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 The names of the cultivars are protected by the seed company and the seed company also asked for its name not to be published.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.

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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.

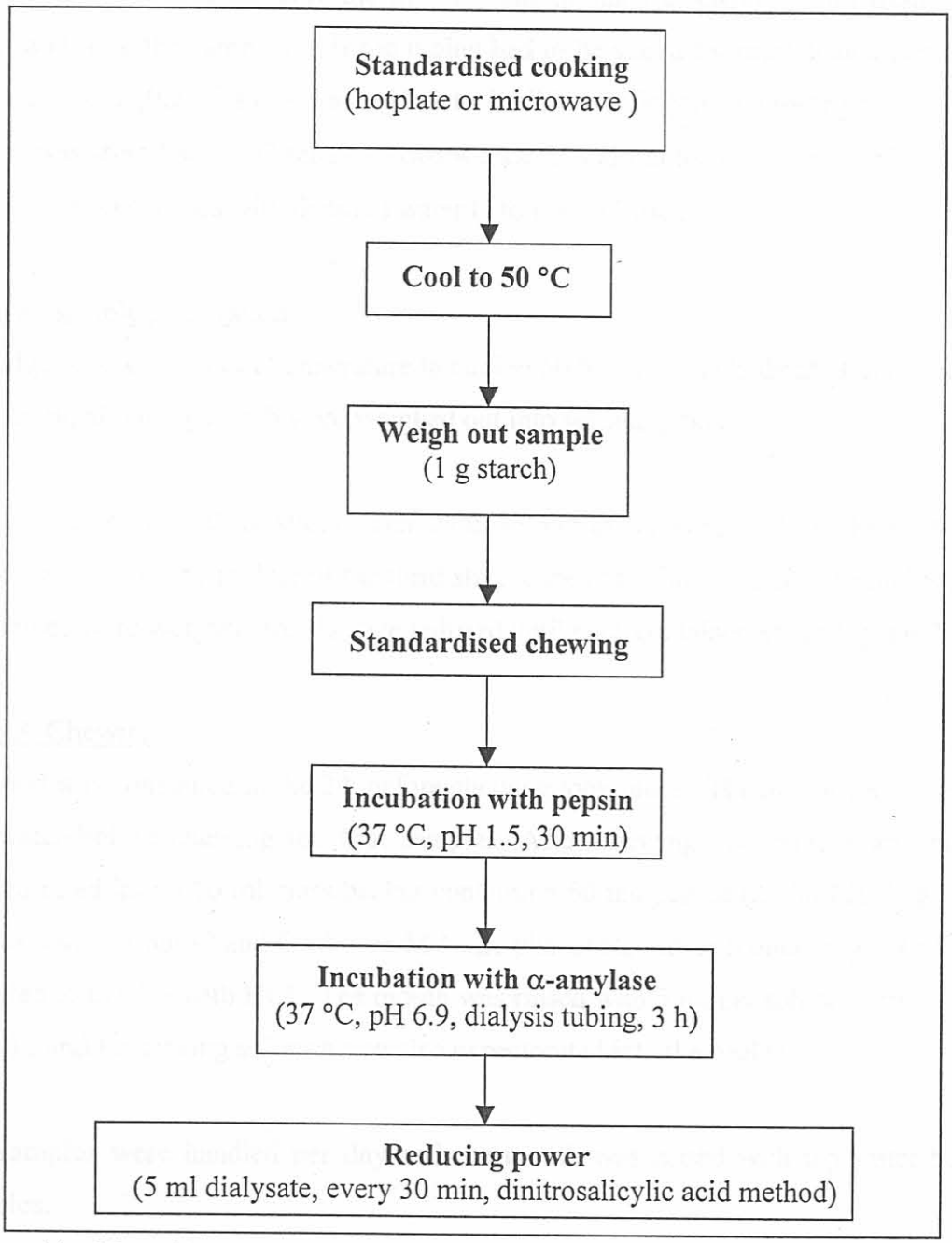

Figure 11: Flow diagram of the procedure used to determine the *in vitro* starch digestibility of porridge

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.