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).

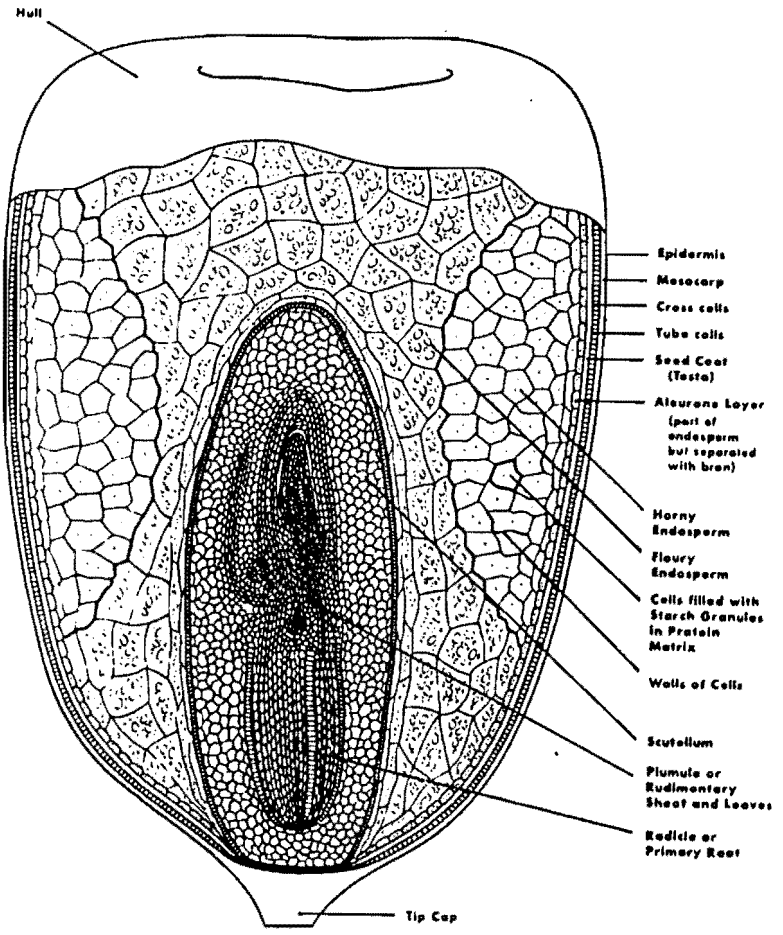

Figure 1. Longitudinal section of maize kernel (Hoseney,1994)

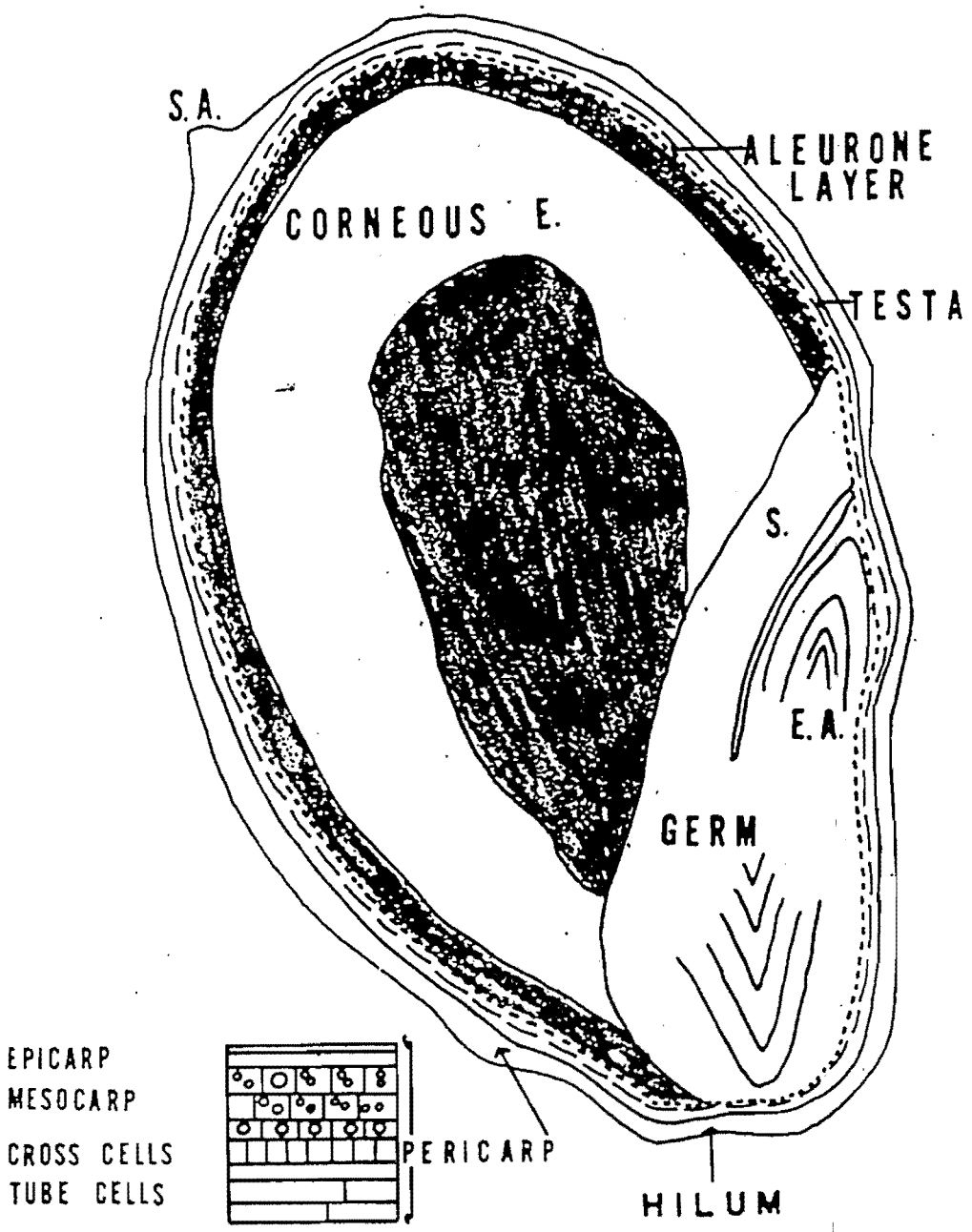

Figure 2. The structure of sorghum kernel (Hoseney, 1994)

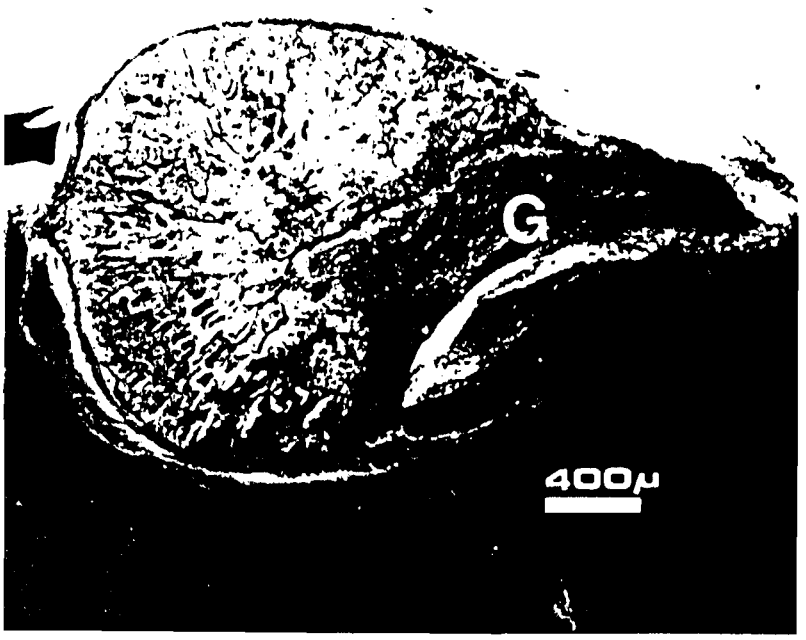

Figure 3. Low magnification of a longitudinal section of a pearl millet kernel (Hoseney, 1994).

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.

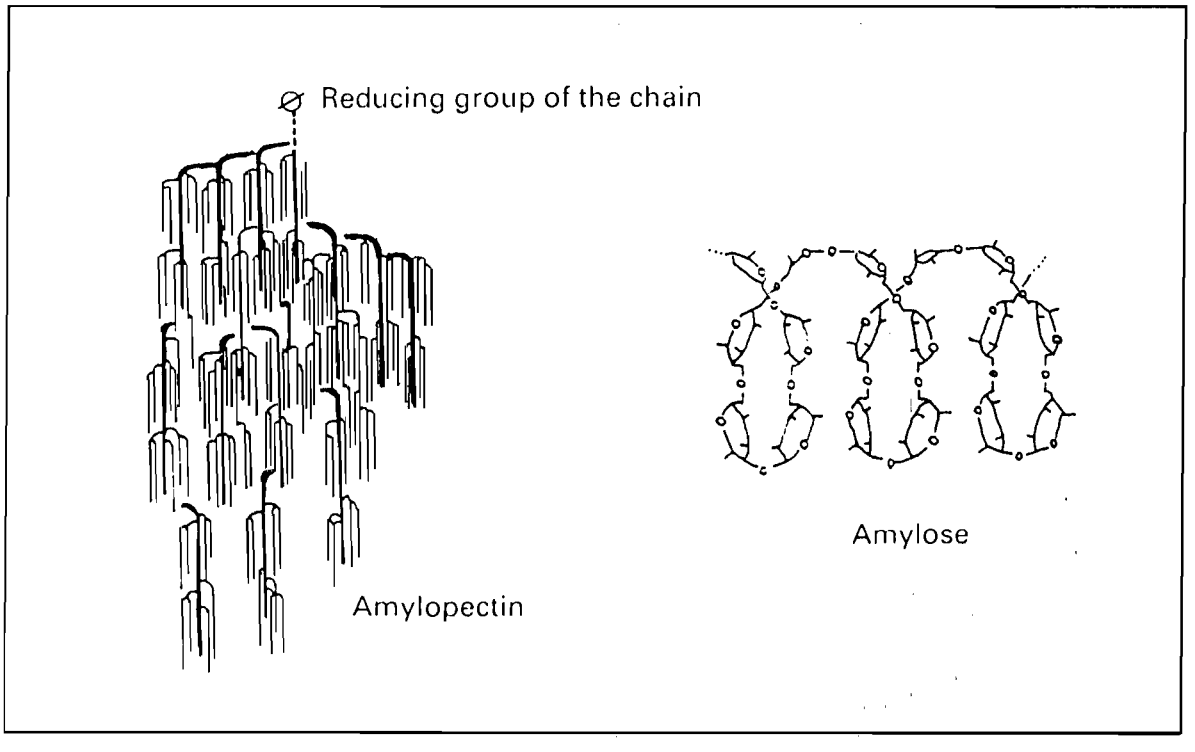

Figure 4. The structures of amylose and amylopectin (Alais and Linden, 1991)

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).

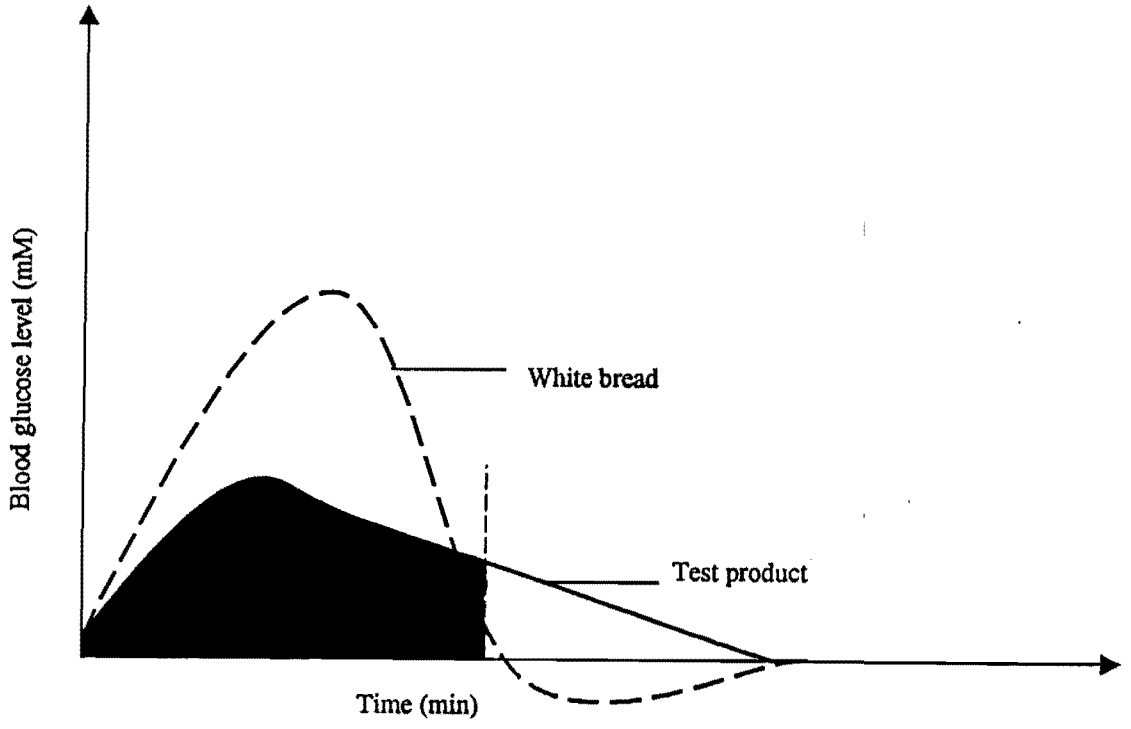

Figure 5: Calculation of the glycaemic index (GI) of a food product (Bjorck and Asp, 1994; adapted).

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.

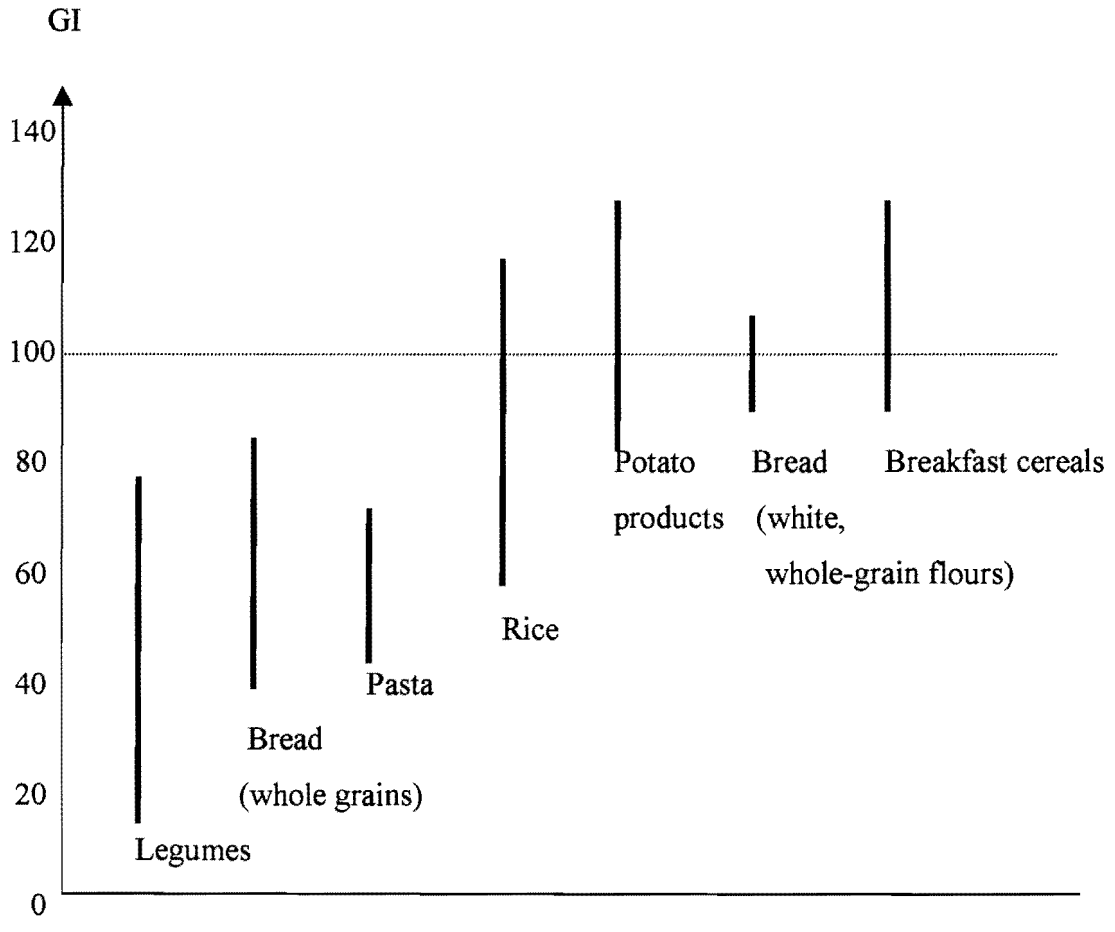

Fig. 6. Approximate ranges in GI for some of the main sources of starch in a Western-type diet (Bjorck and Asp, 1994).

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.