

## CHAPTER 2

### LITERATURE REVIEW

In this review, the incidence of diabetes in the South African Black population and the link between diet and diabetes will be discussed. Because carbohydrate digestibility plays a major role in the management of diabetes, starch and how starch digestibility is determined will also be discussed. Special mention will be made of the characteristics of starch in maize endosperm. An attempt will be made to judge if traditional stiff maize porridge could possibly play a role in the low incidence of diabetes in the rural Black population and if it is a food that could be suitable for consumption by diabetic people.

#### 2.1 Diabetes

##### 2.1.1 *What is diabetes?*

Diabetes mellitus (DM) is a chronic disorder that is characterized by major derangement in the metabolism of glucose and abnormalities in the metabolism of fat, protein and other substances (Wright, 1993a; Anderson & Geil, 1994; De Villiers, 1995). The disease is caused by either a deficiency or defective action of the hormone insulin.

In a healthy body blood transports glucose to the cells (De Villiers, 1995). The glucose is converted to energy with the help of the hormone insulin. Insulin “unlocks” the cells to allow glucose to enter and be used for energy. In diabetics, either the pancreas is unable to produce sufficient insulin, or the cells become insensitive or resistant to the insulin that is produced. The glucose does not get utilized, build up in the blood and is excreted in the urine. At the same time the body cells remain starved of energy.

Clinically, four forms of diabetes have been identified: primary, secondary, impaired glucose tolerance and gestational diabetes (Anderson & Geil, 1994). In primary DM, no

associated disease is present. There are two types of this form of diabetes, namely insulin-dependent diabetes (IDDM or type I diabetes) and non-insulin dependent diabetes (NIDDM or type II diabetes). IDDM usually manifests early in life and require daily insulin injections, while NIDDM usually develops after the age of 40, particularly under the obese and unfit and people with a family history of DM (Anderson & Geil, 1994; De Villiers, 1995; Osman, 1995). In secondary DM, some other identifiable condition causes or allows a diabetic syndrome to develop, e.g. pancreatic disease, endocrine abnormalities, insulin receptor abnormalities or drugs (Wright, 1993a). Gestational diabetes is glucose intolerance that develops during pregnancy (Anderson & Geil, 1994).

Attention will be paid to NIDDM, because 90-95% of all people with diabetes have NIDDM (Health.co.za, 1998). This type of diabetes is considered to be a disease of lifestyle (Zouvanis, 1997) and since it only develops later in life and could be related to diet (Thorburn, Brand & Truswell, 1987), there is a possibility for it to be prevented or controlled by diet.

### *2.1.2 Life-style factors associated with diabetes*

Generally, the lowest incidence of NIDDM is found in underdeveloped, rural communities in Africa and Asia (Wright, 1993a). Extremely high rates are found in certain ethnic groups (e.g. Pima Indians in Arizona, Micronesian population in Nauro, Blacks in America and Aborigines in Australia) when they rapidly change from a traditional lifestyle to a more affluent, “Western” lifestyle (Wursch, 1989; Wright, 1993a; Anderson & Geil, 1994; Cannan & Walker, 1997; FAO, 1997a).

The change in lifestyle involves many factors, one of them being that the carbohydrate source change from slow digestible to conventional Western type (Wursch, 1989). Thorburn *et al.*, (1987) found that the carbohydrate in traditional “bush foods” of Australian Aborigines are more slowly digested and absorbed than the Western foods eaten by the urbanised Aborigines. It has been suggested that the Aborigines possess a “thrifty genotype” which gives the advantage of metabolic efficiency when food is in short supply (Thorburn *et al.*, 1987). They, however, seem to have evolved without the

ability to cope with the fast-release carbohydrate foods that are typical of present Western diets. With urbanisation and the associated changes in diet, the thrifty metabolism of these people can lead to obesity, insulin resistance and eventually NIDDM.

The same is thought to be true for the groups of people in Africa, India and North America who traditionally had slowly digestible staple foods and now show a high incidence of NIDDM after they have urbanised (Thorburn *et al.*, 1987; NIDDK, 1998).

### 2.1.3 Diabetes in South Africa

It is estimated that about 2 million South Africans have diabetes, and that there is about just as many cases of undiagnosed diabetes (Health.co.za, 1998). Diabetes takes a large human and financial toll each year (Anderson, Gustafson, Bryant & Tietzen-Clark, 1987, Walker & Walker, 1991). It is a common disease in affluent societies, affecting from one to three percent of populations, and often five to ten percent of those over 40 years of age (Gresse, 1991). Figure 2 (Data from unpublished preliminary report of the Department of Health, South Africa, 1999) shows the prevalence of self-reported DM in South Africa.

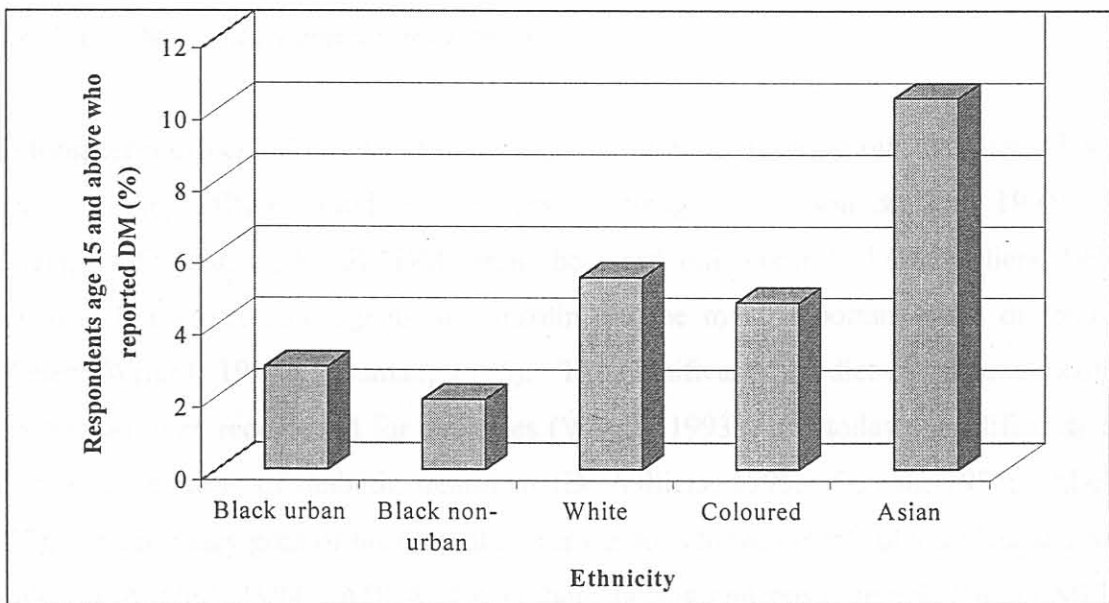


Figure 2: Self reported diabetes mellitus (DM) in South Africa (Data from unpublished preliminary report, Department of Health, South Africa, 1999)



The prevalence of diabetes (self-reported) in South Africa is approximately 5% amongst Whites and Coloureds . It is most widespread under the Indian population and the lowest prevalence is still in the Black population. What is important to note however, is that the prevalence of diabetes in the urbanised Black population is about 50 % higher than that of the rural Black population.

There are indications that DM is increasing in black South Africans. In agreement with the self-reported numbers, many researchers reported that diabetes was rare or uncommon in rural, traditionally living, African Blacks, but is higher in urban dwellers (Walker & Walker, 1991; Omar, Seedat, Motala, Dyer & Becker, 1993; Walker & Walker, 1994). Like the Aborigines, the South African Black population's carbohydrate source changed with the transition from a traditional rural to an urbanised lifestyle. In the case of the Black population, the change was mainly from unrefined maize porridge to bread (Mmakola *et al.*, 1997). It may be that traditional maize porridge is more slowly digestible than bread and that the Blacks in South Africa also possess the "thrifty genotype" that can make them genetically susceptible to the development of diabetes when they change to a carbohydrate staple that is digested more rapidly.

#### ***2.1.4 The effect and treatment of diabetes***

The long-term effects of untreated or poorly controlled DM are serious and include heart disease, kidney failure, blindness and nerve damage (Anderson & Geil, 1994; De Villiers, 1995; Osman, 1995). DM cannot be cured, only controlled (De Villiers, 1995). Diet, oral hypoglycaemic agents and insulin are the most important ways of treating diabetes (Wright, 1993b; Osman, 1995). The significance of diet in the treatment of diabetes has been recognized for centuries (Wright, 1993b) and today a modified diet is still the cornerstone of diabetic treatment (De Villiers, 1995; Osman, 1995; ADSA, 1997). The primary goal of nutritional therapy is to achieve normal blood glucose levels (Anderson & Geil, 1994; ADSA, 1997), both fasting and postprandial (Brand Miller, 1994). A diet high in complex carbohydrate (specifically starch and fibre) and low in fat and simple sugars is recommended (Anderson & Geil, 1994; De Villiers, 1995; Osman, 1995; ADSA, 1997).



After returning to a traditional diet and lifestyle for as short as seven weeks, diabetic Aborigines experienced a marked improvement in their condition (O'Dea, 1984). The slow-release nature of the carbohydrates in the traditional diet might have played a role in the improvement. This led Thorburn *et al.* (1987) to suggest that low-fat, slowly digestible carbohydrate traditional staple foods should be recommended as part of the dietary treatment of diabetes for Australian Aborigines.

Venter *et al.* (1990) indicated that traditional maize porridge might also be useful in the management of diabetes. Gresse, Vorster, Dauth, Welgemoed & Crowter (1993) studied the effect of returning to a rural African diet on the metabolic control of black NIDDM patients. The results, however, did not show any clinically significant improvement in glycaemic control. Possible reasons, according to the authors, for this disappointing result could be that the experimental period was too short, that the dietary fibre content of the test diet was lower than the typical African diet or, less likely, that the patients reduced their medication without reporting it.

Zouvanis (1997) stressed the importance of culture, taste and financial situation in recommending a diet for diabetic Africans. Although diabetes is the same metabolic illness for all people, it does not mean that the same treatments will be suitable for everyone. Treatments are more effective if the dietary and cultural habits of the people are taken into account (Rossouw & Kloppers, 1987). Since maize porridge is a traditional African staple food, it is definitely worthwhile to further examine its potential in the prevention or nutritional treatment of African diabetics.

## 2.2 Maize

### 2.2.1 *Production and consumption of maize in South Africa*

Maize is a staple food for large numbers of people in Latin America, Asia and Africa. In some African countries maize may account for 80-90% of the energy intake (Uhlig and Bhat, 1979). Maize is traditionally ground or lightly refined; however, the popularity and use of highly refined maize is increasing (Latham, 1979). Maize porridges and maize based starchy alcoholic and non-alcoholic beverages are very important foods consumed extensively in Africa (Latham, 1979). In South Africa maize is primarily eaten as a stiff porridge, but soft and crumbly porridges are also eaten (according to the author's knowledge).

Maize is the most important crop in South Africa (Elliott, 1991). In 1996, South Africa produced 13 815 000 metric tons of cereals, of which 10 351 000 was maize (FAO, 1997b). Maize is an important staple food for the majority of the South African population, but about 60 % of the maize grown in South Africa is used for animal feed (Elliott, 1991).

The carbohydrate market in South Africa consists mainly of the maize, wheat, potato and rice markets (Von Bach & Van Zyl, 1994). Maize accounts for 46 %, bread 25 %, potatoes 23 % and rice 5 % of the market. Bread and maize meal are generally regarded as the two staple foods of the South African population, but ethnical, demographical and socio-economical factors influence the consumption patterns of these foods (Mmakola *et al.*, 1997). The Black population consumes 94 % of all the human food maize meal products produced in South Africa (Elliott, 1991). Rural Black people consume approximately one and a half times as much maize meal products as urban Black people. In rural areas, maize seems to be the most important carbohydrate source, while in urban areas, it is bread that is consumed more often (Elliott, 1991; Von Bach & Van Zyl, 1994).

### 2.2.2 Maize kernel morphology and composition

Based on kernel characteristics maize is divided into different types. The five types of maize are flint, dent, floury, pop and sweet. Flint maize has a hard kernel due to the presence of a large and continuous volume of horny (hard) endosperm. Floury maize contains practically only floury (soft) endosperm. Since dent maize is a derivative of flint and flour crosses, it shows significant differences in the ratio of horny to floury endosperm. Dent varieties are grown most widely in the USA and South Africa (Watson, 1987a; Pedersen, Knudsen & Eggum, 1989).

Figure 3 (Hoseney, 1994) illustrates the morphology of a maize kernel.

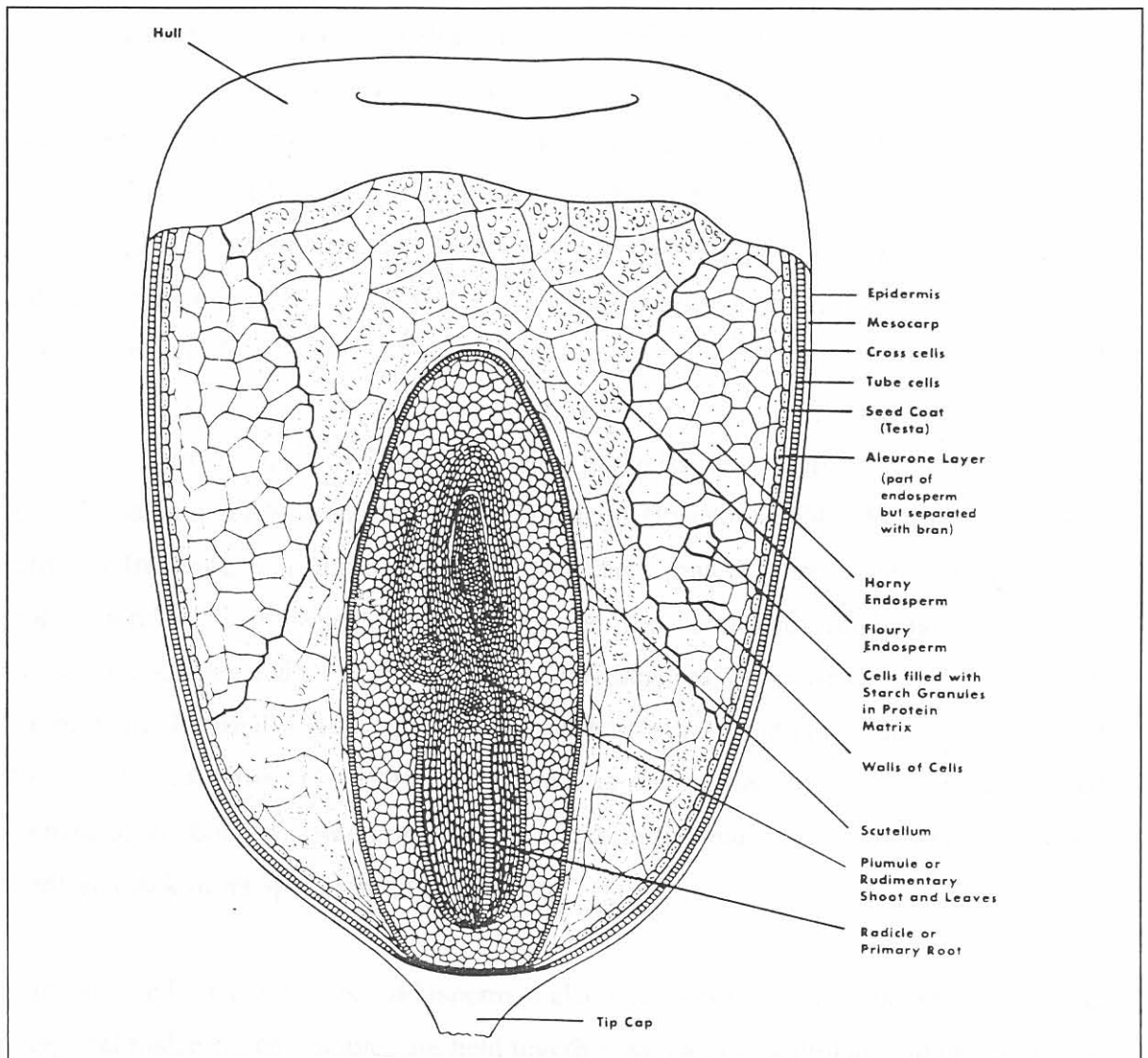


Figure 3: Longitudinal section of a maize kernel (Hoseney, 1994)



The maize kernel consists of four main parts. Expressed as a percentage of the whole kernel (on dry weight basis), these components are the germ, 12 %; endosperm, 82 %; hull or bran (pericarp and seed coat), 5.2 % and tip cap, 0.8 % (Peterson & Johnson, 1978). The endosperm contains 86-89% starch and about 8% protein. The endosperm cells are packed with starch granules embedded in a protein matrix (Pedersen *et al.*, 1989).

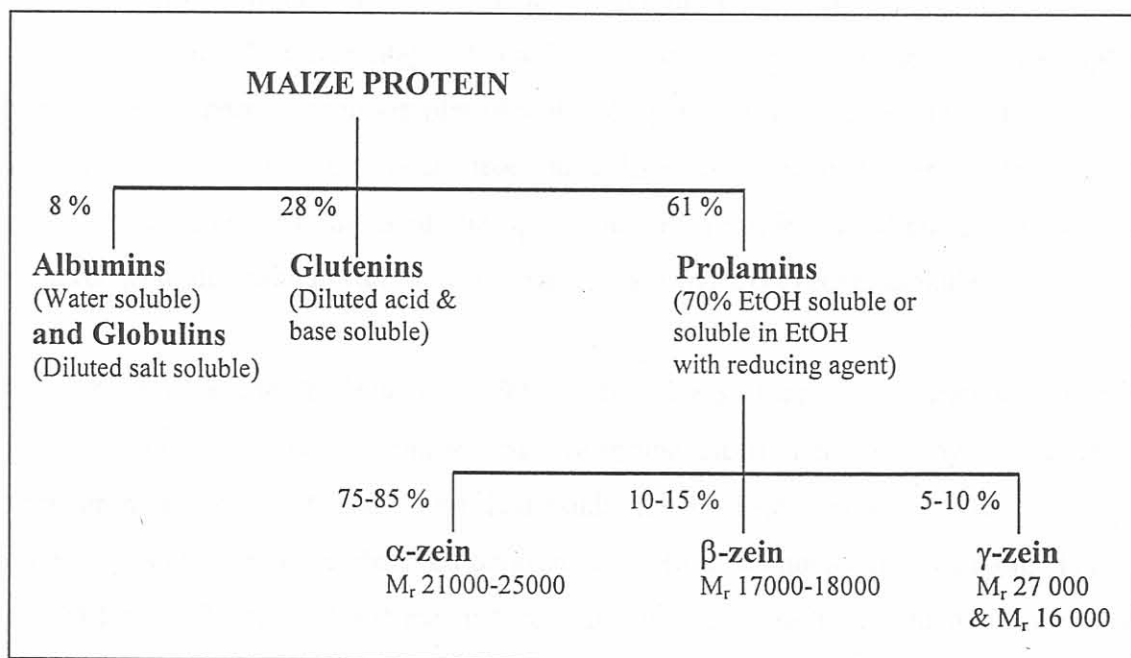
### 2.2.3 Maize endosperm vitreousness

Unlike in wheat where the endosperm in a kernel is either horny or floury, both horny and floury endosperm are found in a single maize kernel (Hoseney, 1994). The periphery of the kernel contains the horny endosperm (refer to Figure 3, Hoseney, 1994). Maize endosperm texture, properly known as endosperm vitreousness, is not well defined and no official test exists for measuring it. Various terms are often used interchangeably in describing kernel properties: *hard*, *vitreous*, *translucent*, *corneous*, *flinty* and *horny* are used as synonyms as are *soft*, *floury*, *mealy* and *opaque* (Dombrink-Kurtzman & Bietz, 1993; Hoseney, 1994).

In wheat, vitreousness is associated with hardness and high protein content and opaqueness with softness and low protein content (Hoseney, 1994). When a hard wheat kernel is fractured (e.g. during milling), the starch granules break and not the starch-protein bonds. In soft wheat, the bond between the starch and protein is broken easily and less force is needed to break the kernel. Nevertheless, the causes for vitreousness and hardness are different and it is possible to get hard wheat that is opaque and soft wheat that is vitreous (Hoseney, 1994). Hardness is caused by the genetically controlled strength of the bonds between protein and starch in the endosperm and vitreousness is a result of a lack of air spaces in the endosperm.

In maize, the horny (vitreous) endosperm is also tightly compact with no air spaces. The polygonal maize starch granules are held together with a matrix protein and protein (zein) bodies are present (Hoseney, 1994). In the floury endosperm the starch granules are spherical and are covered with matrix protein that does not contain zein bodies. The

floury endosperm contains many air spaces, which give it an opaque appearance (Hoseney, 1994; Kent & Evers, 1994). Differences in hardness are correlated with differences in ratio of horny to floury endosperm (Watson, 1987b). Maize with a higher proportion of horny endosperm is typically harder by mechanical measures of hardness (Dorsey-Redding, Hurburgh, Johnson & Fox, 1991). Dorsey-Redding *et al.* (1991) found that there was a correlation between maize kernel protein content and hardness (measured by Steinvert hardness test). The horny endosperm has a thicker protein matrix and thus a higher protein content than the floury endosperm (Pedersen *et al.*, 1989). The classification and nomenclature of maize proteins are summarized in Figure 4 (information from Esen, 1987; Hoseney, 1994; Mestres & Matencio, 1996).



**Figure 4: Classification and nomenclature of maize protein (information from Esen, 1987; Hoseney, 1994; Mestres & Matencio, 1996)**

Maize protein bodies consist mainly of the prolamin zein and the protein matrix consists mainly of glutelin (Peterson & Johnson, 1978). Dombink-Kurtzmann & Bietz (1993) used the resistance to hand drilling as the criterion for hardness and found that hard endosperm fractions contain more α-zein than soft endosperm fractions. Within individual kernels, hard endosperm also contained more total alcohol-soluble proteins than did soft endosperm fractions. In contrast, soft endosperm contained more 27 kDa γ-zein than hard endosperm of the same genotype. These differences in protein



composition suggest that actual composition of protein bodies in hard and soft maize endosperm fractions may be correlated with endosperm texture in normal maize lines. In apparent contrast with the results above, Mestres & Matencio (1996) found that vitreousness was related to the proportion of the two  $\gamma$ -zein fractions and that  $\alpha$ -zeins and salt extractable proteins were related to the milling characteristics of maize kernels.

The characteristics of horny and floury endosperm suggest that there may be a fundamental difference in their respective cells (Dombrink-Kurtzman & Bietz, 1993). Different cell lineages or different stages of differentiation could be present, with the more highly differentiated cells occurring in the horny endosperm. Dombrink-Kurtzman & Knutson (1997) found that there was a correlation between endosperm hardness and amylose content. The percentage of amylose in starch was always greater in samples from hard endosperm than in samples of soft endosperm. They suggested that the cells in the soft endosperm are less mature than the cells in the hard endosperm. The higher amylose concentration in the hard endosperm may result in increased compressibility of the starch granules, which leads to a compacted state and a polygonal granule shape.

Dombrink-Kurtzmann & Knutson (1997) studied the surface characteristics of starch granules from hard and soft endosperm. Scanning electron microscopy showed that starch granules from soft endosperm had randomly distributed pores on their surfaces, while very few pores were observed on granules from hard endosperm. Fannon, Hauber & BeMiller (1992) proved that the surface pores on starch granules, which are observed when scanning electron micrographs of certain starches are taken, were a natural feature and not 1) artifacts formed by preparation of the samples for microscopy; 2) caused by drying the kernel or after isolation; or 3) produced by *in situ* amylases or by amylases produced during wet milling. The size of the pores is approximately 100 nm in diameter, which means that they are large enough to allow very large molecules, including enzymes, direct access to the granule interior (Fannon *et al.*, 1992). The pores may therefore be related to control of starch conversion during germination. If it is true that these surface pores make the granules more susceptible to enzyme attack, it can be a reason for soft endosperm to be more easily digestible than hard endosperm, because



starch granules in the soft endosperm contain more surface pores than starch granules in the hard endosperm (Dombrink-Kurtzmann & Knutson, 1997).

Maize hardness is especially of interest to processors. Dry millers prefer hard kernel maize, because grits is derived from the horny parts of the endosperm (Kent & Evers, 1994). The soft endosperm breaks down too easily into flour. In contrast, wet millers prefer soft kernel maize, because it requires a shorter steeping time and gives better starch-protein separation (Wu & Bergquist, 1991). Numerous indirect tests for breakage susceptibility and hardness have been developed, including kernel density, work required to grind a sample in a laboratory hammer mill, grinding resistance (maximum torque necessary to crush individual kernel under compression), average particle size resulting from grinding (measured by sieving ground kernels) or evaluation by near-infrared reflectance of milled product (Mestres, Louis-Alexandre, Matencio & Lahlou, 1991).

Maize users and breeders usually judge maize vitreousness by considering that a flint grain has a vitreous endosperm and a dent maize kernel has a floury endosperm (Louis-Alexandre, Mestres & Faure, 1991). Vitreousness can also be judged by sectioning a kernel and estimating the vitreousness visually (Mestres *et al.*, 1991). Both of these methods are subjective and the latter depends on the observer's experience. Louis-Alexandre *et al.* (1991) measured the vitreous and total endosperm areas of sectioned kernels and found a high correlation between this vitreousness index and true vitreousness determined by dissection.

Felker & Paulis (1993) used video image analysis as a non-destructive, objective method of determining vitreousness. The kernel vitreousness was estimated by viewing it on a light box with a monochrome video camera. The video signal was captured to a computer and the brightness was quantified. A disadvantage of this method was that each kernel had to be surrounded by modelling clay to exclude excess light. Erasmus, Kuyper & Esterhuyzen (1997) improved the method by adapting the lightbox in such a way that sample preparation was not necessary any more.

## 2.3 Starch

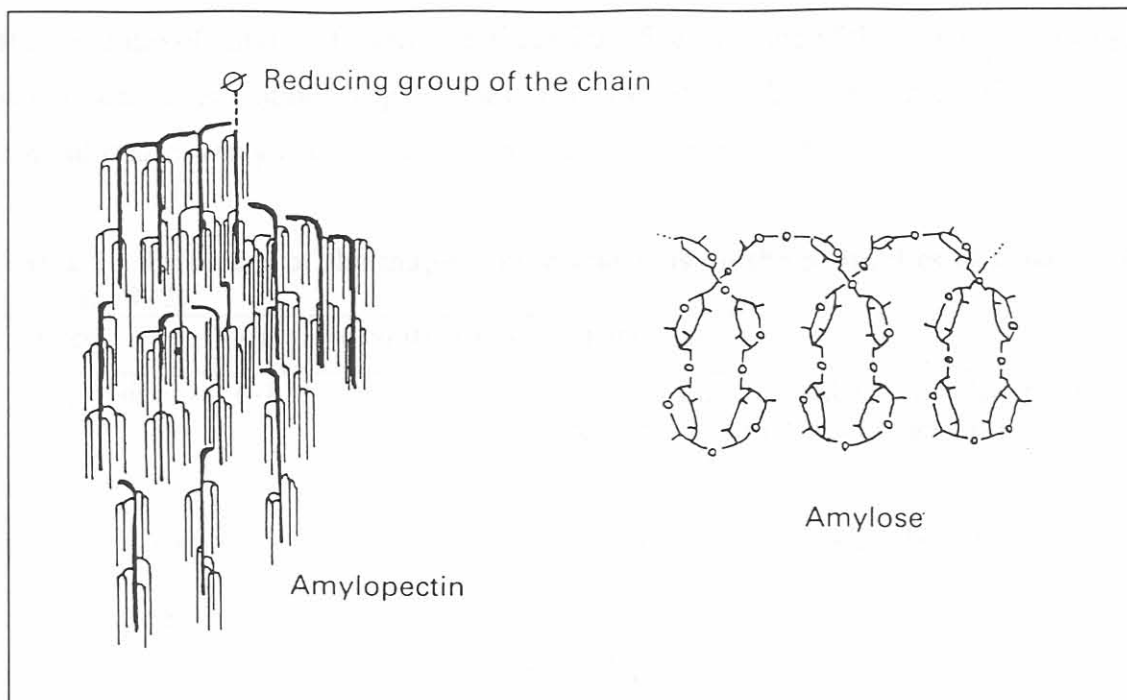
### 2.3.1 *Composition and structure of starch granules*

Starch is found in the form of granules in the organs of many plants (reviewed by Annison & Topping, 1994). 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 & BeMiller, 1997).

Starch is the most abundant carbohydrate in cereal grains (Kent & Evers, 1994). The size of the granules range from 1 to 100  $\mu\text{m}$  in diameter and together with the shape, it is characteristic of the plant species that the starch is derived from (reviewed by Wursch, 1989). The granules are relatively dense, are insoluble and hydrate only slightly in water at room temperature (Whistler & BeMiller, 1997).

Starch is a condensation homopolymer of glucose. The glucose units are linked to one another through the C-1 oxygen, thus forming glycosidic bonds (Mathews & Van Holde, 1990). The glucose unit at the end of the polymeric chain has a latent aldehyde group and is known as the reducing end group (Alais & Linden, 1991). Although starch consists only of glycosidically linked glucose units, it is by no means a uniform substance (Zobel & Stephen, 1995).

Two types of glucose polymers can be distinguished, namely amylose and amylopectin, (Figure 5, Alais & Linden, 1991).



**Figure 5: The structures of amylose and amylopectin (Alais & Linden, 1991)**

Amylose is a predominantly linear, but not straight, molecule linked by  $\alpha$ -D-(1 $\rightarrow$ 4) bonds. This axial $\rightarrow$ equatorial bonding arrangement gives the molecule a helical shape (Alais & Linden, 1991). The inside of the helix contains mostly hydrogen atoms and is lipophylic, while the hydroxyl groups are positioned on the outside of the coil. Many amylose molecules have some  $\alpha$ -D-(1 $\rightarrow$ 6) branches, but only about 0.3-0.5% of the total linkages (Whistler & BeMiller, 1997). Amylose molecules are relatively small, molecular weights range from about 150 000 to 600 000 Da (Alais & Linden, 1991).

Amylopectin, the second and more abundant polymer, is a large and highly branched molecule (Whistler & BeMiller, 1997). The linear regions have  $\alpha$ -D-(1 $\rightarrow$ 4) and the branched points  $\alpha$ -D-(1 $\rightarrow$ 6) glycosidic bonds (Mauro, 1996). An amylopectin molecule consists of a main chain, called the “C” chain, which carries the one reducing end-group and numerous branches, called “B” chains. “A” chains are a third layer of chains that are linked via their reducing ends to the “B” chains (Whistler & BeMiller, 1997). Molecular weights for amylopectin have been reported as high as 500 MDa (Mauro, 1996).



It is the ratio of amylose to amylopectin and the fine structure of these polymers that give native starch its distinctive properties (Mauro, 1996). The properties of starch from normal maize, waxy maize and amylo maize are compared in Table 1.

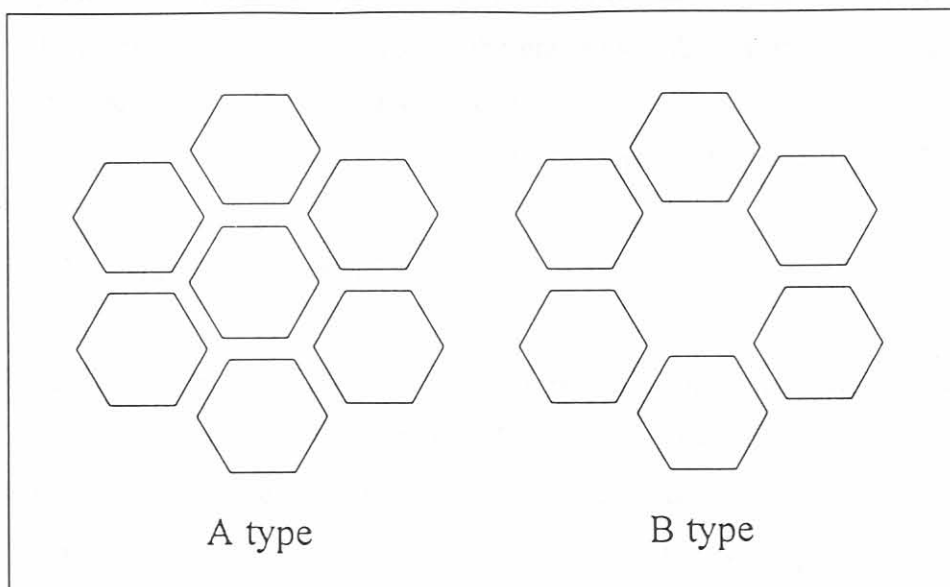
**Table 1: Influence of the major starch fractions on the properties of maize starch (Oates, 1997)**

Source	Amylose content (%)	Properties
Waxy maize	0-1	Non-gelling, low-setback and clear paste; paste that is resistant to syneresis; elastic and stringy paste.
Normal maize	27	Firm gel; opaque paste; short paste texture.
Amylo maize	50-70	Granule that is resistant to swelling; rigid gel; opaque paste; high paste temperature.

When undamaged starch granules are examined under polarised light, maltose crosses can be seen. This phenomenon is called birefringence and is the result of the polarised light being bent as it crosses a region of high molecular order (Jackson, 1993). The fact that starch granules also exhibit X-ray patterns, gives a further indication of the crystallinity of starch. These crystalline structures in starch granules are believed to be mostly amylopectin, because the steeping of starch granules in water leaches out amylose, leaving both the amylopectin and the crystallinity intact (Zobel, 1992). Starch molecules in a starch granule are arranged in a radial direction, containing crystalline and noncrystalline regions in alternating layers. The clustered branches of amylopectin occur as packed double helices. It is these double helical structures that form the crystalline regions in the starch granules (Whistler & BeMiller, 1997).

Three types of X-ray patterns (named A, B and C) have been observed in intact native starch granules (Hoseney, 1994). Type A is characteristic of cereal starches (Whistler & BeMiller, 1997). It indicates parallel double helices of starch separated by interstitial water. Tuber and root starches, high amylose starches, as well as retrograded starch produce B type patterns. In starches with a B pattern, a column of water replaces one of the starch double helices. Figure 6 (Whistler & BeMiller, 1997) gives a diagrammatic representation of the starch helices in the A and B type patterns. The C type pattern is

characteristic of legumes, e.g. smooth pea and bean starches. The C pattern is considered to be an intermediate form consisting of mixtures of the A and B types (Hoseney, 1994).



**Figure 6: Diagrammatic representation of the arrangement of six parallel double helices in starches that give a A-type pattern and starches that give a B-type pattern. Water molecules replace the centre double helix in B-type starches (Whistler & BeMiller, 1997)**

### 2.3.2 Starch gelatinisation

Gelatinisation refers to the disruption of molecular order within starch granules as they are heated in the presence of water (Whistler & BeMiller, 1997). During gelatinisation, several changes take place in the properties of starch granules. Firstly, the starch granules take up water and swell. If a suspension of starch in excess water is heated, the swelling will become irreversible when the temperature reaches around 60 °C (Kent & Evers, 1994), because heating provides sufficient energy to disrupt the weak hydrogen bonds in the crystalline regions of the starch granules (Wong, 1989). Around 60-80 °C, starch granules also lose their birefringence (Jackson, 1993). The temperature at which this starts is different for different starches, e.g. around 65 °C for wheat starch and 71 °C for maize starch (Zobel, 1984). Amylose leaches out of the starch granule during gelatinisation. Leaching also takes place at temperatures below the gelatinisation temperature, because the amylose is located in the noncrystalline regions of the granule

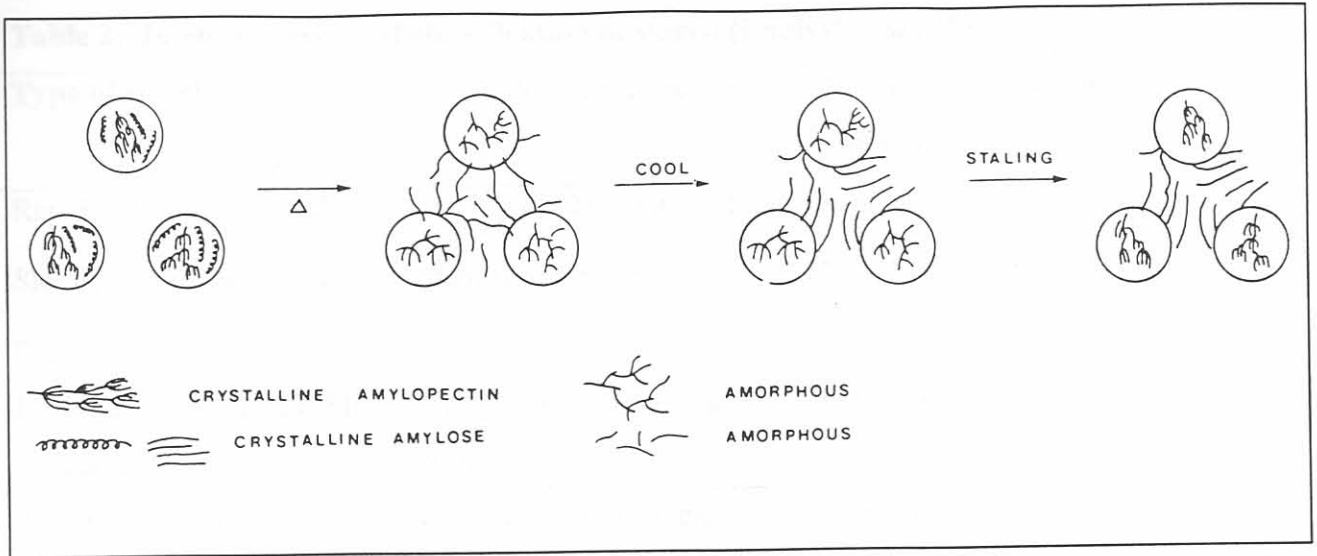
and it is such a small, linear molecule (Whistler & BeMiller, 1997). During gelatinisation, the viscosity of the starch suspension increases. This is initially a result of the swelling and water uptake of the granules. When heated further while stirring, starch granules are disrupted and soluble starch is released into the solution. A viscous mass, called a paste, is formed (Hoseney, 1994; Whistler & BeMiller, 1997).

### *2.3.3 Starch retrogradation*

The starch paste or solution obtained after gelatinisation is not stable. When a diluted gelatinised starch solution is cooled, the linear amylose molecules realign themselves by hydrogen bonding into an insoluble precipitate (Wong, 1989). When the concentration of starch in the solution is higher (5-10%), a gel is formed. These structural transformations are called “setback” or retrogradation. The solidified paste becomes cloudy and opaque with time and eventually releases water as the solubility of the starch decreases (Smith, 1982). Amylopectin also undergoes retrogradation, but much more slowly than amylose. Amylose retrogradation is believed to be largely complete by the time the product has cooled to room temperature. Amylopectin retrogradation involves primarily the association of outer branches and occurs over time after the product has cooled (Whistler & BeMiller, 1997). This could take hours or days, whereas amylose retrogradation could take place within minutes or hours.

Figure 7 (Wong, 1989) summarises the changes that take place in starch granules during heating and subsequent cooling.





**Figure 7: Structural changes in a starch granule during heating and subsequent cooling (Wong, 1989)**

Upon heating, the granules swell and lose crystallinity. The smaller molecules (amylose) dissolve and leach out of the granule. Upon cooling, amylose molecules reassociate to form a precipitate or a gel. During long-term storage, crystallization continues slowly and amylopectin retrogradation will also take place (Smith, 1982).

### 2.3.4 Resistant starch

Uncooked starch granules have long been known to be relatively resistant to hydrolysis by digestive enzymes. However, until recently, it had been assumed that essentially all the starch in our diets would be digested and absorbed once the granules had been fully gelatinised by cooking or processing (Englyst, Kingman & Cummings, 1992).

The European Resistant Starch research group (EURESTA) defined resistant starch (RS) as “the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals” (Asp, 1992). The main forms of resistant starch are physically enclosed starch, e.g. within intact cell structures (RS1), some raw starch granules (RS2) and retrograded amylose (RS3) (Englyst *et al.*, 1992). Table 2 (Englyst *et al.*, 1992) gives an *in vitro* nutritional classification of starch.

**Table 2: *In vitro* nutritional classification of starch (Englyst *et al.*, 1992)**

Type of starch	Example of occurrence	Probable digestion in small intestine
Rapidly digestible starch	Freshly cooked starchy food	Rapid
Slowly digestible starch	Most raw cereals	Slow but complete
Resistant starch:		
1. Physically inaccessible starch	Partly milled grains and seeds	Resistant
2. Resistant starch granules	Raw potato and banana	Resistant
3. Retrograded starch	Cooled, cooked potato, bread and corn flakes	Resistant

The proportion of starch in a food or meal that reaches the large intestine will vary with the source and processing of the food. In spite of the variability, it is said that resistant starch may represent a greater and more important supply of fermentable carbohydrate to the colon than non-starch polysaccharides (Stephen, 1994).

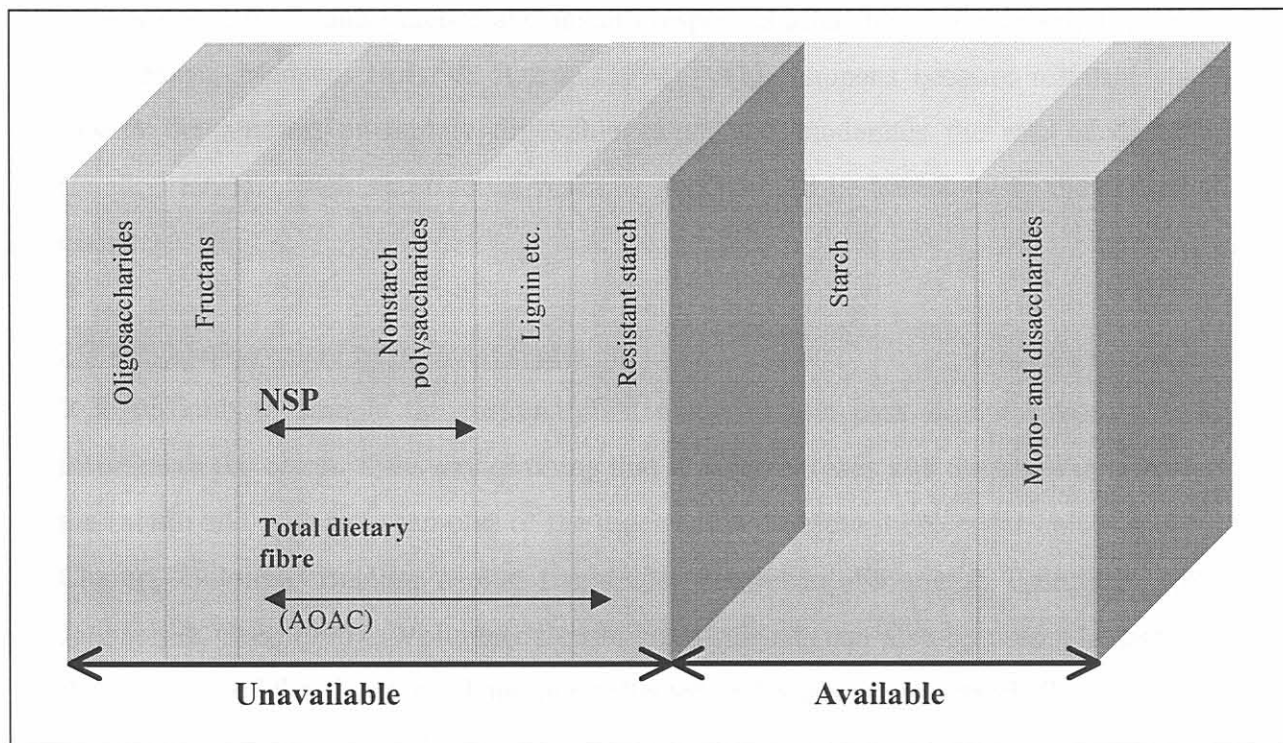
Starch is seldom present in the faeces of humans and experimental animals. Resistant starch is more or less completely fermented by the microflora in the colon (Asp & Björck, 1992). Energy and a number of by-products, including the gases methane and hydrogen, and short chain fatty acids (particularly acetate, propionate and butyrate) are produced (Stephen, 1994).

#### 2.3.4.1 The physiological role of resistant starch

Phillips, Muir, Birkett, Lu, Jones & O'Dea (1995) found that resistant starch had a significant impact on putative markers of colonic health in humans, including increased fecal bulk, increased concentrations of short chain fatty acids and lowered faecal pH. This indicates that undigested starch may have an important role in the prevention of bowel diseases, including colorectal cancer. These effects are comparable with those of non-starch polysaccharides (dietary fibre). In fact, the enzymatic gravimetric AOAC



(Association of Official Analytical Chemists) methods for total dietary fibre include one important form of resistant starch, retrograded starch (Asp, 1996). Figure 8 (Asp, 1995) is a diagrammatic representation of the relationship between non-starch polysaccharides, total dietary fibre and unavailable carbohydrates.



**Figure 8: Relationship between non-starch polysaccharides (NSP), total dietary fibre, and unavailable carbohydrates (Asp, 1995)**

There is special interest in the short chain fatty acid butyrate, because it is the main energy source of the colonic mucosa epithelial cells (Asp & Björck, 1992; Asp, 1996). Butyrate also reduces the risk of malignant tumour formation in the colon and is therefore thought to play a key role in the prevention of colon cancer (Annison & Topping, 1994; Asp, 1996). Starch fermentation produces proportionally more butyrate than the fermentation of dietary fibre (Stephen, 1994). Including resistant starch in the diet could play an important role in this regard.

When absorbed into the bloodstream, the short chain fatty acids formed during colonic fermentation may influence lipid and glucose metabolism (Björck & Asp, 1994).



Propionate is metabolised in the liver, where it is thought to inhibit cholesterol synthesis and suppress glucose release (Wolever, Spadafora & Eshuis, 1991).

The replacement of digestible starch in a meal with resistant starch results in significant reductions in the blood glucose and insulin responses after the meal (Raben, Tagliabue, Christensen, Madsen, Holst & Astrup, 1994). This happens because resistant starch lowers the amount of rapidly digestible starch and moderates the rate of digestion (Faulks, 1993). Such an effect of resistant starch could be beneficial in the control of diabetes.

#### 2.3.4.2 Measurement of resistant starch

It is difficult to sample the residual food materials that pass from the human small intestine to the colon. One way of doing that is to insert tubes into the small intestine via the mouth and withdraw samples of the digested food before it enters the colon (Faisant, Champ, Colonna, Buléon, Molis, Langkilde, Schweizer, Flourie & Galmiche, 1993; Johnson & Gee, 1996). Volunteer ileostomised subjects can also be used. In that case the contents of the ileostomy bags are collected and analysed for starch (Faisant *et al.*, 1993; Englyst, Kingman, Hudson & Cummings, 1996; Johnson & Gee, 1996).

*In vivo* experiments with rats have also been done. The rats are either treated with antibiotics to prevent fermentation of starch in the colon (Björck, Nyman, Pedersen, Siljeström, Asp & Eggum, 1986) or the rats' colons are surgically removed (Marlett & Longacre, 1996).

Resistant starch can be determined *in vitro* by digesting samples with enzymes and quantifying either the digestion products or the residue (Englyst *et al.*, 1992; Muir & O'Dea, 1992; Marlett & Longacre, 1996). Björck *et al.* (1986) and Saura-Calixto, Goñi, Bravo & Mañas (1993) measured the starch content of dietary fibre residues, which would be only type 3 (retrograded) resistant starch.

### 2.3.5 Damaged starch

During the process of dry milling, some starch granules are damaged. In some aspects, these mechanically damaged starch granules are similar to gelatinised starch (Kent & Evers, 1994). When damaged starch granules are placed in cold water, they swell spontaneously and lose their birefringence. Nevertheless, in any population of granules that contains damaged starch, there are undamaged native starch granules and also birefringent remnants of granules that have been damaged only partially (Tester, Morrison, Gidley, Kirkland & Karkalas, 1994). The amorphous damaged starch forms a translucent gel that consist mainly of intact amylopectin, lipid-free amylose, lipid-complexed amylose and a soluble fraction which consist of low molecular weight fractions of amylopectin and lipid free amylose (Tester & Morrison, 1994). In gelatinised starch the loss of organization is achieved without reducing the size of the starch molecules (Kent & Evers, 1994).

All wheat flours contain a certain amount of damaged starch because of the milling process (Jones, 1940). The amount of damaged starch in wheat flour is important in bread making, because it affects the amount of water needed to make a dough of the required consistency. Also, damaged starch is more susceptible to enzyme attack than intact native starch granules (Kent & Evers, 1994; Tester & Morrison, 1994). The amount of damaged starch in the flour can be controlled to some extent during milling (Tester & Morrison, 1994). In roller milling, smooth rolls and moderate pressures cause lower levels of damaged starch than rougher rolls and heavier pressures (Jones, 1940).

Physical hardness of the endosperm also affects the extent of starch damage during milling (Jones, 1940). More starch damage takes place during the milling of hard wheat (Mok & Dick, 1991). No information is available on the effect of endosperm hardness on starch damage in maize, but it seems like maize and wheat flours with similar particle sizes have similar levels of damaged starch. Phegelo (1998) found that wheat flour with 94 % of the particles < 150  $\mu\text{m}$  had about 10 AACC units damaged starch and maize flour with 100 % of the particle < 150  $\mu\text{m}$  had about 12 AACC units damaged starch.

## 2.4 Glycaemic index and carbohydrate digestibility

The digestion of dietary carbohydrates starts in the mouth, where salivary  $\alpha$ -amylase initiates starch degradation (FAO, 1997a). Chewing is an important step in the process of digestion (Würsch, 1989). Because it breaks the food down into smaller pieces, it increases the surface area available for enzyme attack. Starch digestion is continued in the small intestine by pancreatic  $\alpha$ -amylase. The products of digestion of starch by  $\alpha$ -amylase are glucose, maltose, maltotriose and maltotetraose (Faulks & Bailey, 1990). Only monosaccharides can be absorbed from the digestive tract (FAO, 1997a). The brush-border enzymes maltase and isomaltase hydrolyse the oligosaccharides to form glucose (Whistler & BeMiller, 1997). Glucose is absorbed into the blood stream and causes the blood glucose concentration to increase (FAO, 1997a). The extent and duration of the blood glucose rise after a meal is dependent on the rate of absorption, which in turn depends on factors such as gastric emptying, rate of hydrolysis and diffusion of hydrolysis products in the small intestine.

The molecular size of carbohydrates in itself is a poor indicator of the likely metabolic response (Björck & Asp, 1994). In the early 1900s people used to think that the small molecules of simple sugars are digested and absorbed more rapidly than the large molecules of starch, causing larger increases in the blood glucose concentration after a meal (Asp, 1996). Based on this, low molecular weight carbohydrates were restricted in the diets of people with diabetes. From the mid-1980s onwards this view changed (Björck & Asp, 1994; Kalergis, Pacaud & Yale, 1998). The nutritional properties of starch in foods are largely related to its availability for digestion and/or absorption in the gastrointestinal tract (Table 3, Björck & Asp, 1994).

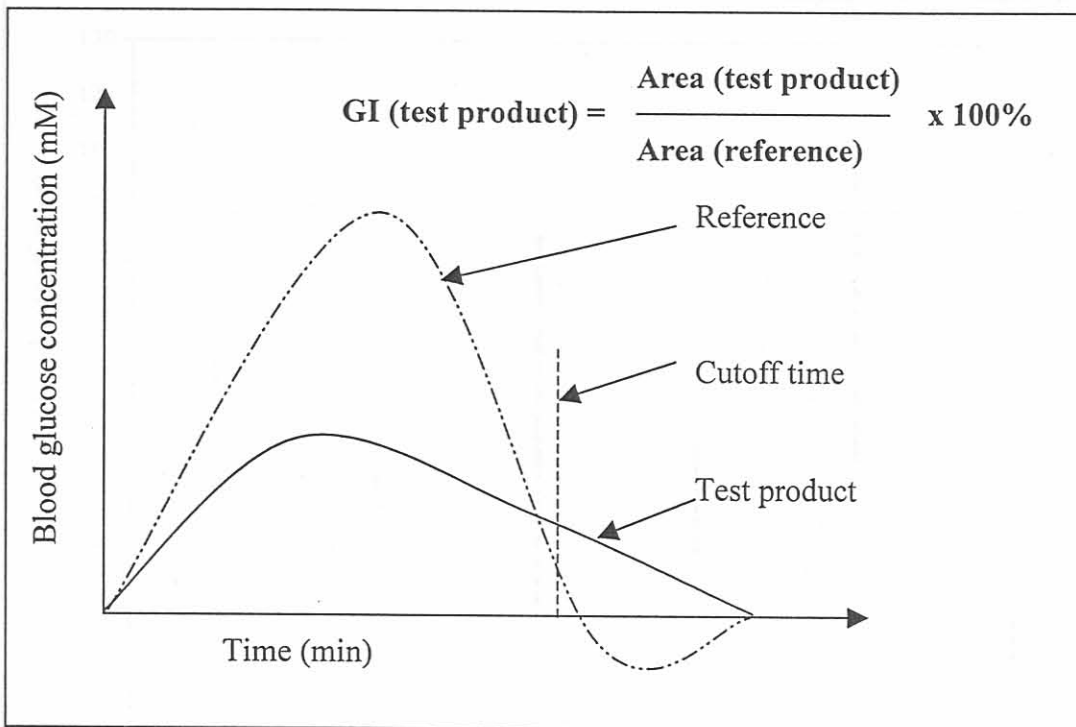


**Table 3: Nutritional indexes related to the availability of starch in the gastrointestinal tract (Björck & Asp, 1994)**

Property	Location	Nutritional indexes
Rate of digestion	Mouth	Delivery of fermentable maltodextrins to the microorganisms in dental plaque
Rate of digestion and absorption	Small intestine	Rate of glucose delivery to the bloodstream
Extent of digestion and absorption	Small intestine	Delivery of starch and starch hydrolysis products to the large bowel (resistant starch)
Fermentability of resistant starch	Large bowel	Microbial formation of short-chain fatty acids

#### *2.4.1 Definition and calculation of the glycaemic index*

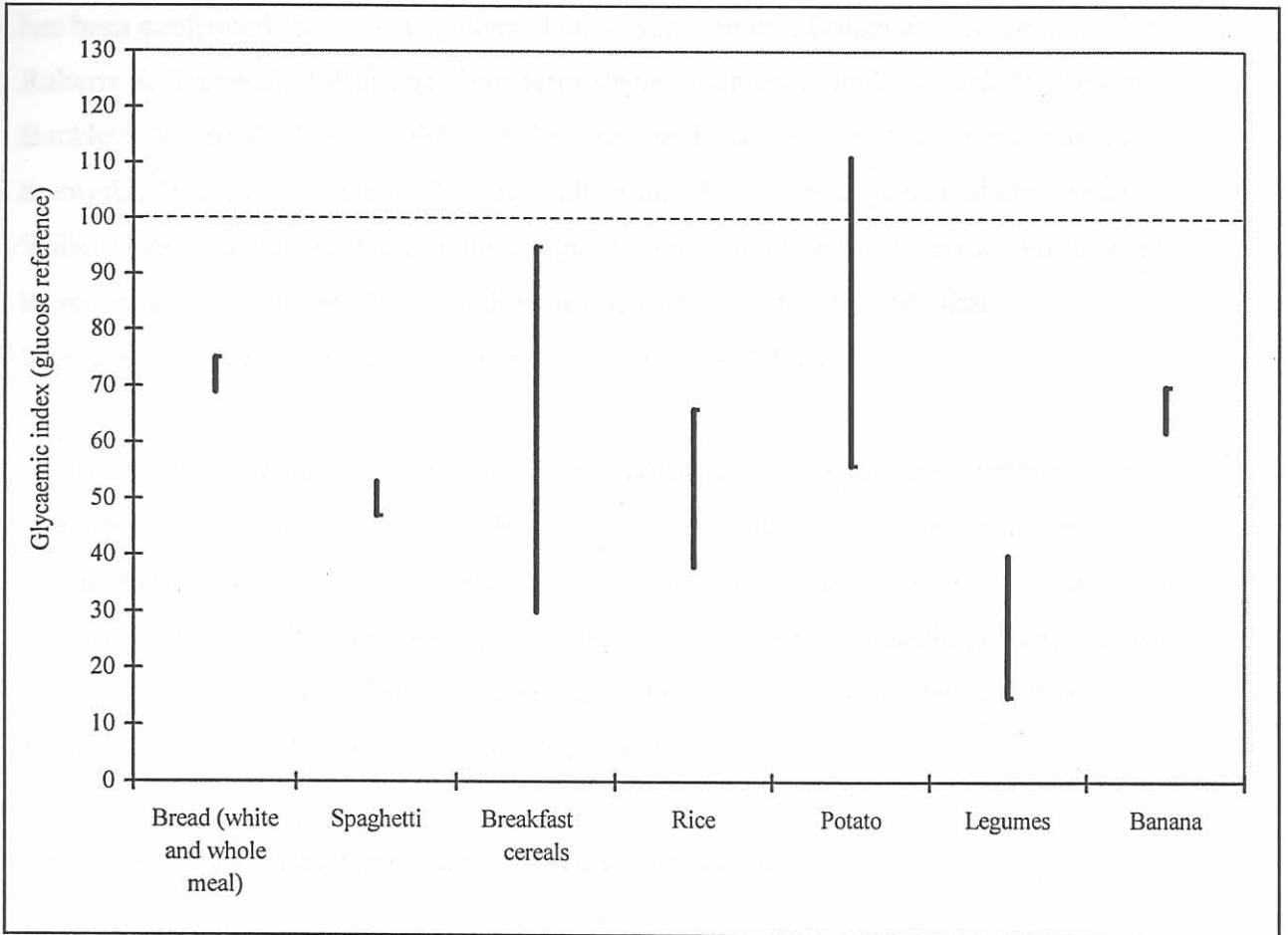
Foods can be classified based on their blood glucose raising potential using the glycaemic index. The glycaemic index (GI) is defined as the incremental area under the blood glucose response curve of a 50 g carbohydrate portion of a test food expressed as a percentage of the response to the same amount of carbohydrate from a standard food taken by the same subject (Vorster, Venter & Silvis, 1990; FAO, 1997a). Either white bread or glucose can be used as standard (Foster-Powell & Miller, 1995). When glucose is used as a standard, the GI of white bread is 70. Figure 9 (Björck & Asp, 1994; adapted) shows how GI is calculated.



**Figure 9: Calculation of the glycaemic index (GI) of a food product (Björck & Asp, 1994; adapted)**

The area of the test product is the part of the area under the test product curve that is left of the time cutoff (dashed line). The area of the reference sample is the area reference curve that is left of the time cutoff. The GI is calculated as the percentage that the area of the test product makes out of the area of the reference. The reference food is defined as having a GI of 100. A product that releases glucose more rapidly than the reference sample has a GI greater than 100 and one that releases glucose more slowly than the reference, a GI of less than 100. The cutoff time is usually two or three hours (Vorster *et al.*, 1990; Truswell, 1992; Björck & Asp, 1994).

The approximate ranges in GI (glucose standard) of some starchy foods eaten by South Africans are shown in Figure 10 (GI values were obtained from Foster-Powell & Miller, 1995).



**Figure 10: Approximate ranges in glycaemic index (glucose standard) for some starchy foods eaten by South Africans (GI values obtained from Foster-Powell & Miller, 1995)**

Starchy foods cover the whole range from slow (GI equal or less than 55) to rapid glucose release (GI equal or greater than 70) (Perlstein, Willcox, Hines & Milosavljevic, 1997). For example, legumes and pasta are digested slowly and puffed breakfast cereals are digested rapidly.

#### 2.4.2 The application and use of the glycaemic index

The GI, in conjunction with information about food composition, can be used to guide food choices (Vorster *et al.*, 1990). Meals containing low GI foods reduce both postprandial blood glucose and insulin responses (Brand Miller, 1994; FAO, 1997a). Low GI foods help to improve control over blood glucose concentration, which is important in the management of diabetes (Perlstein *et al.*, 1997). This beneficial effect



has been confirmed for diabetic patients both in long (Brand, Colagiuri, Crossman, Allen, Roberts & Truswell, 1991) and short term studies (Wolever, Jenkins, Vuksan, Jenkins, Buckley, Wong & Josse, 1992). Salmerón, Ascherio, Rimm, Spiegelman, Jenkins, Stampfer, Wing & Willett (1997) and Salmerón, Manson, Stampfer, Colditz, Wing & Willett (1997) found that a diet with a high glycaemic load and low cereal fibre content increases the risk of NIDDM in both men and women. This suggests that low GI, high fibre diets may decrease the risk of non-insulin dependent diabetes.

GI information should not be used in isolation, but rather be combined with macronutrient recommendations. Misuse of GI would be for a diabetic person to consider chocolate to be suitable and potato to be unsuitable just because chocolate has a low GI and potato a high GI. Chocolate is not recommended for diabetic people, because it is high in saturated fat. Potato is recommended despite the high GI, because it is low in fat and high in carbohydrate (Perlstein *et al.*, 1997).

### ***2.4.3 Factors affecting the measured glycaemic index values***

#### 2.4.3.1 Methodological variability

The method used to assess the GI and the way the results are presented affects the GI values. The variables that are known to have an effect are (Perlstein *et al.*, 1997):

- the standard food used (glucose or white bread);
- the size of the portion (in low carbohydrate foods 25 g available carbohydrate and not 50 g may be used, because the volume that would supply 50 g 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 glucose response curve;
- whether the subjects have glycaemic control problems or not; and
- the fasting blood glucose levels of the subjects .

#### 2.4.3.2 Variability in the sample

Any factor that will influence the rate of digestion and/or absorption of carbohydrates will influence the glycaemic response (Perlstein *et al.*, 1997). There is a positive relationship between the rate at which foods liberate their digestion products and the extent to which they raise the blood glucose concentration (Jenkins, Ghafari, Wolever, Taylor, Jenkins, Barker, Fielden & Bowling, 1982). Factors that influence the rate of digestion of starch will be discussed in detail in section 6.

The rate of absorption of carbohydrate is affected by the rate of gastric emptying (Jenkins *et al.*, 1982). Fat and protein (Perlstein *et al.*, 1997) and viscous forms of dietary fibre like guar gum and pectin (Jenkins, Wolever, Leeds, Gassull, Haisman, Dilawari, Goff, Metz & Alberti, 1978; Jenkins *et al.*, 1982) decrease the rate of gastric emptying. When the rate of gastric emptying is delayed, the glycaemic response curve flattens (Jenkins *et al.*, 1982).

#### 2.4.3.3 Physiological factors

There are variations in the measured GI of a food product between individuals and within the same individual on different occasions (Vorster *et al.*, 1990). Exercise, physical fitness and background diet affect GI. Other factors are age, sex and race. Also, GI values obtained in healthy subjects may differ from that obtained in people who suffer from diabetes.

#### **2.4.4 The GI of maize porridge**

Walker & Walker (1984) reported the GI of unrefined maize porridge to be 71 (using glucose as control), which is in the same order of magnitude as brown bread. Refined maize porridge had a GI of 73, which was similar to that of white bread, but not statistically different from unrefined maize porridge. Venter *et al.* (1990) studied the effects of refined maize meal porridge consumed at different temperatures on blood glucose levels. It was found that the GI (using glucose as control) of cooled maize porridge was lower than that of hot maize porridge (50.0 against 66.2). Reheated maize

porridge had a slightly higher GI (55.7) than cooled maize porridge. These differences were explained in terms of starch retrogradation during the cooling of the maize porridge. When the cooled porridge was reheated, starch retrogradation was partially reversed and the digestibility of the porridge increased in comparison with cooled porridge that had not been reheated.

Looking at the study of Walker & Walker (1984), it would seem like maize porridge is a high GI food with a GI similar to that of bread. The results of the study by Venter *et al.* (1990) suggest that maize porridge is an intermediate to low GI food, depending on the temperature it is consumed at.

The difference in GI between the two studies could be the result of the different recipes, cooking methods, the type of maize meal used, or the subjects used. In both studies, the composition of the maize meal was obtained from food composition tables. It is not clear what the difference between refined and unrefined maize meal was in terms of dietary fibre, starch content, fat content and particle size. Regarding recipes and cooking methods, in the study by Walker & Walker (1984), 25% maize meal was used and the porridge was cooked for 20-30 min. Venter *et al.* (1990) used 29 % maize meal and microwave cooked the porridge at maximum power (microwave oven power not specified) for three periods of 2 minutes each. The longer cooking time and higher moisture content in the first study may be a reason for the higher GI. Panlasigui, Thompson, Juliano, Perez, Yui & Greenberg (1991) found that increasing the cooking time of rice increased the GI and *in vitro* starch digestibility. The longer cooking probably resulted in more disruption of starch granules and a higher degree of starch solubilisation. The amount of water available in the porridge of Venter *et al.* (1990) could have been slightly limited (see section 2.5.2.4). When water is limited, the temperature at which gelatinisation (or melting of the crystallites) takes place is higher and disruption and solubilisation of starch may be limited (Colonna, Leloup & Buleon, 1992).



The nature of the cooking method could also affect the degree of starch gelatinisation and disruption. In the study by Walker & Walker (1984), conventional heating was used. During conventional cooking heat is conducted from the source (e.g. hotplate) to the food. Venter *et al.* (1990) used microwave cooking with which the heat is generated within the food.

Walker & Walker (1984) used black school children as subjects, while Venter *et al.* (1990) used white adults. The black school children were said to be representative of adolescents of rural Third World populations. It is not clear what the socioeconomic background of the white adults was, but it can probably be assumed that they had an urbanised Western lifestyle. These physiological differences (race and background diet) could have contributed to the difference in measured GI.

## **2.5 Factors affecting the rate of starch digestion**

Starch digestibility is limited by the degree of gelatinisation, granule size, amylose content, starch-protein interactions, starch-lipid complexes and degree of crystallinity, including that formed by retrogradation during processing (Whistler & BeMiller, 1997). Generally any treatment that destroys starch crystallinity (e.g. gelatinisation) or the integrity of the plant cell or tissue (e.g. milling) will increase the digestibility and reduce the resistant starch content (Asp & Björck, 1992).

Factors affecting the digestibility of starch can be divided into intrinsic (product) and extrinsic (processing) factors.

### ***2.5.1 Intrinsic factors affecting starch digestibility***

#### **2.5.1.1 Starch source**

The botanical origin of the starch will influence the digestibility thereof. Starches with B-type X-ray patterns are less susceptible to hydrolysis by enzymes (Faisant, Buléon, Colonna, Molis, Lartigue, Galmiche & Champ, 1995). This means that generally, cereal

starches are more susceptible to enzymes than tuber, root and legume starches. Most raw cereal starches are slowly, but completely, digestible. Raw potato and banana starches on the other hand are indigestible (Englyst *et al.*, 1992).

Wen, Lorenz, Martin, Stewart & Sampson (1996) found that wheat endosperm hardness affected the digestibility of starch in steamed bread. The digestibility of bread made from soft wheat flour was higher than that made from hard wheat flour. Since the sample size was kept constant, they explained this in terms of the lower protein content (thus higher starch content) and higher  $\alpha$ -amylase activity of soft wheat flours.

#### 2.5.1.2 Amylose/amylopectin ratio

A high amylose starch is digested more slowly than normal or low amylose starch and also yield more resistant starch in food products (Ring, Gee, Whittam & Johnson, 1988; Granfeldt, Björck, Drews & Tovar, 1992; Muir *et al.*, 1995; Xue, Newman & Newman, 1996; Åkerberg, Liljeberg & Björck, 1998).

#### 2.5.1.3 Starch granule size

The smaller the starch granules, the greater the extent of digestion *in vitro* (Annison & Topping, 1994). This is probably because the smaller granules have a larger surface area that the enzymes can attack.

#### 2.5.1.4 Natural enzyme inhibitors

Anti-nutrients such as tannins, lectins and phytic acid reduce the glycaemic response (Perlstein *et al.*, 1997). These compounds inhibit enzymes that digest proteins or carbohydrates. Snow & O'Dea (1981) found that stoneground wholemeal wheat flour was digested more slowly than white flour, whereas standard wholemeal flour that was reconstituted after milling was digested at a rate similar to that of white flour. They suggested that the stoneground wholemeal was less digestible, because of the presence of a natural amylase inhibitor that is found in the germ of wheat grains.

## 2.5.2. Extrinsic factors affecting starch digestibility

### 2.5.2.1 Physical form

Starch digestion is slowed down 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, or if rigid cell walls inhibit swelling and dispersion of starch, as in legumes (Würsch, 1989). When starch is very densely packed in a food such as spaghetti (Granfeldt & Björck, 1991), or if proteins encapsulate the starch granules (Annison & Topping, 1994), the digestibility is also reduced. When the rate of starch digestion is decreased, postprandial glucose and insulin responses are reduced or delayed.

Salmerón *et al.* (1997a & 1997b) suggested that grains should be consumed in a minimally refined form to reduce the risk of diabetes. Refining grains minimally would mean leaving more physically enclosed (type 1) resistant starch in the product. Also, Liljeberg, Granfeldt & Björck (1992) and Liljeberg & Björck (1994) found that including intact wheat, rye and barley kernels in bread reduced the glycaemic response, which is useful in the management of diabetes.

Several studies (*in vitro* as well as *in vivo*) showed that reducing the particle size of the sample increases the digestibility of starch (Snow & O'Dea, 1981; Holm & Björck, 1992; Granfeldt, Liljeberg, Drews, Newman & Björck, 1994). The digestibility increases, because the surface area increases as the particle size becomes smaller and that gives a greater contact area between the sample and the digestive enzymes (Colonna *et al.*, 1992; Annison & Topping, 1994).

Starch granules that have been damaged during milling or extraction are more susceptible to enzyme attack than intact granules. Physical damage can cause dislocations in the surface of the granule which allow the enzyme greater access to the free glycosidic chains (Oates, 1997).



### 2.5.2.2 Degree of gelatinisation

When starch granules are fully gelatinised and dispersed, the starch becomes easily digestible. Gelatinisation greatly increases the digestibility of starch, probably because it destroys the crystallinity and increases the porosity of the starch granules (Holm, Björck, Asp, Sjöberg & Lundquist, 1985; Holm, Lundquist, Björck, Eliasson & Asp, 1988; Bornet, Fontvieille, Rizkalla, Colonna, Blayo, Mercier & Slama, 1989; Eerlingen, Jacobs & Delcour, 1994a; Oates, 1997).

Starch granules present in high-amylose maize (amylomaize) are highly resistant to gelatinisation and need high pressure and temperatures up to 170 °C for complete granule disruption. These temperatures are not achieved with conventional cooking and large amounts of intact high-amylose maize starch granules can leave the small intestine undigested (Muir *et al.*, 1995).

### 2.5.2.3 Formation of retrograded starch

The degree of starch retrogradation after heat processing will determine the amount of type 3 resistant starch formed. The rate of retrogradation depends on the ratio of amylose to amylopectin, and the structures of the molecules. The higher the amylose content, the more retrograded starch is formed (Muir *et al.*, 1995). The structures of the molecules are determined by the botanical source of the starch, the temperature, the starch concentration and the presence and concentration of other ingredients, such as surfactants and salts (Whistler & BeMiller, 1997).

Amylose and amylopectin gels exhibit very different thermostabilities as shown by their respective melting temperatures ( $T_m$ ) ~125 and ~46 °C (Leloup, Colonna & Ring, 1992). The melting transition of amylopectin gels start around 36.1-37.3 °C, suggesting that the gels will have entered their melting transition *in vivo*. Amylose gels are highly thermoresistant, which make them resistant to digestion.

#### 2.5.2.4 The presence of other ingredients

The amount of moisture present during the heating of the starch plays a very important role (Annison & Topping, 1994). When spaghetti was dried at different temperatures (50, 80 and 90 °C), the *in vitro* digestibility of the starch did not change much (Casiraghi, Brighenti & Testolin, 1992). The digestibility of the spaghetti dried at 90 °C was slightly lower than that dried at 50° C. If the available water was not limited (i.e. more than three times the mass of the starch on dry base), the wheat starch would gelatinise at 53-64 °C (Whistler & Daniel, 1985; Colonna *et al.*, 1992). The spaghetti dried at 90 °C would then have a higher digestibility than that dried at 50° C, because gelatinisation of the starch would have taken place at the higher temperature. At high temperatures and limited water, protein cross-linkage increases. This leads to a higher degree of starch encapsulation by proteins and subsequently a decrease in starch digestibility (Casiraghi *et al.*, 1992).

Salts, sugars and any other compounds that bind water strongly will limit starch gelatinisation by lowering the water activity (Whistler & Daniel, 1985). Eerlingen, Van den Broeck, Delcour, Slade & Levine (1994b) found that the presence of sucrose, glucose, ribose or maltose (at concentrations of about 31%) decreased the amount of resistant starch formed in wheat starch gels, but increased the resistant starch in amylo maize starch gels.

Before an enzyme can start hydrolysing a starch granule, it must attach itself to the surface of the granule. Proteins and lipids can reduce surface accessibility by blocking the adsorption sites and in such a way reduce the susceptibility of the starch to enzyme attack (Oates, 1997). Conversely, lipids can also form complexes with amylose and thereby inhibit the formation of retrograded starch (Annison & Topping, 1994; Mauro, 1996). Usually the digestibility of starch increase as the amount of resistant starch decreases, but not with amylose-lipid complexes. Amylose-lipid complexes are digested more slowly than free amylose (Annison & Topping, 1994).

## 2.6 Determining starch digestibility *in vitro*

Why use *in vitro* methods to determine starch digestibility instead of using *in vivo* techniques like glycaemic index? *In vivo* studies are laborious in many aspects. Several motivated subjects are needed for a long period of time (Granfeldt *et al.*, 1992). When one uses human subjects, control is difficult. People may not follow test diets strictly and make changes in lifestyle or medication without reporting it. It is difficult to decide how long the experimental period should be in order to see the effect of the change in diet. For example, Gresse *et al.* (1993) suspected that the experimental period of 5 months used could have been too short to show the true effect of a typical African diet on the metabolic control of NIDDM in black patients.

Also, the facilities needed for *in vivo* studies are also often not available in laboratories involved in food research (Granfeldt *et al.*, 1992).

An ideal *in vitro* procedure should simulate conditions *in vivo* as far as possible (Asp & Björck, 1992; Champ, 1996). It is not so easy to mimic the *in vivo* situation though (Granfeldt *et al.*, 1992). Various methods for determining *in vitro* starch digestibility have been published. They vary in the way the sample is prepared, the enzymes that are used and the conditions of incubation (time, temperature and restriction). Table 4 summarises some of the methods that have been used to determine starch digestibility *in vitro*.



**Table 4: Some examples of conditions used when determining starch digestibility *in vitro***

Variable	Details of procedure	Reference
Sample preparation	None	Snow & O'Dea (1981); Holm <i>et al.</i> (1988); Englyst <i>et al.</i> (1992)
	Mincing	Englyst <i>et al.</i> (1992); Kingman & Englyst (1994)
	Grinding	Jenkins <i>et al.</i> (1982); Thorburn <i>et al.</i> (1987)
	Chewing	Granfeldt & Björck (1991); Granfeldt <i>et al.</i> (1992); Liljeberg <i>et al.</i> (1992); Liljeberg & Björck (1994)
	Homogenization	Englyst <i>et al.</i> (1992); Wen <i>et al.</i> (1996); Goñi, Garcia-Alonso & Saura-Calixto (1997)
	Starch isolated	Faulks & Bailey (1990); Xue <i>et al.</i> (1996)
Enzymes used	Pancreatin, invertase, amyloglucosidase	Englyst <i>et al.</i> (1992); Kingman & Englyst (1994)
	Salivary $\alpha$ -amylase, pepsin, pancreatic $\alpha$ -amylase	Granfeldt & Björck (1991); Granfeldt <i>et al.</i> (1992); Liljeberg <i>et al.</i> (1992); Liljeberg & Björck (1994)
	Salivary $\alpha$ -amylase, porcine pancreatin	Thorburn <i>et al.</i> (1987)
	Pancreatic $\alpha$ -amylase	Holm <i>et al.</i> (1988); Faulks & Bailey (1990); Xue <i>et al.</i> (1996)
	Salivary $\alpha$ -amylase	Wen <i>et al.</i> (1996)
	Salivary $\alpha$ -amylase, human pancreatic $\alpha$ -amylase	Jenkins <i>et al.</i> (1982)
	$\alpha$ -amylase, amyloglucosidase	Snow & O'Dea (1981)
	Pepsin, $\alpha$ -amylase	Goñi <i>et al.</i> (1997)
Time incubated	30 minutes	Snow & O'Dea (1981)
	60 minutes	Holm <i>et al.</i> (1988); Xue <i>et al.</i> (1996)
	120 minutes	Englyst <i>et al.</i> (1992); Kingman & Englyst (1994)
	180 minutes	Wen <i>et al.</i> (1996)
	210 minutes	Granfeldt & Björck (1991); Granfeldt <i>et al.</i> (1992); Liljeberg <i>et al.</i> (1992); Liljeberg & Björck (1994)
	240 minutes	Thorburn <i>et al.</i> (1987); Faulks & Bailey (1990); Goñi <i>et al.</i> (1997)
	300 minutes	Jenkins <i>et al.</i> (1982)

(Table 4 continues...)

**Table 4: Some examples of conditions used when determining starch digestibility *in vitro* (continued)**

Variable	Details of procedure	Reference
Temperature of incubation	37 °C	Jenkins <i>et al.</i> (1982); Thorburn <i>et al.</i> (1987); Holm <i>et al.</i> (1988); Faulks & Bailey (1990); Granfeldt & Björck (1991); Englyst <i>et al.</i> (1992); Granfeldt <i>et al.</i> (1992); Liljeberg <i>et al.</i> (1992); Kingman & Englyst (1994); Liljeberg & Björck (1994); Wen <i>et al.</i> (1996); Xue <i>et al.</i> (1996); Goñi <i>et al.</i> (1997)
	40 °C	Goñi <i>et al.</i> (1997)
	50 °C	Snow & O'Dea (1981)
Restriction	None	Snow & O'Dea (1981); Thorburn <i>et al.</i> (1987); Holm <i>et al.</i> (1988); Faulks & Bailey (1990); Englyst <i>et al.</i> (1992); Kingman & Englyst (1994); Goñi <i>et al.</i> (1997)
	Dialysis tubing	Jenkins <i>et al.</i> (1982); Thorburn <i>et al.</i> (1987); Granfeldt & Björck (1991); Granfeldt <i>et al.</i> (1992); Liljeberg <i>et al.</i> (1992); Liljeberg & Björck (1994); Wen <i>et al.</i> (1996)
Agitation	None	Thorburn <i>et al.</i> (1987)
	Type not specified	Holm <i>et al.</i> (1988); Wen <i>et al.</i> (1996)
	Shaking water bath	Snow & O'Dea (1981); Thorburn <i>et al.</i> (1987); Englyst <i>et al.</i> (1992); Kingman & Englyst (1994); Goñi <i>et al.</i> (1997)
	Stirred water bath	Jenkins <i>et al.</i> (1982); Granfeldt & Björck (1991); Granfeldt <i>et al.</i> (1992); Liljeberg <i>et al.</i> (1992); Liljeberg & Björck (1994)
	Magnetic stirrer to stir dialysis tubing	Granfeldt <i>et al.</i> (1992)
	Constant stirring	Faulks & Bailey (1990)
Measurement of digestion products	3,5-dinitrosalicylic acid method	Holm <i>et al.</i> (1988); Granfeldt & Björck (1991); Granfeldt <i>et al.</i> (1992); Liljeberg <i>et al.</i> (1992); Liljeberg & Björck (1994)
	Hexokinase method	Thorburn <i>et al.</i> (1987); Faulks & Bailey (1990)
	HPLC	Thorburn <i>et al.</i> (1987)
	Phenol and sulphuric acid	Wen <i>et al.</i> (1996)
	Parahydroxybenzoic acid hydrazide	Snow & O'Dea (1981)
	GOD/POD method	Jenkins <i>et al.</i> (1982)
Glucose GOD-PAP reagent	Englyst <i>et al.</i> (1992); Kingman & Englyst (1994); Goñi <i>et al.</i> (1997)	

### 2.6.1 Sample preparation

In most of the methods, the botanical structure of the food is disrupted mechanically in the first step. Granfeldt *et al.* (1992) developed an *in vitro* method based on chewing. The great advantage of this method is that the food can be analysed “as eaten”. Physically enclosed (type 1 resistant) starch will not be released to any great extent. Another group of researchers (Muir & O’Dea, 1992) also developed an *in vitro* procedure that use chewing as the first step, but specifically to determine resistant starch in food.

Englyst *et al.* (1992) agreed with Granfeldt *et al.* (1992) and Muir & O’Dea (1992) on the importance of the way that the food sample is divided and used different preparation steps for different foods. Most foods were analysed without pretreatment, but samples like pasta and rice were minced and whole wheat grains were milled or homogenized. It was claimed that it was not necessary to imitate chewing too closely. A criticism of using chewing was that the extent to which a food is chewed depended on its texture, the degree of hunger of the consumer, the presence of other foods, dental health and individual chewing habits. Englyst *et al.* (1992) favoured mechanical means of dividing the sample, because it resulted in smaller standard deviations than chewing.

Muir & O’Dea (1992) showed that the amount of resistant starch measured with their method decreased as the number of times that the sample was chewed increased. They also noted that people chewed ground samples of rice, cornflakes and chickpeas less than they chewed the food in when it was given in the whole form. They determined the average number of times that a particular food was chewed and kept it constant. This approach gave good results and it was found that there were no significant differences in the amounts of resistant starch measured when different people chewed the same foods a similar number of times.

Like Muir & O’Dea (1992), Granfeldt *et al.* (1992) standardised the chewing step. Subjects were told not to eat in the 1 to 2 hours prior to the experiment. They rinsed their mouths with water and then chewed the sample 15 times during 15 seconds. Björck and



co-workers applied this method successfully in several studies subsequently (Liljeberg *et al.*, 1992; Granfeldt *et al.*, 1994; Liljeberg & Björck, 1994; Åkerberg *et al.*, 1998).

The digestibility of food is affected by the number of times it is chewed and the chewing is affected by the texture and form of the food. Therefore, if a satisfactory standardised chewing technique is used, such a method could give results that are more useful than that of a method where the sample is not prepared or where the structure is disrupted mechanically.

### ***2.6.2 Enzymes used to digest sample***

Enzymes used to digest the sample may consist of amylases only or a combination of proteolytic enzymes and amylases. Granfeldt *et al.* (1992) included a pre-digestion step with pepsin to simulate the time that the food spends in the stomach. Granfeldt & Björck (1991) found that incubation with pepsin prior to incubation with  $\alpha$ -amylase increased the starch digestibility of bread and pasta slightly, but did not affect the differences between the samples in relation to one another. Kingman & Englyst (1994) used amyloglucosidase to prevent possible inhibition of the action of the  $\alpha$ -amylase by the products of digestion. Amyloglucosidase is not a mammalian enzyme though, therefore its suitability for *in vitro* digestibility experiments may be debated.

### ***2.6.3 Incubation conditions***

Incubation times vary, but so do the sample size and the concentrations of the enzymes that were used. Generally, the incubation time must be long enough for the digestibility curve to start flattening. Faulks & Bailey (1990) incubated for 240 minutes, because that is approximately the time it takes for food to pass through the human small intestine.

In most cases, incubation took place at 37 °C, which is body temperature, but the enzyme used will mostly determine the temperature.

Agitation ranged from none at all to constant stirring (Faulks & Bailey, 1990) and the use of shaking water baths.

The use of dialysis tubing offers certain advantages compared to unrestricted systems (Granfeldt *et al.*, 1992). The viscosity inside the dialysis sack will affect the rate of the appearance of maltose in the dialysate. Since the rate of absorption is also a factor that affects the glycaemic response of a food when eaten, the use of a restricted system can give useful information. A restricted system can also prevent the enzymes being inhibited by their end products (Boisen & Eggum, 1991). The end products of digestion are small molecules that will diffuse out of the dialysis sack into the surrounding solution.

#### *2.6.4 Measurement of digestion end products*

The products of starch digestion by  $\alpha$ -amylases are glucose, maltose, maltotriose and maltotetraose (Faulks & Bailey, 1990). Faulks & Bailey (1990) digested pea, maize, bean and rice starch with porcine  $\alpha$ -amylase and found that in all four cases maltose and maltotriose were present in the largest concentrations. Some researchers hydrolysed the digestion products with concentrated acid (Jenkins *et al.*, 1982; Thorburn *et al.*, 1987) or amyloglucosidase (Kingman & Englyst, 1994; Goñi, Garcia-Alonso & Saura-Calixto, 1997) before quantifying them. Others determined the digestion products individually by HPLC (Thorburn *et al.*, 1987; Faulks & Bailey, 1990). Another practice is to use the 3,5-dinitrosalicylic acid (Holm *et al.*, 1988; Granfeldt & Björck, 1991; Granfeldt *et al.*, 1992; Liljeberg *et al.*, 1992; Liljeberg & Björck, 1994). In this instance, the reducing hemiacetal groups in the digestion products are measured. The glucose chain lengths in the starch sample affect the results. Faulks & Bailey (1990) found that the percentage maltotriose decreased as the amylose content of the starches increased. The maltotriose was probably formed due to the  $\alpha$ -1-6 branching points in amylopectin.

Despite the large variation in conditions used, many researchers have found that *in vitro* starch digestibility correlated positively with *in vivo* glycaemic response (Jenkins *et al.*, 1982; Thorburn, 1987; Holm *et al.*, 1988; Bornet *et al.*, 1989; Granfeldt & Björck,

1991; Granfeldt *et al.*, 1992; Liljeberg *et al.*, 1992; Granfeldt *et al.*, 1994; Englyst, Veenstra & Hudson, 1996; Goñi *et al.*, 1997). Simple, reliable methods that determine the rate of starch digestibility *in vitro* can be useful in screening the starch properties of food, because the rate of digestion is a critical factor in determining the metabolic response after a meal (O'Dea, Snow & Nestel, 1981). Using dialysis tubing can also take into account some of the factors that will affect the rate of absorption of glucose in the digestive tract (e.g. viscosity of the food).

## 2.7 Conclusions

Diabetes could become a serious problem in the Black South African population as more and more of them adapt an urbanised lifestyle. There are clear similarities between the incidence of diabetes in the Australian Aboriginal population and that in the Black South African population. It was shown that reverting to a traditional lifestyle and diet improved diabetic Aborigines' condition (O'Dea, 1984). Although Gresse *et al.* (1993) could not prove the same for Black South Africans with their study, there is still a possibility that traditional stiff maize porridge could play a role in the low incidence of diabetes in rural Black people.

Traditional stiff maize porridge could contain type 1 (physically entrapped) and type 3 (retrograded) resistant starch. The hardness of the maize endosperm could affect the amount of physically entrapped starch present, because the starch granules are packed more tightly in the hard endosperm than in the soft endosperm. The limited amount of water that is available in stiff porridge could also prevent all the starch from gelatinising and raw cereal starch is digested more slowly than gelatinised starch. This indicates that maize porridge could possibly be a low GI food. *In vivo* studies to determine the digestibility of traditional stiff maize porridge have yielded conflicting results. According to Walker & Walker (1984) maize porridge is a high GI food, but according to Venter *et al.* (1990) it is an intermediate to low GI food.



There is little analytical nutritional data available on the starch-based foods that South Africans eat. Only 20 % of the data in the 1991 MRC Food Composition Tables is of local origin (Langenhoven, Kruger & Van Twisk, 1996). These data do not necessarily reflect local food composition, because of differences in cultivars, agricultural conditions and practices. Another problem is that the carbohydrate figures in food tables were in most cases obtained by difference. This means that there is little information on the composition and availability of the carbohydrate.

Using a simple, reliable *in vitro* method to determine the digestibility of starch in maize porridge in comparison to bread could help to clear up the discrepancies in the results of previous studies, for example Walker & Walker (1984) and Venter *et al.* (1990). This data could give useful information on the composition of a starch based staple food and help to enable researchers to: 1) possibly lower the risk for the development of DM in Africans and 2) to provide modified products better suited to existing diabetics' nutritional needs.