MAIZE PORRIDGE STARCH DIGESTIBILITY

BY

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SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

MSc (Agric) Food Science and Technology

IN THE
DEPARTMENT OF FOOD SCIENCE
FACULTY OF NATURAL, AGRICULTURAL AND INFORMATION SCIENCES
UNIVERSITY OF PRETORIA
SOUTH AFRICA

DECEMBER 1999

DECLARATION

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

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ABSTRACT

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The incidence of diabetes mellitus is very low in rural, traditionally living South African Black people, but higher in urbanised Black people. The carbohydrate staple of rural Black people is maize porridge, but with urbanisation maize porridge is often replaced by bread. This change in carbohydrate staple could have contributed to the higher incidence of diabetes in urban Black people.

An *in vitro* method involving pre-chewing of the food followed by digestion with pepsin and α-amylase in dialysis tubing was used to determine starch digestibility. The starch digestibility of traditional stiff maize porridge made from cultivars with different endosperm hardness was compared to white wheat bread. A hydrolysis index was calculated and used to predict the glycaemic index. The effect of different preparation parameters (particle size, cooking time, hotplate and microwave cooking) on the digestibility of maize porridge was determined, as well as the microstructures of the two food products and the starch digestibilities of maize, wheat and oat flour porridges.

Maize porridge had a lower rate ($p < 0.001$) and extent ($p < 0.001$) of *in vitro* starch digestibility than bread. Possible reasons for the difference are the dense microstructure of porridge with physically enclosed starch, and the high amylose content of South African maize. Wheat flour porridge was less digestible than bread ($p < 0.001$), which showed that the nature of the heat treatment process and the presence of other ingredients (e.g. fat) could have a great effect on starch digestibility.

Wheat flour porridge and oat flour porridge had lower starch digestibilities than maize flour porridge ($p < 0.05$), which indicated that there were intrinsic differences between the starch digestibility of the endosperm material from different cereals. These differences can possibly be attributed to other endosperm constituents, gluten in wheat and β-glucans in oats.

The starch digestibility of hotplate cooked porridge was positively correlated with endosperm hardness ($p < 0.01$). This phenomenon cannot be explained in terms of differences in composition or particle size. The composition of the endosperm from different cultivars was similar. The hard cultivars had more large particles than the soft cultivars, but it was shown that reducing the particle size of maize meal to maize flour did not have a significant effect on starch digestibility.

Interestingly, both decreasing and increasing the cooking time decreased the starch digestibility of maize porridge significantly. Cooking porridge shorter probably disrupted starch granules to a lesser extent while cooking longer probably solubilised more starch, which led to the formation of more retrograded amylose during cooling. The rate and extent of starch digestibility of microwave cooked maize porridge was similar to that of hotplate cooked porridge, but the differences between cultivars were not related to endosperm hardness. This difference between the effect of microwave and conventional cooking may be due to the faster heat transfer during microwave cooking.

The mean predicted glycaemic index for maize porridge (glucose standard) was 44, which implies that maize porridge is a slow carbohydrate release food that may be useful in the dietary management of diabetes. The fact that bread is digested faster than maize porridge could be a contributing factor in the increased prevalence of diabetes in the urban compared to the rural Black South Africans.

UITTREKSEL

MIELIEPAP STYSELVERTEERBAARHEID

deur

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Die voorkoms van diabetes is baie laag onder plattelandse Suid-Afrikaanse Swartmense met 'n tradisionele lewenswyse, maar hoër onder verstedelikte Swartmense. Die koolhidraat stapelvoedsel van plattelandse Swartmense is mieliepap, maar met verstedeliking word mieliepap dikwels vervang met brood. Hierdie verandering in koolhidraat stapelvoedsel kon bygedra het tot die hoër voorkoms van diabetes onder verstedelikte Swartmense.

Styselverteerbaarheid is bepaal met 'n *in vitro* metode wat begin met die kou van die monster gevolg deur vertering met pepsien en α-amilase in 'n dialise buis. Die styselverteerbaarheid van tradisionele stywe mieliepap gemaak van kultivars met verskillende endosperm hardheid is vergelyk met witbrood. 'n Hidrolise indeks is bereken en gebruik om die glukemiese indeks te voorspel. Die effek van verskillende voorbereidingsparameters (partikelgrootte, kooktyd, stoofplaat en mikrogolf kook) op die verteerbaarheid van die pap is bepaal, asook die mikrostruktuur van die twee voedselprodukte en die styselverteerbaarhede van koring- en hawermeelpap.

Mieliepap het 'n laer tempo ($p < 0.001$) en mate ($p < 0.001$) van *in vitro* styselverteerbaarheid as brood. Moontlike redes vir die verskil is die digte mikrostruktuur van mieliepap met fisies ingeslote stysel, en die hoë amilose-inhoud van Suid-Afrikaanse mielies. Koringmeelpap was minder verteerbaar as brood ($p < 0.001$), wat aandui dat die aard van die hittebehandeling en die teenwoordigheid van

ander bestanddele (bv. vet) 'n groot effek kan hê op styselverteerbaarheid. Koringmeelpap en hawermeelpap was minder verteerbaar as mieliemeelpap ($p < 0.05$) wat aandui dat daar intrinsieke verskille in verteerbaarheid tussen die endosperm materiaal van verskillende grane is. Hierdie verskille is moontlik veroorsaak deur wesentlike bestanddele van die endosperm, gluten in koring en β-glukane in hawer.

Daar was 'n positiewe korrelasie ($p < 0.01$) tussen die styselverteerbaarheid van stoofplaat gekookte mieliepap en endosperm hardheid. Hierdie verskynsel kan nie verklaar word deur verskille in samestelling of partikelgrootte nie. Die harde kultivars het meer groot parikels gehad as die sagte kultivars, maar daar is gewys dat 'n verkleining van die partikelgrootte vanaf mieliemeel na mielieblom nie 'n betekenisvolle effek op styselverteerbaarheid gehad het nie.

Interessant genoeg het beide 'n verkorting en verlenging van die kooktyd die styselverteerbaarheid van die mieliepap betekenisvol verlaag. Om die pap korter te kook het waarskynlik die styselkorrels minder ontwrig, terwyl langer kook waarskynlik meer stysel in oplossing gebring het, wat gelei het tot meer geretrogradeerde stysel gedurende afkoeling. Die tempo en mate van styselverteerbaarheid van mikrogolf gekookte mieliepap was soortgelyk aan stoofplaat gekookte mieliepap, maar die verskille tussen kultivars was nie verwant aan endosperm hardheid nie. Hierdie verskil tussen die effek van mikrogolf- en konvensionele verhitting mag verwant wees aan die vinniger hitte-oordrag gedurende mikrogolfverhitting.

Die gemiddelde voorspelde glukemiese indeks vir mieliepap (glukose standaard) was 44, wat impliseer dat mieliepap 'n kossoort is wat koolhidrate stadig vrystel. Dit kan nuttig wees in die dieetbehandeling van diabetes. Die feit dat brood vinniger verteer as mieliepap kon 'n bydraende faktor wees in die hoë voorkoms van diabetes onder verstedelikte in vergelyking met plattelandse Swart Suid-Afrikaners.

ACKNOWLEDGEMENTS

I would like to sincerely thank the following people:

Prof. J.R.N. Taylor, my study leader, for guidance, advice and positive criticism throughout the project.

Mrs. C. Erasmus, my co-leader for her role in the planning of the project, the image analysis and milling of the maize, and for her critical review of my dissertation.

The Council for Scientific and Industrial Research (CSIR) and National Research Foundation (NRF) for funding the project.

Mr. C.F. van der Merwe and Mr. A.N. Hall from the Laboratory for Microscopy and Microanalysis, University of Pretoria for assistance with the light microscopy and scanning electron microscopy work.

Dr. M.J. van der Linde from the Department of Information Technology and Prof. H.T. Groeneveld from Department of Statistics, University of Pretoria for advice and assistance in statistical analysis of data.

Mr. P. Rankhumise and Mrs. R. Mathibe for their important roles in the development of my maize porridge recipes.

My best friend and husband, Jacobus, for his support and encouragement.

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CHAPTER 1

INTRODUCTION

Carbohydrates are the single most important source of food energy in the world. They make up 40 to 80 percent of total food energy intake, depending on locale, cultural considerations and economic status. Those persons with high carbohydrate diets are often in the lower economic strata as foods high in carbohydrate, such as cereal grains, are most often the least expensive (FAO, 1997a).

For many years, starch was considered little more than fattening bulk (Johnson & Gee, 1996). For over thirty years there has been a misconception in North America that starch is fattening and should be excluded from the diet (Stephen, 1994). Some people also avoid eating starchy foods, because they believe it is an indicator of non-affluence (Stephen, Sieber, Gerster & Morgan, 1995). Throughout the 1980s, however, there was a major re-evaluation of the importance of starchy foods in Western diets. This was partly because they came to be seen as a major source of dietary fibre and partly because it was recognized that, despite their bulk, cereals and starchy vegetables were low in fat and therefore also relatively low in energy (Johnson & Gee, 1996).

In South Africa remarkable changes are taking place in the diet of African Blacks during their transition from a traditional rural to an urbanised lifestyle. With their traditional lifestyle in past generations, degenerative Western diseases like diabetes were nearly absent in the Black population. Now the prevalence of some of these diseases are higher than in the White population (Cannan & Walker, 1997). It is predicted that in South Africa, diabetes will become an increasing public health burden; especially among the local Black urban dwellers rising in socioeconomic state (Walker & Walker, 1991). Since Blacks constitute 76% of the total population in South Africa (Directorate: Agricultural Statistics and Management Information, 1998), this problem can have far-reaching consequences.

It is suspected that traditionally prepared and cooked maize porridge (the staple food of many rural Blacks in South Africa) could have some beneficial health effects that could be useful in the management of diabetes (Venter , Vorster, Van Rooyen, Kruger-Locke & Silvis, 1990). Traditional stiff maize porridge is shown in Figure 1 together with an example of the maize meal used in this study

Figure 1: Traditional stiff maize porridge (left) and laboratory milled maize meal (right)

With urbanisation maize porridge is often replaced by bread (Mmakola, Kirsten & Groenewald, 1997). This change in carbohydrate staple is one of the factors that could have played a role in increasing the incidence of diabetes among South African Black people.

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 & Tietyen-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.

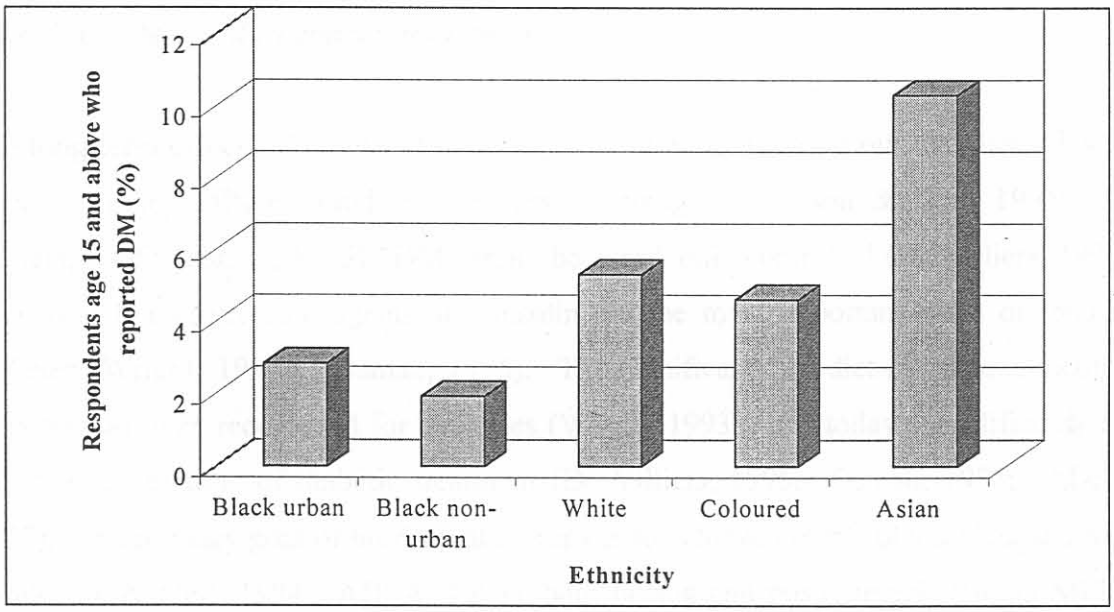

Figure 2: Self reported diabetes mellitus (DM) in South Africa (Data from unpublished preliminary report, Department of Health, South Africa, 1999)

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 (Elliot, 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.

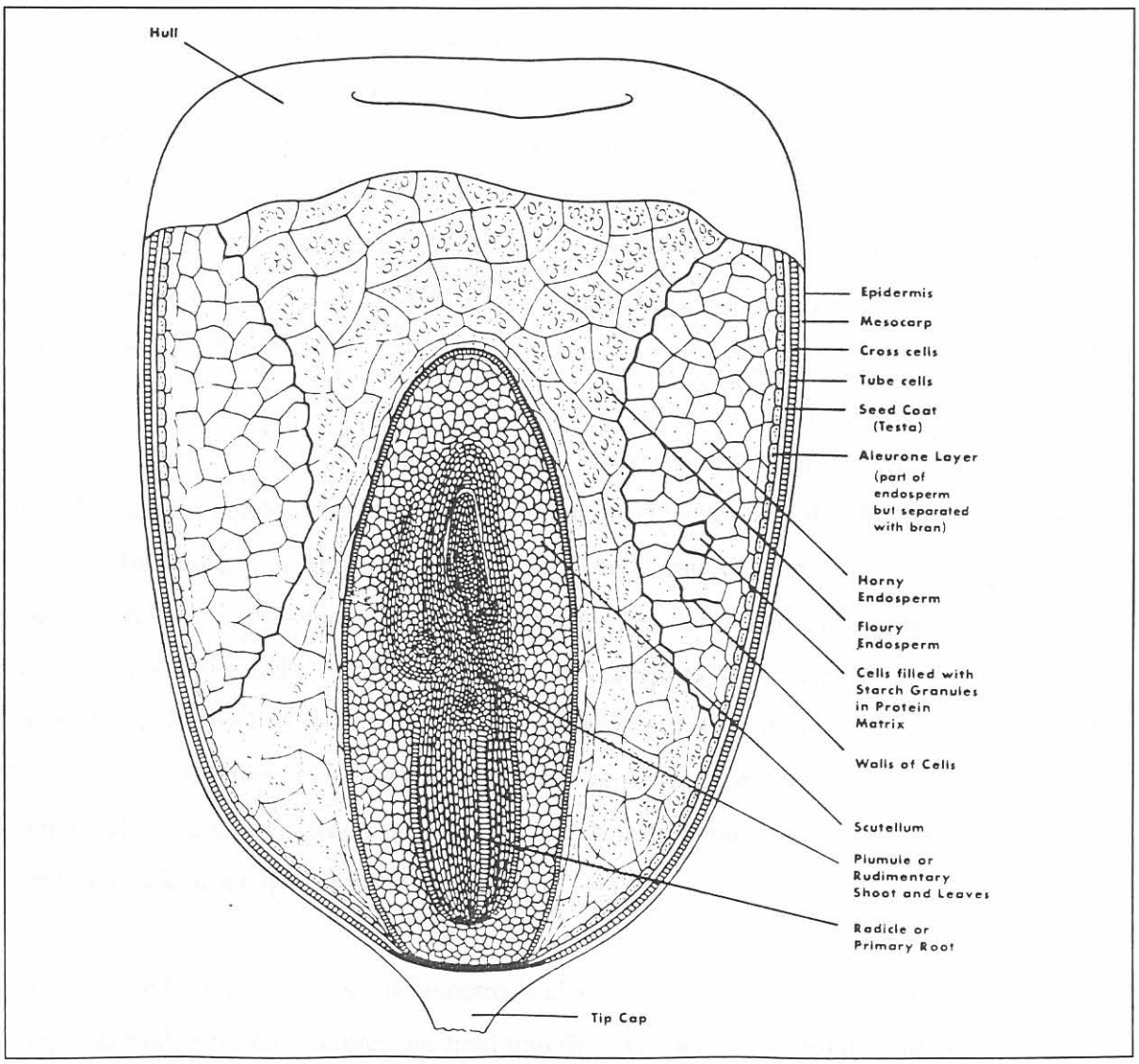

Figure 3: Longitudinal section of a maize kernel (Hoseney, 1994)

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).

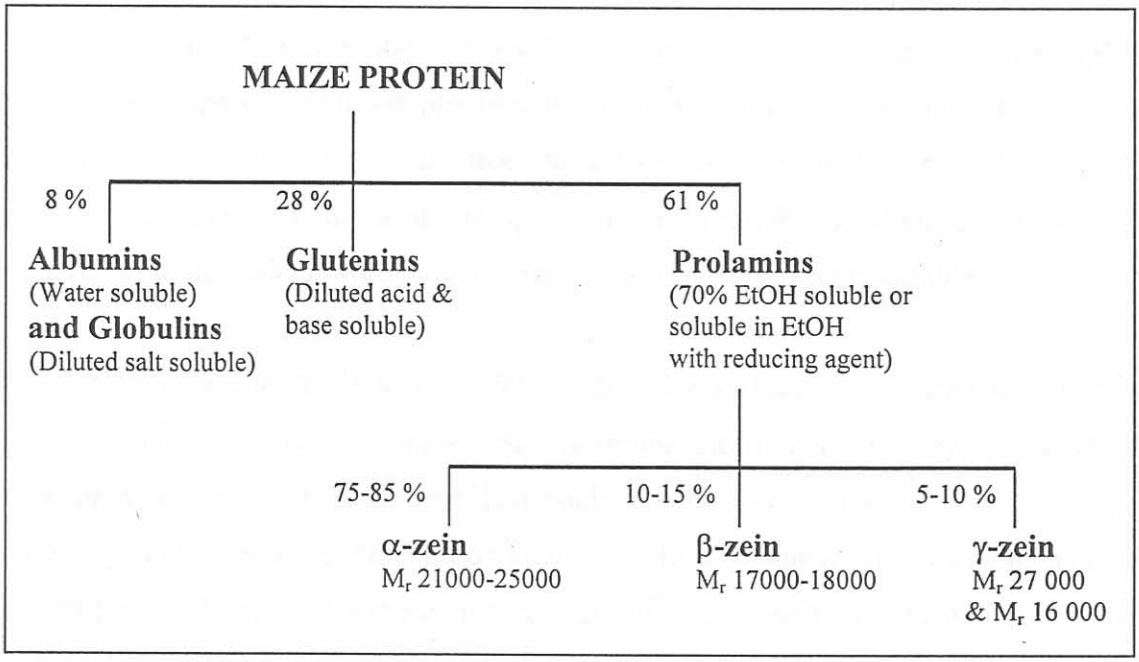

Figure 4: Classification and nomenclature of maize protein (information from Esen, 1987; Hoseney, 1994; Mestres & Matencio, 1996)

Maize protein bodies consist mainly of the prolamin zein and the protein matrix consists mainly of glutelin (Peterson & Johnson, 1978). Dombrink-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 γ-zein fractions and that α-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 estmating 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 neccesary 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 μm 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).

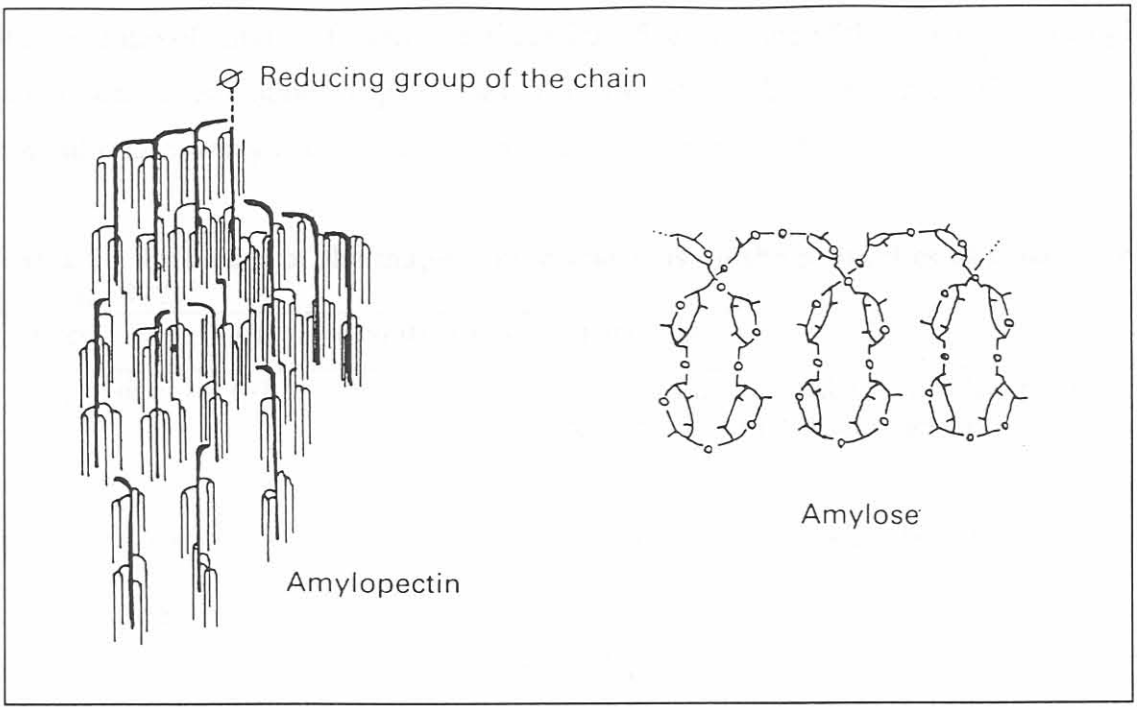

Figure 5: The structures of amylose and amylopectin (Alais & Linden, 1991)

Amylose is a predominantly linear, but not straight, molecule linked by α-D-(1→4) bonds. This axial→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 α-D-(1→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 α-D-(1→4) and the branched points α-D-(1→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 amylomaize 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.
Amylomaize	50-70	Granule that is resistant to swelling; rigid gel; opaque paste; high paste temperature.

When undamaged starch granules are examined under polarised light, maltese 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).

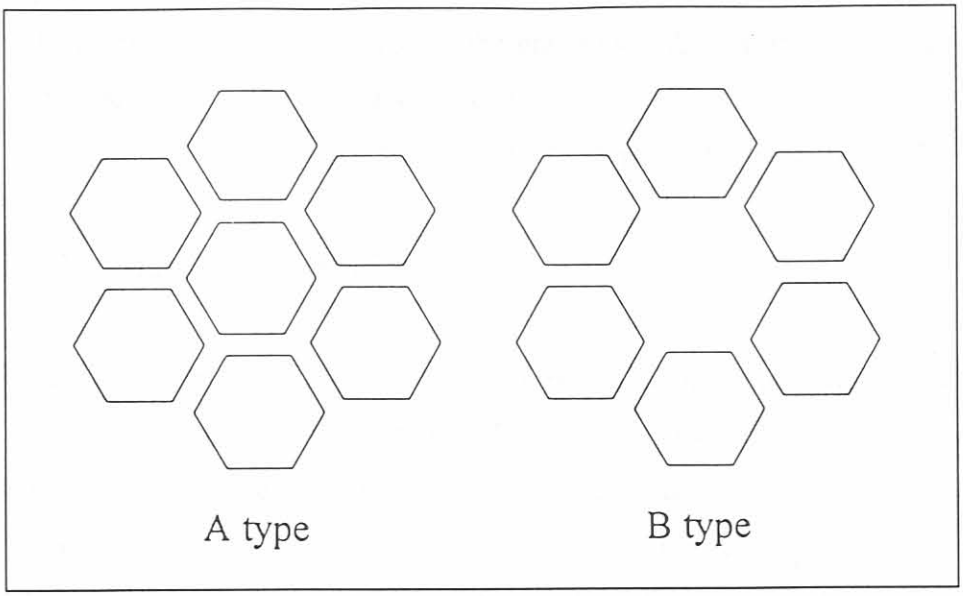

Figure 6: Diagrammatic representation of the arrangement of six parallel double helices in starches that give a A-type pattern and starches that give a B-type pattern. Water molecules replace the centre double helix in B-type starches (Whistler & BeMiller, 1997)

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 strarch (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.

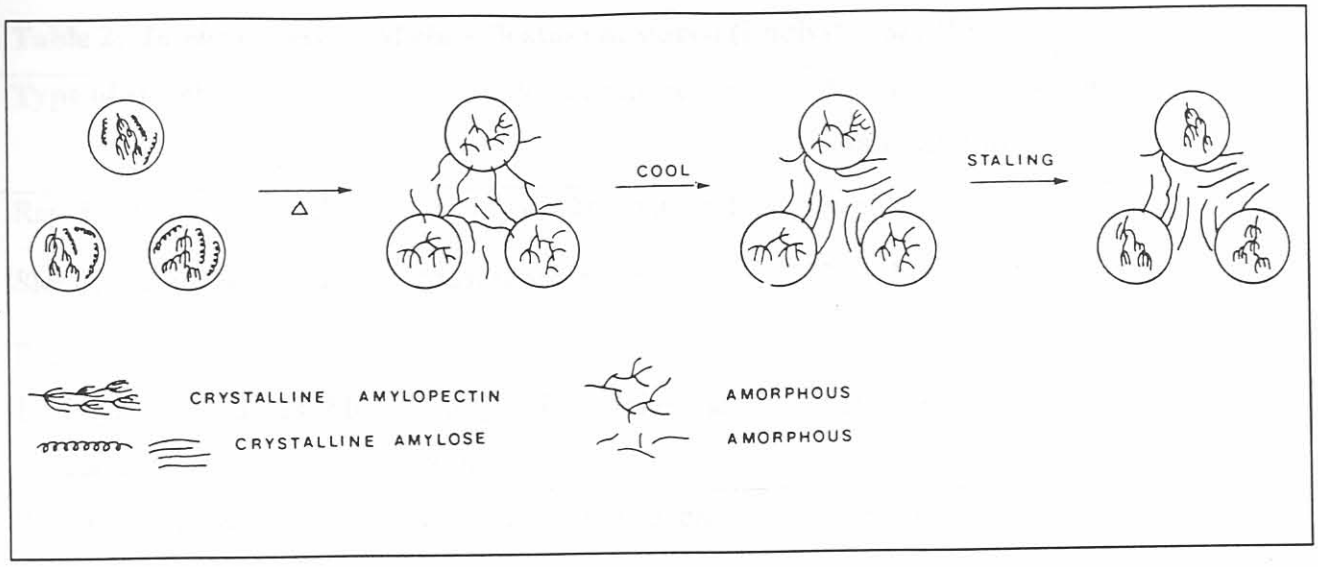

Figure 7: Structural changes in a starch granule during heating and subsequent cooling (Wong, 1989)

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.

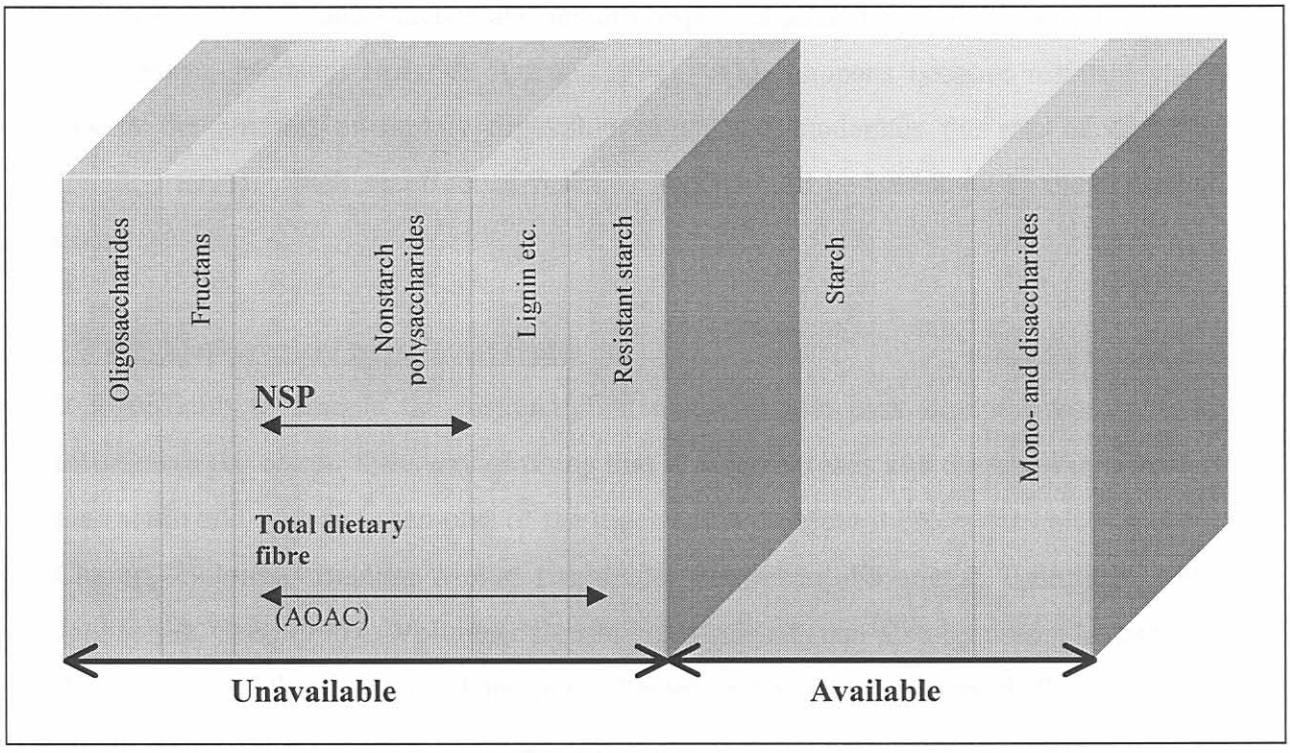

Figure 8: Relationship between non-starch polysaccharides (NSP), total dietary fibre, and unavailable carbohydrates (Asp, 1995)

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 μm had about 10 AACC units damaged starch and maize flour with 100 % of the particle < 150 μm 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 α-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 α-amylase. The products of digestion of starch by α-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.

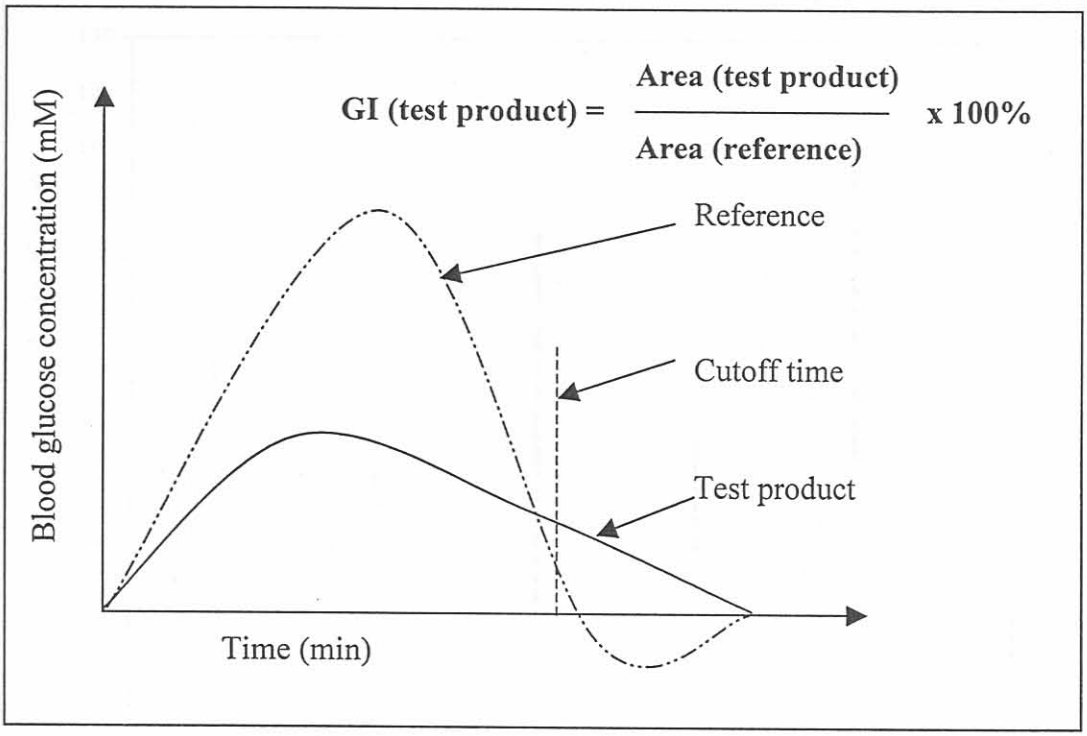

Figure 9: Calculation of the glycaemic index (GI) of a food product (Björck & Asp, 1994; adapted)

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).

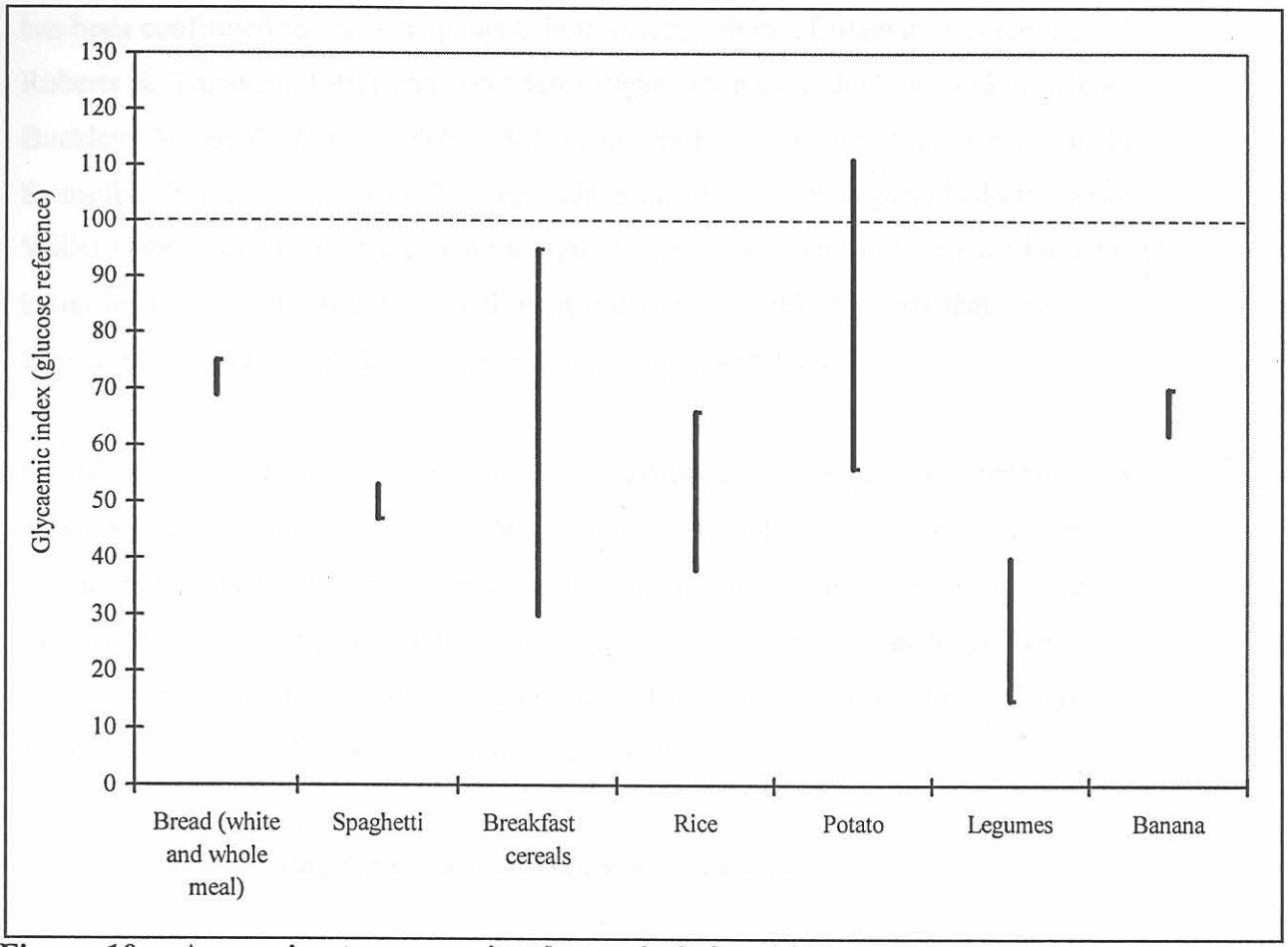

Figure 10: Approximate ranges in glycaemic index (glucose standard) for some starchy foods eaten by South Africans (GI values obtained from Foster-Powell & Miller, 1995)

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 & Willet (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 statistictically 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 section2.5.2.4). 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 α-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 amylomaize 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 *et al.* (1988); Englyst *et al.* (1992)
	Mincing	Englyst *et al.* (1992); Kingman & Englyst (1994)
	Grinding	Jenkins *et al.* (1982); Thorburn et al. (1987)
	Chewing	Granfeldt & Björck (1991); Granfeldt *et al.* (1992); Liljeberg *et al.* (1992); Liljeberg & Björck (1994)
	Homogenization	Englyst *et al.* (1992); Wen et al. (1996); Goñi, Garcia-Alonso & Saura-Calixto (1997)
	Starch isolated	Faulks & Bailey (1990); Xùe *et al.* (1996)
Enzymes used	Pancreatin, invertase, amyloglucosidase	Englyst *et al.* (1992); Kingman & Englyst (1994)
	Salivary α-amylase, pepsin, pancreatic α-amylase	Granfeldt & Björck (1991); Granfeldt *et al.* (1992); Liljeberg *et al.* (1992); Liljeberg & Björck (1994)
	Salivary α-amylase, porcine pancreatin	Thorburn *et al.* (1987)
	Pancreatic α-amylase	Holm *et al.* (1988); Faulks & Bailey (1990); Xue *et al.* (1996)
	Salivary α-amylase	Wen *et al.* (1996)
	Salivary α-amylase, human pancreatic α-amylase	Jenkins *et al.* (1982)
	α-amylase, amyloglucosidase	Snow & O'Dea (1981)
	Pepsin, α-amylase	Goñi *et al.* (1997)
Time incubated	30 minutes	Snow & O'Dea (1981)
	60 minutes	Holm *et al.* (1988); Xue *et al.* (1996)
	120 minutes	Englyst *et al.* (1992); Kingman & Englyst (1994)
	180 minutes	Wen *et al.* (1996)
	210 minutes	Granfeldt & Björck (1991); Granfeldt *et al.* (1992); Liljeberg *et al.* (1992); Liljeberg & Björck (1994)
	240 minutes	Thorburn *et al.* (1987); Faulks & Bailey (1990); Goñi *et al.* (1997)
	300 minutes	Jenkins *et al.* (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 *et al.* (1982); Thorburn *et al.* (1987); Holm *et al.* (1988); Faulks & Bailey (1990); Granfeldt & Björck (1991); Englyst *et al.* (1992); Granfeldt *et al.* (1992); Liljeberg *et al.* (1992); Kingman & Englyst (1994); Liljeberg & Björck (1994); Wen *et al.* (1996); Xue *et al.* (1996); Goñi *et al.* (1997)
	40 °C	Goñi *et al.* (1997)
	50 °C	Snow & O'Dea (1981)
Restriction	None	Snow & O'Dea (1981); Thorburn *et al.* (1987); Holm *et al.* (1988); Faulks & Bailey (1990); Englyst *et al.* (1992); Kingman & Englyst (1994); Goñi *et al.* (1997)
	Dialysis tubing	Jenkins *et al.* (1982); Thorburn *et al.* (1987); Granfeldt & Björck (1991); Granfeldt *et al.* (1992); Liljeberg *et al.* (1992); Liljeberg & Björck (1994); Wen *et al.* (1996)
Agitation	None	Thorburn *et al.* (1987)
	Type not specified	Holm *et al.* (1988); Wen *et al.* (1996)
	Shaking water bath	Snow & O'Dea (1981); Thorburn *et al.* (1987); Englyst *et al.* (1992); Kingman & Englyst (1994); Goñi *et al.* (1997)
	Stirred water bath	Jenkins *et al.* (1982); Granfeldt & Björck (1991); Granfeldt *et al.* (1992); Liljeberg *et al.* (1992); Liljeberg & Björck (1994)
	Magnetic stirrer to stir dialysis tubing	Granfeldt *et al.* (1992)
	Constant stirring	Faulks & Bailey (1990)
Measurement of digestion products	3,5-dinitrosalicylic acid method	Holm *et al.* (1988); Granfeldt & Björck (1991); Granfeldt *et al.* (1992); Liljeberg *et al.* (1992); Liljeberg & Björck (1994)
	Hexokinase method	Thorburn *et al.* (1987); Faulks & Bailey (1990)
	HPLC	Thorburn *et al.* (1987)
	Phenol and sulphuric acid	Wen *et al.* (1996)
	Parahydroxybenzoic acid hydrazide	Snow & O'Dea (1981)
	GOD/POD method	Jenkins *et al.* (1982)
	Glucose GOD-PAP reagent	Englyst *et al.* (1992); Kingman & Englyst (1994); Goñi *et al.* (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 α-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 α-amylase by the products of digestion. Amyloglucosidase is not a mamalian 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 α-amylases are glucose, maltose, maltotriose and maltotetraose (Faulks & Bailey, 1990). Faulks & Bailey (1990) digested pea, maize, bean and rice starch with porcine α-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 α-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 Austaralian 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.

CHAPTER 3

RESEARCH OBJECTIVES

Objective 1

To determine the *in vitro* starch digestibility of traditional stiff maize porridge compared to that of white bread in order to establish whether maize porridge is a slow, intermediate or fast carbohydrate release food.

Objective 2

To determine the effect of cooking conditions (time and hotplate vs. microwave) on the digestibility of starch in maize porridge. The two groups of researchers who obtained conflicting results (Walker & Walker, 1984; Venter *et al.*, 1990) used different cooking conditions to prepare the maize porridge.

Objective 3

If the starch digestibility of maize porridge differs from that of bread, attempt to establish whether the difference is due to intrinsic or extrinsic factors.

Objective 4

To determine the effect of endosperm vitreousness on the *in vitro* digestibility of maize porridge. The hypothesis was that maize porridge made from cultivars with a hard endosperm would have a lower rate and extent of digestion than porridge from cultivars with a softer endosperm. Because the starch granules of hard endosperm are tightly packed in the protein matrix, expansion would be limited and it would be difficult to gelatinise the starch. In the soft endosperm, where the starch granules are more loosely packed and there are many air spaces, gelatinisation would take place more easily. Native starch is less susceptible to enzyme digestion than gelatinised starch (Holm *et al.*, 1985).

Objective 5

To determine the effect of particle size on the *in vitro* digestibility of maize porridge. The hypothesis was that digestibility would increase with a decrease in particle size, because a decrease in particle size would increase the surface area of the particles which would be in contact with the enzymes (Colonna *et al.*, 1992).

CHAPTER 4

EXPERIMENTAL

4.1 Materials

4.1.1 Maize

Twelve samples of South African bred white maize kernels from different hybrid dent cultivars were used. The cultivars were obtained from a South African seed company and represented a wide range of endosperm hardness (vitreousness). The maize was evaluated for milling properties at the Division of Food Science and Technology of the Council for Scientific and Industrial Research (Foodtek, CSIR). The names of the cultivars are protected by the seed company and the seed company also asked for its name not to be published. The cultivars therefore had to be coded. From the twelve cultivars, six were selected on grounds of endosperm hardness (refer to 4.3.1) for digestibility experiments. The selected cultivars were sorted in order of increasing endosperm hardness and numbered A to F. Table 5 compares the endosperm hardness (% translucency) of the selected maize cultivars to that of a soft standard (SR 52, an industry standard) and a hard standard (maize cultivar that is known by the industry to yield a high percentage of grits on dry milling).

Table 5: Endosperm hardness (% translucency) of maize from selected[1] cultivars compared to a hard and soft standard[2]

Cultivar	Number of replicates (n)	Mean translucency (%)
A	48	$25^{3,d}$ (23)[4]
B	48	$45^{b,c}$ (23)
C	48	$49^{3,b}$ (19)[4]
D	48	53^{b} (21)
E	48	72^{a} (15)
F	48	74^{a} (19)
Hard standard	48	73^{a} (12)
Soft standard	48	$34^{c,d}$ (22)

1 The two cultivars with the highest and the two with the lowest % translucency were selected, as well as two cultivars with values in between.

2 The soft standards was SR 52 and the hard standard a maize cultivar that is known by the industry to yield a high percentage of grits on dry milling.

3 Values with different letters in superscript are statistically significantly different ($p < 0.05$).

4 Values in brackets are the standard deviations of the measurements.

The results of the Tukey grouping ($p < 0.05$) indicated that cultivar E and F were similar to the hard standard and cultivar A and B to the soft standard. Cultivars C and D were similar neither to the hard standard nor to the soft standard. A Tukey grouping excluding the hard and soft standards (not shown) indicated that there was no significant difference between cultivars E and F and no significant difference between B, C and D, but cultivar A was significantly different ($p < 0.05$) from the other cultivars. To summarise, the hardness of cultivar A was significantly lower than that of B, C and D, which were on their turn were significantly lower than E and F.

Table 5: Endosperm hardness (% translucency) of maize from selected[1] cultivars compared to a hard and soft standard[2]

Cultivar	Number of replicates (n)	Mean translucency (%)
A	48	$25^{3,d}$ (23)[4]
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Hard standard	48	73^{a} (12)
Soft standard	48	$34^{c,d}$ (22)

1 The two cultivars with the highest and the two with the lowest % translucency were selected, as well as two cultivars with values in between.

2 The soft standards was SR 52 and the hard standard a maize cultivar that is known by the industry to yield a high percentage of grits on dry milling.

3 Values with different letters in superscript are statistically significantly different ($p < 0.05$).

4 Values in brackets are the standard deviations of the measurements.

The results of the Tukey grouping ($p < 0.05$) indicated that cultivar E and F were similar to the hard standard and cultivar A and B to the soft standard. Cultivars C and D were similar neither to the hard standard nor to the soft standard. A Tukey grouping excluding the hard and soft standards (not shown) indicated that there was no significant difference between cultivars E and F and no significant difference between B, C and D, but cultivar A was significantly different ($p < 0.05$) from the other cultivars. To summarise, the hardness of cultivar A was significantly lower than that of B, C and D, which were on their turn were significantly lower than E and F.

4.1.2 Wheat

Wheat flour (Snowflake White Bread Flour, Premier Milling, Newtown, South Africa) was bought from a local supermarket.

4.1.3 Oats

Whole kernel rolled oats (Tiger Oats, Jungle Oats Company, Maitland, South Africa) was bought from a local supermarket.

4.1.4 White bread

White pan-style wheat bread was bought from Pick 'n Pay supermarket (Hatfield, Pretoria). The bread was baked at the in-store bakery and it was bought freshly baked on each day of analysis.

The composition of the maize meal samples is shown in Table 6, and that of white bread, wheat flour and oat flour in Table 7.

Table 6: Proximate composition of maize meal from different cultivars

Cultivar	Moisture (%)	Ash (% dry basis)	Fat (% dry basis)	Protein[1] (% dry basis)	Starch (% dry basis)
A	15.8[2,a] (0.2)[3]	0.454[b] (0.031)	1.15[b] (0.01)	8.03[a,b] (0.04)	85.1[b] (2.2)
B	15.5[a] (0.1)	0.529[a] (0.014)	1.36[a] (0.05)	8.39[a] (0.07)	85.2[b] (1.7)
C	16.8[a] (0.1)	0.426[b] (0.027)	1.02[c] (0.02)	7.92[b,c] (0.08)	87.1[a,b] (1.8)
D	16.4[b] (0.2)	0.505[a] (0.026)	1.19[b] (0.02)	6.88[d] (0.18)	88.7[a] (2.5)
E	15.1[a] (0.1)	0.367[c] (0.033)	1.00[c] (0.02)	8.18[a,b] (0.03)	84.5[b] (2.2)
F	16.4[b] (0.1)	0.411[b,c] (0.016)	1.06[c] (0.03)	7.48[c] (0.08)	88.6[a] (2.4)

1 N x 6.25

2 Values with different letters in superscripts in columns are statistically significantly different ($p < 0.05$)

3 Values in brackets are the standard deviations of the measurements

Table 7: General composition of bread, wheat flour and oat flour

Sample	Moisture (%)	Ash (% dry basis)	Fat (% dry basis)	Protein[1] (% dry basis)	Starch (% dry basis)
White bread	45.3 (0.9)[2]	2,94[3]	2.77[4]	13.1[4]	73.8 (1.4)
Wheat flour	13.7 (0.0)	0.65 (0.92)	1.56[5]	13.7 (0.1)	72.7 (1.4)
Oat flour	10.8 (0.1)	1.32 (0.02)	11.02[3]	12.3 (0.0)	60.4 (0.7)
			8.06[6]		

1 N x 5.70 for wheat, N x 5.83 for oats

2 Values in brackets are the standard deviations of the measurements

3 According to Van Heerden, Anderson, Van Niekerk & Wight (1990)

4 According to South African food composition tables (Langenhoven, Kruger, Gouws & Faber, 1991)

5 According to the manufacturer

6 According to Kent & Evers (1994)

The starch content of the maize meal ranged between 84.5 and 88.6 %, the protein content between 6.9 and 8.4, the fat content between 1.00 and 1.32, the ash content between 0.38 and 0.53 and the moisture content between 15.0 and 16.8. There were small, but statistically significant differences between some of the cultivars in all the components measured.

The ash and fat content of oat flour were higher than that of wheat flour, but the starch content was lower. On a dry basis, white bread and wheat flour had similar starch and protein contents. The fat content of white bread is higher than that of wheat flour.

4.2 Methods

4.2.1 Degerming maize

The main objective of degerming was to separate the germ and bran from the maize kernel so that an endosperm-rich, low fat meal would be obtained. Six maize cultivars were selected on basis of vitreousness. Before degerming, the moisture content of the maize kernels was increased to toughen the germ and bran, which makes it easier to remove them from the endosperm (Uhlig & Bhat, 1979). This conditioning was done in two steps: overnight to 14 % moisture and then a 10 min conditioning to a final moisture content of approximately 18 %.

Degerming was done with a small-scale maize degermer (designed by the CSIR in collaboration with the South African Maize Board) which simulates the action of an industrial-scale Robinson or Beall degermer. According to Uhlig & Bhat (1979), the Beal degermer "consists of a cast iron, cone shaped rotor mounted on a rotating horizontal shaft in a conical cage. Part of the cage is fitted with perforated screens and the remainder with plates having conical protrusions on the inner surface. The cone has similar protrusions over most of the surface and the small end of the cone has spiral corrugations to move the maize forward. Attached to the large end is a short cylinder corrugated in an opposing direction to retard the flow. Clearance between the tips of the

rotor protrusions and the perforated screens is about half an inch". Because of the relatively large clearance (12.7 mm) with respect to the size of a maize kernel, the action of the degermer is mainly due to kernel rubbing against kernel.

4.2.2 Milling maize to meal

A Miac laboratory scale roller mill (model C, 1960, Mühlenbau und Industrie, Braunschweig, Germany) with two rollers was used to mill the samp (degermed maize) to maize meal. The first few steps in the milling sequence released the germ and bran that had remained with the endosperm after degerming. This germ and bran were separated from the endosperm by sieving the maize meal after the second step (Table 8).

Table 8: Maize milling procedures in terms of the size of the gap between the rollers, the number of times milled and the aperture of the sieve used to sieve the meal after the milling step

Step number	Gap between rollers (mm)	Times milled	Sieve aperture (mm)
1	1.00	1	*
2	0.50	1	3.5 mm
3	0.20	2	*
4	0.05	3	1.01 mm
5	0.05	1	1.01 mm

(* not sieved)

The gap between the rollers was decreased progressively. Milling was continued until the meal passed through a 1.01 mm sieve. After step 4, all the particles of the soft and medium endosperm maize passed through a 1.01 mm sieve. The hard cultivars required an additional milling step before the desired particle size was reached.

The maize meal produced by this procedure is what some authors would refer to as "highly refined". Unrefined maize meal would be a wholemeal in which the bran and germ are retained. Lightly refined refers to a semi-sifted meal in which part of the bran

and germ are removed. In a highly refined or super-sifted meal the germ and bran is removed as far as possible.

4.2.3 Milling maize and oats to flour

For the experiment with maize flour, the maize meal from cultivar C was milled with a laboratory hammer-mill fitted with an 800 µm screen. The resulting flour was sieved with a 212 µm South African Bureau of Standards approved laboratory test sieve. The fraction with particle sizes larger than 212 µm was milled again and the procedure was repeated until about 95 % of the original sample passed through the 212 µm sieve. The remaining fraction of particles larger than 212 µm was discarded. The same procedure was followed to mill the rolled oats to flour.

4.2.4 Maize, wheat and oat porridge cooking procedure

The recipe was derived from a traditional African recipe. Mr. P. Rankhumise (Tswana man, aged 65), was asked to show how he usually cooked his stiff maize porridge. All quantities were measured and the process was timed. For cooking porridge in the laboratory, the quantities had to be decreased. A microwave cooking procedure with the same decreased quantities was also developed. Mrs. R. Mathibe (Tswana woman, aged 52) was asked to taste the porridge in both cases and confirm that it was similar to the traditional stiff maize porridge that she was used to.

4.2.4.1 Hotplate cooking

One quantity of porridge was cooked per day. The cultivar was chosen randomly and two replicates were done for each cultivar on two different days. Salt (0.71 g) tap water (94.00 g) was weighed into a 250 ml plastic beaker. The salt was dissolved and the solution poured into a saucepan. The saucepan was covered and put on a hotplate (double solid hotplate with settings "on", 1, 2, 3, 4 and "full") with the temperature setting on "full". The solution was brought to boiling point (approximately 6 min), after which the temperature setting was turned down to exactly half way between 2 and 3. A

stop watch was started, counting down from 10 min 30 sec. Immediately, 38.39 g maize meal was added to the water/salt solution. The mixture was stirred well and the saucepan was covered again. At 7 min remaining, 31.00 g tap water was added. The mixture was stirred well and the saucepan was covered. At 6 min remaining, the temperature setting was turned down to "on". The porridge was stirred well at 3 min 30 sec and 1 min remaining. When the time expired, the porridge was transferred to a 250 ml plastic beaker. The beaker was covered with paper towel to prevent the surface of the porridge of drying out and left to cool at room temperature.

4.2.4.2 Microwave oven cooking

Six samples were cooked simultaneously. Three replicates were done on three different days. A 900 W microwave oven was used. Tap water (94.00 g) and salt (0.71 g) were weighed into each of six 250 ml plastic beakers. The beakers were covered with cling wrap and heated for 5 min on full power to bring the salt/water solution to boiling point. Maize meal (38.39 g) was added to each beaker and mixed well. The beakers were covered and heated for 2 min 30 sec on full power. Tap water (31.00 g) was added, well mixed into the porridge and the beakers was covered. The porridge was cooked for 3 min 30 sec on full power and left in the microwave oven for 10 min. It was then taken out of the microwave oven, stirred well and left to cool at room temperature.

4.3 Analyses

4.3.1 Hardness of maize kernels

Before milling, the % translucency of the maize kernels was determined by an image analysis method developed by Erasmus *et al.* (1997). This was done at Foodtek, CSIR as part of the evaluation of the milling properties requested by the seed company. The image analysis system converts the optical image from the macroviewer with a charged couple device camera into an electric signal. Inside the camera an electron beam scans the image line by line. As each line is being scanned, the output signal changes according to the scene illumination. Each line is divided into a number of pixels, which

are calibrated against a fixed size. After the feature to be measured is detected, the amount of pixels is integrated to give a total value for the area detected.

The image analyser determined the following attributes of the maize kernel: spherical dimensions (length, height, width and thickness), top surface area, size of germ and tip cap and translucency area. The % translucency was used to quantify the hardness of the kernel.

$$\text{Hardness (\% translucency)} = \frac{\text{Area of translucent parts}}{\text{(Total kernel area – area of germ and tip cap)}} \times 100$$

Soft and hard control samples were included. The soft control was SR 52, an industrial soft standard. The hard control was a cultivar known by the industry to yield a high percentage grits on dry milling.

4.3.2 Particle size distribution

Particle size distribution was determined by using South African Bureau of Standards approved laboratory test sieves with apertures of 1000, 500, 250 and 150 μm.

4.3.3 Proximate analysis

The proximate composition of the samples was expressed on dry weight basis. Values calculated were converted to dry basis by the following equation:

$$\text{Component (\% dry basis)} = \frac{\text{component (\% as is)} \times 100}{\text{(100 – moisture content (\%))}}$$

4.3.3.1 Ash

AACC Method 08-01 (American Association of Cereal Chemists, 1983a) was used to determine the ash content of the maize meal and the wheat and oat flours.

Approximately 4 g sample was weighed accurately into a silica ashing crucible which had previously been ignited, cooled in a dessicator and weighed. The samples were incinerated in a muffle furnace until a light grey ash was obtained, cooled in a dessicator and weighed. Ash content was calculated as follows:

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

4.3.3.2 Moisture

AACC Method 44-15A (American Association of Cereal Chemists, 1983b) was used. For samples that with moisture content of less than 13 % (maize meal, maize flour, wheat flour, oat flour, standard amylose, standard amylopectin and dried isolated starch of 4.3.4.1), the one-stage air oven method was used. Approximately 15 g of well mixed sample was placed into a moisture dish that had previously been dried, cooled in a dessicator and weighed. The dish was covered with its lid and weighed. The sample was put in an air oven at 103 ± 1 °C. The lid was put under the dish. The sample was heated for 72 h, the dish was covered with its lid and the sample was placed in a dessicator to cool to room temperature. The moisture content was determined as a loss in moisture using the following equation:

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

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

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

was then reduced using a clean, dry food liquidiser. The one-stage procedure described above was then followed. The total moisture content was calculated with the following equation:

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

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

4.3.3.3 Total starch

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

The analysis included solubilisation of the starch with dimethyl sulphoxide, digestion with thermostable α-amylase and digestion with amyloglucosidase. The formed glucose was then determined with a glucose oxidase/peroxidase reagent (GOPOD) and the absorbance read at 510 nm. The total starch was calculated with the following equation:

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

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

4.3.3.4 Total protein

Samples were analysed for crude protein using a Kjeldahl method (modified AACC Method 46-12, American Association of Cereal Chemists, 1983c). Approximately 0.5 g sample was weight accurately into a digestion tube. One Kjeltab (Thompson & Capper, Cheshire, England, UK), a 5 g tabled consisting of 100 parts K_2SO_4, 6 parts $CuSO_4.5H_2O$ and 2 parts selenium was added. To that, 20 ml of concentrated H_2SO_4 was added.

Samples were digested for approximately 2 h using a Büchi 430 Digestor (Büchi, Flavil, Switzerland). Distillation of ammonia, reaction with boric acid and titration with standard HCl (0.1 M) were done with a Büchi 322 Distillation Unit (Büchi, Flavil, Switzerland). The crude protein content was calculated using the following equation:

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

For maize, the factor used was 6.25, for wheat flour 5.70 and for oat flour 5.83.

4.3.3.5 Crude fat

The crude fat content of the maize meal was determined with AACC Method 30-25 (American Association of Cereal Chemists, 1983d). The maize meal was milled with a laboratory hammer mill to pass through a 500 µm sieve. Approximately 5 g of well-mixed sample was weighed accurately onto a filter paper. The filter paper was folded to prevent escape of the meal and placed in a thimble. A piece of fat-free absorbent cotton wool was placed on top to distribute solvent as it dropped on the sample. Sample was extracted with petroleum ether (condensation rate of 5-6 drops per sec) for 5 h in a Soxhlet extractor. The solvent was evaporated on a water bath. The flask with fat was then dried completely in an oven at 103 °C for 30 min. The flask was cooled in a dessicator and weighed. Before the extraction, the same flask had been dried in an oven, cooled in dessicator and weighed. The crude fat content was calculated as follows:

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

4.3.4 Amylose / Amylopectin ratio

4.3.4.1 Isolation of starch

A 20 % (m/m) maize meal in water slurry (containing 100 p.p.m. NaN_3) was steeped for 24 h at room temperature. The slurry was put through a wet mill (Retsch, Haan,

Germany) with a 250 µm sieve 20 times. It was then centrifuged at a relative centrifuging force (g)of 2,000 for 10 min. The protein layer was scraped off and the starchy pellets combined. The starchy pellets were diluted with water and centrifuged. Again the protein layer was scraped off. The procedure was repeated until no protein layer was formed when the slurry was centrifuged. The isolated starch was dried in an air oven at 55 °C for 48 h.

4.3.4.2 Amylose content of starch

The method of Faulks & Bailey (1990) was used. The isolated starch sample was boiled in a reagent containing dimethyl sulphoxide and iodine. This severe treatment solubilised the starch. Aliquots of this solution was diluted and the absorbance was measured at 620 nm. The amylose content was determined by comparison with a standard curve. Mixtures of pure amylose and amylopectin (Sigma) in different rations between 0 and 100 % was used to create the standard curve.

4.3.5 Damaged starch

Chopin type SD 4 starch damage determination instrument (Chopin, Villeneuve-La-Garenne, France) was used. The principle of operation of the instrument is that damaged starch absorbs iodine more rapidly than undamaged starch. The quantity of iodine absorbed is measured amperometrically. Iodine absorption is inversely proportional to the current flowing between the two poles of an amperometric electrode. The amount of damaged starch was expressed in AACC units. South African Wheat Board standard samples (7.5 and 12.9 AACC units respectively) were used to calibrate the instrument and more Wheat Board standard samples (12.5, 14.5 and 15.5 AACC units) were also analysed.

4.3.6 In vitro starch digestibility

A procedure involving pre-chewing of the food (Granfeldt *et al.*, 1992) was used, with slight modifications. The flow diagram (Figure 11) summarises the procedure. Three samples were taken from each batch of hotplate cooked porridge. From the microwave cooked porridge, only one sample was taken from each cultivar.

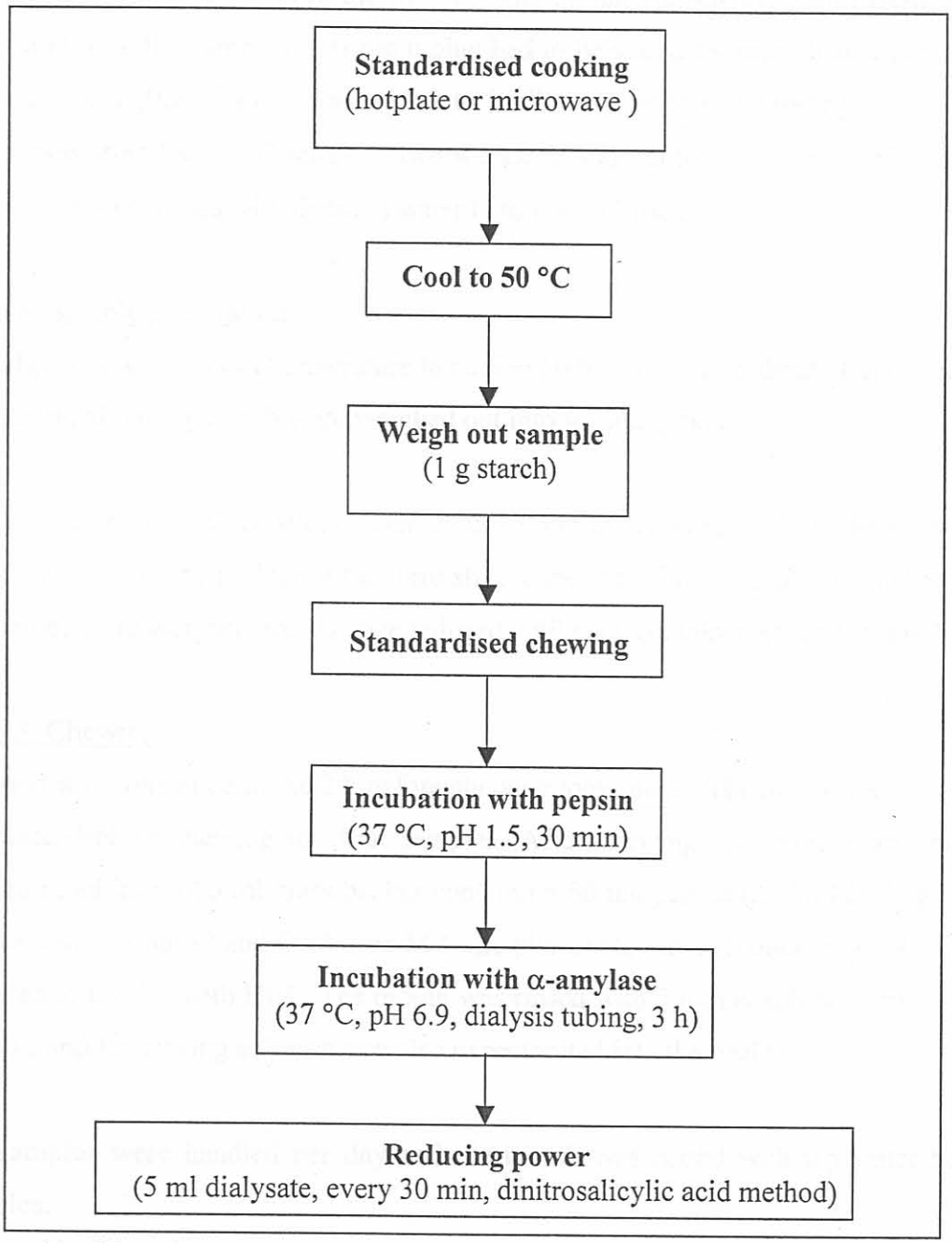


Figure 11: Flow diagram of the procedure used to determine the *in vitro* starch digestibility of porridge

4.3.6.1 Preparation of dialysis tubing

Dialysis tubing (Visking ex Labretoria, Pretoria) with a dry flat width of 45 mm and a molecular weight cut-off of 12-14 kDa was cut into 13 cm strips. The tubing was soaked in distilled water at 15 °C overnight. One end of the tube was then closed by tying it with a piece of string. The tubing, as well as extra pieces of string later used to close the other end of the tube, were then boiled in distilled water for 5 min to remove the sulphur that manufacturers add to preserve the tubing. The tubing was covered with fresh distilled water and used the same day. If the tubing had to be stored for more than a day, sodium benzoate acid (0.2 % m/v) was added to inhibit cellulolytic micro-organisms and the tubing was stored at 10 °C for up to two weeks. If sodium benzoate was used, the tubing was rinsed three times with distilled water before it was used.

4.3.6.2 Sample preparation

Porridge was left at room temperature to cool to 50 °C. This took about 20 min. Samples containing about 1 g starch were weighed out into weighing boats.

Just before analysis, three slices, each about 15 mm thick, were cut from the white wheat bread loaf. From the middle of the third slice, cubes of about 20 x 20 x 15 mm were cut. The cubes were weighed and the size reduced until each contained about 1 g starch.

4.3.6.3 Chewing

No food was consumed in the 2 h before chewing took place. The mouth was rinsed with tap water before chewing the first sample. After chewing, the sample was carefully expectorated into a 50 ml glass beaker containing 50 mg pepsin (2 000 FIB-U/g, Merck, Darmstadt, Germany) and 6 ml 0.05 M Na,K-phosphate buffer (containing 0.4 g/l NaCl) adjusted to pH 1.5 with HCl. The mouth was rinsed with 5 ml phosphate buffer (pH 6.9) for 30 s and the rinsing solution was also expectorated into the beaker.

Six samples were handled per day and the mouth was rinsed with tap water between samples.

4.3.6.4 Enzyme incubation

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

After this, the pH was adjusted to 6.9 with 2 M NaOH. Porcine pancreatic α-amylase (A 6255 Sigma) was then added. The enzyme (70 µl) was dissolved in 7 ml phosphate buffer and 1 ml of this solution was added to the sample. This represented 237 Sigma units per g of starch.

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

4.3.6.5 Measurement of products of digestion

Every 30 min a 5 ml aliquot of the dialysate was removed after stirring the contents of the beaker well with a glass rod. It was analysed for reducing power by the 3,5-dinitrosalicylic acid (DNS) method. The aliquot was added to 5 ml DNS reagent (1% DNS in 0.4 M NaOH containing 30 % sodium potassium tartrate) in a 25 ml volumetric flask. The flask was immersed in a boiling water bath for 5 min to develop the colour, cooled and the sample was made up to volume. Absorbance was measured at 540 nm. A maltose standard curve was also constructed and used to convert the absorbancy readings to maltose concentration (mg/ml). The following calculations were done to calculate starch digestibility:

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

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

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

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

4.3.6.6 Blanks and reference sample

With every set of samples, a blank was run in duplicate. The blank sample was not chewed, but transferred to the beaker containing pepsin and buffer (6 ml, pH 1.5) immediately. Buffer (5 ml, pH 6.9) was added and the sample was broken down slightly with a glass rod. From there the blank was treated the same as the samples, but instead of adding 1 ml of enzyme solution, 1 ml of phosphate buffer (pH 6.9) was added.

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

4.3.6.7 Calculation of hydrolysis index (HI) and predicted GI

A hydrolysis index (HI) was calculated as described by Granfeldt *et al.* (1992):

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

According to Åkerberg, *et al.* (1998), Granfeldt (in her Ph.D. thesis, 1994) found a significant correlation (r = 0.826) between HI and GI. The equation used to predict GI was the following:

$$GI = 0.862HI + 8.198$$

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

4.3.7 Microscopy

4.3.7.1 Light microscopy

The porridge was allowed to cool to room temperature. A piece of porridge was cut out, approximately 5 x 20 x 20 mm. It was fixed with glutaraldehyde (2.5 % in 0.075 M phosphate buffer) for 30 min. The porridge was cut into smaller blocks, approximately 2 x 3 x 5 mm. It was fixed in glutaraldehyde for 4 – 24 h. The sample was rinsed three times with phosphate buffer (0.075 M, pH 6.9). It was then dehydrated with 30, 50, 70, 90 and three times 100 % ethanol. The sample was impregnated with 50 % LR White resin (in 100 % ethanol) for 2-3 h and then with 100 % LR White resin overnight on a rotator. The sample was put into a gelatine capsule and covered with LR White resin. The resin was polymerised overnight at 75 °C. The gelatine capsule was removed and ultra-thin sections (0.4 µm) was made. The section was put on a water droplet on a microscope slide and dried. It was stained with Toluidine Blue O.

4.3.7.2 Scanning electron microscopy (SEM)

The sample preparation for SEM was similar to that for light microscopy, up to the point was rinsed with phosphate buffer. For SEM, the sample was then fixed in osmium tetroxide (2.5 % in water). It was rinsed three times with water and then dehydrated in ethanol, the same procedure as for light microscopy. After ethanol dehydration, the sample was dried using critical point dehydration in liquid carbon dioxide. The sample was then mounted and covered with gold vapour and examined.

4.3.8 Statistical analysis

Statistical analysis was done using Statistica for Windows Release 5.0 (StatSoft Inc. 1984-1995, Tulsa, USA) and Microsoft Excel 97 (Microsoft Corporation, 1985-1997). Dr. M.J. van der Linde (Department of Information Technology, University of Pretoria) and Prof. H.T. Groeneveld (Department of Statistics, University of Pretoria) were consulted regarding the experimental design and statistical analysis.

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

$$\text{test quantity} = \frac{b_1 - b_2}{(S^2_{b1} - S^2_{b2})^{1/2}}$$

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

To determine the significance of a correlation, Pearson product moment correlation distribution was used to compute p from r.

CHAPTER 5

RESULTS

5.1 Particle size distribution of maize meal made from different cultivars

Figure 12 shows the particle size distribution of the maize meal made from the endosperm of different maize cultivars. Within each particle size category the cultivars are numbered from A to F with A the cultivar with the softest endosperm (lowest % translucency) and F the cultivar with the hardest endosperm (highest % translucency).

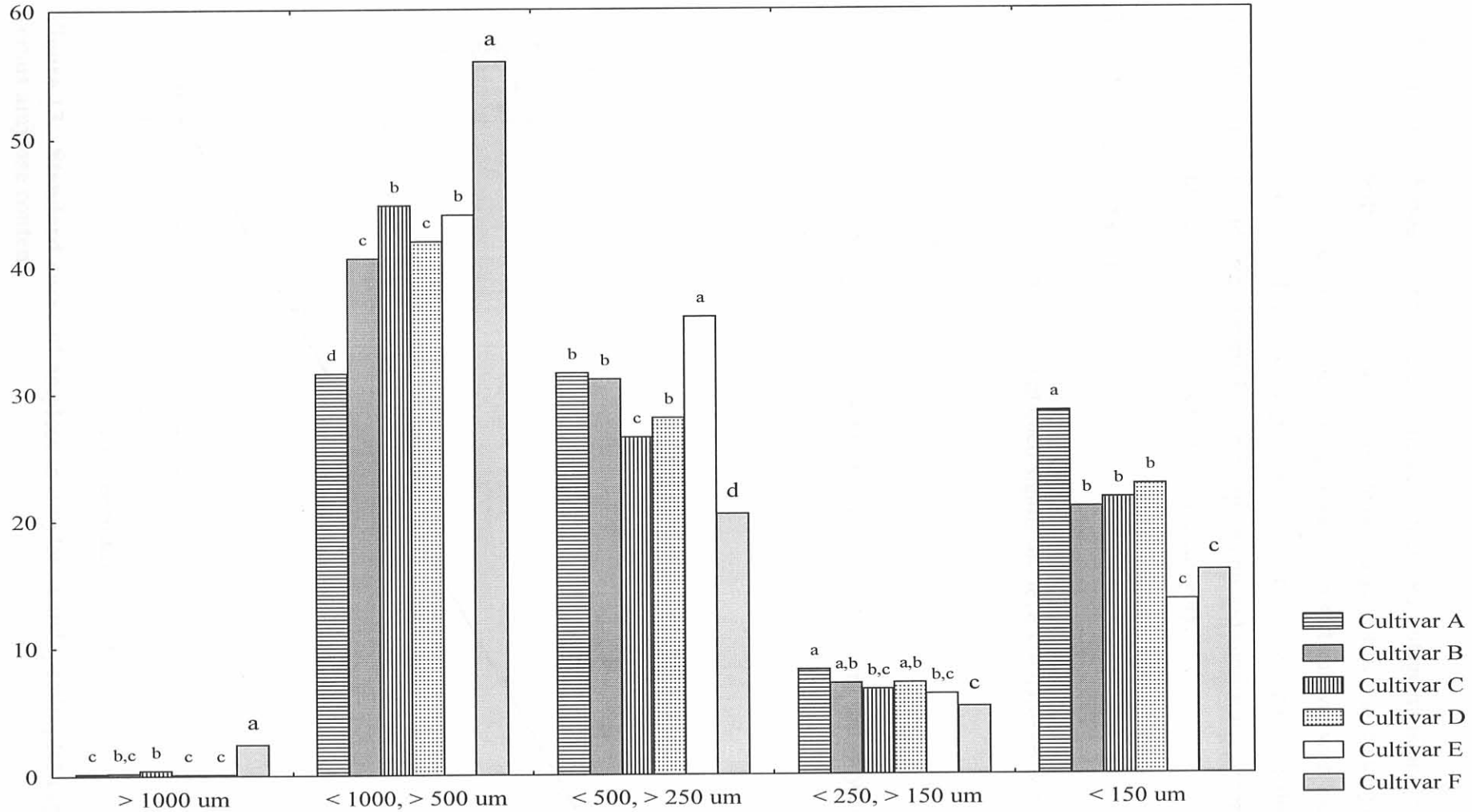


Figure 12: Particle size distribution of maize meal from cultivars with different endosperm vitreousness represented per particle size category to show significant differences[1] between cultivars within particle size categories

1 Columns with different letters in a particle size category are statistically significantly different ($p < 0.05$)

Cultivar F, which was a hard cultivar with the highest percentage translucency of the six samples, had significantly more particles in the two larger particle size categories than the other samples. On the contrary, cultivar A, which was a soft cultivar with the lowest percentage translucency of the six samples, had significantly less particles than the other samples in the second largest particle size category and had significantly more particles than the other samples in the smallest particle size category.

5.2 Damaged starch

None of the maize meal samples contained significant levels of damaged starch.

5.3 Amylose content

Figure 13 shows the relationship between absorbance (at 620 nm) and amylose content in mixtures of amylose and amylopectin.

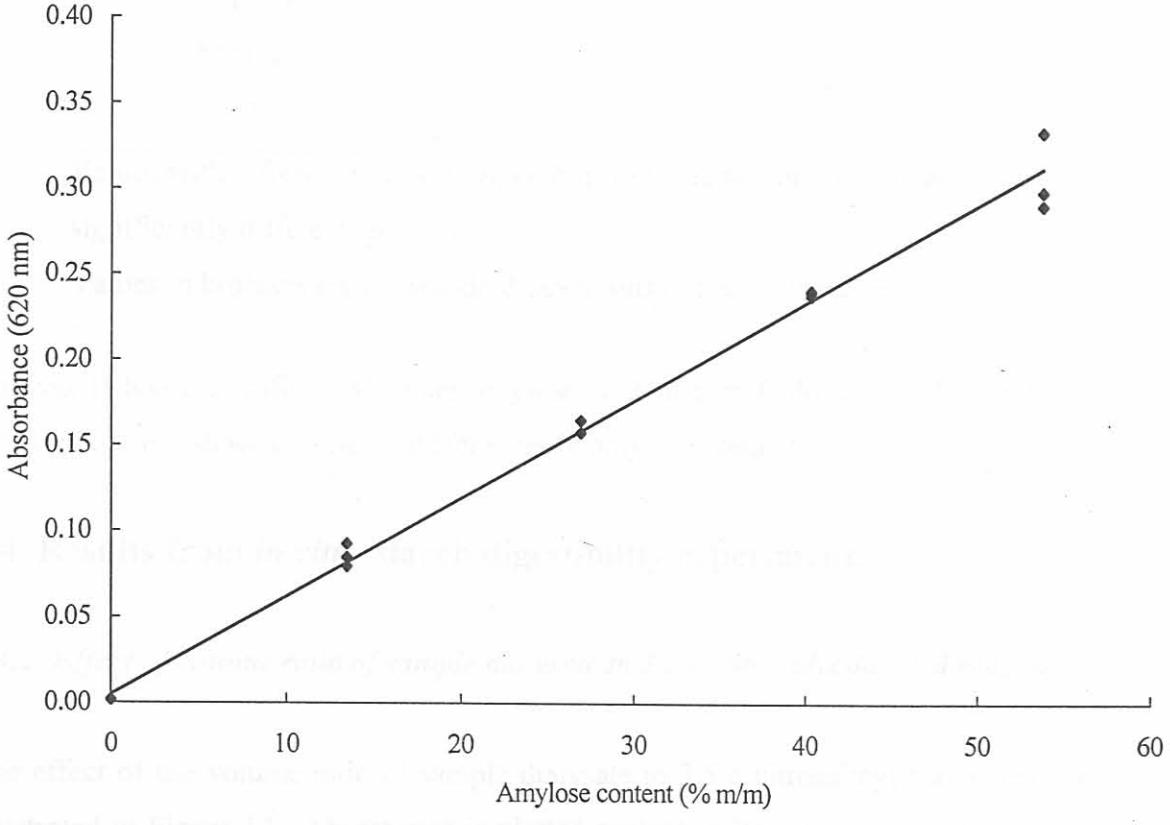


Figure 13: Standard curve of amylose in amylose:amylopectin plotting absorbance versus amylose content

A linear model was fitted to the data. The resulting regression equation was:

$y = 0.0057x + 0.0047$

where y = absorbance (620 nm) and x = amylose concentration (%)

An R^2 of 0.992 was obtained.

Table 9 shows the amylose content of the starch in the endosperm of the different maize cultivars.

Table 9: Amylose content of starch in maize meal from different cultivars

Cultivar	Amylose content (% of total starch)
A	$37.5^{1,a,b}$ $(1.6)^2$
B	39.9^{a} (1.5)
C	37.1^{b} (1.5)
D	$37.5^{a,b}$ (0.9)
E	$38.0^{a,b}$ (1.3)
F	$37.9^{a,b}$ (0.6)

1 Values with different letters in superscripts in columns are statistically significantly different ($p < 0.05$)

2 Values in brackets are the standard deviations of the measurements

Cultivar B had a significantly higher amylose content than Cultivar C. The rest of the cultivars did not show significant differences in amylose content.

5.4 Results from *in vitro* starch digestibility experiments

5.4.1 Effect of volume ratio of sample dialysate to 3,5-dinitrosalycilic acid reagent

The effect of the volume ratio of sample dialysate to 3,5-dinitrosalicylic acid reagent is illustrated in Figure 14. Absorbance is plotted against maltose (mg) and a linear model was fitted.

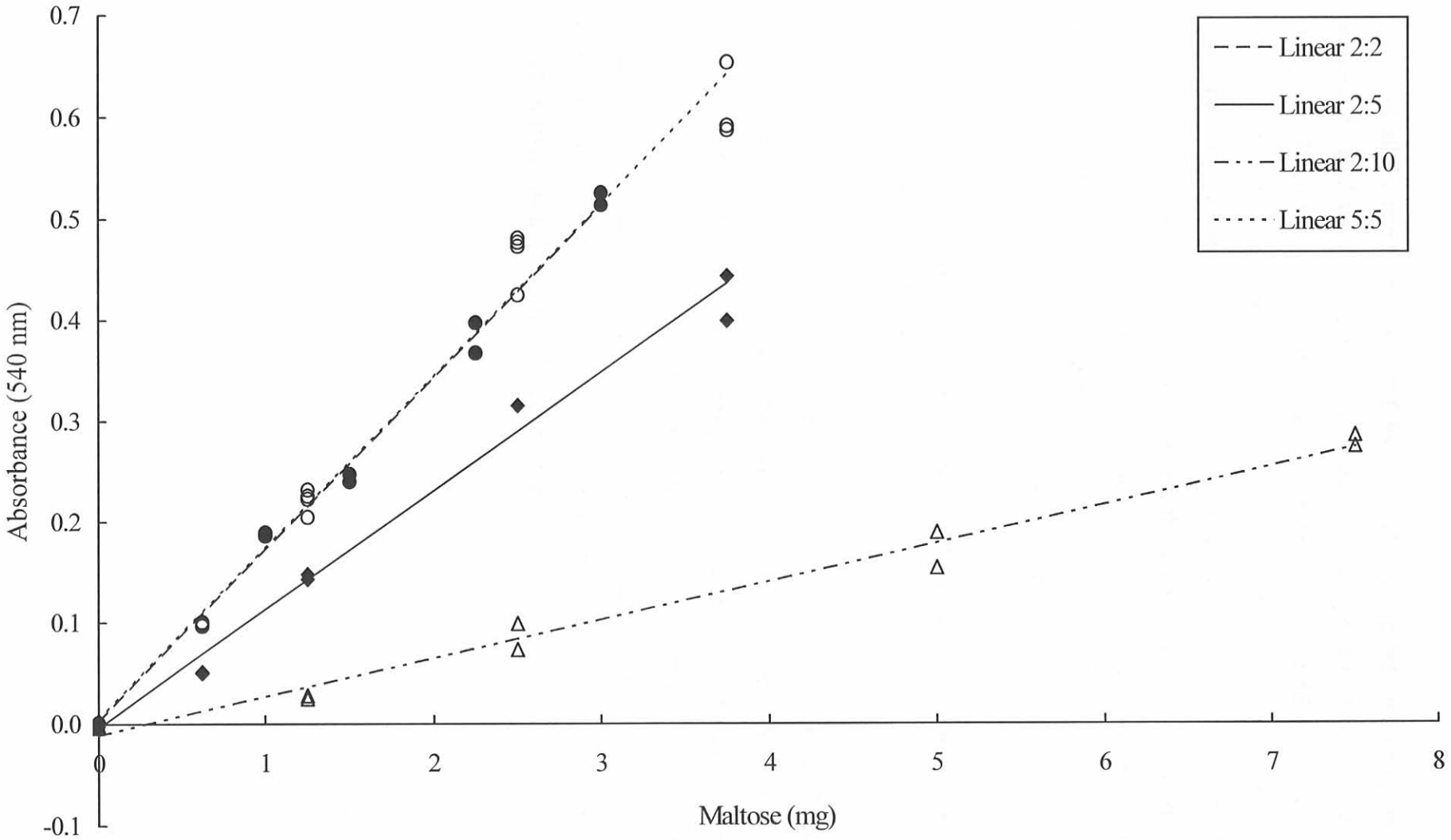


Figure 14: Effect of the ratio of dialysate to 3,5-dinitrosalycilic acid reagent (DNS) on the relationship between absorbancy at 540 nm and maltose (mg) DNS:dialysate 2:2 (●), 5:5 (○), 2:5 (◆) and 2:10 (Δ)

The R^2 of all the regressions were > 0.99. The relationship between absorbance and mg maltose changed significantly as the volume ratio of dialysate to 3,5-dinitrosalicylic acid changed. When the volume ratio was 1:1, there was no significant difference in the relationship between absorbance and mg maltose, even if the actual volumes were not the same (i.e. 2 ml dialysate and 2 ml reagent or 5 ml dialysate and 5 ml reagent). However, when the volume of dialysate was increased, the gradient of the relationship between absorbance and mg maltose decreased significantly ($p < 0.001$). For the 1:1 volume ratio, the slope was 0.17 for both 2 ml and 5 ml volumes. For the 2:5 ratio, the slope was 0.12 and for the 2:10 ratio the correlation coefficient was 0.04.

5.4.2 Starch digestibility of white bread and porridge made from maize cultivars with different endosperm hardness

After chewing, the bread was in the form of a dense lump, but expanded again into a porous structure when coming in contact with the liquid enzymes. With maize porridge, a part of the sample broke up into endosperm grit particles, while most of it remained in 1 to 3 lumps. After 180 min of incubation with α-amylase, the maize porridge sample had broken down into smaller lumps and more loose endosperm grit particles. Figure 15 compares the *in vitro* starch digestibility of white bread to that of porridge made from maize cultivars with different endosperm hardness.

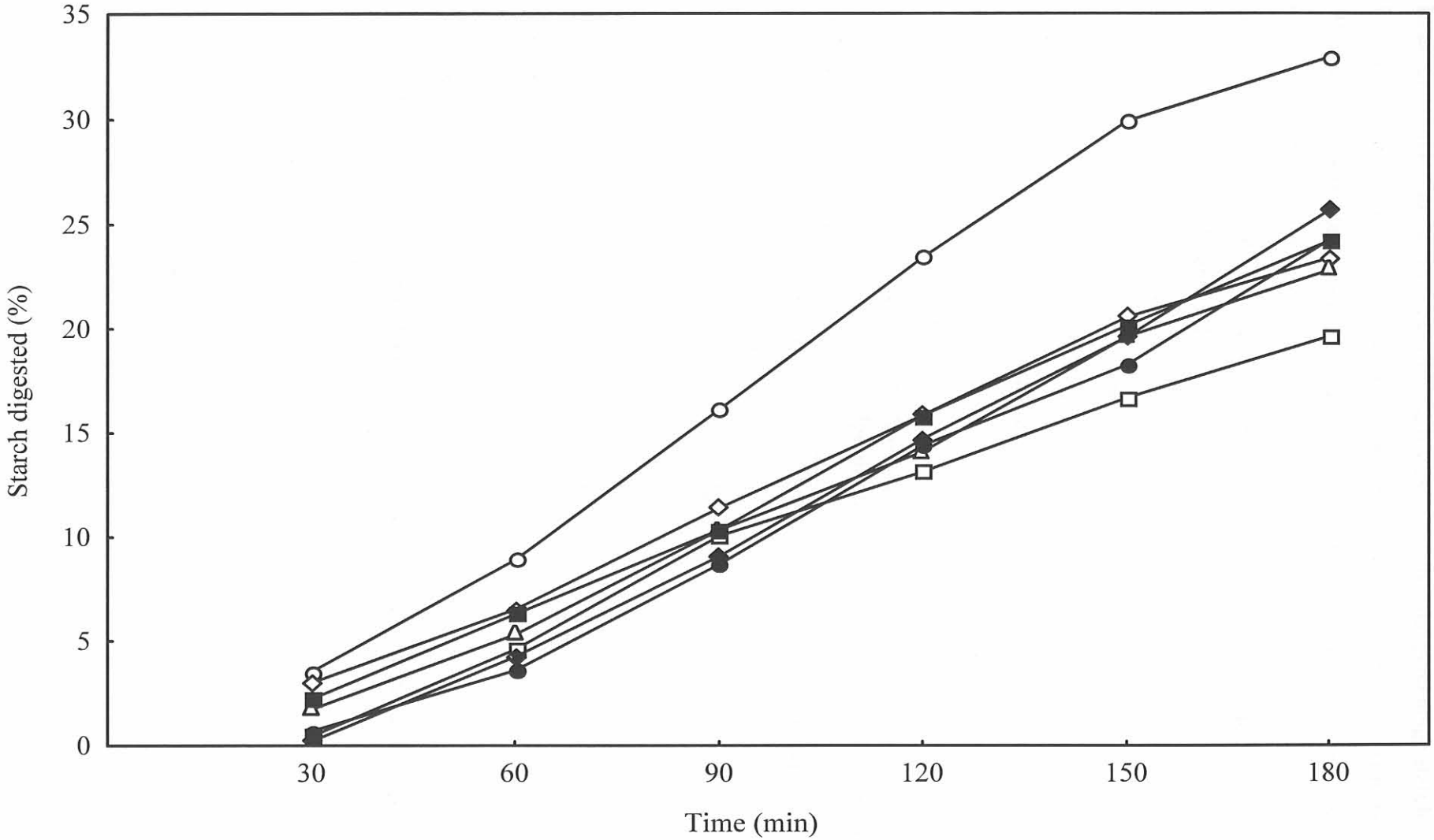


Figure 15: ***In vitro*** **starch digestibility of six maize cultivars with different endosperm hardness compared to white bread, cultivar A (□), B (◊), C (Δ), D (●), E (■), F (◆) and White bread (○)**

In all the starch digestibility experiments it was found that the percentage starch digested increased significantly ($p < 0.001$) from 30 to 180 min after incubation with α-amylase. White bread was statistically significantly ($p < 0.001$) more digestible than maize porridge. There were also small, but statistically significant ($p < 0.05$) differences between the cultivars at 30, 60 and 180 min after incubation with α-amylase. To aid in highlighting these differences, linear models were fitted on the data of the white bread and maize cultivars. The model was $y = mx + c$, where y is starch digested (%), x is time (min), m is the slope of the line and c the intercept. Table 10 gives the regression statistics of the fitted models and Figure 16 shows the fitted lines for starch digested over time of maize porridge made from cultivars with different endosperm hardness compared to white bread.

Table 10: Regression statistics of the linear models fitted to the data of digestibility over time for white bread and porridge made from maize cultivars with different endosperm hardness

Sample	Coefficient of determination (R^2)	Slope	Intercept
Cultivar A	0.932	$0.129^{1,a}$	-2.78
Cultivar B	0.973	0.141^{b}	-1.41
Cultivar C	0.947	0.145^{b}	-2.90
Cultivar D	0.979	0.158^{c}	-5.02
Cultivar E	0.917	$0.151^{b,c}$	-2.64
Cultivar F	0.974	0.170^{d}	-5.65
White wheat bread	0.966	0.207^{e}	-2.59

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

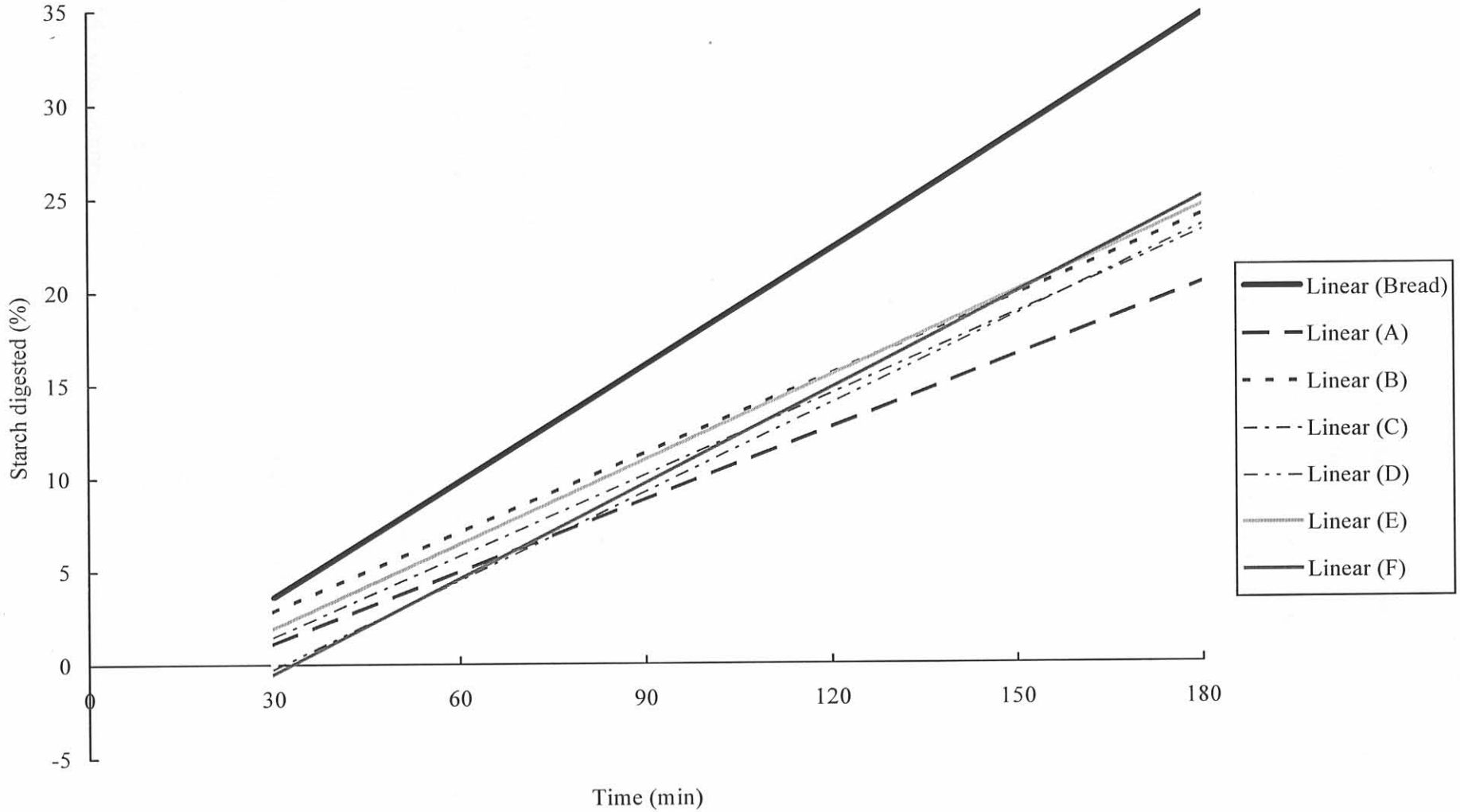


Figure 16: Fitted linear models of percentage starch digested over time in maize porridge made from cultivars with different endosperm hardness compared to white bread

White bread was digested significantly faster than the maize porridge from all the cultivars ($p < 0.001$). The digestibility rate of the maize porridge increased in the order A, B, C, E, D, F. A correlation was done between % starch digested in porridge after 180 min and maize kernel endosperm hardness. The correlation is shown in Figure 17.

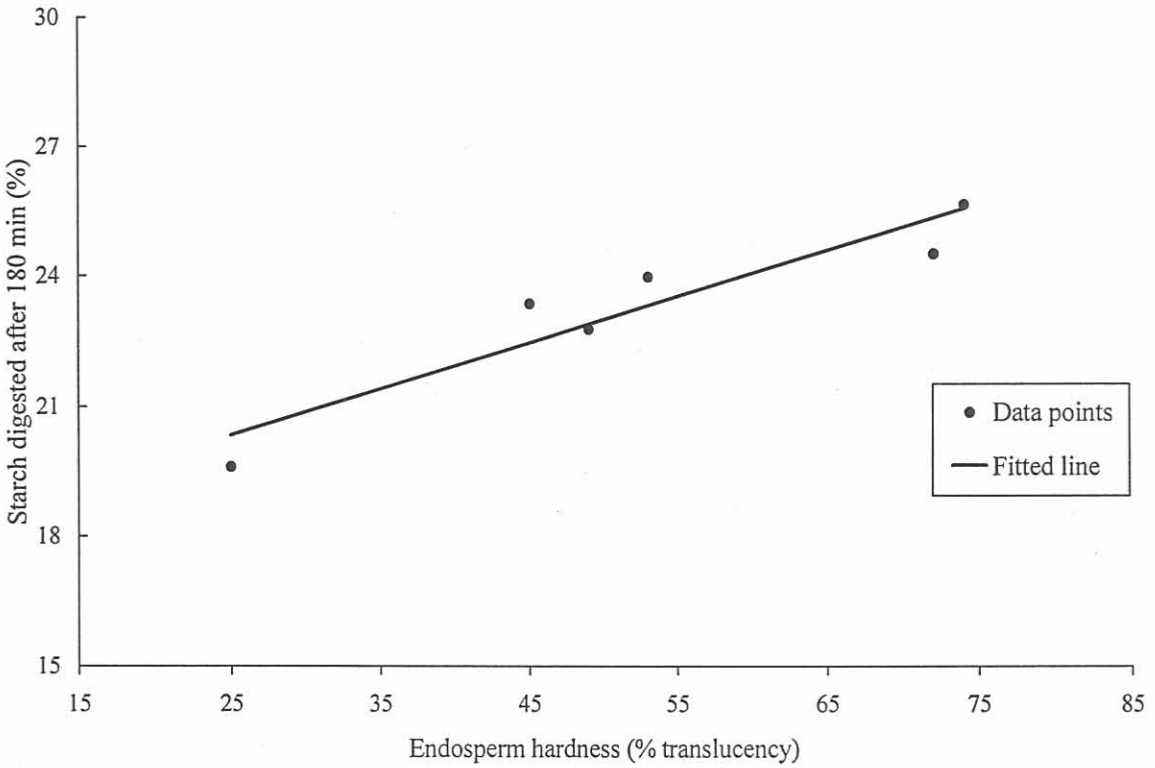


Figure 17: Correlation between % starch digested in maize porridge after 180 min and maize kernel endosperm hardness

With r (correlation coefficient) = 0.94, the correlation was found to be statistically significant ($p < 0.01$). There was also a significant correlation ($p = 0.05$) between the rate of starch digestibility (slopes of fitted lines in Figure 16) and maize endosperm hardness.

5.4.3 Hydrolysis index and predicted GI of maize porridge

The average hydrolysis index of maize porridge was 64 and the predicted GI value 63. (with white bread as reference). Converting this to glucose as a reference, a predicted GI of 44 was obtained.

5.4.4 Effect of particle size

The starch digestibility of maize meal compared to maize flour of cultivar C is shown in Figure 18.

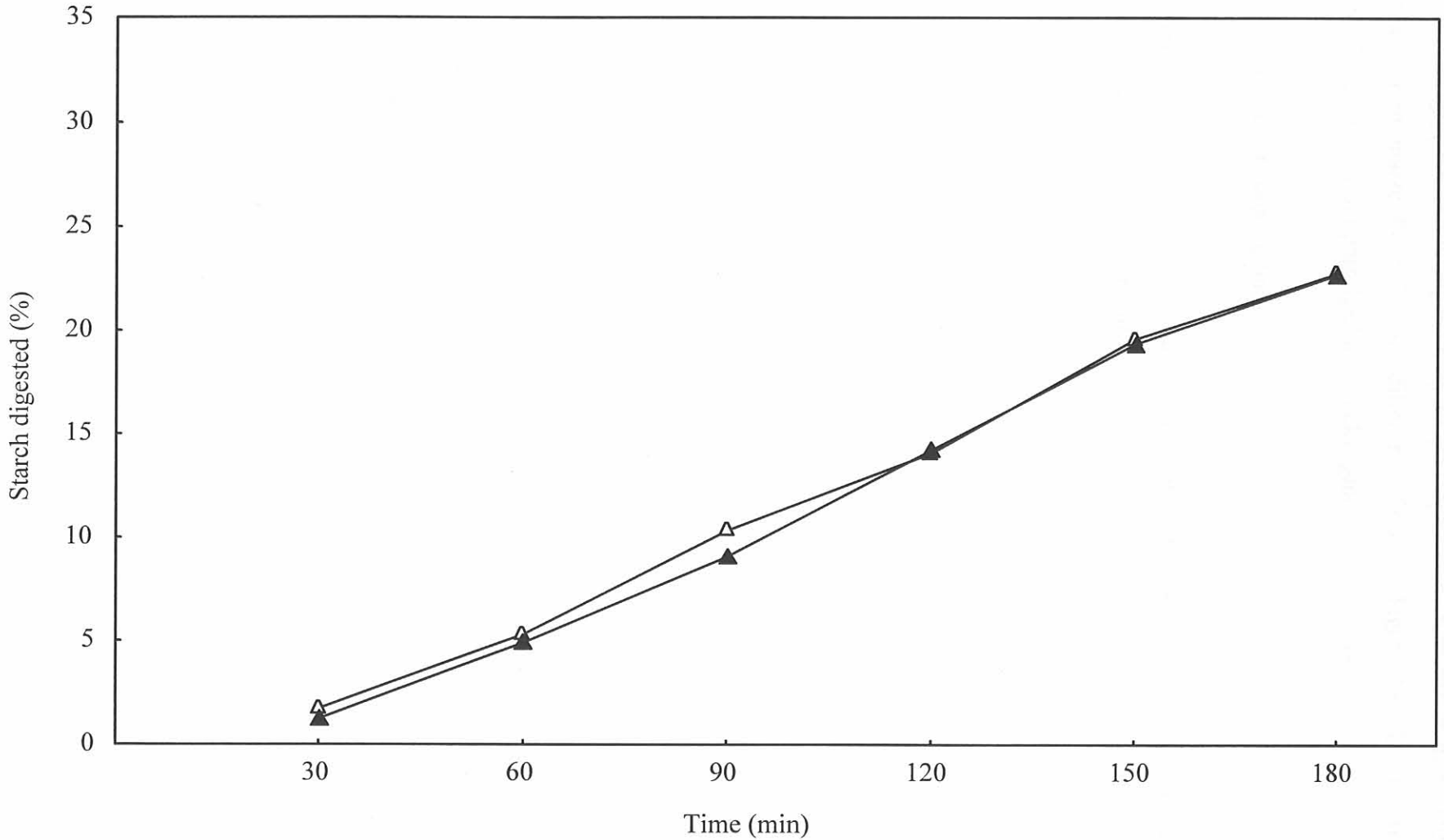


Figure 18: ***In vitro*** **starch digestibility of maize meal (Δ) and maize flour (▲) hotplate cooked maize porridge made from cultivar C maize meal**

Surprisingly, the starch digestibility of porridge made from maize meal and porridge made from maize flour did not differ significantly (Figure 18). Unlike maize meal porridge, maize flour porridge had a sticky, glue-like consistency.

5.4.5 Effect of cooking time

Figure 19 compares the starch digestibility of short, standard and long cooked porridge made from cultivar C maize meal.

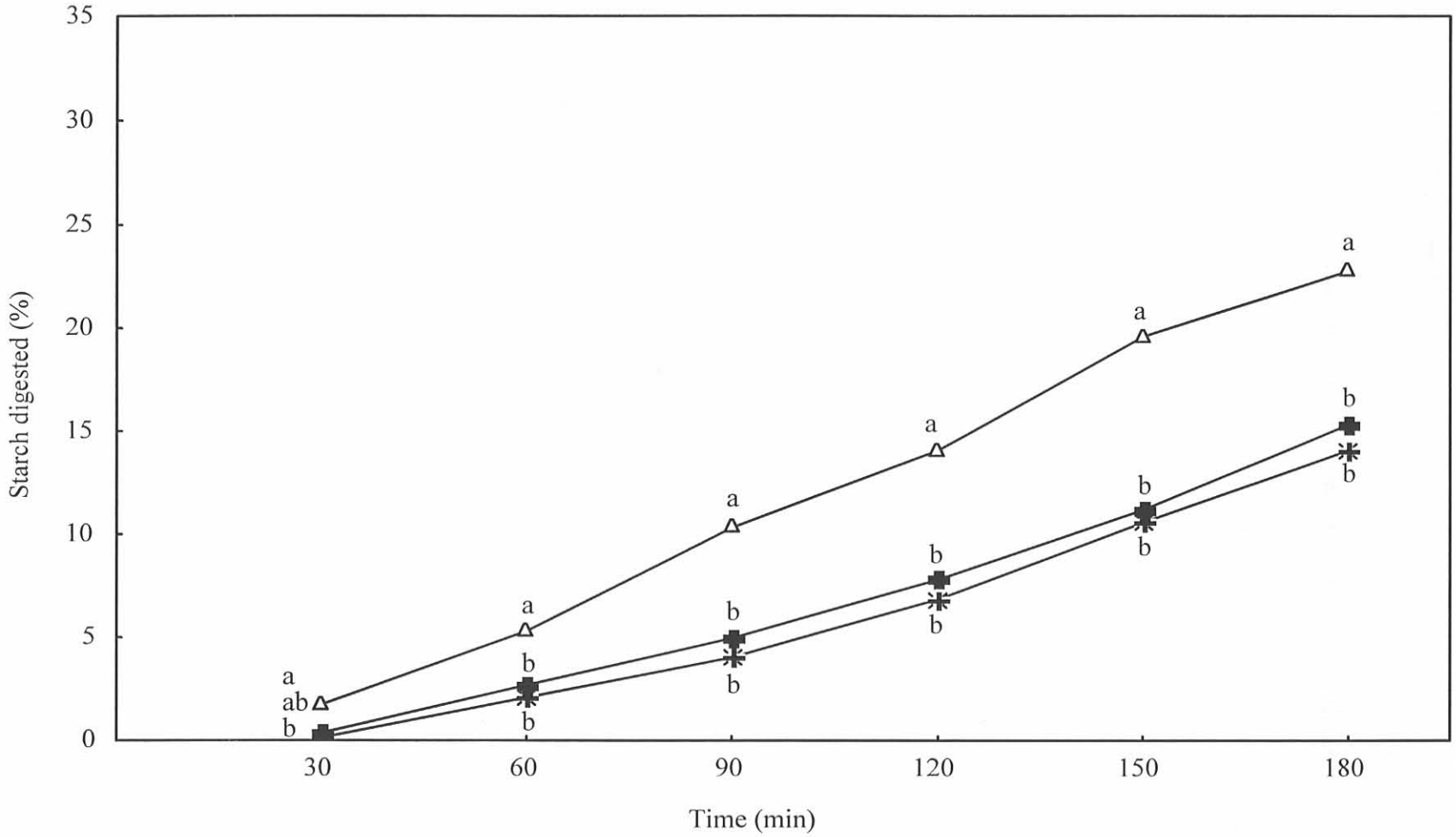


Figure 19: ***In vitro*** **starch digestibility of short (✚), standard (Δ) and long (✳) hotplate cooked maize porridge made from cultivar C maize meal (at each time, means not sharing the same letter are significantly ($p < 0.05$) different)**

Both increasing and decreasing the cooking time decreased the starch digestibility significantly. There was no significant difference between the digestibility of the long and short cooked porridge.

5.4.6 Starch digestibilities of maize, wheat and oat flour porridges

Figure 20 compares the *in vitro* starch digestibility of maize, wheat and oat flour with that of white bread.

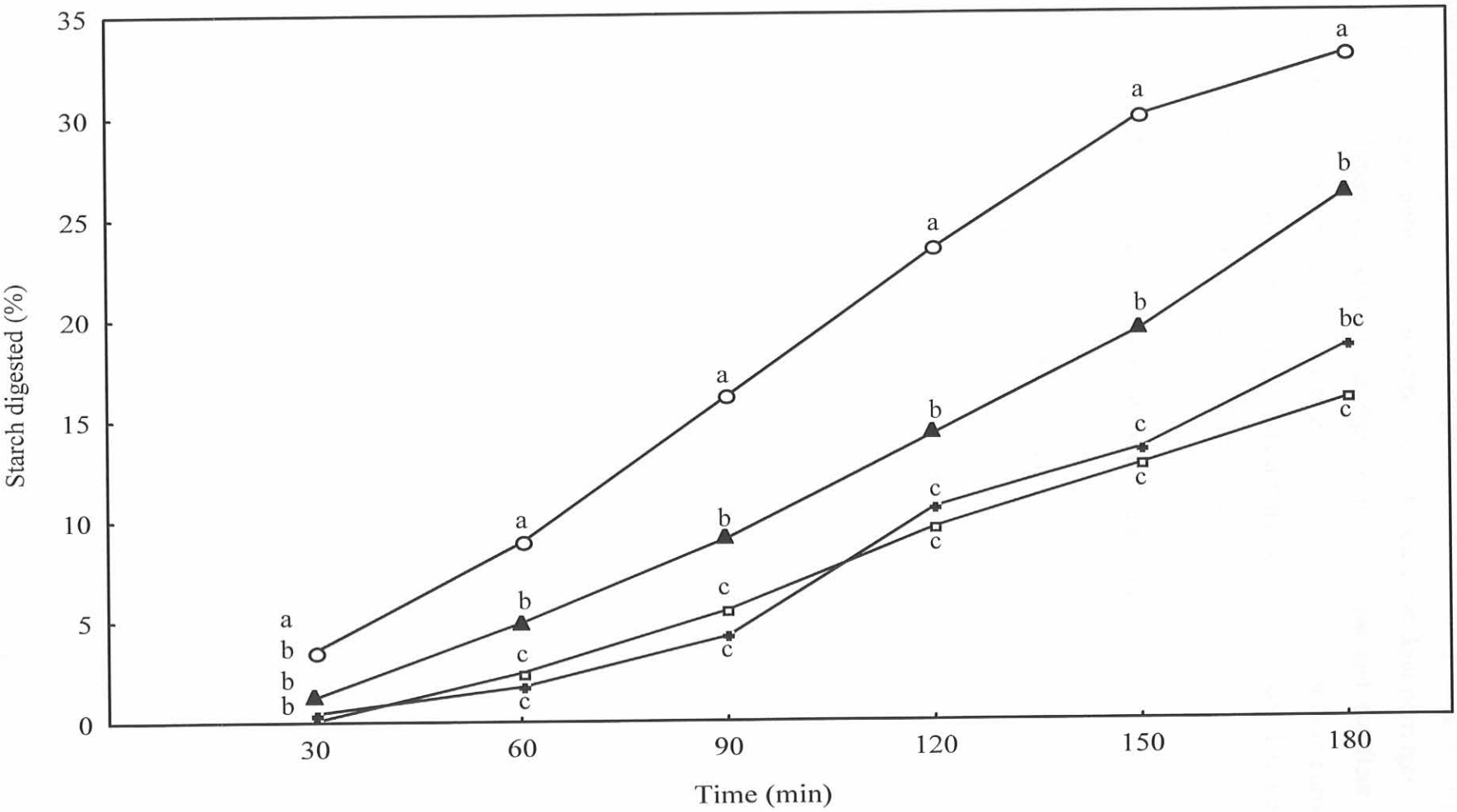


Figure 20: ***In vitro*** **starch digestibility of standard hotplate cooked porridge made from cultivar C maize flour (▲), wheat flour (□) and oat flour (+) compared to white bread (○) (at each time, means not sharing the same letter are significantly ($p < 0.05$) different)**

White bread was significantly more digestible than all the porridges. Maize flour porridge was significantly more digestible than wheat and oat flour porridges. There was no significant difference between the digestibility of wheat and oat flour porridges. During the experiment it was observed that both wheat flour and oat flour porridges were more viscous than maize flour porridge. Wheat flour porridge, and oat flour porridge to a lesser extent, had an almost elastic and rubbery consistency.

5.4.7 Starch digestibility of microwave cooked maize porridge

The digestibility of microwave cooked porridge from maize of cultivars with different endosperm hardness is shown in Figure 21.

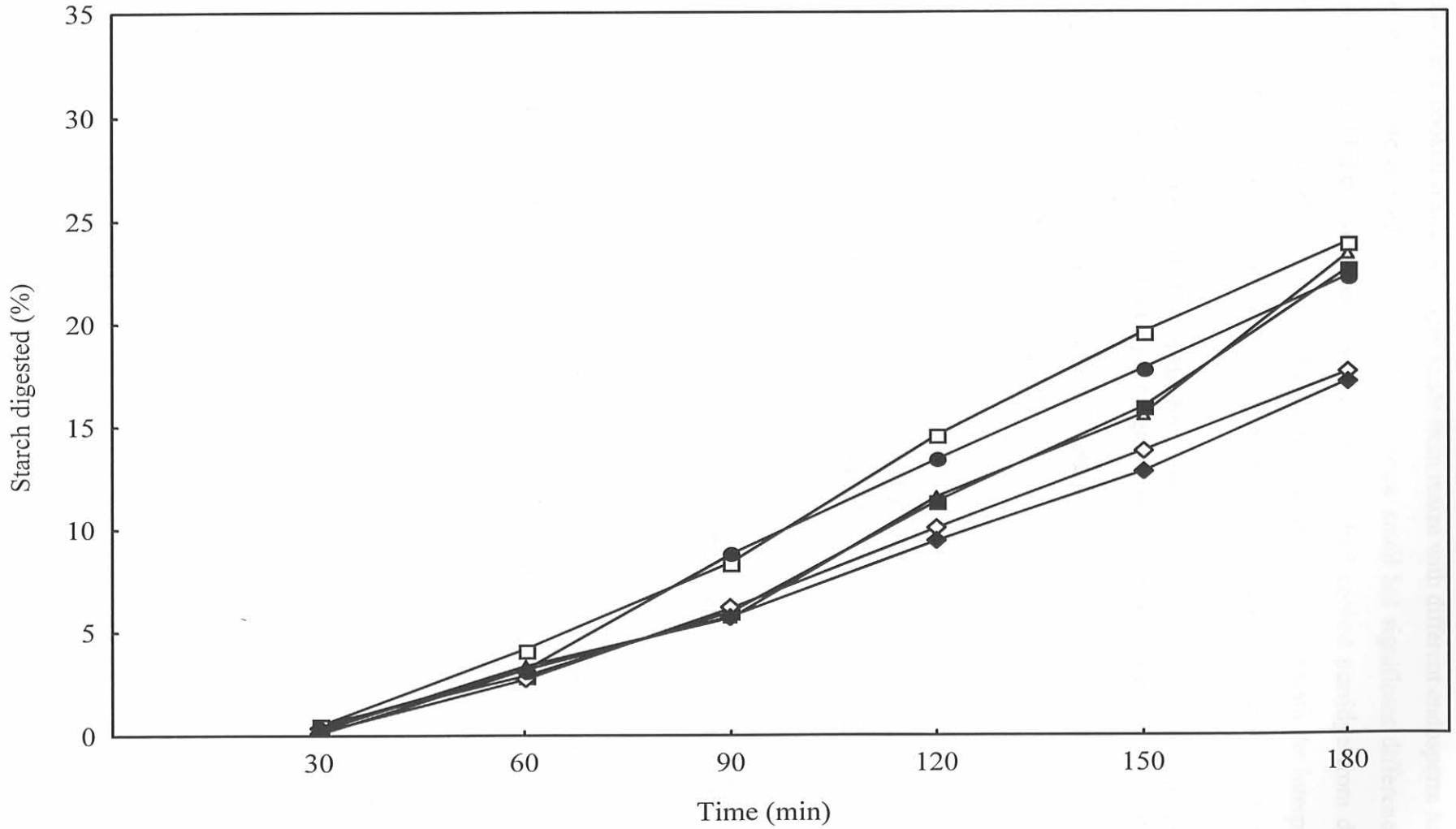


Figure 21: The *in vitro* starch digestibility of microwave cooked porridge made from meal of different maize cultivars. Cultivar A (□), B (◊), C (Δ), D (●), E (■) and F (◆)

Microwave cooked maize porridges made from maize with different endosperm hardness, like the hotplate cooked porridges, showed some small but significant differences ($p < 0.05$) in digestibility over time. As with the standard cooked porridge from different cultivars, linear models ($y = mx + c$) were fitted to the data to aid with the interpretation of the differences between the cultivars. The regression statistics are shown in Table 11.

Table 11: Regression statistics of the linear models fitted to the data of digestibility over time for microwave cooked porridge made from maize cultivars with different endosperm hardness

Sample	Coefficient of determination(R^2)	Slope	Intercept
Cultivar A	0.936	$0.161^{1,a}$	-5.11
Cultivar B	0.948	0.118^{b}	-4.02
Cultivar C	0.924	0.150^{a}	-5.82
Cultivar D	0.936	0.152^{a}	-5.02
Cultivar E	0.940	0.147^{a}	-5.62
Cultivar F	0.921	0.111^{b}	-3.50

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

Figure 22 shows the fitted lines for starch digested over time in microwave cooked maize porridge made from cultivars with different endosperm hardness.

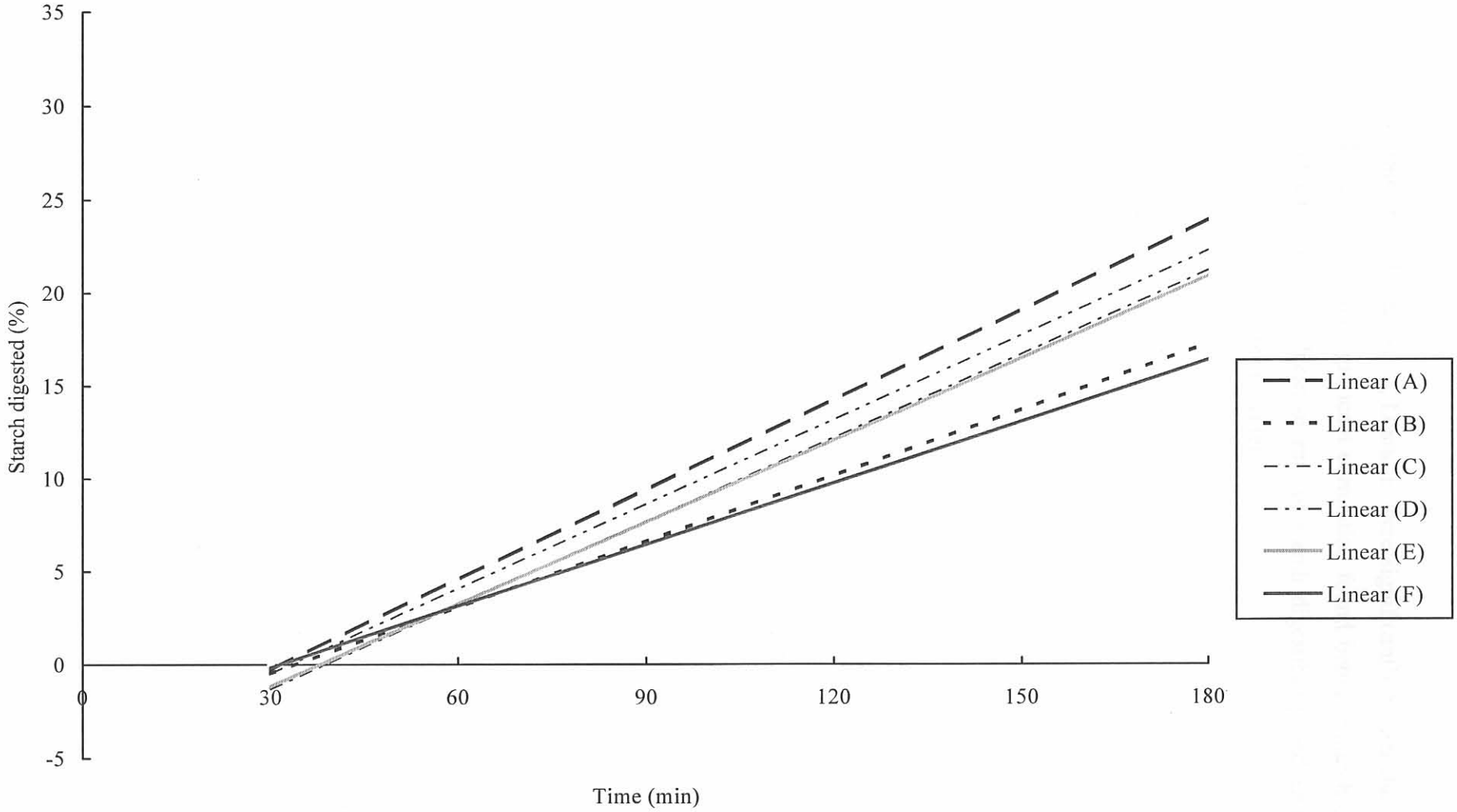


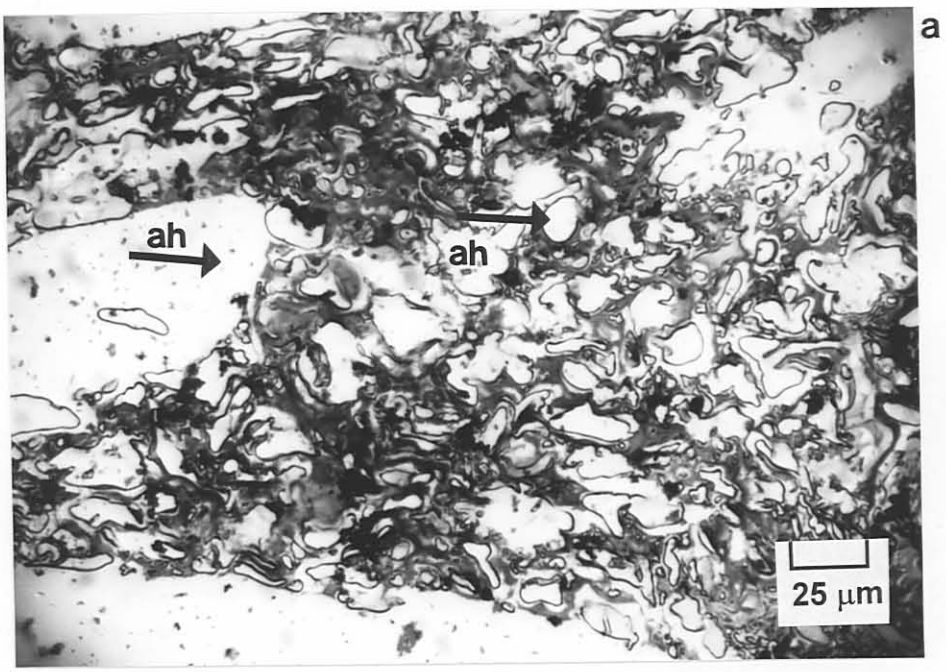
Figure 22: Fitted linear models of percentage starch digested over time in microwave cooked maize porridge made from cultivars with different endosperm hardness

The digestibility rates of cultivars A, C, D and E were significantly higher than that of cultivars B and F. There was no significant correlation found between starch digested after 180 min and endosperm hardness or rate of starch digestibility and endosperm hardness for microwave cooked maize porridge.

5.5 Microscopy

5.5.1 White bread

Figures 23a-c are light micrographs of white wheat bread before and after digestion with pepsin and α-amylase.



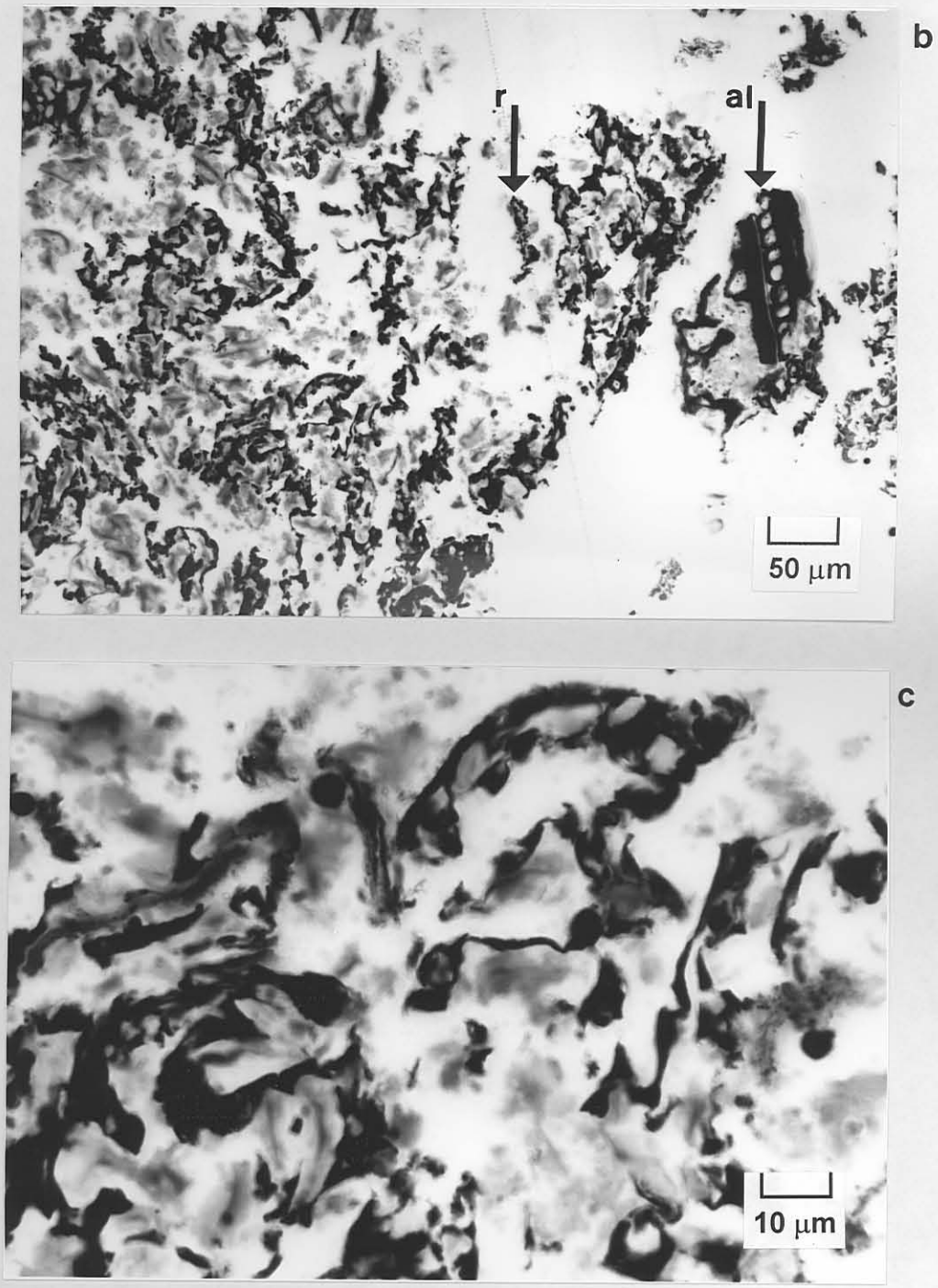
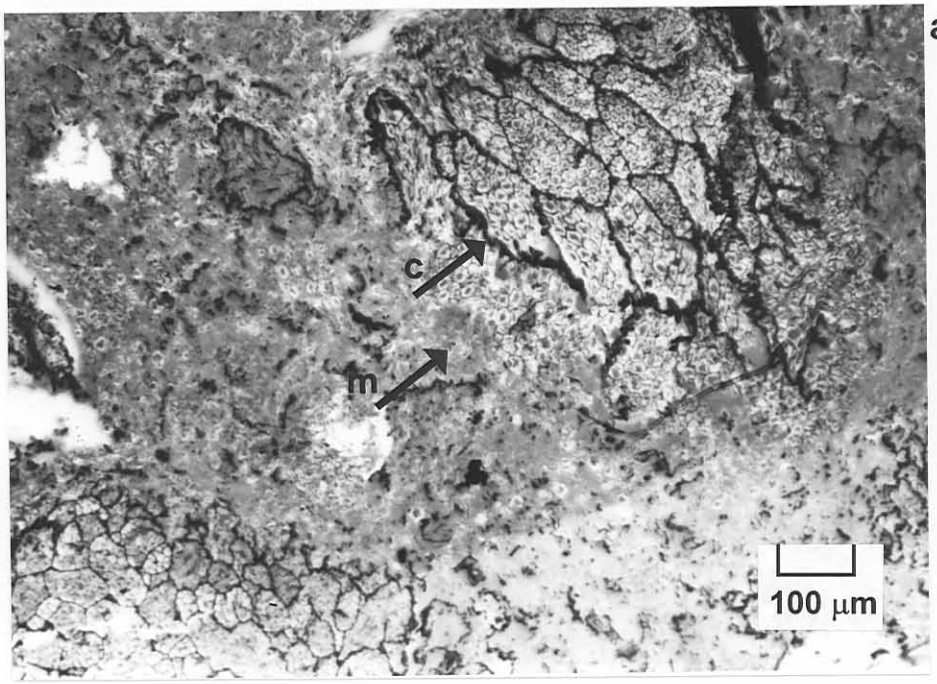


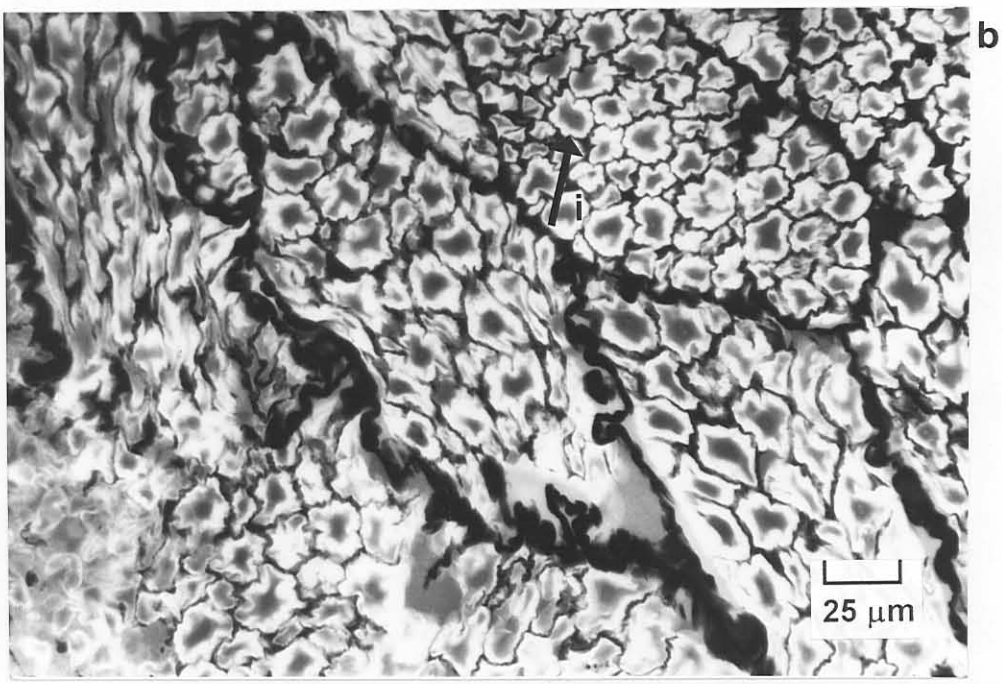
Figure 23: Light micrographs of white bread before (a) and after (b,c) digestion with pepsin and α-amylase (c is a higher magnification of one of the non-cellular areas in b)

Before digestion, the structure of bread was open and there were a large number of air holes (ah). No endosperm cell structures or intact starch granules were visible. After digestion with pepsin and α-amylase, the structure was basically amorphous consisting of cell wall remnants (r), except for a piece of aleurone layer (al) here and there.

5.5.2 Maize porridge

Figure 24a-c are light micrographs of maize porridge before digestion.





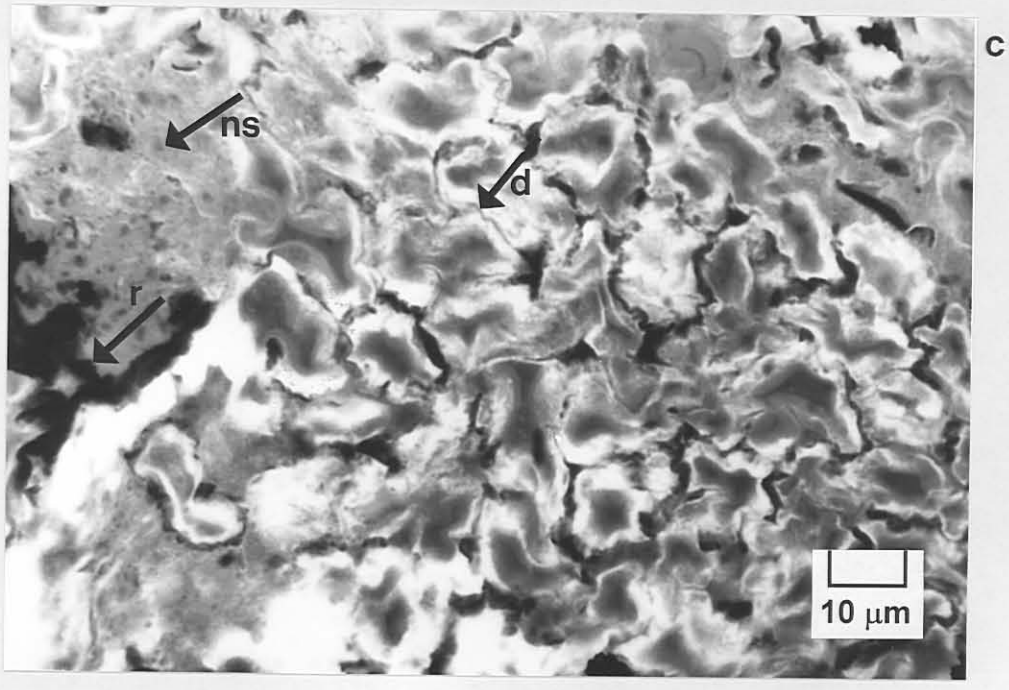
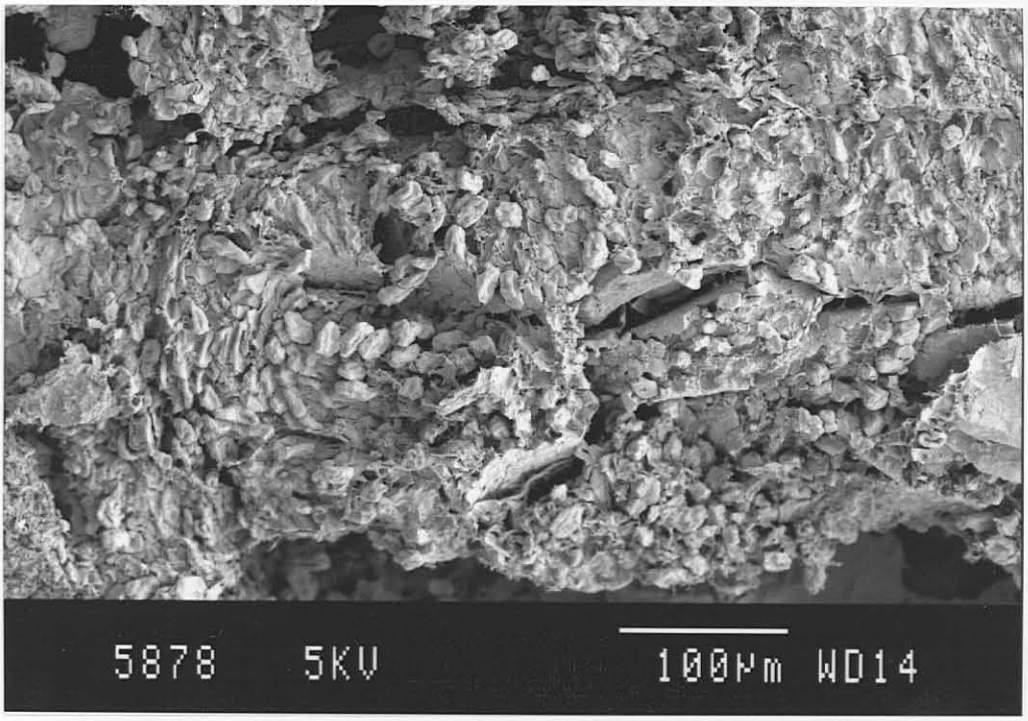


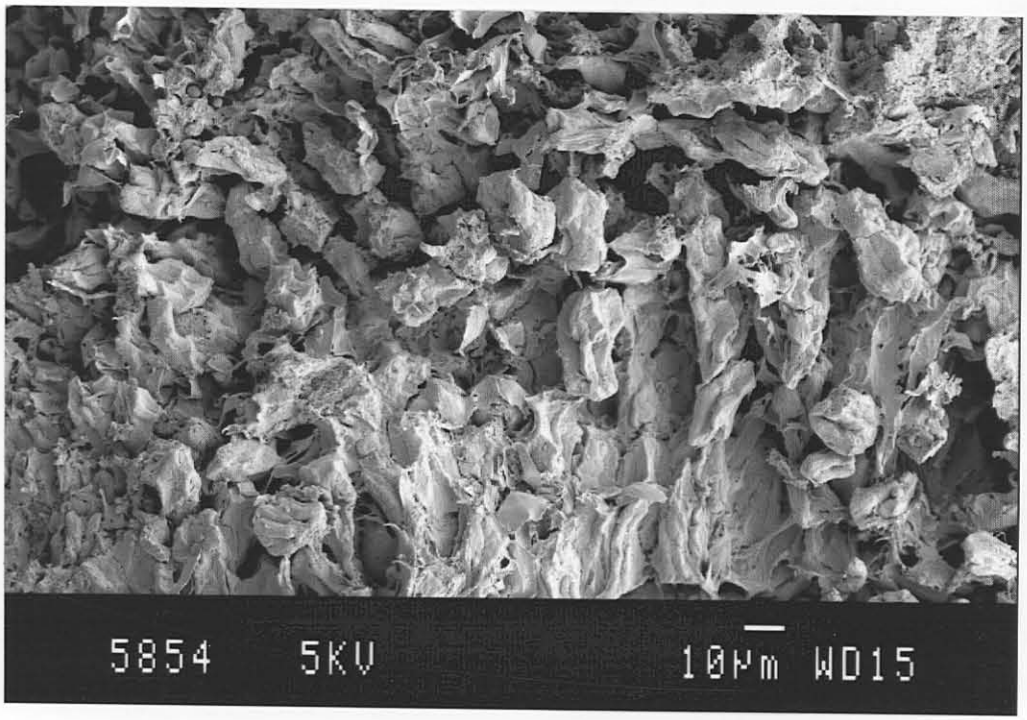
Figure 24: Light micrographs of maize porridge before digestion (a, low magnification; b, higher magnification of cellular area; c, higher magnification of amorphous area)

The structure of maize porridge was very dense, there were no air holes. The maize porridge consisted of amorphous (m) and cellular (c) areas. The cellular areas will be called endosperm grit particles and the amorphous areas surrounding the porridge particles will be called the porridge matrix. Magnification of a cellular area (23b) revealed that the endosperm grit particles consisted of swollen, but in many cases still intact (i) starch granules in the cells. When the starch granules were viewed under polarised light, almost all the granules showed a lack of birefringence. The porridge matrix (Figure 24c) consisted of swollen, distorted starch granules (d), cell wall remnants (r) and areas that stained the same as starch, but showed no structure (ns) under 1000 times magnification. The shown light micrographs are all of cultivar C maize porridge. No difference could be observed between the microstructure of maize meal porridge made from cultivars with different kernel endosperm hardness.

Figure 25a-d are SEM micrographs of maize porridge before digestion.



a



b

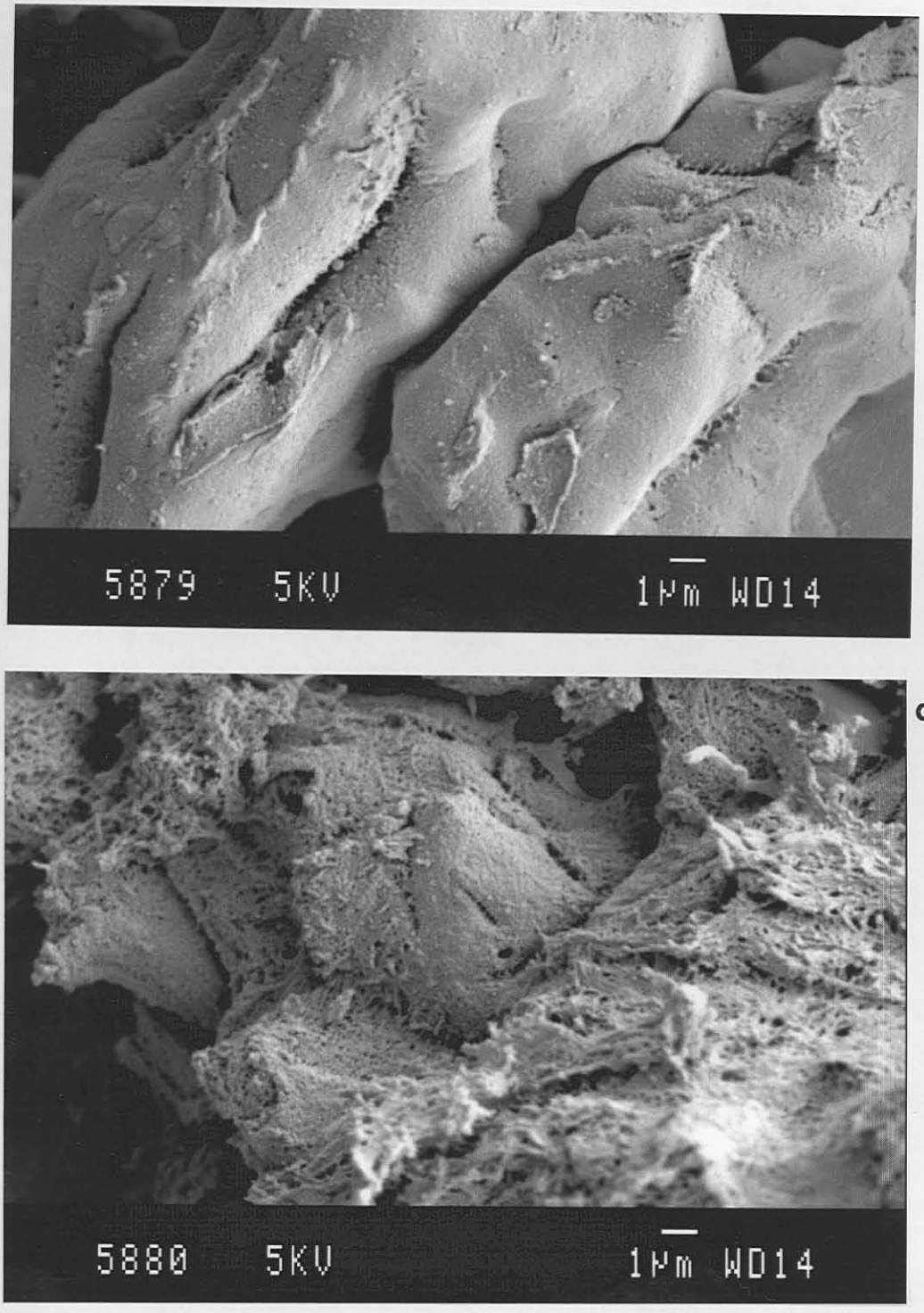
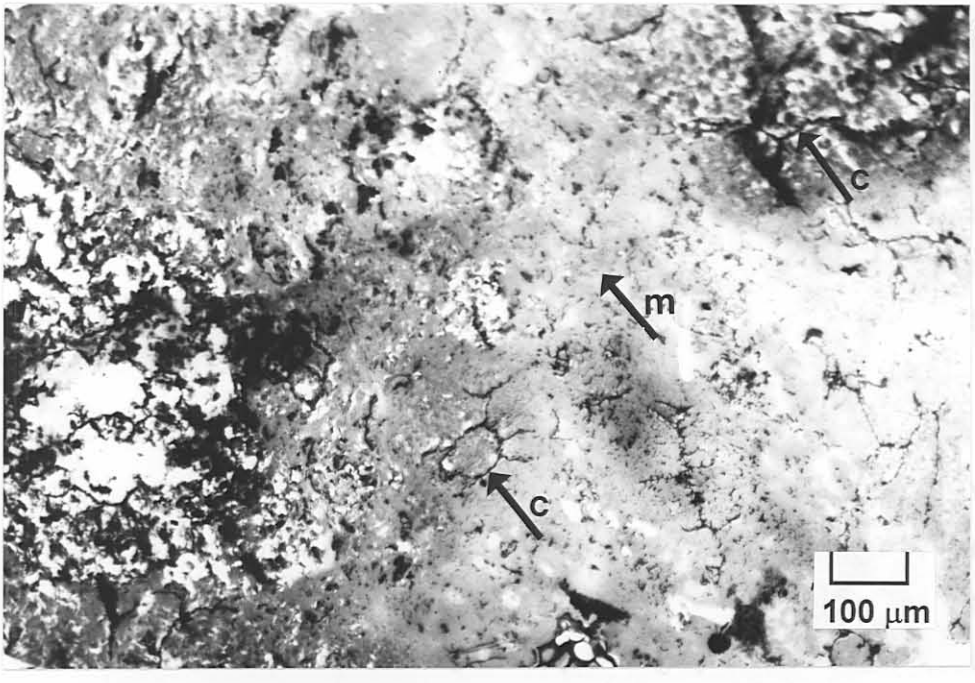


Figure 25: SEM micrographs of maize porridge before digestion with pepsin and α-amylase (a, showing starch granules in cells; b, showing loose starch granules in the porridge matrix; c, showing two intact starch granules on the surface of the porridge particle; d, showing disrupted starch granules on the surface of the porridge matrix)

Observing the surface of maize porridge by SEM showed that some starch granules were still partially or completely contained in cells (Figure 25a). These starch granules were located on the surface of porridge particles. The starch granules on the surface of the porridge matrix were not contained in cells (Figure 25b, 4 times higher magnification than 25a). At higher magnification (25c and d) it could be seen that many starch granules on the surface of the porridge particles were still intact, but on the surface of the porridge matrix many starch granules were disrupted and had a spongy appearance.

Figure 26a-c are light micrographs of maize porridge after digestion with pepsin and α-amylase.



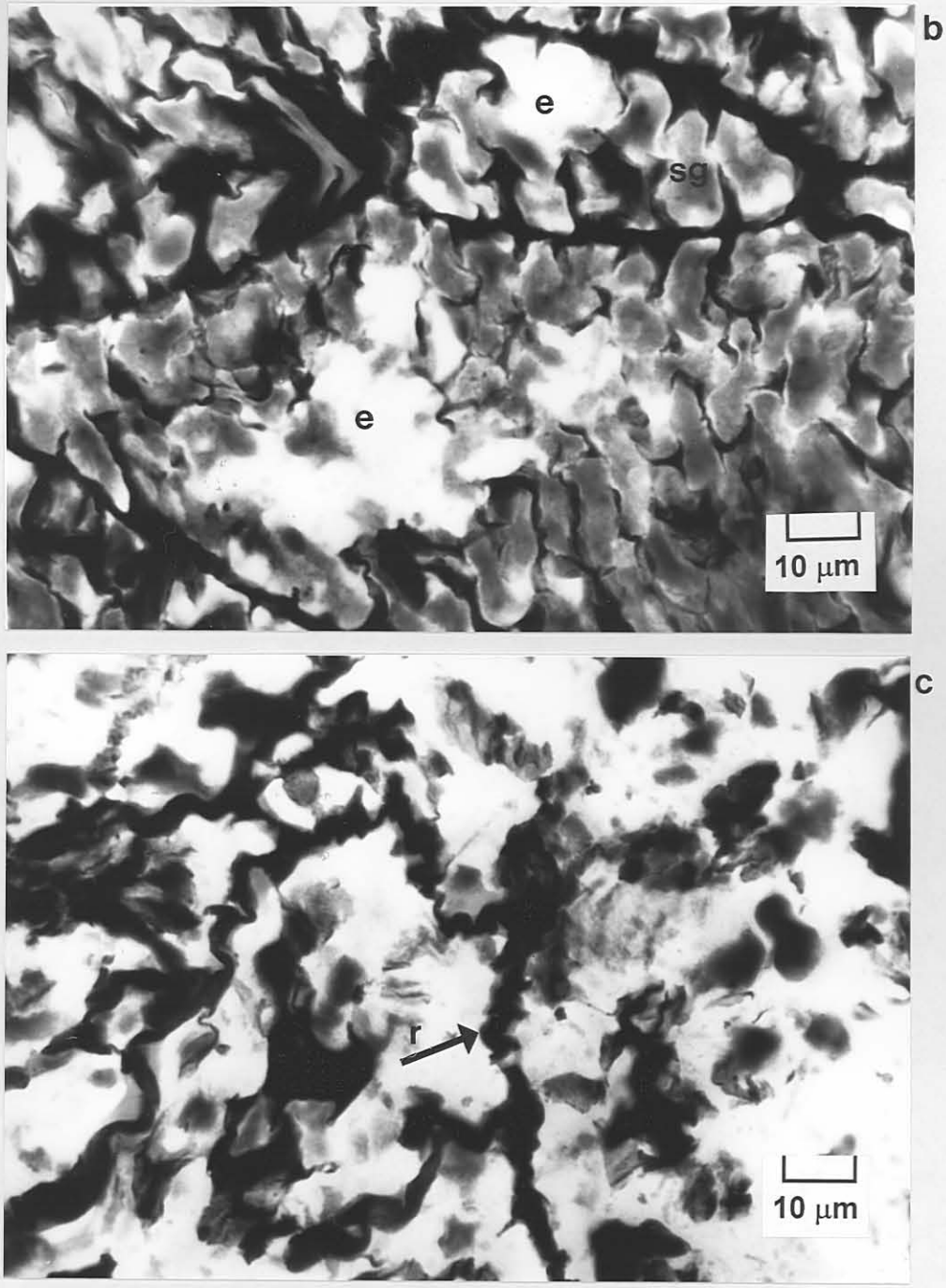


Figure 26: Light micrographs of maize porridge after digestion with pepsin and α-amylase (a, low magnification; b, magnification of cellular area; c, magnification of non-cellular area)

After digestion with pepsin and α-amylase, there were still amorphous (m) and cellular (c) areas in the residue (26a). There were also areas consisting of cell wall remnants (r). At higher magnification it could be seen that the cellular areas (26b) consisted of cells with starch granules (sg) inside. There were also empty spaces (e) in the cells. The amorphous areas (26c) consisted mainly of cell wall remnants

CHAPTER 6

DISCUSSION

The word "hardness" is often used loosely to describe the endosperm texture of cereal grains. In the following discussion, if the word "hard" is used to describe maize endosperm, it will refer to endosperm with a vitreous texture, like that of flint maize. In maize, hard endosperm is translucent, unlike wheat endosperm where it is possible to get hard endosperm that is opaque (Hoseney, 1994). If the word "soft" is used, it will refer to endosperm with a floury texture, like that of floury maize. The floury endosperm is opaque due to air holes that refract light. A maize cultivar will be classified as a hard cultivar if the mean percentage of translucent endosperm was high and similar to that of the hard standard. A soft maize cultivar was a cultivar with a low mean percentage of translucent endosperm and similar to that of the soft standard. Taking into consideration the fact that cultivar B had a significantly higher percentage translucency than A and that B was not significantly lower than C and D, A was classified as a soft cultivar, B, C, and D as intermediate and E and F as hard

The composition of the maize meal used in this study was typical (compared to literature values reported by Peterson & Johnson, 1979) and similar to the standard for commercial "Super" maize meal (South Africa, 1984) in terms of fat content and particle size distribution. The starch digestibility of highly refined maize meal and not unrefined maize meal was determined, because the popularity of highly refined maize meal seems to be increasing.

Unlike Björck and co-workers (Granfeldt & Björck, 1991; Granfeldt *et al.*, 1992; Liljeberg *et al.*, 1992; Liljeberg & Björck, 1994) who used volunteers to chew the samples, in this study the researcher did the chewing herself. There were two reasons for this decision; firstly that there were not enough trustworthy volunteers available on a regular basis to do the chewing and secondly that differences between the way that

people chew would be eliminated.

Based on personal experience and conversation with other people who eat porridge regularly, a stiff maize porridge sample containing 1 g of starch (about 5 g porridge) was chewed about five times in five seconds before being swallowed. A bread sample containing 1 g of starch weighed about 2.4 g. Using the same approach as with porridge, it was found that such a sample was chewed about seven times in seven seconds. Considering this, all porridge samples were chewed five times in five seconds and all bread samples seven times in seven seconds.

During digestion with α-amylase, the sample was inside a dialysis tube and suspended in a beaker with buffer that was placed in water bath. The water in the water bath was circulated. The buffer solution in the beaker was stirred before samples of the dialysate were taken (every 30 minutes). The lack of constant agitation in the form of a shaking water bath or magnetic stirrer bar inside the beaker with buffer could be thought to have an effect on the rate of starch digestibility. Because the maltose had to diffuse from inside to outside the dialysis tube, the unstirred buffer solution surrounding the dialysis tube could have slowed down diffusion to the outside as the concentration of maltose in that surrounding layer increased. This has been found not to be the case (Wong *et al.*, 1985). Increasing the shaking rate of a shaking water bath from 1 to 120 oscillations per minute did not increase the starch digestibility of red kidney beans.

When the *in vitro* starch digestibility determinations were done initially, very low absorbancy values were obtained. The volume of dialysate was increased in order to try to increase the absorbancy values. The absorbancy values did not increase. It was suspected that the absorbancy was not only dependent on the volume of the dialysate, but also the volume ratio of dialysate to dinitrosalysilic acid reagent. The results shown in Figure 14 confirm this. The relationship between absorbance and maltose (mg) weakened when the volume of maltose solution was increased, even though the mg maltose in the solution was kept constant. When the volume ratio of reagent and maltose solution was kept constant, however, the relationship between absorbance and maltose

(mg) remained constant, even if the actual volumes were 2 ml in the one case and 5 ml in the other. It appears that concentration of the reagent is very important and that if the sample volume is increased without increasing the reagent volume accordingly, it dilutes the reagent to such and extent that the colour development is decreased.

The dinitrosalicylic acid method determines reducing power (Granfeldt *et al.*, 1992). Even though a maltose standard curve was used to convert absorbance to maltose concentration, it really measures reducing equivalents and not maltose concentration. Faulks & Bailey (1990) determined the relative percentages of the products of maize starch digestion by HPLC (high performance liquid chromatography). The results are shown in Figure 27.

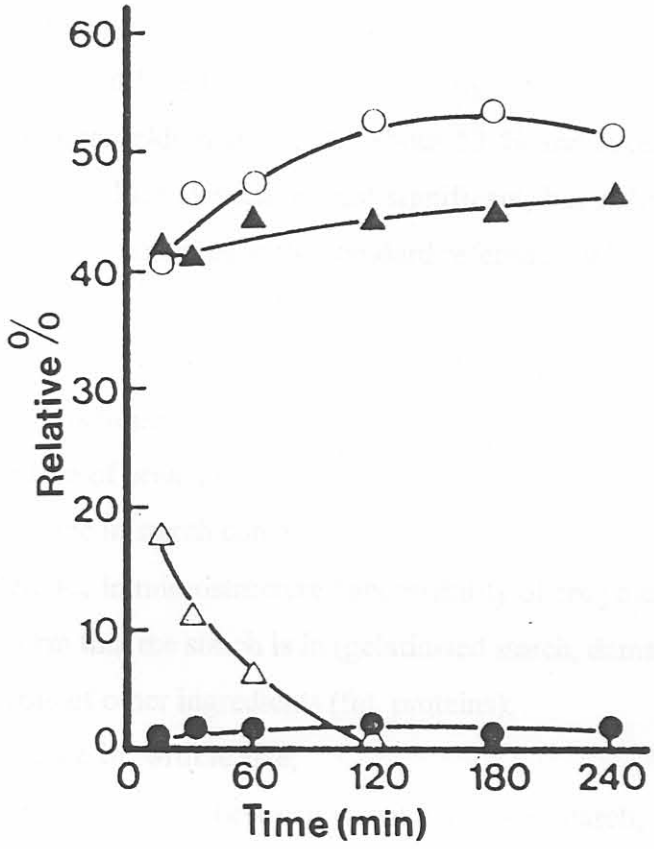


Figure 27: Relative percentage composition of the hydrolysate from maize starch treated with α-amylase. Glucose (●); maltose (○); maltotriose (▲); maltotetraose (△). (Faulks & Bailey, 1990)

The digestion products of maize starch consisted of about 50 % maltose, 40% maltotriose and a small amount of glucose after 180 min. Since the amylose content and glucose chain length of starch from different origin may differ, the relative percentages of the products of digestion may differ. This might have had an effect on the results obtained, because only the reducing power of the products of digestion and not the products themselves are determined. On the positive side, maltose is the largest component of the digestion products and the relative proportion of maltose remains relatively constant over the period of digestion.

Considering the effect that sample preparation, enzymes used, incubation conditions, and method of measuring the end products have on the results of *in vitro* starch digestibility experiments, it is important to interpret the results accordingly. In this study the starch in white wheat bread was about 33 % digestible, while Granfeldt & Björck reported about 46 %, Granfeldt *et al.* (1992) about 53 % and Åkerberg *et al.* (1998) about 50 %. The absolute values as such are not significant, but rather the relation of the values obtained for different samples to the standard reference (white wheat bread).

The rate of starch digestibility of white bread was significantly higher than that of all the maize porridge samples. There could be several possible reasons for the higher rate of digestion of bread, including:
difference in starch content;
difference in microstructure / accessibility of enzymes to substrate;
the form that the starch is in (gelatinised starch, damaged starch, retrograded starch);
the role of other ingredients (fat, proteins);
difference in particle size;
intrinsic difference between wheat and maize starch; and
difference in preparation and type of heat treatment.

When substrate and not enzyme is limited, the velocity of an enzyme reaction is increased with increased substrate concentration (Mathews & Van Holde, 1990). In the current study the enzyme was not limited. The difference in starch content between bread and

maize porridge could not have affected the starch digestibility, because all the analyses were done on samples containing approximately 1 g of starch.

The microstructure of white bread and maize porridge differed. White bread had an open structure with many air holes. Other researchers who studied the microstructure of bread using SEM also observed an open structure (Freeman & Shelton, 1991; Brennan, Blake, Ellis & Schofield, 1996). This porous structure would greatly increase the surface area of the bread sample. Maize porridge, on the other hand, had a dense structure that consisted of maize endosperm grit particles suspended in a matrix of gelatinised starch granules and free starch. The endosperm grit particles consisted of cells with starch granules inside that were generally still intact. Figure 28 is a simplified diagram of a lump of porridge showing endosperm grit particles in the porridge matrix.

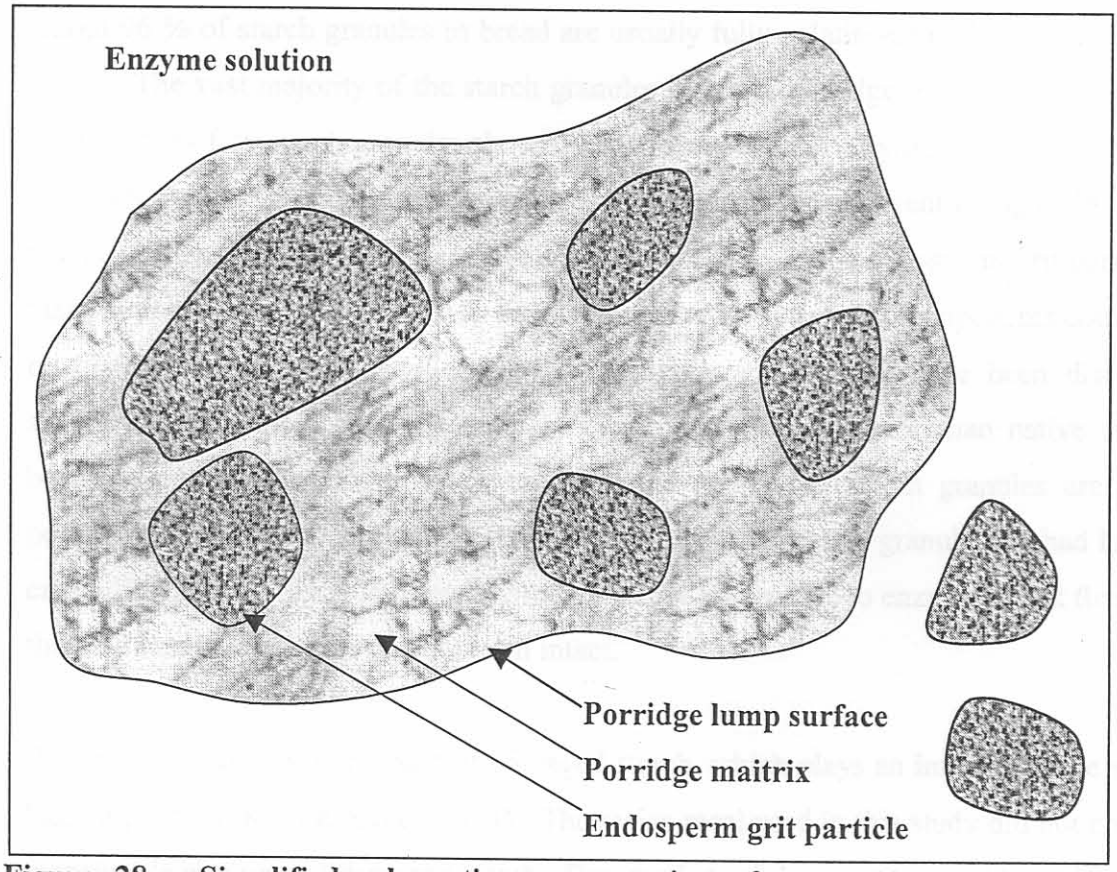


Figure 28: Simplified schematic representation of a porridge lump showing porridge matrix and endosperm grit particles

In the case of bread, the enzyme solution would fill the pores and have a large contact surface area with the substrates. With maize porridge, the enzymes would be in contact with the surface of the porridge lumps and the endosperm grit particles. Initially the surface area would be very small, but as the porridge matrix was digested, more endosperm grit particles would be released and the contact surface area of the enzymes with the substrate would increase. Much of the starch in the porridge particles was however still enclosed in cells. Physically enclosed starch is type 1 enzyme resistant starch (Englyst *et al*, 1992). This was in contrast to starch granules in bread, which could not even be seen as intact entities in the white bread (using light microscopy). The fact that starch in white bread was physically more accessible to the enzymes than the starch in maize porridge, could have contributed to the higher rate of starch digestibility of white bread compared to maize porridge.

About 96 % of starch granules in bread are usually fully gelatinised (Whistler & Daniel, 1985). The vast majority of the starch granules in maize porridge were also gelatinised, because very few starch granules showed birefringence under polarised light. The extent to which the starch granules were distorted or disrupted was different though. In maize porridge, the starch granules were swollen, but especially in the endosperm grit particles, many granules were still intact. In the bread the starch and gluten components could not be distinguished, which implied that the starch granules must have been disrupted severely. Gelatinised starch is more susceptible to enzyme attack than native starch, because gelatinisation destroys crystallinity and gelatinised starch granules are more porous than native starch granules (Holm *et al.*, 1985). A starch granule that had lost its crystallinity and is disrupted severely may be more susceptible to enzyme attack than one that had lost its crystallinity, but is still intact.

Bread flour contains an amount of damaged starch, which plays an important role in the baking process (Kent & Evers, 1994). The maize meal used in this study did not contain measurable amounts of damaged starch. Damaged starch is more susceptible to digestion by enzymes than intact native starch granules (Tester & Morrison, 1994). The fact that the starch in bread is gelatinised fully during the baking (Whistler & Daniel, 1985) may, however, cause the effect of damaged starch to be insignificant.

After cooking, the porridge was allowed to cool to 50 °C at room temperature. During this period retrogradation of amylose would take place (Whistler & BeMiller, 1997), thus forming type 3 enzyme resistant starch (Englyst *et al.*, 1992). Starch retrogradation also takes place in bread after baking (Coultate, 1996). Because the maize porridge and bread were analysed shortly after they were prepared, mainly amylose retrogradation and not amylopectin retrogradation would have taken place, as according to Whistler & BeMiller (1997) amylose retrogradation takes place in minutes or hours, but amylopectin retrogradation in hours or days.

On a dry basis, bread contains approximately 3 % fat, but the maize meal used in this study contained only about 1% fat. Bread contains more fat, because fat is added as an ingredient of bread (Kent & Evers, 1994). Amylose can form complexes with lipids (Czuchajowska, Sievert & Pomeranz, 1991). These amylose-lipid complexes are digested more slowly than free amylose (Annison & Topping, 1994). Retrograded amylose, on the other hand, is resistant to digestion (Englyst *et al.*, 1992). The formation of amylose-lipid complexes competes effectively with the formation of resistant starch (type 3) (Czuchajowska *et al.*, 1991). The higher fat content of bread could thus result in lower levels of enzyme resistant starch and higher starch digestibility compared to maize porridge.

Wheat, maize and oat starch generally have the same amylose content, roughly 25 % (Hareland, 1993). The amylose content of the cultivars analysed in this study was in the region of 37 %. It is known that South African dent maize cultivars typically have high amylose contents (Mrs. C. Erasmus, Foodtek CSIR, South Africa, Personal Communication, 1999). High amylose starch is digested more slowly than normal or low amylose starch and also yields more resistant starch (type 3) in food products (Muir *et al.*, 1995). The high amylose content of the maize starch compared to the wheat starch could therefore have lead to higher levels of retrograded amylose and slower starch digestion in the maize porridge than in the bread.

Wheat flour used for baking bread has a particle size of < 212 μm (Kent & Evers, 1994), which is much finer than the particle size of the maize meal that was used (< 1mm). It was seen as a possibility that the smaller particle size of the cereal in bread could cause the higher starch digestibility, because several researchers had found that decreasing particle size increased digestibility (Snow & O'Dea, 1981; Holm & Björck, 1992; Granfeldt, *et al.*, 1994). To test this hypothesis, the maize meal of cultivar C was milled down to a flour with a particle size of < 212 μm. The digestibility of this maize flour porridge was ,however, not significantly different from the maize meal porridge from the same cultivar. This finding agrees with Nelles, Dewar & Taylor (1999), who attempted to increase the degree of solubilisation and enzyme susceptibility of the starch in maize grits adjunct used in the sorghum beer brewing industry. It was found that decreasing the particle size of the maize grits did not have a significant effect on the amount of starch that was solubilised after digestion with malt enzymes when the maize grits had been cooked under well-stirred conditions. The maize porridge in this study was also stirred well during cooking

It is possible that the starch digestibility of the porridge was not so much related to the particle size of the endosperm grit particles in the porridge, but more to the size of the porridge lumps after chewing. In the porridge lumps the surface area of the individual endosperm grit particles could be less important than the surface area of the lump itself (refer to Figure 28).

Another possible explanation is that the particle size of the maize meal was already reasonably small compared to the maize flour. Snow & O'Dea (1981) compared the *in vitro* starch digestibility of raw oats, wheat, barley and rye in the rolled and ground forms. The ground cereals were significantly more digestible than the rolled cereals. Cooked ground rice was also significantly more digestible than cooked whole rice. Holm & Björck (1992) found that substituting 80% of the wheat flour in a white wheat bread recipe with intact wheat kernels decreased the GI significantly. Granfeldt *et al.* (1994) compared the metabolic response of boiled whole barley kernels with barley flour porridge. The barley flour porridge had a significantly higher GI and *in vitro* starch

digestibility than cooked whole barley kernels. In all these studies the particle size of the cereal was reduced dramatically, whereas the reduction in particle size from maize meal to maize flour is not that dramatic.

The difference between the digestibility of bread and maize porridge might be due to intrinsic differences between wheat starch and maize starch, or different preparation methods for bread and porridge (baking and wet cooking). To test the hypothesis, the starch digestibility of maize flour porridge and wheat flour porridge was compared (thus keeping the particle size and preparation method constant). Oat flour porridge was also tested to see how the digestibility of oat flour porridge would be in relation to the other two cereal flour porridges. The fact that wheat flour porridge was much less digestible than wheat bread (even less digestible than maize porridge), indicated that the preparation method had an enormous effect on starch digestibility. This result is in agreement with work done by other researchers. For example, the starch digestibility of steam-cooked and popped wheat were significantly higher than that of flaked wheat (Holm *et al.*, 1985); bread baked from spaghetti ingredients was significantly more digestible than spaghetti mixed into a porridge and spaghetti porridge significantly more digestible than spaghetti (Granfeldt & Björck, 1991).

The starch digestibility of maize flour porridge was higher than that of wheat and oat flour porridges, but was the difference due to intrinsic differences between the starches? Faulks & Bailey (1990) studied the digestibility of isolated starch from different sources after being fully gelatinised. After 240 min incubation with α-amylase, it was found that maize starch was 77 % hydrolysed and wheat starch 72 % (no data for oat starch). The difference in the digestibility of the pure, gelatinised starches could have contributed to the higher starch digestibility of maize flour porridge compared to wheat flour porridge. Yet the cereal porridges in the present study were not made from pure starch, but from cereal endosperm flours. The non-starch components (protein, non-starch polysaccharides, cell wall remnants and lipids) in wheat and oat flour could also have contributed to the low starch digestibility.

It is probable that the gluten in the wheat porridge covered the starch granules and by doing that reduced accessibility of the enzymes to the starch. This would make the starch less susceptible to enzyme attack (Oates, 1997) and hence reduce the rate of digestion. The wheat flour porridge had a dense and elastic texture. Of course the starch granules in bread are also covered with gluten, but because the structure of bread is porous the starch in bread could be more accessible to enzymes than the starch in wheat flour porridge. Wheat flour porridge is not a food that is usually consumed in the form that it was prepared in this study. The wheat flour porridge studied here had an unpalatable elastic texture. According to the author's knowledge, wheat flour or meal is only used to prepare porridge after being toasted. Porridge made from toasted wheat flour has a pleasant consistency (not elastic at all). This change in consistency is probably caused by a breakdown of glutenin and gliadin during the toasting, which will then prevent the formation of gluten during the cooking. Without the gluten, the texture will not be elastic and rubbery. The starch in the porridge will most probably be more easily digestible too, because the starch would not be covered by protein.

The starch digestibility of oat flour porridge was also lower than that of maize flour porridge, but oat flour does not contain gluten (Kent & Evers, 1994). However, the fat content of oat flour is very high (8.1 %, Kent & Evers, 1994; 11.0 %, Langenhoven *et al.*, 1991) compared to wheat flour (1.6 %, manufacturer) and maize flour (1.1 %, this study). The fat content of oat starch itself is also known to be higher than other cereal starches (Paton, 1986). These lipids could reduce the surface accessibility of starch to enzymes, and thereby reduce starch digestibility (Oates, 1997).

Oats are also rich in β-glucans (Hareland, 1993). β-glucans are gums and responsible for the high viscosity of oat porridge (Hareland, 1993). The increased viscosity could reduce the rate of diffusion of the digestion products out of the dialysis tube and in that way reduce the measured starch digestibility.

The oat flour used in this study was milled from rolled oats. Whole oats has to be steamed to plasticise it before it can be rolled (Kent & Evers, 1994). During steaming,

some starch gelatinisation will take place. According to Kent & Evers (1994) about 30 % of the starch in commercial rolled oats is gelatinised. This pre-gelatinised starch could cause an increase in starch digestibility, because gelatinised starch is more susceptible to enzymes than raw starch (Holm *et al.*, 1985). Upon storage, gelatinised starch retrogrades (Whistler & BeMiller, 1997). This implies that there would also be some enzyme resistant starch (type 3) in rolled oats before cooking porridge. To minimise the effect of pre-gelatinised starch, the rolled oats chosen for this study was a product made from whole oat groats and hence had received minimum processing.

Bread baking involves the mixing of ingredients (wheat flour, water, yeast, salt, fat, etc.) to form an elastic dough (Kent & Evers, 1994). The dough is aerated by carbon dioxide formed by yeast fermentation and then baked at 220-230 °C for 30 min (Kent & Evers, 1994). To cook stiff maize porridge, maize meal is added to boiling water. After cooking it for a few minutes, more water is added and the porridge is simmered for about six minutes before being served. Cooking porridge takes less than eleven minutes in total and because it is a wet heat process, the temperature will not exceed the boiling point of water. During bread baking the temperature of the crust will exceed the boiling point of water when all the water in the crust had evaporated. The starch granules in bread flour would have had time take up water during the dough formation, whereas the starch in maize porridge would not have been hydrated before the heat treatment. Baking bread takes longer than cooking porridge and together with the fact that bread baking is a dry heat process and porridge cooking a wet heat process, this could cause the starch granules in bread to be more disrupted than the starch granules in maize porridge.

To summarise, white bread had a higher rate and extent of starch digestibility than traditional stiff maize porridge. This difference is probably due to the difference in microstructure, the higher fat content of bread and the higher amylose content of maize porridge and the less distorted starch granules in maize porridge. The difference in starch content, particle size and levels of damaged starch probably did not play an important role. The intrinsic difference in digestibility between maize and wheat starch could play a small role, but the most important reasons were probably extrinsic, namely the

difference in the ingredients of bread and porridge recipes, the difference in the preparation (mixing, proofing) and the difference in the heat treatments.

The average predicted GI of 44 (glucose reference) for hotplate cooked maize porridge definitely fell into the slow carbohydrate release group (GI less than 55) if the classification of Perlstein *et al.* (1997) is used. This predicted GI of stiff maize porridge is only an estimation making use of the *in vitro* starch digestibility results obtained in this study, combined with correlations between *in vitro* an *in vivo* results obtained in studies done by other researchers who used the same *in vitro* method. Björck and co-workers obtained close correlations between GI and hydrolysis index for various starchy food products, e.g. pasta, bread and legumes (Granfeldt *et al.*, 1992) and wheat, rye, oats and barley bread products (Liljeberg, Granfeldt & Björck, 1992); Other researchers using different *in vitro* methods also found close correlations between GI and starch digestibility (O'Dea *et al.*, 1981 for rice, Bornet *et al.*, 1989 for wheat, tapioca, manioc, smooth pea and mung bean starches), therefore it is valid to use the hydrolysis index to predict GI.

The estimated GI is in agreement with the *in vivo* study by Venter *et al.* (1990), in which it was found that traditional stiff maize porridge was a slow to intermediate carbohydrate release food with a GI of 50-66. This confirmation that starch in maize porridge is digested slowly, opens up exciting possibilities for the treatment and prevention of diabetes in South African Black people. It seems like if the South African Black people do indeed change from a slow carbohydrate release staple food (maize porridge) to a fast carbohydrate release food (bread) when they convert from a rural to an urbanised lifestyle. South African Black people consume more brown bread than white bread (Jooste, Langenhoven, Wolmarans & Benade, 1994), but this fact is of no significance regarding starch digestibility (Würsch, 1989).

The situation of urbanising South African Black people is similar to that of the Australian Aborigines, who also changed from slow to a fast carbohydrate release foods with urbanisation. In the case of diabetic Aborigines, it was shown that returning to a traditional diet (rich in slowly digested starchy tubers, roots and seeds) and lifestyle

improved their carbohydrate and lipid metabolism (O'Dea, 1984). Returning to a diet with traditional stiff maize porridge as the main carbohydrate staple could possibly aid in preventing the development of diabetes in rapidly urbanising Black South Africans.

This slow carbohydrate release food could also be useful in the dietary treatment of people who already suffer from diabetes. It is usually very difficult to convince people to change their eating habits. For people suffering form diabetes, a diet high in complex carbohydrate and dietary fibre and low in fat is one of the most important ways of managing the illness (De Villiers, 1995). Traditional stiff maize porridge is rich in starch (a complex carbohydrate) and very low in fat. The present study indicates strongly that maize porridge is high in resistant starch (type 1 and 3). Since resistant starch is part of dietary fibre (Asp, 1995), this would imply that traditionally prepared stiff maize porridge could contain a considerable amount of dietary fibre. The advantage of promoting the consumption of traditional stiff maize porridge in South Africa, is that it is a food product that is already known and used widely. According to MacIntyre, Venter & Vorster (1999), maize meal is something that can be found in almost every Black South African household, even though upper-class urban Black people consume very little maize porridge and rural Black people consume it as their main staple.

The advantages of consuming stiff maize porridge do not only apply to South Africans or people who suffer from diabetes. The principles of a healthy diet are really the same for all people (De Villiers, 1995) and including maize porridge, which is rich in complex carbohydrates and low in fat in the diet could benefit anyone.

Although this study indicated clearly that traditional stiff maize porridge was not a fast carbohydrate release food as is bread, Walker & Walker (1984) reported a GI similar to that of bread. Possible reasons for this discrepancy between the results of Walker & Walker (1984) and the results of the present study and Venter *et al.* (1990) could be that different raw materials and preparation methods were used. To explain, the effect of maize cultivar and cooking method on the *in vitro* starch digestibility of maize porridge will now be discussed.

With standard hotplate cooked porridge, the rate of starch digestibility increased as endosperm hardness increased ($p = 0.05$). Possible reasons could include:
differences in composition of the endosperm;
different levels of damaged starch;
difference in particle size distribution;
differences in starch gelatinisation;
differences in microstructure; and
unidentified texture-related factors.

Some researchers attempted to relate maize kernel composition and other properties with endosperm vitreousness (Dorsey-Redding *et al.*, 1991; Dombrink-Kurtzman & Bietz, 1993; Dombrink-Kurtzman, 1994; Dombrink-Kurtzman & Knutson, 1997). Results were sometimes contradictory (see Chapter 2, Literature Review, 2.2.3). In this study protein content did not seem to increase with increased endosperm hardness. The highest protein content was found in a soft cultivar (cultivar A, 8.03 %) and the lowest protein content in a medium cultivar (cultivar D, 6.88 %). This is in contrast with the study by Dorsey-Redding *et al.* (1991) who found a significant correlation between protein content and hardness with the hard cultivars having a higher protein content. The latter study was however done on 183 maize hybrids that were tested in two consecutive years, while the present study was done on only six maize cultivars.

Muir *et al.* (1995) found that maize variety affected the amount of resistant starch escaping the small intestine with varieties high in amylose yielding more resistant starch. Dombrink-Kurtzman & Knutson (1997) dissected maize kernels by hand and found that hard endosperm fractions contained 23.0 % amylose, compared to 20.5 % amylose in soft endosperm fractions. In this study the highest % amylose (39.0) was found in the one of the soft endosperm cultivars (B), but the second lowest % amylose (36.6) was also found in a soft endosperm cultivar (A). The amylose content did not vary much and could not explain the differences in starch digestibility. Panlasigui *et al.* (1991) found that rice varieties with similar high amylose contents had different starch digestibility rates and attributed the differences to differences in physiochemical properties between rice varieties.

Generally in this study, small, but significant differences in composition between cultivars were found, but except for particle size distribution, these differences did not seem to be related directly to endosperm vitreousness. It seemed like the harder cultivars had more large particles than the softer cultivars and the softer cultivars more small particles than the hard cultivars. This was expected, because hard endosperm needs more mechanical force to be broken (Dorsey-Redding, *et al.*, 1991) and yields more grits (Kent & Evers, 1994) than soft endosperm which breaks down to flour very easily (Wu & Bergquist, 1991). This was also experienced during the milling of the maize grits when the grits from the hard endosperm cultivars (E and F) had to be put through the roller mill one extra time before the desired particle size (< 1.01 mm) was obtained.

In hard wheat, it is the starch granules that break when the kernel is fractured and not starch-protein bonds (Hoseney, 1994). If the same were true for maize (no information in this regard could be found in the literature), then one would expect that maize meal from hard endosperm would contain more damaged starch than maize meal from soft endosperm. The fact that the hard cultivars had to be milled an extra time, meaning more severe milling, could also increase the amount of damaged starch in the meal (Tester & Morrison, 1994). If the hard cultivars had contained more damaged starch, then they should have had a higher starch digestibility (Kent & Evers, 1994) than the soft cultivars in the raw form. However, none of the maize meal samples contained measurable levels of damaged starch. Phegelo (1998) found no damaged starch in maize flour with a particle size of > 150 μm, but 11.7 AACC units in maize flour with a particle size of < 150 μm. The maize meal samples used in this study all contained 14-29% particles with a particle size of < 150 μm. The particle size of maize meal was relatively large and did not need such a severe milling as is needed to produce a flour, therefore it could be expected that the damaged starch content would be low (Jones, 1940). Considering the absence of measurable amounts of damaged starch, the effect of damaged starch on the digestiblilty of maize porridge from cultivars with different endosperm hardness can be ignored.

The particle size distribution of cultivars with different endosperm hardness differed, but that could not have caused the differences in starch digestibility, because reducing the particle size of the maize meal of cultivar C to maize flour (discussed earlier) did not change the starch digestibility of that cultivar as had been expected.

The initial hypothesis (Objective 4) was that it starch in hard endosperm would be difficult to gelatinise because of the tight packing in the protein matrix. It is also said that the protein matrix is thicker in the hard than in the soft endosperm of maize (Pedersen *et al.*, 1989). Starch in soft endosperm would be easier to gelatinise, because of the more loose packing of the starch granules in the cells. The higher degree of starch gelatinisation would then increase the digestibility of maize porridge made from a soft cultivar compared to a hard cultivar. This hypothesis does not explain the differences in digestibility between cultivars and is not valid, because virtually al the starch in stiff maize porridge was gelatinised.

It may well be that the starch in the softer cultivars was more easily disrupted than the starch in hard cultivars where distortion and disruption could have been limited by the protein matrix. During the cooling period, however, the cultivars with more disrupted starch granules (solubilised amylose leached out) could have formed more retrograded amylose (type 3 enzyme resistant starch). Raben *et al.* (1994) found that the glycaemic response is reduced if digestible starch is replaced by resistant starch. The soft cultivar porridges could possibly have had a lower rate of starch digestion than the hard cultivar porridges because possibly more enzyme resistant retrograded amylose had formed in the soft cultivar porridges than the hard cultivar porridges.

No difference could be observed between the microstructure (as observed with light microscopy) of porridge made from hard and porridge made from soft endosperm cultivars. This means that there were probably no obvious differences in microstructure that could have affected the starch digestibility of different maize cultivars.

Maize endosperm hardness is not well defined and understood (Dombrink-Kurtzman & Bietz, 1993). The differences in starch digestibility between the cultivars could also have been caused by endosperm texture related physiochemical properties that are not identified and understood yet.

As expected, decreasing the cooking time by half decreased the starch digestibility of maize porridge. With the shorter cooking time, the starch granules were probably gelatinised to a lower extent and less disrupted than with the standard time. The ungelatinised or partially gelatinised starch is less susceptible to enzyme digestion (Holm *et al.*, 1985), which decreased the digestibility.

Surprisingly, the opposite (doubling the cooking time) did not increase the digestibility rate as was expected. In fact, it reduced digestibility. Many researchers reported that gelatinisation increased starch digestibility, for example Holm *et al.*, 1985 (raw, cooked, popped, flaked and steam cooked wheat); Holm *et al.*, 1988 (wheat starch); Bornet *et al.*, 1989 (wheat, manioc and smooth pea starch) and Eerlingen *et al.*, 1994a (waxy maize starch). The reduced digestibility of long cooked maize porridge can be explained as follows: Increasing the cooking time would increase the degree of starch granule disruption, especially because of the increased number of times that the porridge was stirred during the extended cooking time. This could have lead to more starch molecules being released from starch granules. The increased degree of starch solubilisation could have lead to the formation of more retrograded amylose (type 3 enzyme resistant starch) during the cooling period than was the case with the porridge cooked for the standard period of time. If more type 3 resistant starch had formed, then starch digestibility rate could have been decreased (Raben *et al.*, 1994).

It is interesting to see how people prepare the same food differently by culture. Black South Africans seem to prefer stiff maize porridge and use relatively short cooking times. The porridge studied here was a stiff porridge. Black South Africans also eat a crumbly porridge. The crumbly porridge is made by adding more dry maize meal later in the cooking process (Mr. P. Rankhumise, Tswana man aged 65, Personal Communication, 1998). In the crumbly porridge the water would be limited, which would in turn limit

starch gelatinisation at cooking temperatures (Colonna *et al.*, 1992). Mr. Rankhumise remarked that the crumbly porridge kept hunger away for longer than the stiff maize porridge. The lower degree of gelatinisation would decrease the rate of starch digestion, because native maize starch is slowly, but completely digestible (Englyst *et al.*, 1992). Because of the lower moisture content, the crumbly porridge also has a higher nutrient density, which could increase satiety.

Traditionally, White South Africans use longer cooking times in the preparation of maize porridge. De Villiers (1992), in her book on traditional South African cooking, remarked that the longer porridge is cooked, the better it tastes. This was referring to a recipe for a thin porridge with a maize meal to water ratio of about 1:10. If such porridge was cooked for an hour or more (as suggested by the author), the starch would probably be fully gelatinised. This porridge would probably be highly digestible, because the gelatinised starch granules would be disrupted during the long cooking time in the presence of excess water. Very little starch would probably retrograde before the porridge is eaten, because White South Africans eat their porridge hot. In this study the stiff maize porridge was chewed when it was lukewarm, because it is usually consumed at a lukewarm temperature (Mr. P. Rankhumise, Tswana man aged 65, Personal Communication, 1998). Black South Africans also often eat cold porridge, in which a considerable amount of retrograded starch would have formed, especially when left over night (Venter *et al.*, 1990).

The starch digestibility of microwave cooked porridge was very similar to traditional hotplate cooked porridge. This is an advantage, because it means that more affluent, urbanised people with their busy lifestyles could use this convenient way of preparing the porridge and still enjoy the benefits of slow starch digestibility. In contrast with hotplate cooked porridge, the starch digestibility of microwave cooked porridge made from different maize cultivars did not increase with increased endosperm hardness. In fact, there was no correlation between starch digestibility and endosperm hardness. Cultivars B and F had a lower rate of digestibility than the rest. Cultivar B was a soft endosperm cultivar and cultivar F a hard endosperm cultivar.

Not much research had been done on the difference in the effect of microwave and conventional heating on starch. Lewandowicz, Fornal & Walkowski (1997) reported that the changes that occur in moist tuber starches on microwave heating were similar to those brought about by conventional heat moisture treatment. According to Marsono & Topping (1993) the effects of cooking rice in a rice cooker and microwave oven were similar and generally produced similar amounts of resistant starch upon cooling. On the other hand, Huang, Hess, Weber, Purcell & Huber (1990) examined potatoes after microwave and conductive heating and found that the swelling patterns of the starch granules were different. Heated to the same temperature, the starch granules in microwave heated samples were less hydrated and disrupted than conventionally heated samples. Yui, Weisz & Wood (1991) found that the starch in microwave cooked rolled oat porridge was less dispersed than porridge prepared by the conventional method. This difference was, however, ascribed to the fact that the microwave cooked porridge was stirred less. In the present study the effect of stirring would be minimal.

The reason for this difference or absence in trend was probably related to the way that energy is transferred during conventional and microwave cooking. The kinetics of heating during conventional and microwave heating are not comparable (Tomasik & Zaranyika, 1995). During conventional cooking, energy is conducted from the surface to the inside of the food. With microwave cooking, the heat is generated within the product (Potter & Hotchkiss, 1995). Because the microwaves generated the heat very fast and inside each endosperm grit particle, the endosperm texture related properties that may have caused the differences in digestibility in the hotplate cooked porridge, may have been destroyed.

Neither Walker & Walker (1984) nor Venter *et al.* (1990) gave full details on the nature of the maize meal that they used to cook the porridge from, it was just called "refined" or "unrefined" maize meal respectively. The two groups used different cooking methods. Walker & Walker did not specify, but considering that it was done 15 years ago it was probably a form of conventional heating (e.g. stove or hotplate). Venter *et al.* (1990) used microwave cooking. Walker & Walker (1984) gave the ratio of maize meal to water, but did not say whether that was a mass or volume ratio. They gave the cooking

time, but not the settings on the stove or the actual size of a batch of porridge cooked. Because of a lack of information, it is difficult to determine the real reason for the conflicting results obtained by different groups of researchers. This emphasizes the importance of specifying the raw materials and preparation methods in detail to enable comparison with other studies.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

Traditional stiff maize porridge contains two types of enzyme resistant starch: physically enclosed starch (type 1) and retrograded amylose (type 2). Resistant starch is an important part of dietary fibre (Asp, 1996). A diet rich in starch and dietary fibre and low in fat is recommended for people who suffer from diabetes (ADSA, 1997). Traditional stiff maize porridge fits these criteria. It can be useful in the dietary management of diabetes, because of the low rate of starch digestion. The predicted GI value is low (44 with glucose reference) which is an indication that traditional stiff maize porridge will not cause a sudden, sharp rise in blood glucose concentration, but rather a small increase over a longer period.

The change in carbohydrate staple food from slowly digested maize porridge to rapidly digested bread when South African Black people urbanise could be a contributing factor to the higher prevalence of diabetes (situation similar to that of Australian Aborigines as discussed by Thorburn *et al.*, 1987). The change in staple food is, however, merely one of the diet and lifestyle factors that changes. It is recommended that the results of the Transition, Health and Urbanisation in South Africa (THUSA) study (currently being done by Prof. Vorster and co-workers at the Department of Nutrition, Potchefstroom University for Christian Higher Education) be used to compare the glycaemic load of the diets of rural and urban Africans in order to determine to which extent the change in carbohydrate staple affected the glycaemic load of the diet.

The higher rate of starch digestion in bread compared to stiff maize porridge is probably due to the more open structure of bread, which gives α-amylase a larger surface contact area with the starch molecules. The higher fat content of bread may reduce amylose retrogradation in bread to some extent, whereas the high amylose content of South African maize will cause slower starch digestion and the formation of more retrograded amylose (type 3 enzyme resistant starch) in maize porridge. The presence of physically enclosed starch granules in maize porridge and the fact that starch granules in maize

porridge are less distorted than starch granules in bread contributes to the lower rate of digestion of porridge compared to bread. Intrinsic ingredients of the cereal endosperm (e.g. gluten in wheat and β-glucans in oats) probably have an important effect on the digestibility of starch in cereal flour porridges.

The digestibility of maize porridge was affected more by the duration of cooking than by maize cultivar, the particle size of the maize meal or the type of cooking (hotplate or microwave). Decreasing the cooking time may cause less disruption of the starch granules and thereby decrease the susceptibility of the starch to enzymes. Increasing the cooking time, together with more stirring, probably increases the disruption of starch granules and solubilisation of starch molecules. This may lead to the formation of more retrograded starch during the cooling, which decreases digestibility.

Reducing the particle size of maize meal to maize flour does not affect starch digestibility, because the surface area of the porridge lumps after chewing may have a greater effect than the size of the individual endosperm grit particles. Also, the particle size difference between meal and flour is relatively small.

The increase in the starch digestibility of hotplate cooked porridge with increase in endosperm hardness cannot be explained in terms of differences in degree of gelatinisation, because virtually all the starch granules in maize porridge are gelatinised. The difference in particle size distribution between cultivars is also ruled out, because it was shown that particle size did not affect digestibility. The proximate composition of the maize meal from different cultivars was also very similar. It is possible that soft maize cultivar porridge contained more retrograded amylose, or that the differences in digestibility are caused by unidentified endosperm texture related properties.

Because the starch digestibility of microwave cooked porridge is similar to hotplate cooked porridge, it possible to prepare the porridge in a convenient way that can even suit the more affluent urban dweller's lifestyle without losing the advantages of slow starch digestibility. With microwave cooked porridge there is no correlation between

digestibility and endosperm hardness as is the case with hotplate cooked porridge. During microwave cooking the high rate of heat transfer may destroy the endosperm texture related properties.

CHAPTER 8

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