed maize grain, while from sorghum and pearl millet was produced by grinding decorticated grains.

Fat contents of the refined flours of both maize varieties used in this study were less than 0.8% which is the amount suggested by Shukla (1981) for high quality refined maize flour. The amount of fat in the refined maize flour was six times lower than that contained in the refined flour of pearl millet which was about 4% and three times lower than that contained in the refined flour of sorghum which was about 2%. According to Kent and Evers (1994) a decortication rate of 20% for sorghum and millet was recommended by the FAO for good consumer acceptance. Owing to the differences in kernel structure and composition between maize and both sorghum and pearl millet, it is impractical to employ the same refinement process trying to achieve the same quality of the end product. Maize being large, flat and also having a relative large germ compared to other cereals, its pericarp and germ are generally separated much easier by the Beall degerminator at high moisture content of 20 to 21% (Alexander, 1987; Hoseney, 1994; Kent and Evers, 1994). In the case of sorghum and pearl millet, decortication appears to be the best method for removing the outer layers (bran) because these grains are nearly round and do not have a crease (Hoseney *et al.*, 1987)

The major problem with the decortication process for sorghum and pearl millet is that a high proportion of the germ is often left with the endosperm. The presence of the high-fat germ, apart from causing rancidity (Hoseney, 1994), can also reduce starch digestibility as will be discussed later.

Sorghum KAT 369 (white) and NK 283 (red) varieties which were used in this study were tannin-free sorghums (Duodu, Tang, Grant, Wellner, Belton and Taylor, in press). The two pearl millet varieties SDMV 89004 and SDMV 91018 used in this study were also tannin-free. Tannins are known to inhibit the activity of α-amylase and consequently reducing starch digestibility (Alonso *et al.*, 2000). In sorghum grain (Rooney *et al.*, 1980) and in pearl millet grain (Reichert *et al.*, 1980) polyphenols are mainly located in the pericarp and testa. Flavonoids, which are present in large amounts in pigmented sorghums, and the polyphenols present in pearl millet might inhibit the activity of α-amylase and consequently reduce the rate of starch digestibility. It is also known that non-tannin polyphenols in sorghum bind strongly to the starch (Reviewed by Bravo, 1998; Beta, Corke, Rooney and Taylor, 2000). This might also reduce starch digestibility.

The grains used in this study were assessed in terms of grain hardness by a visual characterization method. According to a review by Chandrashekar and Mazhar (1999) the physical structure of grains, as in many materials, determines many of their physical properties such as crushing strength, particle size index (PSI) and density. In some cereals like sorghum, the grain hardness or strength can be measured visually by comparing the proportions of the vitreous and floury endosperm.

All the grains used in this study fell in an intermediate category. According to Chandrashekar and Kirleis (1988) the protein bodies from hard grain cooked sorghum flour remained intact and the structure comprising the protein body and matrix remained rigid, with many partially gelatinised starch granules still surrounded by protein. On the other hand, the protein matrix in the softer grains expanded during cooking and liberated starch granules. This suggests that the effect of protein body and matrix in hard grain sorghums on starch gelatinization and probably on starch digestibility might not pose any significant effect in sorghum porridges prepared in this study.

The stiff porridges produced were left to cool to about 50°C in a covered container to avoid rapid cooling and drying up before weighing. By the time the portions of the stiff porridges were chewed they were already at room temperature. During cooling, starch

retrogradation takes place when mainly solubilised and leached-out amylose chains re-associate and form crystalline aggregates and a gelled texture (Baghurst *et al.*, 1996; Thomas and Atwell, 1999). With amylose, unlike amylopectin, retrogradation may be largely complete by the time the product has cooled to room temperature (Whistler and BeMiller, 1997). The crystalline structure resulting from starch retrogradation is of the B-type pattern, which is more resistant to digestion by pancreatic amylase (Englyst *et al.*, 1992; Annison and Topping, 1994). It is therefore likely that amylose was mainly responsible for the formation of retrograded starch in the stiff porridges. This implies that the starch digestibility of stiff porridges containing higher proportions of amylose would probably be lower than those with lower proportions of amylose.

Flour from sorghum NK 283 had a significantly lower ($p < 0.05$) proportion of amylose in the starch than those from all the other varieties of maize, sorghum and pearl millet. According to Hoseney (1994) the ratio of amylose to amylopectin from different cereals is relatively constant and the proportion of amylose in various cereals is about 23 ± 3%. Kent and Evers (1994) reported almost the same proportion of amylose in cereals, i.e. 20 to 35%. The amylose proportion in the flours used in this study fell in the normal range, with sorghum NK 283 flour at the lower limit and all the rest very close to the upper limit. The lower proportion of amylose in sorghum NK 283 starch could render the porridge prepared from more digestible than the other porridges, as a result of less formation of retrograded amylose, as discussed above.

Briefly about the *in vitro* digestibility assay; volunteers were not used to chew the samples, it was done by the researcher. The reasons for not using volunteers were as follows:- There were no reliable volunteers available on a regular basis to do the chewing, and, it eliminated the variation which would have been caused by using different people.

The sizes of the porridge portions chewed from unrefined flours were bigger than those from the refined flours. This was because all portions contained approximately the same amount of starch, and the unrefined flours contained more bran and part of the germ.

Concerning the number of times the samples were chewed; this was done according to the method of Granfeldt *et al.* (1992) chewing 15 times in approx. 15 s. The number of chewing times seemed to be appropriate for bread and maize but was a little high for the softer stiff porridges of sorghum and pearl millet. However, to maintain uniformity, all the porridges and the bread were chewed 15 times. The softer porridges of sorghum and pearl millet disintegrated easily. This probably exposed more surface area and mixed up with more salivary α-amylase on chewing than with the maize porridges possibly increasing starch digestibility. Maize porridges not only were firmer than those of sorghum and pearl millet, but they formed lumps on chewing. Lumps in maize stiff porridges could have reduced the surface area in contact with enzyme solutions (pepsin and α-amylase) and consequently reduced the starch digestibility.

Unlike Granfeldt *et al.* (1992) who used a magnetic stirrer to achieve constant stirring of the buffer solution in which dialysis tubes were suspended, in this study it was stirred for 20 – 30 s before drawing aliquots of the dialysate after every 30 min for analysis. The lack of constant stirring of the buffer solution may possibly have adversely affected the rate of the amount of maltose diffusing from the dialysis tubing to the buffer solution. The reason for this view is based on the assumption that the maltose which was diffusing from inside to the outside of the dialysis tube, could have built up around the tube to a high concentration, due to the lack of stirring and hence slowed down diffusion to the outside. However, according to Wong *et al.* (1985) there was no significant difference between the rate of *in vitro* starch digestibility of red kidney beans carried out in stationary water bath and the one carried out in shaking water bath with 120 oscillations per min. Possible factors which might have contributed to the differences in the rate of *in vitro* starch digestibility of the white wheat bread between this study and Granfeldt *et al.* (1992) study might be the following: different properties of the breads in terms of the endosperm physico-chemical properties of the wheat and the formulation used to make the breads.

Owing to different conditions and small variations in the procedures and the materials used, different researchers have obtained varying results on the rate of *in vitro* starch

digestibilities. In this study, like in other studies, a universal reference material (white wheat bread) was incorporated in each *in vitro* experiment of starch digestibility. This acted as a yardstick against which comparison with other experimental materials were made to check for reproducibility. An example of variation in the results of *in vitro* starch digestibility as a result of using different procedures can be seen between the results of this study and that of Van der Merwe *et al.* (2001). The *in vitro* starch digestibility of white wheat bread measured in this study was 46% while in that of Van der Merwe *et al.* (2001) it was 33%. A major reason for the lower value obtained in the study of Van der Merwe *et al.* (2001) might be the lower number of times of chewing of the bread. As stated in this study, the method of Granfeldt *et al.* (1992) of chewing 15 times in approx. 15 s was used, while in the study of Van der Merwe *et al.* (2001) the bread was chewed only 7 times in approx. 7 s. The higher number of chewings might have broken the bread sample into smaller pieces and resulted in a larger surface area of contact with the enzyme solution. As a result of this, the bread chewed higher number of times would probably have higher *in vitro* starch digestibility.

Concerning the effect of cereal species on the *in vitro* starch digestibility of the stiff porridges compared with bread, the overall results showed that the stiff porridges prepared from the unrefined and refined flours from maize, sorghum and pearl millet of all varieties except that from refined sorghum NK 283 had a lower rate ($p < 0.05$) of *in vitro* starch digestibility than that of the white wheat bread.

The results of this study are in agreement with the results of Van der Merwe *et al.* (2001) who observed that all the maize porridges had a significantly lower ($p < 0.001$) rate of *in vitro* starch digestibility than the white bread. Van der Merwe *et al.* (2001) found a dense structure with no air holes in maize porridges, while bread had an open structure with many air holes. According to Pyler (1992) the crumb structure of bread consists of a porous and resilient protein-structure-lipid matrix that encloses, in honeycomb fashion, minute gas cells that make up most of the loaf volume. Open structure in starchy foods increases the susceptibility to enzymatic action because it increases surface area in contact with the enzyme (Colonna *et al.*, 1990; Seneviratne and Biliaderis, 1991).

In case of this study, there is a possibility that the higher rate of starch digestibility of white bread compared to porridges might have to some extent resulted from different impacts of chewing between the two foods. The impact of chewing bread might be higher than that of chewing porridges, meaning that the break up of the bread portions was higher than that of the porridges, probably due to the open structure of the bread and hence resulting in more contact surface area with the enzyme solutions (pepsin and α-amylase).

Van der Merwe *et al.* (2001) observed lower digestibility of wheat flour porridge than that of wheat bread. This indicates that the preparation method has a great effect on starch digestibility. Bread flour is made into a dough and left to ferment for a period of time, depending on the production technique being used before it is baked in an oven. In South Africa the mechanical dough development process is mainly used for bread making with a fermentation time of about 1 hour. In the process of dough making and during the fermentation period, starch granules become hydrated and probably make heat transfer more efficient and uniform during baking. It is also suggested that the different cooking methods i.e., baking for bread and wet cooking for porridges affects the structure of the food macro-nutrients differently. Baking could probably distort the structure of the starch granules more severely than wet cooking and render the food more digestible due to the fact that it is carried out in an enclosure and the surrounding temperatures are constant and much higher than those in the interior of the food.

As discussed, particle size and hence surface area of the food, does affect the starch digestibility. However, only studies which involved food materials with a large difference in particle size such as whole kernel versus flour have yielded significant differences in starch digestibility (Snow and O'Dea, 1981; Granfeldt *et al.*, 1992). In all the studies done, bread made from grain flours had significantly higher rates of *in vitro* starch digestibility than those made from whole kernels.

In a limited study done by Van der Merwe *et al.* (2001) using one variety of maize, no significant difference in the rate of *in vitro* starch digestibility between stiff porridge

made from maize meal of particle size (< 1 mm) and that made from maize flour of the same particle size as that of bread flour (< 212 μm). The finding of Van der Merwe *et al.* (2001) was in agreement with the observation made by Nelles, Dewar and Taylor (1999), whereby, decreasing the particle size of the maize grits showed no significant effect on the amount of starch that was solubilised after digestion with malt enzymes. From these findings, it seems that, the differences in particle size between the bread flour and the porridge flours (< 800 μm) was not a contributing factor to the significantly higher rate ($p < 0.05$) of *in vitro* starch digestibility of the bread than that of the stiff porridges.

It is possible that there is a point at a certain level of milling of cereal grains whereby almost all of the intact cell walls are disrupted and therefore making them ineffective in resisting the amylase enzymes. This means that any milling of the cereal grains below a certain particle size does not affect the rate of *in vitro* starch digestibility.

The overall results on the effect of cereal species for unrefined and refined flours on the *in vitro* starch digestibility of the stiff porridges showed that there was no effect of cereal species (maize, sorghum and pearl millet).

Possible reasons for the absence of effect of cereal species between maize, sorghum and pearl millet on the *in vitro* starch digestibility of the stiff porridges prepared from unrefined or refined flours could be the following:- The proximate compositions of the three cereals were not very different from one another. According to Hoseney (1994) and Kent and Evers (1994) the three cereals have in common the following important similarities; have similar endosperm structure divided into two parts i.e., corneous and floury parts; have the same 50% gelatinisation temperature of about 67°C; have starch granules with the same shape i.e., polygonal and round. All the three are tropical cereals which utilize C4 photosynthetic pathway (Kent and Evers, 1994).

However, there was a one clear difference in the porridge texture between maize and both sorghum and pearl millet. The results of the porridge texture showed that sorghum and pearl millet flours gave softer porridges than maize. These results agree with the research

of Taylor *et al.* (1997) that sorghums normally produce softer porridges compared to maize. The softer sorghum and pearl millet porridges might be explained by the shear thinning properties of their starches. Subramanian, Hoseney and Bramel-Cox (1994) observed higher shear shinning for cooked sorghum starch than for cooked maize starch. The reasons for differences in shear shinning are not well understood but Jane and Chen (1992) suggested that the fine structure and the molecular weights of amylose and amylopectin in sorghum might be responsible.

In this study it was observed that the macrostructure of the maize porridges seemed to be held together by stronger cohesive forces, and as a result it was more compact, as opposed to sorghum and pearl millet porridges which were loose. As discussed earlier, this situation led to the formation of lumps in maize porridges. Apart from the possibility, that lumps reduced surface area in contact with the enzyme solution, and hence reducing starch digestibility, they also, tightly enclosed some starch granules. The starch granules at the middle of these lumps might have been less accessible to the α-amylase thus composing enzyme-resistant starch Type 1 (RS_1) as a result of physical entrapment (Englyst *et al.*, 1992). This situation, might have also slowed down the rate of *in vitro* starch digestibility in maize porridges.

The results on the effect of cereal species (maize, sorghum and pearl millet) on *in vitro* starch digestibility of stiff porridges were contrary to two of the hypotheses established in this study. One of these hypotheses was that sorghum stiff porridge would have lower rate and extent of *in vitro* starch digestibility than those of maize and pearl millet, due to the rigid protein body and matrix in sorghum imbedding the starch granules and thus limiting both gelatinisation and enzyme accessibility. The second hypothesis was that stiff porridge from unrefined pearl millet, would have lower rate and extent of *in vitro* starch digestibility than its maize and sorghum counterparts, due to its high levels of fat, which forms complexes with amylose and hence lowering starch susceptibility to α-amylase.

Zhang and Hamaker (1998) observed lower starch digestibility in cooked sorghums flours than in cooked maize flour. This was not the case in this study, probably due to the type of sorghums, maize and methods of preparation used; notably, the starch concentration used by Zhang and Hamaker (1998) was 4%, while in this study it was about 20% (1 g of starch was contained in about 5 g of the stiff porridges portions) Zhang and Hamaker (1998) used hard and soft sorghum grains and a maize variety with unidentified properties. Sorghum and maize varieties used in this study were all intermediate and probably the two varieties of sorghum were not affected by the rigid protein body and matrix cover around the starch granules as it would have been the case for hard grain sorghum (Chandrashekar and Kirleis 1988). Also one of the sorghum used, NK 283, had the highest amylopectin-amylose ratio, which is associated with high rate of starch digestibility (Sagum and Arcot, 2000).

The higher fat content of pearl millet was not reflected in the results of *in vitro* starch digestibility of the stiff porridges. One reason might be the slow digestion of the amylose-lipid complex with sufficient enzyme and time (Holm *et al.*, 1983; Seneviratne and Biliaderis, 1991). The presence of complexing lipids affect the re-association behaviour of amylose upon retrogradation of starch and thus reduce the formation of resistant starch Type 3 (RS_3) (Czuchajowska *et al.*, 1991). In a study on the effect of fat on the *in vitro* starch digestibility of lentils and potatoes by Wong *et al.* (1985), they did not find significant difference between the control and fat added samples, even after adding amount of fat equivalent to 50% of the weight of starch contained in the samples. Another possible reason might be the method of *in vitro* starch digestibility determination used. Chewing of the samples before digesting them might have made sorghum and pearl millet more digestible, because they disintegrated easily into cooked flour particles, unlike maize which formed lumps. Lumps formed in maize porridges might have reduced the rate of *in vitro* starch digestibility in maize porridges such that the rates of *in vitro* starch digestibility of both maize and pearl millet were not significantly different.

Concerning the effect of variety on the *in vitro* starch digestibility of the stiff porridges, sorghum NK 283 was more digestible than sorghum KAT 369. The probable reason for

the stiff porridge made from refined flour of sorghum NK 283 to have a significantly higher rate ($p < 0.05$) of *in vitro* starch digestibility could be the significantly lower ($p < 0.05$) proportional of amylose in the starch than in all the other flours. Probably related to this was the significantly softer ($p < 0.05$) porridge from sorghum NK 283 than all the other porridges.

Due to the lower proportion of amylose relative to amylopectin in the flour of sorghum NK 283, it is probable that during cooling, lower amount of enzyme-resistant starch Type 3 (Englyst *et al.*, 1992) was formed in the stiff porridge of NK 283 than in maize and pearl millet stiff porridges. As a result of this condition it is probable that more starch in sorghum NK 283 was available to α-amylase than in maize and pearl millet stiff porridges, hence higher rate of *in vitro* starch digestibility. Sagum and Arcot (2000) observed a negative correlation between amylose content and the rates of starch digestibility of three varieties of rice (Japonica, Inga and Doongara). They attributed the significantly higher rates ($p < 0.05$) of *in vitro* starch digestibility of Inga and Japonica rice to their lower amylose content.

As stated, the higher proportion of amylopectin in sorghum NK 283 was also probably the major reason for the stiff porridge prepared from refined sorghum NK 283 being significantly softer. Mohamed and Hamid (1998) observed a decreased in firmness as the ratio of amylopectin to amylose was increased in rice cakes. Mohamed, Hamaker and Aboubacar (1993) found a significant correlation between sorghum porridge firmness and amylose content. Bello *et al.* (1990) reported that increased amounts of solubilised and retrograded amylose in stiff porridge result in increased firmness. Solomonsson and Sundberg (1994) suggested that high amylose content and longer amylopectin chains may give a firmer texture. Probably highly branched and shorter amylopectin chains which form a "tumbleweed-like" structure (Thomas and Atwell, 1999), as opposed to long chains linear amylose which are more compact is the reason for the softness and easy penetration of the starch digestive enzyme (α-amylase) to the gelatinised starch granules which resulted in higher *in vitro* starch digestibility of sorghum NK 283 porridge.

Porridge prepared from the unrefined flour of sorghum NK 283 did not differ significantly ($p < 0.05$) in the rate of *in vitro* starch digestibility from the other stiff porridges prepared from unrefined flours. The probable reason for this is the relatively high levels of polyphenols in the unrefined flour. As stated, tannins are known to inhibit activity of α-amylase and consequently lowering the rate of starch digestibility (Alonso *et al.*, 2000). It is possible that other polyphenols also inhibit α-amylase activity to some extent. As also stated, non-tannin polyphenols in sorghum are known to bind strongly to the starch (Reviewed by Bravo, 1998; Beta *et al.*, 2000). This could inhibit the hydrolysis of the starch by α-amylase and consequently lowering starch digestibility.

Overall, refinement of the cereal grain flours had no effect on the rates of *in vitro* starch digestibility of the stiff porridges. However, porridges prepared from unrefined flours of sorghum NK 283 and pearl millet SDMV 91018 were significantly lower ($p < 0.05$) in the rates of *in vitro* starch digestibility than those prepared from the refined flours. It should be noted that the unrefined flours of both these grains had relatively high levels of polyphenols.

The results of the effect of refinement on *in vitro* starch digestibility were both contrary to and in agreement with one of the hypotheses of this study which stated the following; the refinement of the cereal grain flour would improve starch digestibility because the bran (pericarp, testa and aleurone layer), antinutritional substances and the fat from the germ which might interfere with the accessibility of the amylase enzymes to starch granules would be completely or partially removed.

The reasons for the refinement of the cereal grain flours in general having no effect on the *in vitro* starch digestibility of the stiff porridges might be the following: Despite the great reduction in fat in refined flours of maize, there were no significant differences in the rates of *in vitro* starch digestibility between the stiff porridges prepared from unrefined and refined flours. This suggests that, lower amount of fat in the refined flours as opposed to large amount of fat in the unrefined flours, did not affect the rates of *in vitro* starch digestibility between stiff porridges prepared from unrefined and refined

flours. Interaction between amylose and lipids (fat) has already been discussed in this chapter. Despite the fact that amylose-lipid complex is highly resistant to α-amylase *in vitro*, compared to free amylose in solution, complete digestion of the complex is obtained when sufficient enzyme and time is used (Holm *et al.*, 1983; Seneviratne and Biliaderis, 1991). It might be that the amount of enzyme and 3 hours of incubation used were enough for complete digestion of the amount of complexes formed, and hence there were no differences between stiff porridges prepared from unrefined and refined flours.

Concerning fibre, using ash as a rough indicator for the bran content in the varieties used in this study, it can be seen that the highest reduction of the bran was achieved in the refined flours in maize. However, there were no significant differences in the rates of *in vitro* starch digestibility between the stiff porridges prepared from unrefined and refined flours. This suggests that, lower amount of the bran in the refined flours as opposed to the large amount of the bran in the unrefined flours, did not affect the rates of *in vitro* starch digestibility between stiff porridges prepared from unrefined and refined flours.

These results are in agreement with a related and limited study by Zhang and Hamaker (1998). Using only one sorghum cultivar with different levels of decortication, they did not find significant differences in cooked starch digestibility due to decortication, even when the level of decotication was raised from 0% (whole flour) with a corresponding total starch content of 80% to the level of 30% with corresponding total starch content of 93%. A significant difference was only observed when the materials removed from decortication were pooled together to form a concentrate of which the total starch content was 55%. This shows that bran can reduce starch digestibility when it is in a concentrated form, or when the bran present in the unrefined flours is supplemented with the bran from external source.

The results, also, agree with the findings of Snow and O'Dea (1981) who observed that whole brown rice was hydrolyzed more slowly ($P < 0.001$) than whole white rice, showing that, the intact bran was responsible for the lower hydrolysis rate. But when brown rice was ground it was hydrolyzed at the same higher rate as ground white rice,

despite the presence of the same amount of fibre. The fact that both brown and white rice after milling had higher and the same starch digestibility, led these authors to conclude that cereal fibre by itself, does not appear to affect the rate of starch digestibility. It only does so, when it forms a physical barrier limiting access of the hydrolytic enzymes to the starch. Similar observations have been made between the wholemeal and white breads (Snow and O'Dea, 1981; Granfeldt *et al.*, 1992; Bravo *et al.*, 1998).

However, Liljeberg *et al.* (1992) and Granfeldt *et al.* (1992) observed that whole kernel breads made from wheat, rye and barley displayed significantly lower GI than did the corresponding breads prepared from milled flour. This phenomenon shows that the presence of an organized kernel structure is more critical than the naturally occuring soluble dietary fibre in oats and barley. Intact structures provide starch that is physically inaccessible to amylase enzymes due to the barrier created by the cell walls and hence causing lower digestibility (Snow and O'Dea 1981; Colonna *et al.*, 1992; Bravo *et al.*, 1998).

From the above discussion regarding the cereal grain fibre, it appears that the milling process of cereal grains, which reduces the whole kernels to coarse grain and eventually to flour, also break up the bran and the cell walls. At the end of this process, it is possible that only few intact cell walls are left, which cannot resist swelling during gelatinization and restrict penetration of the hydrolytic enzymes during digestion. As a result of this the cereal bran which was present in the unrefined flours in higher proportion than in the refined flours seemed to have no effect on the rates of *in vitro* starch digestibility of the stiff porridges.

However, refinement of the cereal grain flours with a relatively high levels of polyphenols did have effect on the rates of *in vitro* starch digestibility of the stiff porridges. The probable reason for the stiff porridges made from refined flours of sorghum NK 283 and pearl millet SDMV 91018 having significantly higher rates ($p < 0.05$) of *in vitro* starch digestibility than those prepared from unrefined flours of the same varieties could be the following:

As discussed earlier in this chapter, all the varieties of sorghum and pearl millet used in this study were tannin-free. However, the analysis of polyphenols indicates that, despite of being generally low, the amounts of polyphenols in the flours used in this study were relatively highest in the unrefined flours of sorghum NK 283 and pearl millet SDMV 91018. However, in the refined flour of pearl millet SDMV 91018 polyphenols were reduced by 85% and in the refined flour of sorghum NK 283 reduced by 40% through the decortication process. Tannins are known to inhibit the activity of α-amylase and consequently lowering starch digestibility (Alonso *et al.*, 2000). These non-tannin polyphenols might also reduce α-amylase activity. Thus, the reduction of these non-tannin polyphenols in these two refined flours might be the reason for the stiff porridges prepared from these flours to have higher rate ($p < 0.05$) of *in vitro* starch digestibility than those prepared from unrefined flours with higher levels of polyphenols. As stated, non-tannin polyphenols in sorghum are also known to bind strongly to the starch (Reviewed by Bravo, 1998; Beta *et al.*, 2000). This could inhibit the hydrolysis of the starch by α-amylase and consequently lowering starch digestibility.

As stated, in nutritional studies of starch digestibility there are two commonly used indices; these are hydrolysis index (HI) used for *in vitro* and glycaemic index (GI) used for *in vivo* studies. Definitions of GI and HI were given earlier. According to Mendosa (1999) GI is especially useful to diabetics who want to plan their diets to minimize the incidence of high blood sugar, or spikes.

All the average predicted GIs based on glucose standard of the porridges from the three cereals (maize, sorghum and pearl millet) measured in this study fell into an intermediate group (GI between 56 and 69) if the classification of Perlstein *et al.* (1997) is used. As it can be seen on page 96 and in Table 14, using the equation GI = 0.862HI + 8.198 gave the bread a GI of 66 (glucose reference) which is a somewhat lower value than the expected. The predicted GI of 66 for bread would make it an intermediate GI food instead of high GI food and therefore misleading. In this study the classification of Perlstein *et al.* (1997) is not applicable due to a slightly low correlation value between HI and GI (r =

0.862) used in the equation to predict GI and as a result led to obtaining lower figures than those obtained from *in vivo* studies. In the case of maize stiff porridge, the predicted GI was within the range observed by Venter *et al.* (1990) which is from 50 to 66 showing that maize porridge is a slow to intermediate starchy digested food. However, the predicted GI for maize was slightly higher than that found by Van der Merwe *et al.* (2001) of 44, probably due to the lower number of chewing times of the stiff porridge used by these authors. The predicted GIs of the porridges from the three cereals and the bread are only estimations making use of the *in vitro* starch digestibility results obtained in this study, combined with correlations between *in vitro* and *in vivo* results obtained in studies done by other researchers who used the same *in vitro* method.

The bread had higher ($p < 0.05$) GI than all the stiff porridges except that from refined flours of sorghum NK 283 and maize PAN 6043. The reasons for the bread to have higher GI are the same as those discussed previously on the rate of *in vitro* starch digestibility results. There was no effect of species on the GIs, however stiff porridge from refined sorghum NK 283 and PAN 6043 had a higher ($p < 0.05$) GI than the other stiff porridges. The probable reason for the higher GI of the stiff porridge prepared from refined flour of sorghum NK 283 than the other porridges, has been discussed previously as due to its high amylopectin-amylose ratio. Also, already discussed, the reason for the lower GI of the stiff porridge prepared from unrefined flour of sorghum NK 283 than that prepared from refined flour of sorghum NK 283 could be due to the relatively high levels of non-tannin polyphenols in the unrefined flour. The probable reason for the higher GI of the stiff porridge prepared from refined flour of maize PAN 6043 than the other porridges is attributed to the methodology of computing hydrolysis index which only considers the ratio of the area under the curve for the text product to the area under the curve for the reference (bread). As it has been discussed previously, the rate of *in vitro* starch digestibility of stiff porridge prepared from refined flour of maize PAN 6043 was not different from the other stiff porridges. Significant differences between the rates of *in vitro* starch digestibility of different stiff porridges were calculated by using equation involving the differences of the slopes of two straight lines after linear regression. The steeper the line the higher the rate.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

All the stiff porridges prepared from unrefined and refined flours of the three cereals used in this study (maize, sorghum and pearl millet), except that from refined flour of sorghum NK 283, were less digestible than that of the white wheat bread.

Cereal species (maize, sorghum and pearl millet) does not affect the rates of *in vitro* starch digestibility of the stiff porridges.

Probably due to the higher proportion of amylopectin in the starch, porridge from refined flour of sorghum NK 283 was more digestible than the porridges from other varieties. However, the stiff porridge made from the unrefined flour did not show this effect probably due to relatively high levels of non-tannin polyphenols in the flour.

Non-tannin polyphenols appear to lower starch digestibility of the stiff porridges prepared from flours containing high levels of polyphenols.

Refinement of cereal grain flours does not improve the rates of *in vitro* starch digestibility of the stiff porridges prepared from low non-tannin polyphenol grains but it does so for the stiff porridges prepared from relatively high non-tannin polyphenol grains.

Based on the findings of this study, diabetic people can use maize, sorghum or pearl millet in unrefined or refined forms without discrimination as none of them differ significantly in-terms of GI. None of the three cereals can be claimed as more suitable than the others in diabetes management. However, if there are varieties known to have a high amylopectin/amylose ratio in their starches, like sorghum variety NK 283, they should be avoided as a diet for diabetic people, because this type of starch is associated with higher rate of starch digestibility and hence higher GI which is unsuitable for diabetics. On the other hand, varieties known to contain relatively high levels of non-

tannin polyphenols may be useful for diabetics in the unrefined form, as these grains have shown both lower starch digestibilities and GIs.

The major difference between this study and that of Zhang and Hamaker (1998) was that in this study no significant differences in the rates of *in vitro* starch digestibility were found, neither between the stiff porridges prepared from the intermediate grains of maize and sorghum, nor, between maize and pearl millet. This appears to be contrary to the findings of who found that cooked sorghum flours had lower starch digestibility (15 – 25%) than normal maize flour, regardless of whether the endosperm type was floury, dense floury or vitreous. Regarding the differences, more studies are required on how different porridge preparations methods (procedure, water addition, starch concentration, cooking, cooling rate, storage time) impacts on the formation of more enzyme-resistant starches. Also studies on how various preparation steps might interact with grain components (or types) with different hardness levels are required before considering possible relationships between varying levels of hardness of different grains and the GI.

CHAPTER 8

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