CHAPTER 3

OBJECTIVES

A two phased approach was used in this study. In the first phase, the effects of irradiation on the physico-chemical properties of maize flour, bean flour and their 70/30 composite flours were determined. The effects of irradiation on starch digestibility of raw maize and bean flours and on porridges made from these flours were determined *in vitro*. Effects of irradiation on protein digestibility of porridges made from maize and beans were also determined *in vitro* using pepsin and multi-enzyme assays. The primary objectives were:

1) To determine the effect of irradiation on viscosity of porridges made from maize flour, bean flour and their 70/30 maize:bean composite flours.
2) To determine the effect of irradiation on *in vitro* starch digestibility of raw maize or bean flours and of cooked porridges made from them.
3) To determine the effect of irradiation on *in vitro* protein digestibility of porridges made from maize and bean flours.

Based on the results of the first phase, the second phase was introduced to investigate changes in molecular properties of starch of maize and bean flours that could have been caused by irradiation that is responsible for changes in their digestibility. The secondary objective was to determine the effects of irradiation on molecular properties of starch of maize and bean flours that may alter their digestibility.

HYPOTHESES

Phase 1 Effects of irradiation on physico-chemical properties of maize flour, bean flour and of their 70/30 composite flours

1) Irradiation reduces viscosity of maize or bean porridges by depolymerisation and debranching of amylopectin molecules.
2) Irradiation may affect starch digestibility of raw maize and bean flours and of

porridges made from them by increased or decreased accessibility of starch molecules to amylase enzymes.

Phase 2 Effects of irradiation on molecular properties of maize, bean flour and on their starches that may lead to reductions in their starch digestibility at doses higher than 2.5 kGy

1) Irradiation may cause Maillard (browning) reactions in maize and bean flours that may result in productions of compounds, which inhibit amylase and proteolytic enzymes.
2) Irradiation of maize or bean flours at high doses results in molecular changes in starch structure promoting higher crystallinity of amylopectin fraction of starch in maize and bean flours.
3) Irradiation of maize or bean flours results in the formation of indigestible β (1-2) β (1-3) β (1-4) and β (1-6) glycosidic bonds in their starches which could lead to reduced starch digestibility at high doses.
4) Irradiation of maize or bean flours results in the production of easily retrogradable short chain amylose molecules from debranched amylopectin molecules resulting in reduced starch digestibility of raw flours and of porridges.

CHAPTER 4

MATERIALS AND METHODS

4.1 Phase 1 Effects of irradiation on the physico-chemical properties of maize flour, bean flour and of their 70/30 composite flours

1) Determination of the effects of irradiation on viscosity of porridges made from maize flour, bean flour and their 70/30 maize:bean composite flours.
2) Scanning electron microscopy to determine any visible changes in the structure of starch in irradiated maize and bean flours as is without any extraction of starch.
3) Determination of the effects of irradiation of maize and bean flours at 0, 2.5, 5, 7.5, and 10 kGy on the *in vitro* starch digestibility of raw flours and of porridges made from them.
4 Determination of the effects of irradiation of maize and bean flours at 0, 2.5, 5, 7.5 and 10 kGy on the *in vitro* protein digestibility of their porridges.

4.1.1 *Procurement and preparation of flours*

Dehulled and degermed maize flour at 14% moisture content and of particle size less than 500 µm was kindly donated by Maizecor, Pretoria. This was subdivided into 500 g portions and sealed in moisture and air-proof polythene bags and stored at -20°C. Dry speckled sugar beans (Pinto beans) were kindly donated by the Dry Bean Producers Organisation of South Africa. The beans were soaked in 0.01% sodium metabisulphite solution for 12 h at 25°C as described by Hoover, Rorke and Martin (1991). The hulls were then removed manually by rubbing between the fingers (Henshaw, McWaters, Oguntunde and Phillips, 1996). The cotyledons were then dried in a vacuum oven at 50 °C to final moisture content of 9.8%. The dry cotyledons were then pin-milled to final particle size of less than 250 µm, packed in moisture- and air-proof polythene bags and stored at -20°C until required for use.

4.1.2 *Characterisation of raw materials*

Quantitative determinations of percentage contents of moisture, ash, protein, lipids and total starch were performed on treated and untreated maize and bean flours as follows:

4.1.2.1 Moisture

Moisture was determined in triplicate using AOAC method 925.10 (Association of Official Analytical Chemists, AOAC, 1990a). Approximately 5 g of flour samples was accurately weighed into moisture tins pre-dried at 105°C for 14 h. The samples were then dried in a hot air oven at 135°C for 2 h. Dried samples were then cooled in a desiccator. The cooled tins and their contents were then weighed.

Moisture content was calculated as follows:

$$\% \text{ moisture content (as is)} = \frac{\text{Mass of fresh sample} - \text{Mass of dried sample}}{\text{Mass of fresh sample}} \times 100$$

4.1.2.2 Proteins

Maize and bean flour samples were analysed for total protein content (N × 6.25) using a Kjeldahl method (AOAC Method 920.87 of 1990b).

Approximately 200 mg of flour sample was weighed accurately on Whatman No 4 filter paper in triplicate and transferred into Kjeldahl digestion flasks. A Kjeltab tablet (Thompson & Capper, London, UK) and 20 ml conc. H_2SO_4 was added to each flask. The samples were then digested using Büchi 430 digester (Büchi Flavil, Switzerland) for 2 h until the flask contents became a crystal clear, green coloured liquid. In this reaction all the nitrogen in the sample is incorporated in ammonium sulphate (Dahlin and Lorenz, 1993). The digestion flasks and their contents were then allowed to cool at room temperature in the fume chamber.

After cooling, the ammonium sulphate in the digestion flasks was reacted with 32% (w/v) NaOH in the Büchi 322-distillation unit. Released ammonia was trapped in 4% (w/v) boric acid. The trapped ammonia was then released by titrating the ammonium boric acid complex with 0.2M HCl. The end point was determined as the point at which all the ammonia was released from its boric acid complex as indicated by change in colour of the reaction indicator. The amount of HCl used to release trapped ammonia was used to calculate the amount of protein in the original sample. Blanks of Whatman filter paper No 4 were treated the same way as described for the sample.

4.1.2.3 Crude fat

Crude fat contents of maize and bean flours were determined according to the American Association of Cereal Chemists, method 44-15A of 1983. Approximately 5 g of flour was weighed accurately on Whatman No 4 filter paper. The fat was then extracted using 200 ml petroleum ether (boiling point range 30-60°C) heated under reflux for about 5 h. The petroleum ether with dissolved fats was collected in a pre-weighed soxhlet collection flask. The extracted crude fat was recovered from petroleum ether by heating the soxhlet collection flasks over a boiling water bath in a fume cupboard. The dry soxhlet collection flasks and their crude fat contents were further dried in the hot air oven at 105°C for 5 h to remove residual moisture. The soxhlet collection flasks and their crude fat contents were then cooled to room temperature in a desiccator for 12 h and weighed.

Percentage crude fat content (on dry basis, db) was then calculated from the following formula:

$$\% \text{ crude fat (db)} = \frac{\text{Wt of soxhlet flask with crude fat} - \text{Wt of empty soxhlet flask}}{\text{Weight of moisture free flour sample}} \times 100$$

4.1.2.4 Ash

Ash content was determined using the AOAC method 942.05 (Association of Official Analytical Chemists, 1990c). Approximately 5 g of flour was accurately weighed in pre-dried, cooled and weighed ashing crucibles. The samples were then ignited to burn in a fume cupboard to avoid volatilisation in the incinerator. The burnt samples were then

transferred to the incinerator and burnt further at 550°C for 5 h. The ashing crucibles and their ash contents were allowed to cool at room temperature in the incineration chamber for 12 h and weighed. The total ash content on dry basis was then calculated using the following formula:

$$\% \text{ ash (dry basis)} = \frac{\text{Wt of crucible with ash} - \text{Wt of empty crucible}}{\text{Wt of moisture free flour sample}} \times 100$$

4.1.2.5 Total starch

Total starch content of maize and bean flours was determined by the method of Champ (1992). Approximately 1 g of flour of known moisture content was weighed accurately into a 250 ml conical flask. The flour was then dispersed using 3 ml absolute ethanol to avoid clumping. The starch in the flour was then solubilised using 10 ml 2M NaOH at room temperature for 10 min with intermittent shaking to aid in solubilisation. The pH was then adjusted to 4.2 using 2M acetic acid. The total volume was adjusted to 50 ml with distilled water. The solubilised starch was then gelatinised by autoclaving at 121°C for 20 min and cooling to 37°C in a shaking waterbath (Tovar, Bjorck and Asp, 1990). The gelatinised starch was then hydrolysed using 200 Novo units/ml of glucoamylase (EC 3.2.1.3, Novo AMG, Enzyme South Africa, Johannesburg) in 0.1M sodium acetate buffer (pH 4.2). This enzyme hydrolyses starch completely to glucose (Bravo, Siddhuraju and Saura-Calisto, 1998).

The hydrolysis was stopped after 2 h by adding 15 ml 33% (w/v) trichloroacetic acid (TCA). The sample was then centifuged at 2000 × g for 10 min at 5°C. The volume of the supernatant was adjusted to 100 ml to give a final TCA concentration of 5%. Blanks were run on samples with 0 min hydrolysis time. The reducing sugar content of the hydrolysate was determined using 3, 5 dinitrosalicylic acid as described by Holm et al. (1986). Maltose treated the same way as described for the starch samples was used as the standard. Percentage starch on dry basis was calculated using the following formula:

$$\% \text{ starch (dry basis)} = \frac{\text{mg equivalent of maltose released}}{\text{Wt of moisture free flour}} \times 100$$

4.1.3 *Irradiation of flours*

The packed maize flour, bean flour and their 70/30 maize:bean composite flour samples were irradiated at ambient temperature using ^{60}Cobalt γ-rays at Biogam (Nuclear Energy Corporation of South Africa, formerly Atomic Energy Corporation, Pelindaba) at dose rate of 0.5 kGy/h to target doses of 0, 2.5, 5.0, 7.5 and 10 kGy. Samples were irradiated in triplicate on different dates and the actual dose received was confirmed using cobalt glass dosimeters.

4.1.4 *Determination of the viscosity of porridges made from irradiated maize flour, bean flour and 70/30 maize:bean composite flour*

Viscosity of the porridges made from irradiated flours was determined using a Rapid Viscosity Analyser 3 (RVA, Newport Scientific, Narrabeen, Australia). The flours were added to the RVA aluminium canister containing 25 ml of distilled water to give total solids concentrations of 15, 20 and 25% (on dry basis). The RVA program was set to run at 960 rpm for 2 s to disperse the flour completely in the water and to stabilise the temperature. The rest of the test was performed at 160 rpm. The temperature profile used was 2 min at 25°C, the temperature was then increased from 25°C to 92°C in 8 min, held at 92°C for 10 min, and then the temperature was reduced from 92°C to 25°C in 8 min. This temperature profile allowed for the determination of porridge viscosity at 50°C, 40°C and 30°C, which are the temperatures at which the infants and children are most likely to consume the porridge (Lorri, 1993).

4.1.5 *Scanning electron microscopy of irradiated maize and bean flours*

Maize and bean flours irradiated at 0, 2.5, 5, 7.5 and 10 kGy were examined as is under a Jeol scanning electron microscope (JSM 840, Tokyo, Japan). The flours were sprinkled on double edged adhesive tapes mounted on aluminium stubs, coated with gold and examined in the scanning electron microscope at an accelerating potential of 5 KV according to the method described by Hoover and Manuel (1996a).

4.1.6 *Effect of irradiation on in vitro starch digestibility of raw maize and bean flours and of porridges made from them*

Starch digestibility *in vitro* was determined by the method described by Faulks and Bailey (1990) using porcine pancreatic α-amylase (EC 3.2.1.1) (Sigma Chemical Co., St. Louis, MO, USA) in 0.5M saturated sodium chloride containing 3 mM calcium chloride. The concentration of α-amylase was 23.9 mg/ml and the specific activity was 1240 units/mg of proteins. One unit was defined as the α-amylase activity that liberated 1 mg of maltose in 3 min at 20°C at pH 6.9 (as declared on the label of container).

To determine raw starch digestibility, 1 g of flour of known starch content was suspended in 3 ml absolute ethanol to help disperse the flour, 70 ml distilled water added, 20 ml enzyme solution in 0.5M phosphate buffer pH 6.9 added and incubated at 37°C for 15, 30, 60 and 120 min in a shaking water bath. Adding trichloroacetic acid (TCA) to a final concentration of 5% stopped the reaction. After mixing thoroughly the mixture was centrifuged at 2000 ×g for 10 min at 5°C. Blanks were treated in the same way except that the reaction was not allowed to proceed. TCA was added immediately after adding the enzyme solution. The supernatant was then made up to 100 ml using distilled water and assayed for the reducing sugar as described by Holm et al. (1986).

For the cooked samples, 70 ml distilled water was added to the dispersed flour and autoclaved at 121°C for 20 min to gelatinise the starch completely. It was then cooled in a shaking water bath at 37°C. After cooling for about 45 min the enzyme solution was added and it was incubated as described above. Maltose standards treated the same way as above were used to determine the rate of hydrolysis. Percentage hydrolysis was calculated as mg maltose released per 100 mg of starch in the flour (Socorro et al., 1989).

4.1.7 *Effect of irradiation on in vitro protein digestibility of maize and bean porridges*

Two methods were used to determine the *in vitro* protein digestibility of irradiated maize and bean flours.

4.1.7.1 Protein digestibility of cooked irradiated maize and bean porridges by pepsin method

The method described by Hamaker et al. (1987) was used. Irradiated maize or bean flours were dispersed in 3 ml absolute ethanol, and water added to a final ratio of 1:3 flour to distilled water and autoclaved at 121°C for 40 min according to the method described by De Gordinez , Bressani and Melgar (1992). The porridge was then freeze-dried to about 3% moisture content.

The freeze-dried material was then ground to pass through a 250 µm sieve. The protein content of the freeze-dried porridge was determined by the Kjeldahl method. Thirty five ml of daily prepared fresh pepsin solution (105 mg/100 ml 0.1M sodium citrate buffer pH 2.0) was added to 200 mg freeze-dried powder in a 250 ml conical flask and incubated in a shaking water bath at 37°C for 2 h. The reaction was stopped by adding 5 ml of 2M NaOH. The mixture was then filtered through Whatman No 4 filter paper. The residue was washed with 10 ml distilled water. The residue on the filter paper was then analysed for the protein content by the Kjeldahl method. A blank of protein filtered without digestion was used to obtain the soluble protein content of the sample and this was used to calculate the difference in the protein solubility which was not caused by the enzyme digestion. Protein digestibility was calculated from the following formula:

$$\% \text{ protein digestibility} = \frac{X-Y}{X} \times 100$$

where X is the protein content of freeze dried powder and Y is the protein content of the residue on the filter paper above the initial soluble protein content (Hamaker et al., 1987).

4.1.7.2 Determination of protein digestibility of cooked irradiated maize and bean porridges using multi-enzyme method

The multi-enzyme method of Hsu et al. (1977) was also used to determine the protein digestibility of porridges made from irradiated maize and bean flours. Irradiated maize or bean flour (5 g) was dispersed using 3 ml absolute ethanol and suspended in three times its weight in distilled water and autoclaved at 121°C for 40 min as described by De Gordinez et al. (1992). The porridge was then freeze-dried to about 3% moisture content.

The freeze-dried porridge powder was suspended in distilled water to form an aqueous suspension of concentration 6.25 mg/ml. The pH of the mixture was adjusted to 8.0 using 0.1M NaOH or 0.1M HCl in a shaking water bath at 37°C. The enzymes used were porcine pancreatic trypsin type IX of activity 14190 units per mg, bovine pancreatic chymotrypsin (type II) of activity 60 units per mg and porcine intestinal peptidase (grade III) of activity 40 units per mg. All enzymes were from Sigma. A solution was made from 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase per ml of distilled water and maintained in an ice bath and the pH adjusted to 8.0 using 0.1M NaOH or 0.1M HCl. Five ml of the enzyme solution was added to the 50 ml of the test protein solution. A standard solution of casein (Sigma) at the same protein concentration was used to monitor the reactions. The pH of the enzyme protein mixture was recorded after 10 min and the digestibility of the protein calculated from the following formula:

$Y = 210.46 - 18.10X$

where Y is the protein digestibility % and X is the pH of the enzyme protein mixture after 10 min (Hsu et al., 1977).

4.2 Phase 2 Effect of irradiation on molecular properties of maize flour, bean flour and on their starches that may lead to reductions in their starch digestibility at doses higher than 2.5 kGy

1) Determination of the effects of irradiation on the colour of maize and bean flours. It was suspected that irradiation at doses higher than 2.5 kGy might have caused

Maillard reactions which are known to produce compounds which inhibit amylase and proteolytic enzymes.

2) Differential scanning calorimetry (DSC) measurements were performed to establish whether irradiation of maize and bean flours could have altered the crystallinity of amylopectin fractions of the starch in their flours.

3) Irradiation of maize and bean flours could have led to the production of β-bonded starch. Beta bonded starch are only partially digestible by porcine pancreatic α-amylase *in vitro*. The formation of β-bonded starch in irradiated maize and bean flours was investigated using endo (1-3) (1-4)- β-D-glucanase (lichenase) in combination with porcine pancreatic α-amylase.

4) Irradiation of maize and bean flours may have caused depolymerisation and debranching of amylopectin fraction of starch of their flours. Debranching and depolymerisation of amylopectin molecules could have produced the highly retrogradable short chain amylose molecules of DP_n 15-45 and these are resistant to digestion by amylase enzymes *in vitro*. To investigate the molecular weight distribution of starch fractions in irradiated maize and bean flours, starch was isolated by wet milling of the treated flours. The isolated starch was then fractionated using size exculsion high performance liquid chromatography (HPLCSEC).

4.2.1 *Preparation of samples*

During Phase 1, it was established that irradiation up to 2.5 kGy improved the starch digestibility in raw maize and bean flours and poridges made from them. However, irradiation at doses higher than 2.5 kGy resulted in statistically significant ($p \leq 0.05$) reductions in starch digestibility.

Samples were tested for effects of irradiation on starch digestibility of maize and bean flours and on porridges made from them by irradiation of maize and bean flours at 0, 5 and 10, and at higher doses of 20 and 40 kGy.

4.2.2 *Determination of colour of irradiated maize and bean flours.*

Colour was determined using a Gardner XI-23 Tristimulus reflectance colorimeter (Gardner Laboratories, Bethesda, MD, USA) using the method described by Erasmus (1996). The colorimeter was calibrated using a standard white tile to the appropriate L, a and b values as described by Sokhey and Hanna (1993).

4.2.3 *Determination of β(1-3) and β(1-4) bonded starch contents of starches isolated by wet milling from irradiated maize and bean flours*

The scheme for determination of β-bonded starch content of starches isolated from irradiated maize and bean flours is summarised in Figure 8. The irradiated maize and bean flours were wet milled according to the method described by Taylor, Novellie and Liebenberg (1984). Approximately 200 g flour was suspended in 1 l of distilled water and left to stand for 2 h with intermittent stirring. The mixture was then passed four or five times through a Fryma stone wet mill (Reinfelden, Germany) to disrupt the starch protein complex. The slurry was then passed through a 250 μm seive followed by a 75 μm seive three times, each time discarding the residue that remained on the screen (this was mainly the fibre fractions of the flour). The resulting slurry was then centrifuged at 2000 × g for 10 min at 5°C. The supernatant was discarded. The protein fraction appeared as a dark layer on the surface of the starch and was discarded. The white starch layer was resuspended in water and the procedure repeated twice; each time the supernatant was discarded and the protein layer scraped off. The starch was vacuum dried at 50°C to about 1% moisture content and analysed according to the scheme given in Figure 8.

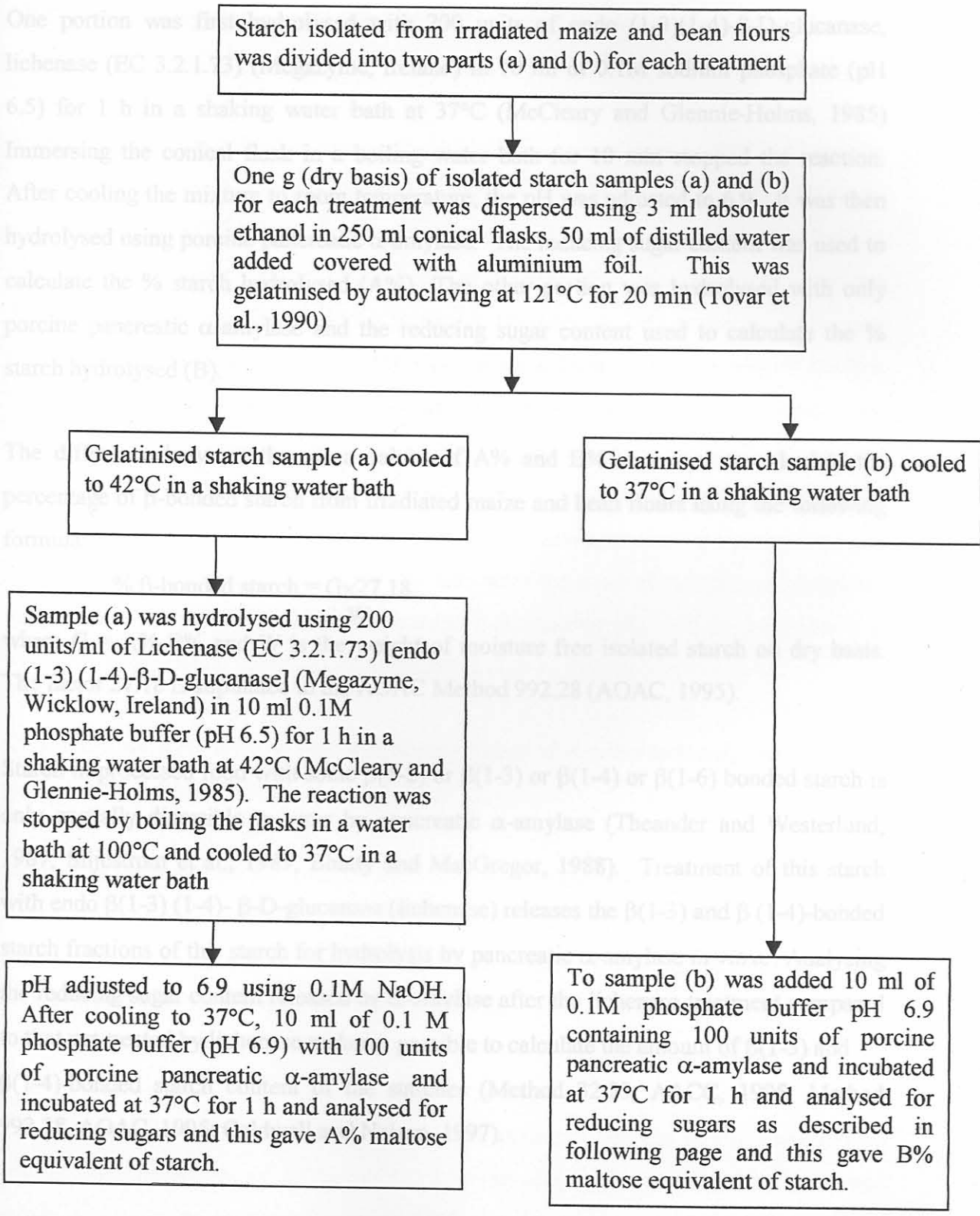

Figure 8 The analytical scheme for determining the β (1-3) and β (1-4) -bonded starch content of starches isolated from irradiated maize and bean flours

One portion was first hydrolysed with 200 units of endo (1-3)(1-4)-β-D-glucanase, lichenase (EC 3.2.1.73) (Megazyme, Ireland) in 10 ml of 0.1M sodium phosphate (pH 6.5) for 1 h in a shaking water bath at 37°C (McCleary and Glennie-Holms, 1985) Immersing the conical flask in a boiling water bath for 10 min stopped the reaction. After cooling the mixture to room temperature, the pH was adjusted to 6.9. It was then hydrolysed using porcine pancreatic α-amylase. The reducing sugar content was used to calculate the % starch hydrolysed (A%) The other portion was hydrolysed with only porcine pancreatic α-amylase and the reducing sugar content used to calculate the % starch hydrolysed (B).

The difference between the mean values of A% and B% was used to calculate the percentage of β-bonded starch from irradiated maize and bean flours using the following formula:

$$\% \text{ β-bonded starch} = \frac{G \times 27.18}{W}$$

where G = A%-B% and W is the weight of moisture free isolated starch on dry basis. The factor 27.18 is stipulated in the AOAC Method 992.28 (AOAC, 1995).

Starch in processed food with some β(1-2) or β(1-3) or β(1-4) or β(1-6) bonded starch is only partially digestible *in vitro* by pancreatic α-amylase (Theander and Westerlund, 1987; Siljestrom et al., 1989; Bhatty and MacGregor, 1988). Treatment of this starch with endo β(1-3) (1-4)- β-D-glucanase (lichenase) releases the β(1-3) and β (1-4)-bonded starch fractions of this starch for hydrolysis by pancreatic α-amylase *in vitro.* Analysing the reducing sugar content released by α-amylase after the lichenase treatment compared to that not treated by lichenase makes it possible to calculate the amount of β(1-3) and β(1-4)-bonded starch content of the starches (Method 32.23, AACC, 1995; Method 992.28, AOAC, 1995; Caldwell and Nelsen, 1997).

4.2.4 *Differential scanning calorimetry (DSC) of starch in irradiated maize and bean flours*

Irradiated flours were dried to constant weights in a vacuum oven at 50°C and quickly sealed in moisture and air-proof polythene bags. Changes in thermal properties of starch in irradiated maize and bean flours were investigated using a Setaram Micro-DSC III type microcalorimeter in the Department of Refrigeration and Livestock Products Technology, Szent Istavan University, Budapest, Hungary. A 20% suspension was made from irradiated flour and from this 750 mg was loaded onto 1 ml size sample holders. This was then scanned from 25 to 115°C at 1°C/min using 750 µl distilled water as the reference standard. After cooling down to 25°C the scanning was repeated again and the difference in the gelatinisation temperature of the second thermogram from the first thermogram gave the retrogradation enthalpy and retrogradation temperature of the irradiated flours. Temperatures of interest here were the onset of endothermic transition temperature (T_o), peak of endothermic transition temperature (T_p) and the temperature at which the endothermic transition was completed (T_c).

4.2.5 *Determination of molecular weight distribution of starches isolated from irradiated maize and bean flours*

Starch was isolated from the irradiated flours by the method described by Glennie (1987). About 10 g of irradiated maize or bean flours was dispersed using 3 ml of absolute ethanol. To this was added 200 ml 90% dimethyl sulphoxide solution and heated in a boiling water bath for 1 h with intermittent shaking. The mixture was then centrifuged at 2000 × g for 10 min at 25°C to remove insoluble non-starch components of the flour.

The supernatant with dissolved starch was then collected and the starch recovered by adding twice its volume of 100% n-butanol. After settling for 30 min, the precipitated starch was collected by centrifuging at 2000 × g for 10 min at 5°C. Flooding with absolute ethanol removed any residual n-butanol washed the precipitate. Finally it was washed with absolute acetone to remove any residual water. The precipitate was freeze-

dried. The dried starch was carefully ground to a fine powder using pestle and mortar to avoid loss of starch as dust in the hammer mill. The starch powder was sealed in moisture and air-proof polythene bags and stored at 5°C.

One gram of dry starch was weighed into 200 ml plastic beakers. About 50 ml water at 90°C was added to the flour and was homogenised for 5 min using Ultra-Turax model T25 homogeniser (Janke & Kunkel, Staufen, Germany). The homogeniser was cleaned with 10 ml boiling distilled water. The starch suspension was centrifuged at 2000 × g for 5 min. The supernatant was dialysed for 12-14h in dialysis tubes of pore size 10,000 to 12,000 Da to remove any of the contaminating low molecular weight chemicals like dimethyl sulphoxide and n-butanol, which had been used to isolate the starch. The supernatant was adjusted to 0.01M NaOH using 0.5M NaOH, and was also adjusted to 0.02% sodium azide (NaN_3) content to avoid microbial contamination. The final volume was made to 100 ml.

The liquid phase was filtered through two filter membranes mounted in series; pre-filter (Machery-Nagel MN 85/90; cat.150597/0) and 1.2 µm pore size AcetatePlus membrane filters (Osmonics Inc.,Sunnyvale, CA, USA, cat A12SPO2500). The amount of starch (and other carbohydrates) dissolved in the liquid phase was determined by the phenol sulphuric method (Dubois, Gilles, Hamilton, Rebers and Smith, 1956).

The filtrate with dissolved starch (400 µl) was then injected onto two TosoHaas HPLC columns G5000PW_{XL} and G6000PW_{XL} (TosoHaas, Tokyo, Japan) connected in series, to give a higher resolution, with a TSK PW_{XL} guard column to protect the main columns. The mobile phase used was 0.01M NaOH with 0.02% NaN_3 at a flow rate of 0.5 ml/min pumped by HPLC pump (Waters model 501; Millipore Corp., Bedford, MA, USA) at room temperature. The columns were joined to a Waters Associates model 401 refractive index detector at sensitivity × 8. The refractive index at any given moment is directly proportional to the solutes dissolved in the mobile phase. The refractive index data collected from the detector was then integrated using Apex version 2.14 Software (Autochrom Inc., Millford, Bedford, MA, USA). The retention time for the peaks of the

main starch fractions was used to calculate their average molecular weights from that obtained from the retention time of pullulan standards, P2: 20,000, P3: 23,700, P4: 48,000, P5: 100,000, P8: 853,000 Da (Showa Denko K. K., Tokyo, Japan). A standard curve was made using Microsoft Excel software relating retention time and the Log_{10} of molecular weight. The retention time was then used to calculate the apparent molecular weights of the starch fractions.

4.3 Statistical analysis

Data was analysed in triplicate for six assays of porridge viscosity, starch digestibility, protein digestibility, β-bonded starch and colour measurements. Mean, standard deviation, one way analysis of variance (ANOVA) followed by the least significant difference (LSD) tests were performed to determine the effects of irradiation at 95% confidence ($p \leq 0.05$) limit using Statistica© 6.0 programme.