Physico-chemical effects of irradiation on starch and protein of maize and bean flours

BY

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I declare that the thesis herewith submitted for the PhD degree at the University of Pretoria, had not been previously submitted by me for a degree at any other University.

ABSTRACT

PHYSICO-CHEMICAL EFFECTS OF IRRADIATION ON STARCH AND PROTEIN OF MAIZE AND BEAN FLOURS

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The high viscosity of starch based weaning foods made from cereals, legumes and tubers, is a major impediment to adequate intake of energy and proteins in developing countries. Infants, with stomach capacity of only 220-250 ml and undeveloped digestive systems, can only consume porridges of viscosity of 1000-3000 cP. To achieve this consistency, traditional weaning foods are normally diluted to about 5-10% total solids content. However, weaning foods need to contain at least 20% total solids content to meet their nutritional requirements. Cereal based weaning foods are also poor in protein quality but this can be improved by combining cereals (e.g. maize) with legumes (e.g. beans) at 70/30 ratio.

To meet the consistency and total solids requirements for infant porridges, maize flour, bean flour and their 70/30 composite flours were irradiated at 0, 2.5, 5.0, 7.5 and 10 kGy. Irradiation significantly reduced the viscosity of porridges made from these irradiated flours. Porridges of 20% total solids content and the desired consistency consumable at 30-50 °C could be prepared from bean flours irradiated at 5.0 kGy while maize and the 70/30 composite flours needed at least 7.5 kGy. The reduction in viscosity of these porridges was probably due to debranching and depolymerisation of amylopectin starch fractions.

Irradiation had significant ($p \leq 0.05$) effects on starch digestibility of maize and bean flours and porridges made thereof. Irradiation of maize flour increased the *in vitro* starch digestibility of porridges at 2.5 kGy by 3.2% but at 10 kGy it decreased by 2.8% compared to the control. In porridges made from irradiated bean flours, there was an increase of 8.8% in the *in vitro* starch digestibility at 2.5 kGy and a decrease of 2.1% at 10 kGy compared to the control. The observed increase in starch digestibility *in vitro* at 2.5 kGy was probably due to increased access of enzymes to starch molecules due to debranching and depolymerisation of amylopectin molecules, reduced viscosity of the porridges and increased solubility of the starch molecules.

Experiments were performed to determine the probable chemical reactions that might have caused decreases in starch digestibility at doses higher than 2.5 kGy. It was established that irradiation caused browning, which may be due to Maillard reactions. Maillard reactions produce chemical species that may inhibit α-amylase enzymes. Using differential scanning calorimetry (DSC) it was established that irradiation at higher doses caused increased crystallinity of amylopectin molecules which could have led to lower degree of gelatinisation and subsequently reduced starch digestibility. Using lichenase and α-amylase enzymes, it was established that irradiation at higher doses led to formation of less digestible β(1-3) and β(1-4)-bonded starch. HPLCSEC experiments showed that higher doses led to more debranching of amylopectin molecules, probably resulting in the production of more retrogradable short chain amylose molecules in maize and bean starches.

Irradiation of maize and bean flours at 0-10 kGy had very little effects on their protein digestibility compared to starch digestibility, probably due to the large size of amylopectin fractions of starches in maize and bean flours.

Irradiation processing shows a high potential for use to increase the total solids content of porridges. However, the small changes in starch digestibility of porridges made from irradiated maize and bean flours should be investigated further.

UITTREKSEL

FISIES-CHEMIESE EIENSKAPPE VAN BESTRALING OP STYSEL EN PROTEÏENE VAN MIELIE- EN BOONTJIEMELE

deur

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Die hoë viskositeit van styselgebaseerde speenpappe, wat van grane, peulgewasse en knolle gemaak word, is geïdentifiseer as 'n belangrike struikelblok vir die opname van energie en proteïene vir suigelinge en jong kinders in die meeste ontwikkelende lande. Suigelinge en jong kinders het 'n baie klein maagkapasiteit van 200-250 ml en as gevolg van hulle onderontwikkelde spysverteringstelsel kan hulle slegs voedsel met 'n viskositeit van 1000-3000 cP inneem. Om hierdie viskositeit te verkry word die tradisionele speenvoedsels normaalweg verdun tot omtrent 5-10 % totale vastestofinhoud. Speenvoedsel moet egter minstens 20% totale vastestofinhoud hê om aan die energie- en proteïenbehoeftes van suigelinge en jong kinders te voldoen. Graangebaseerde speenvoedsels het verder ook 'n lae proteïenkwaliteit maar dit kan verbeter word deur grane (bv. mielies) met peulgewasse (bv. boontjies) te kombineer in 'n 70/30 verhouding.

Mieliemeel, boontjiemeel en die 70/30 mielie:boontjie gemengde mele is bestraal teen 0, 2.5, 5, 7.5 en 10 kGy ten einde aan die konsistensie-en totale vastestofinhoudvereistes van speenvoedsels te voldoen. Hierdie studie bevestig dat bestraling betekenisvolle verlagings in die viskositeit van hierdie mele teweeg bring en dat die vastestofinhoud van die pappe verhoog kon word tot 20%, die vlakke waar dit voldoen aan suigelinge en jong kinders se energie- en proteïenbehoeftes. Deur bestraling van die mieliemeel en 70/30 mengsel van

mielie- en boontjiemeel teen 7.5 kGy kon die totale vastestofinhoud verhoog word tot 20% en steeds inneembaar wees tussen 30 en 50 °C. Boontjiemele moes teen 5 kGy bestraal word om aan bg. vastestofinhoudstandaarde te voldoen by 30 tot 50 °C. Die verlagings in viskositeit van pappe gemaak van mieliemeel, boontjiemeel en hulle 70/30 gemengde meel is waarskynlik toeskryfbaar aan depolimerisasie en onttakking van die amilosepektienstyselfraksies.

Bestraling het betekenisvolle ($p \leq 0.05$) effekte gehad op styselverteerbaarheid van mielie-en boontjiemele asook hul pappe. In pappe gemaak van mieliemeel het bestraling 'n verhoging van 3.2% by 2.5 kGy in *in vitro* styselverteerbaarheid veroorsaak en 'n afname van 2.8% by 10 kGy, in vergelyking met die kontrole. In pappe gemaak van boontjiemeel het bestraling 'n verhoging van 8.8% in *in vitro* styselverteerbaarheid tot gevolg gehad by 2.5 kGy en 'n verlaging van 2.1% by 10 kGy, gemeet aan die kontrole. Die verhoging in *in vitro* styselverteerbaarheid teen 2.5 kGy bestraling was waarskynlik toe te skryf aan die verhoogde beskikbaarheid van styselmolekules aan ensieme, veroorsaak deur die aftakking en depolimerisasie van amilopektienmolekules, verlaagde viskositeit van pappe en verhoogde oplosbaarheid van styselmolekules.

Eksperimente is uitgevoer om te bepaal wat die moontlike chemiese reaksies is wat verantwoordelik was vir die verlaagde styselverteerbaarheid by dosisse hoër as 2.5 kGy. Hierdie studie het bevestig dat bestraling verbruining veroorsaak, wat wel moontlik aan Maillardreaksies te wyte mag wees. Maillardreaksies produseer chemiese spesies wat amilase ensieme inhibeer. Dit is verder bevestig deur differensiële skanderings kalorimetrie (DSC) dat bestraling veranderings in die kristalliniteit van amilopektien veroorsaak in beide boontjie- en mieliemele, waarskynlik a.g.v. die onttakking van die amilopektienmolekules. Verhoogde amilopektienkristalliniteit kon gelei het tot 'n mindere mate van gelatinisasie, en gevolglik verlaagde styselverteerbaarheid. Deur gebruik te maak van ligenase en α-amilase ensieme, is vasgestel dat bestraling by hoër dosisse lei tot die ontstaan van minder verteerbare $\beta(1\text{-}3)$ en $\beta(1\text{-}4)$ gebonde stysel.

Dit is ook bevestig, deur gebruik te maak van grootte-uitsluitings-hoë-verrigtings-vloeistofchromatografie (HPLCSEC), dat bestraling van mielie- en boontjiemeel tot aftakking van amilopektien molekules lei. Afgetakte amilopektienmolekules het die vorming van geretrogradeerde kort-ketting amilose molekules tot gevolg.

Bestraling van mielie- en boontjiemele teen 0-10 kGy het baie min uitwerking gehad op die proteïenverteerbaarheid moontlik a.g.v. die groter grootte van die amilopektienfraksies van stysels in mielie-en boontjiemele.

Bestralingsprosessering beskik oor die hoë potensiaal om die totale vastestofinhoud van pappe te verhoog. Die klein verskille in styselverteerbaarheid in pappe gemaak van bestraalde mielie- en boontjiemele moet egter verder ondersoek word

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CHAPTER 1

INTRODUCTION

It is estimated that one third of the world's children are affected by protein-energy malnutrition (de Onis, Monteiro, Akre and Clugston, 2001). This manifests itself as wasting or stunting (Waterlow, 1994). The causes of protein-energy malnutrition are varied but the majority of cases in tropical Africa are from diets which are inadequate in quality and quantity associated with bulky, starchy weaning foods (Latham, 1990). Malnutrition-induced growth failure occurs mainly during the weaning period when the infants are introduced to foods other than their mother's milk at the age of four to six months (Griener, 1988).

The staple foods consumed in the tropics are mainly cereals, root crops and legumes (Delgado and Serna-Saldivar, 2000). These are starchy foods that have to be cooked to gelatinise the starch to improve their starch digestibility (Walker and Pavitt, 1989). During cooking, the starch absorbs a substantial amount of water, swells and becomes very thick (Weaver, Dibba, Sonko, Bohane and Hoare, 1995). The young children with their undeveloped digestive systems cannot readily consume the thick foods and so their porridge has to be diluted with water to a consistency of between 1000 and 3000 cP (Mosha and Svanberg, 1983). To achieve this consistency, children's porridges are usually served at about 5-10% total solids (Lorri, 1993), and with stomach capacity of 220-250 ml the children are unable to consume enough nutrients from these starchy foods (Brown and Begin, 1993).

The high viscosity of weaning porridges has been identified as a major cause of protein-energy malnutrition (Ngoddy, Nout, Nche, van Zulichem and Stolp, 1994). Typically, a gruel prepared from millet flour containing 50-100 g of solids per litre (l) that meets the required consistency has a very low energy density of only 900-1800 kJ/l (Janssen, O'Deen, Tribelhorn and Harper, 1981; Onofiok and Nnanyelugo, 1998; Food and Agriculture Organisation, FAO, 2002) compared to the energy density of milk at 2860

kJ/l (Brown, 1997) or a typical weaning foods used in the United Kingdom with an energy density of 5980 kJ/l (Walker, 1990). Addition of sugar to increase the energy density is discouraged because it is cariogenic (Hegarty, 1982) and may also raises the osmolality of the gruel which may lead to diarrhoea (Acre, 1989).

Methods proposed to increase the energy density of weaning foods include traditional methods such as germination (Svanberg, 1988), fermentation (Nout, Rambouts, Rambouts and Hautvast, 1989) and roasting of starchy weaning foods (Griffith, Castell-Perez and Griffith, 1998). Reducing the viscosity using traditional methods are time consuming, unhygienic and may even produce toxic foods (Panasiuk and Bills, 1984; Dada and Dendy, 1988). The reduction of viscosity by fermentation is very limited (Lorri, 1993). The pH of most fermented cereals and legumes is about 4.2 which is also the isoelectric point of their proteins, hence higher viscosity due to reduced protein solubility (Wanink, van Vliet and Nout, 1994).

Non-traditional methods proposed to reduce the viscosity of infant gruels include dehulling (Dada and Dendy, 1988), steam roasting (van der Poel, 1990), drum drying (Bressani, 1993) and extrusion cooking (Almeida-Dominguez, Serna-Saldivar, Gomez and Rooney, 1993). The use of irradiation to sterilise infant foods was first suggested by Raffi, Agnel, Thiery, Frejaville and Saint-Lebé (1981d) and they also reported its ability to reduce the porridge viscosity.

Irradiation has been shown to reduce the viscosity of pastes made from starchy foods due to depolymerisation of starch (Sokhey and Hanna, 1993). It has been approved by the Codex Alimentarius Commision (Codex) to improve the safety of infant foods (Codex, 1983; 1984) and a standard for good manufacturing practice of infant foods by irradiation has been formulated (Codex, 1994). However, irradiation, may alter starch and protein bioavailability (Swallow, 1991a; Lagunas-Solar, 1995; Murano, 1995). This research was undertaken to investigate the effect of irradiation on viscosity of porridges made from maize and beans flours and its effect on their starch and protein digestibility.

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CHAPTER 2

LITERATURE REVIEW

2.1 Maize and beans: benefits and problems associated with their use in weaning foods in sub-Saharan Africa

In Africa, maize is the main staple food to which a child is most likely to be introduced (Lorri, 1993). In South Africa, for example, maize is consumed by more than 90% of the rural African communities (Iputo and Makuzeni, 1993). Maize porridge, with a starch digestibility close to 90%, is a good source of energy and is a better source of starch compared with other cereals (Pedersen and Eggum, 1983; Cummings and Englyst, 1985). However, some starch (5-6%) of even well-cooked maize has been shown to escape digestion in the small intestines and is fermented in the large intestines (Englyst and Cummings, 1985). This undigested starch is called resistant starch (RS) (Berry, 1986). Resistant starch has been defined as the sum of starch and products of starch hydrolysis that passes into the colon of healthy subjects and has been found to range from 2-20% even in normal Western diets (Stephen, 1991).

Three forms of RS are currently distinguished (Cummings and Englyst, 1991). RS_1 is starch that is enclosed in plant cell walls and is inaccessible to α-amylase enzymes (de Roos, Heijnen, de Graaf, Woestenenk and Hobbel, 1995). RS_2 starch is native starch in granules and can be made accessible to α-amylase enzymes by gelatinisation (Gee, Johnson and Lund, 1992). RS_3 is retrograded starch that forms after cooling of gelatinised starch (Colonna, Leloup and Buleon, 1992). Up to 50% of RS_3 may escape absorption in the small intestines (Molis, Champ, Flourie, Pellier, Bornet, Colonna, Kowlowski, Rambaud and Galmiche, 1992) and may be fermented in the small intestines (Molis et al., 1992) though less well than RS_2 (Gee et al., 1992).

Maize contains about 10% proteins like other cereals (Phillips, 1997). Maize protein digestibility is about 95% when cooked (Graham, Glover, de Romana, Morales and MacLean, 1980). However, like most other cereals, maize proteins is lacking in lysine

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and it is recommended that maize-based weaning foods be complemented with legumes to improve on the protein quantity and quality (World Food Programme, 1994). In modern milling practice, maize grains are first tempered to about 20% moisture and then processed in a degerminator which removes the bran (pericarp) and the germ (Chung and Pomeranz, 1985). It is well known that the maize germ protein has a higher nutritional value than the endosperm protein due to its better balance in essential amino acids (Latham, 1979). Therefore, whereas degerming extends the shelf life of maize flour, by reducing rancidity, it actually reduces the nutritional quality of maize porridge protein (Pedersen, Bach-Knudsen and Eggum, 1989). When maize meal is supplemented with dehulled legume flour at 70/30 ratio, the resultant protein quality is close to that of casein in value (Yadav and Liener, 1978; Bressani, 1993) (Figure 1).

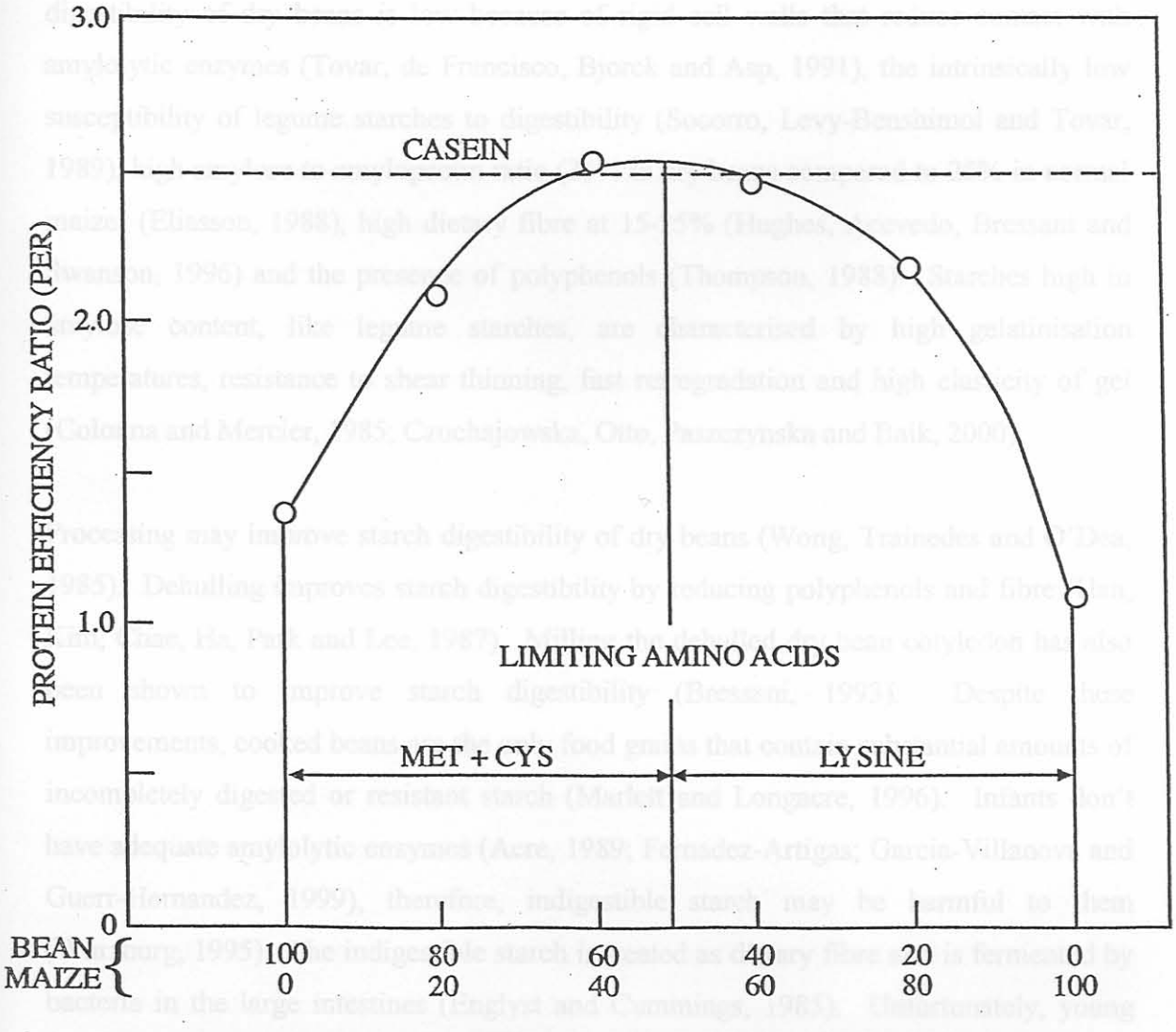

Figure 1 Complementary nutritional effects produced by mixtures of maize and navy bean at protein level of 8.3% (Yadav and Liener, 1978)

A major problem of using maize, beans or their 70:30 composite flours to make weaning porridges is that they are high starchy foods and as discussed in chapter 1, their flours have to be processed to reduce the viscosity of porridges made from them to levels consumable by infants and young children (Harper and Janssen, 1985). Without reducing their viscosity, weaning foods have to be diluted with water in order to achieve the right consistencyThis would reduce the total solids contents of the porridges and may result in protein-energy malnutrition (Lorri, 1993; Weaver et al., 1995).

With a total starch content of 20-56%, dry beans represent a good source of dietary energy (Tovar, Bjorck and Asp, 1990). However, its starch digestibility is very low compared to other starchy foods (Wursch, 1989). It has been suggested that the starch digestibility of dry beans is low because of rigid cell walls that reduce contact with amylolytic enzymes (Tovar, de Francisco, Bjorck and Asp, 1991), the intrinsically low susceptibility of legume starches to digestibility (Socorro, Levy-Benshimol and Tovar, 1989), high amylose to amylopectin ratio (35% in dry beans compared to 25% in normal maize) (Eliasson, 1988), high dietary fibre at 15-25% (Hughes, Acevedo, Bressani and Swanson, 1996) and the presence of polyphenols (Thompson, 1988). Starches high in amylose content, like legume starches, are characterised by high gelatinisation temperatures, resistance to shear thinning, fast retrogradation and high elasticity of gel (Colonna and Mercier, 1985; Czuchajowska, Otto, Paszczynska and Baik, 2000).

Processing may improve starch digestibility of dry beans (Wong, Trainedes and O'Dea, 1985). Dehulling improves starch digestibility by reducing polyphenols and fibre (Han, Kim, Chae, Ha, Park and Lee, 1987). Milling the dehulled dry bean cotyledon has also been shown to improve starch digestibility (Bressani, 1993). Despite these improvements, cooked beans are the only food grains that contain substantial amounts of incompletely digested or resistant starch (Marlett and Longacre, 1996). Infants don't have adequate amylolytic enzymes (Acre, 1989; Fernadez-Artigas; Garcia-Villanova and Guerr-Hernandez, 1999), therefore, indigestible starch may be harmful to them (Wurzburg, 1995). The indigestible starch is treated as dietary fibre and is fermented by bacteria in the large intestines (Englyst and Cummings, 1985). Unfortunately, young

children don't have well-developed bacterial flora in their large intestines, hence, excess resistant starch may cause diarrhoea and even death (Siljestrom and Bjorck, 1990).

Dry beans are an important source of proteins for many people in the tropics (Nielsen, 1991; FAO, 1993; Phillips, 1993) and are widely used to supplement proteins in weaning foods (van Steenbergen, Kusin, Voorhoven and Jansen, 1980; Kusin, van Steenbergen, de Witt and Jansen, 1983). However, several factors impair their protein quality. These include deficiency in sulphur amino acids, namely methionine and cystine (Bressani and Elias, 1980; Aguilera, 1993), presence of heat stable and heat labile anti-nutritional factors (Martinez, Marcos, Macarulla and Lerralde, 1995), as well as poorly digestible protein fractions which may limit amino acid availability (Sgarbieri and Whittaker, 1982; Lanfer-Marquez and Lajolo, 1991; de Gordinez, Bressani and Melgar, 1992). Cooking disrupts the secondary and tertiary structures of legume proteins, exposing their hidden hydrophobic sites to proteolytic enzymes (Moneam, 1990; Wang and Damodaran, 1990a; Clemente, Sanchez-Vioque, Vioque, Bautista and Millan, 1998). Cooking also denatures heat labile antinutritional factors of dry beans, thereby improving their protein digestibility (Marletta, Carbonaro and Carnovalle, 1992). Despite this, protein digestibility of cooked dry beans, at 50-80%, is still lower than that for proteins in most cooked cereal (70-90%) and cooked animal proteins (80-95%) (McDonough, Steinke, Sarwar, Eggum, Bressani, Huth, Barbeau, Mitchel and Phillips, 1990; Joseph and Swanson, 1993).

Irradiation has been proposed as a method to sterilise infant foods (Raffi et al., 1981d) and is known to reduce the viscosity of starchy foods (Rayas-Solis, 1987; Sokhey and Chinnaswamy, 1993). However, its effects on starch and protein bioavailability are not clear.

2.2 **Irradiation and its mode of action in food**

Food irradiation consists of exposing food to high-energy short wavelength electromagnetic waves (Urbain, 1971; Moseley, 1990; Swallow, 1991b). These waves

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remove electrons from atoms and molecules thus changing them into ions; hence the name ionising radiation (Thakur and Singh, 1993). High-energy charged particles, such as electrons, or photons such as X-rays and γ-particles, are examples of ionising radiation. Not all types of ionising radiation are suitable for use in food either due to their low penetrating power (α-rays) or because they cause food to be radioactive (high-energy electrons or X-rays generated above certain energy levels). Therefore a Joint Committee of the Food and Agriculture Organisation (FAO), International Atomic Energy Agency (IAEA) and the World Health Organisation (WHO) has authorised only the following types of ionising radiation for use in food:

(a) Gamma rays from ^{60}Co and ^{137}Cs at an energy level of 5 MeV; (1 eV = 1.6×10^{-19} J).

(b) X-rays generated at an energy level below 5 MeV;

(c) Electrons generated from machine sources operated at below 10 MeV (WHO, 1981).

The effects of ionising radiation on food components can either be direct or indirect. In direct or primary interactions, the ionising radiations affect the molecules directly. In indirect interactions, also called secondary interactions, the ionising radiations first interact with water molecules and the free radicals created from the water molecules interact with those of food molecules.

2.2.1 *Primary effects*

When ionising radiation penetrates into foods, all or part of their energy is absorbed by the food. This absorbed energy (the dose) leads to ionisation or excitation of the atoms and molecules in the medium. Depending on the energy of the electromagnetic waves, a molecule may be ionised by losing an electron $RH \rightarrow RH^{+} + e^{-}$ or be dissociated by splitting $RH \rightarrow {}^{*}R + {}^{*}H$. If the energy associated with the electromagnetic wave is low, it may just cause excitation of the molecule ($RH \rightarrow RH^{*}$).

The electrons with energy in the order of 1 MeV, produced by the primary effects of electromagnetic waves, may produce $3\text{-}4 \times 10^{4}$ additional ionisation processes and between $4.5\text{-}8 \times 10^{4}$ excitations. Secondary (*delta*) electrons produced in this way have

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sufficient energy (100 eV) to cause further ionisation (Hasselmann and Marchioni, 1991). Depending on the energy or the dose, other food molecules can be ionised by losing electrons when they are hit by the electromagnetic waves ($RH \rightarrow RH^{+} + e^{-}$) or be dissociated by splitting ($RH \rightarrow {}^{*}R + {}^{*}H$) (Simic, 1978).

Water, a major component of most foods, absorbs radiation energy and undergoes the following primary reaction: $2H_2O \xrightarrow{\gamma\text{-irradiation}} {}^{*}OH + e^{-} + {}^{*}H + H_3O^{*}$

The primary effects are non-specific and can hit any molecule that is in the radiation's path, without specific preference to a particular atom or groups of atoms (Diehl, 1991). In a small and symmetrical molecule the radiation energy absorbed is uniformly distributed and the number of bonds broken or changed will be the same. In large molecules, however, the absorbed radiation is unevenly distributed in the excited molecule with centres having highest concentration of electrons receiving the highest energy (Hasselmann and Marchioni, 1991). This also occurs during thermal processing with the result that products of irradiation and heat are sometimes similar (Wasik and Bushuk, 1973). Free radicals are produced in the primary effects of irradiation when the high-energy electromagnetic waves strike molecules in the food including water (Thakur and Singh, 1993).

2.2.2 *Secondary effects*

The free radicals produced by primary effects of irradiation are very reactive and short-lived (Wasik and Bushuk, 1973). Free radicals are defined as highly reactive molecular entities with unpaired electrons in the outer orbits of an atom, which is part of the molecule (International Atomic Energy Agency, IAEA, 1982). Free radicals may be produced when molecules are split by heat, light or ionising energy or by catalytic reactions involving enzymes and are commonly found in any food or living system (Zweier, Kuppusamy and Lutty, 1988). They may react with each other or with other food components (Swallow, 1991a). These reactions lead to secondary products of food irradiation, which may lead to the following reactions:

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Combination $^{*}R + {}^{*}H \rightarrow RH$

Dimerisation $^{*}R + {}^{*}R \rightarrow R\text{-}R$

Degradation $RCHNH_2COOH + H \rightarrow R^{*}CHCOOH + NH_3$ as in deamination

or $RCHNH_2COOH \rightarrow RCH_2NH_2 + CO_2$ as in decarboxylation

Electron capture $RH^{+} + e^{-} \rightarrow RH$ (Urbain, 1978; 1986)

The hydrated electrons (e^{-}) are equally reactive and attack the aromatic compounds, carboxylic acids, ketones, aldehydes and SH (thiol) groups (Elias, 1987).

Oxidation: $2\,{}^{*}R\text{-}H + 2O_2 \rightarrow 2R{=}O + H_2O + {}^{*}O$

Hydrogen peroxide formation: $2HO_2 \rightarrow H_2O_2 + O_2$ (Davis, Lin and Pacifi, 1987)

Hydroperoxide formation: $^{*}R + O_2 \rightarrow ROO + RH \rightarrow ROOH + {}^{*}R$

Among the radiolytic products of water, the hydroxyl radical is a powerful oxidising agent and is most reactive towards unsaturated compounds especially the unsaturated fatty acids (Dogbevi, Vachon and Lacroix, 1999a). It can abstract hydrogen from C-H and S-H bonds (Nawar, 1983; Hosova and Sorman, 1991) as shown below:

$R\text{-}H + {}^{*}OH \rightarrow {}^{*}R + H_2O$

$RS\text{-}H + {}^{*}OH \rightarrow RS^{*} + H_2O$

The secondary radicals produced by the reactions of primary radicals are not necessarily the same as the primary radicals produced by the direct effects of ionising radiation on food (Diehl, 1991). The reactivity of the free radicals depend on their ability to diffuse in the medium in which they are formed. In dry solids or deep-frozen foods, diffusion is not

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possible; hence the secondary effects are minimised (Fiddler, Gates, Pensabene, Phillips and Wierbicki, 1981). This results in trapping the primary radicals for a very long time (Wasik and Bushuk, 1973). Once the irradiated dry material absorbs moisture or the irradiated frozen material thaws, the trapped free radicals start moving around and this leads to secondary reactions with each other or other food constituents. All these reactions lead to stable end products (also called radiolytic products) in the food and the whole process is called radiolysis (Urbain, 1986).

Although most irradiation-induced chemical changes are very fast, some reactions continue during the storage of irradiated food. Irradiation of aqueous compounds, for example, produces hydrogen peroxide, which is unstable and may disappear over time. In one experiment, the unstable hydrogen peroxide was reported to oxidise other food constituents and produced new substances that were absent immediately after irradiation (Diehl, 1982). Diehl (1972) reported a loss of 25% in thiamine solution three hours after irradiation and a loss of 48% twenty-four hours after irradiation. Thakur and Arya (1993) reported a decrease in malonaldehyde content of irradiated mango pulp during storage, which indicates that a reaction was going on that reduced the malonaldehyde content. The technological and nutritional significance of these reactions depend on the radiation dose, composition of the irradiated system, presence or absence of oxygen, temperature during irradiation and subsequent storage (Thorne, 1991; Lopez-Gonzalez, Murano, Brennan and Murano, 2000).

2.2.3 *Factors influencing effects of irradiation on food*

2.2.3.1 Dose

The quantity of energy absorbed by a mass of material exposed to ionising radiation is called the dose. The SI unit for irradiation dose is the Gray (Gy), which is equal to the absorption of 1 J/kg. Dose affects the rate of chemical and/or physical changes that occur in the food product. At low doses there is a linear relationship between the products formed and the dose. However, at higher doses, there may be secondary reactions

between products resulting in completely new products formed and the linear relationship between the doses and products ceases to hold true (Taub, 1983; Thakur, Trehan and Arya, 1990).

The initial linear relationship between the products and dose is given by the following equation: $Y = 10^7 \times G \times D \times \rho$; where Y is the yield of product per kilogram, G is the number of molecules changed per 100 ev of the energy transferred to the food, D is the dose in Gray and ρ is the specific gravity of the food. Use of this equation to estimate the dose of ionising energy that has been applied to a food is not feasible because the products formed are also produced naturally in the food or are also produced during thermal processing (Nawar, 1983). It is clear from the equation above that as the dose increases the chemical changes also increase (Urbain, 1978).

Particular use of food irradiation requires specific doses in order to achieve the desired objective. In every application of food irradiation, the basic mechanism involves chemical change and this determines the amount of ionising irradiation to be received by the food (McManus, 1982).

2.2.3.2 Dose rate

Dose rate refers to the rate at which energy in the form of ionising radiation is supplied to food while dose refers to the total energy received by food after treatment with ionising radiation (Thorne, 1991). In general, dose rate is usually not a critical parameter in food irradiation (CAST, 1989). However, at high dose rates, so many free radicals are formed that recombination rather than interaction with other food components is favoured and this reduces secondary effects (Lu, Miller and Loretan, 1989; Hayashi, 1991). Dose rate is also critical where oxygen has to diffuse from the surface to the interior parts of a food. If the dose rate is too high, the demand for oxygen in the inner parts of the food may not be met through diffusion and this leads to secondary reactions different from those at the surface of the food (Hayashi, 1991).

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2.2.3.3 Moisture content

Indirect action (secondary effects) of irradiation on food and other biological systems depends on their water content (Hasselmann and Marchioni, 1991). Liquid water provides a medium for primary products of irradiation (free radicals) to migrate in and to interact with either each other or attack other food components (Kempner and Haigler, 1982). Liquid water, therefore, promotes secondary effects of irradiation. Frozen foods behave as if there is no liquid water present , hence, secondary effects of irradiation are minimised (Hasselmann and Marchioni, 1991). Secondary effects do not occur in foods at moisture content less than 12% (Colonna, Buleon and Mercier, 1987; Rayas-Duarte and Rupnow, 1994), thus dry foods undergo less chemical changes than high moisture foods (Adams, Blankenhorn and Diehl, 1979). In this study dry foods were used (i.e. bean flour at 9.8% moisture content and maize flour at 14% moisture content), therefore not much secondary effects are expected from radiolytic products of ionised water molecules.

2.2.3.4 Temperature

Primary effects of irradiation are independent on the temperature during irradiation (Odamten, Appiah and Langerak, 1985). Secondary effects of irradiation, however, are very much dependent on the temperature during irradiation. The role of temperature is very critical during the sterilisation of high protein foods or animal products (Taub, Kaprielian, Halliday, Walker, Angelini and Merritt, 1979). These foods are usually frozen during irradiation to minimise off-flavour development and the mobility of free radicals is minimal in these frozen foods (CAST, 1989). These foods, therefore, behave like dry foods (Diehl, 1972; Olson, 1998).

2.2.3.5 Atmosphere during irradiation

Free oxygen in the air behaves like a freed radical and combines readily with reactive compounds in the food (Simic, 1983; Davis, Lin and Pacifi, 1987). Oxygen adds to free

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radicals ($^{*}RH$) to give peroxyradicals ($^{*}ROOH$), oxidised products or superoxide radicals ($^{*}O_2$). Many hydroperoxides are unstable and soon oxidise other food components (Giroux and Lacroix, 1998).

Irradiating food in the absence of oxygen leads to decarboxylation, dehydration and polymerisation (Giroux and Lacroix, 1998). Radiolytic products produced include CO_2, CO, hydrogen, hydrocarbons and aldehydes (CAST, 1989).

2.3 **Effect of irradiation on starch**

2.3.1 *Chemical effects of irradiation on starch*

Irradiation effects on carbohydrate molecules have been summarised by Raffi, Agnel, Dauberte and Saint-Lebé (1981c) as being hydrolysis and oxidative degradation. Polysaccharides are depolymerised and smaller molecular weight subunits or smaller oxidative degradation products are produced (Urbain, 1986). The reducing power of barley starch was substantially increased by irradiation doses higher than 5 kGy (Faust and Massey, 1966). This was attributed to depolymerisation of starch molecules by ionising radiation (Adams, 1983). Radiolytic products have been determined in gamma irradiated starches derived from different foods including maize (Raffi, Frejaville, Dauphin, Dauberte, d'Urbal and Saint-Lebé, 1981a), amylomaize, waxy maize (Raffi, Agnel, Thiery, Frejaville and Saint-Lebé, 1981b) wheat, cassava, rice (Raffi, Agnel, Dauberte and Saint-Lebé, 1981c), potato, and dry beans (Raffi, Agnel, Frejaville and Saint-Lebé, 1981d). All the types of starch behave similarly under irradiation (Raffi et al., 1981a; b; c; d; Adams, 1983) (Figure 2).

Both α-hydroxy and α,β-dihydroxy radicals may disproportionate, dimerise or simply lose water. Depending on the position of the C=O formed by dispropotionation or dehydration, the resulting product can be an acid, a ketone or an aldehyde. If irradiated in presence of oxygen, glucose for example, forms gluconic acid, glucono-1,5- lactone,

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saccharic acid, D-arabinose, D-xylose, D-erythrose, glyoxal, dihydroxyacetone and hydrogen peroxide (Adams, 1983).

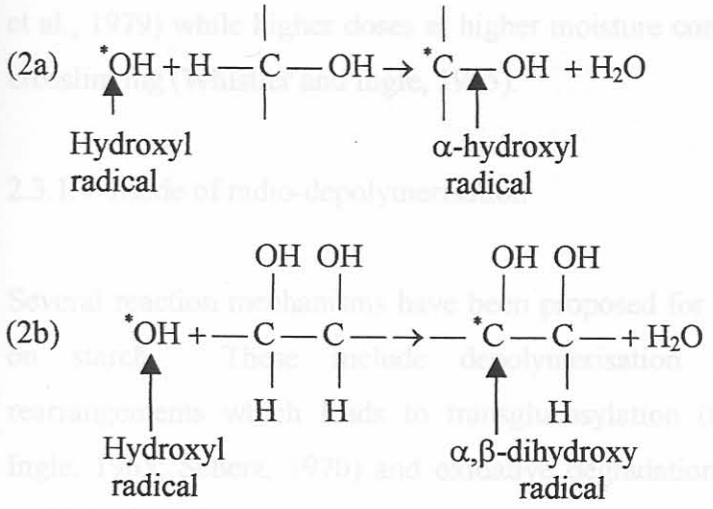

Figure 2 Action of hydroxyl radical on carbohydrates (Hasselmann and Marchioni, 1991)

During irradiation, in the presence of water, carbohydrates are attacked by the hydroxyl radicals which then abstract hydrogen from the C-H bonds forming α-hydroxyl and α,β-dihydroxy radicals (Diehl, 1982; Hasselmann and Marchioni, 1991) (Figure 3).

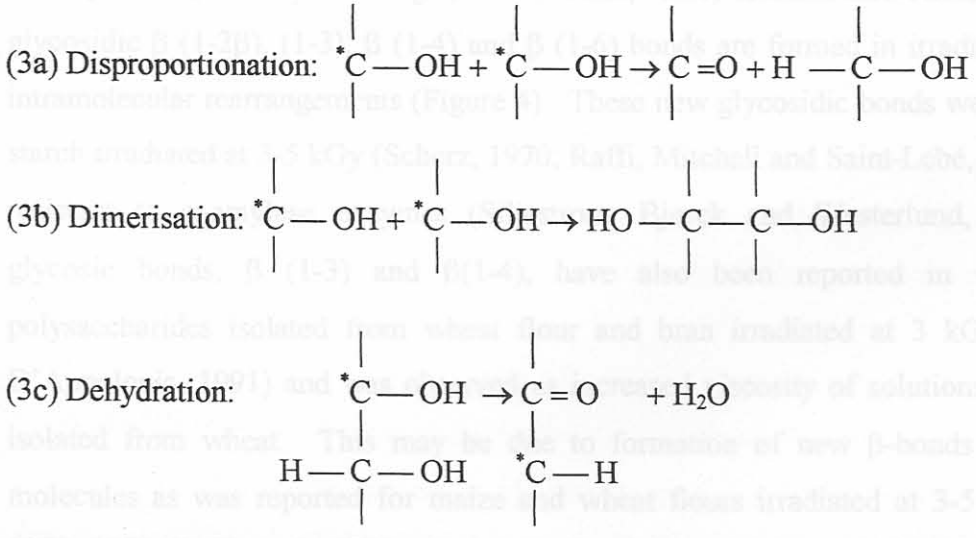

Figure 3 Pathways of carbohydrate degradation on irradiation (Hasselmann and Marchioni, 1991).

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The reaction products formed from secondary reactions of irradiation depend on irradiation dose and the moisture content, but are not significant at moisture content less than 12% (Ehrenberg et al., 1957; Ananthaswamy, Vakil and Sreenivasan, 1970b; Adams et al., 1979) while higher doses at higher moisture contents lead to more debranching or crosslinking (Whistler and Ingle, 1965).

2.3.1.1 Mode of radio-depolymerisation

Several reaction mechanisms have been proposed for the chemical effects of irradiation on starch. These include depolymerisation (Urbain, 1971), intramolecular rearrangements which leads to transglucosylation (transglucosidation) (Whistler and Ingle, 1965; Scherz, 1970) and oxidative degradation (Scherz, 1974; Colonna, Buleon and Mercier, 1987) (Figure 4).

2.3.1.2 Transglucosidation (formation of β-bonded starch)

Apart from hydrolysis and oxidation, irradiated starch may develop crosslinkages by transglucosidation (Whistler and Ingle, 1965; Scherz, 1974). Transglucosidation in starch is not dependent on the moisture content but on the irradiation dose at any water activity level (Whistler and Ingle, 1965; Ghali, Gabr, Ibrahim and Aziz, 1979). New glycosidic β (1-2β), (1-3), β (1-4) and β (1-6) bonds are formed in irradiated starch by intramolecular rearrangements (Figure 4). These new glycosidic bonds were detected in starch irradiated at 3-5 kGy (Scherz, 1970; Raffi, Mitchell and Saint-Lebé, 1980) and are resistant to α-amylase enzymes (Siljestrom, Bjorck and Westerlund, 1989b). The glycosic bonds, β (1-3) and β(1-4), have also been reported in water soluble polysaccharides isolated from wheat flour and bran irradiated at 3 kGy (Grant and D'Appolonia, 1991) and was observed as increased viscosity of solutions of pentosans isolated from wheat. This may be due to formation of new β-bonds in the starch molecules as was reported for maize and wheat flours irradiated at 3-5 kGy (Scherz, 1970; 1974; Raffi et al., 1980). Interestingly enough, similar results were obtained with

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starch of wheat flour (moisture content 10 %) heated at 180°C for 4 hours (Siljestrom et al., 1989a) (Figure 4).

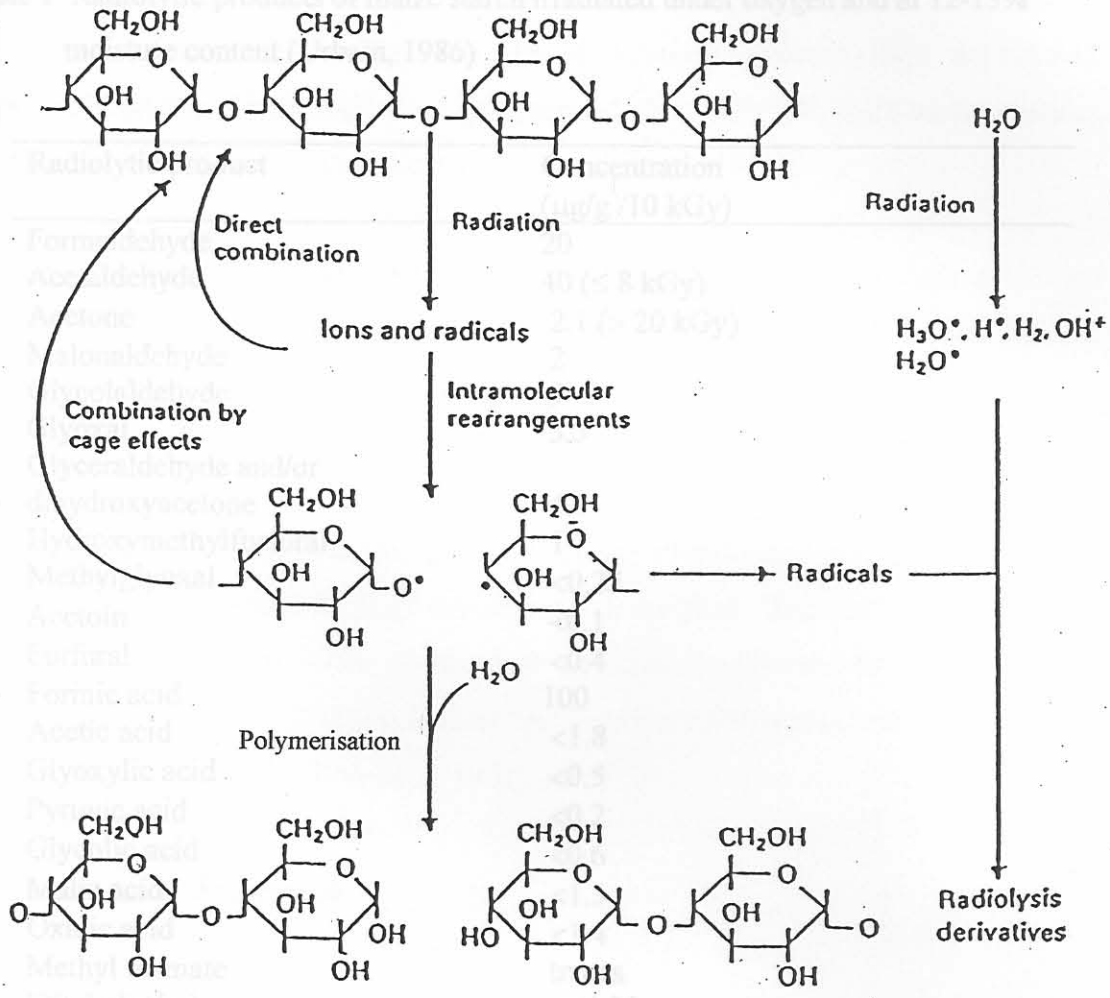

Figure 4 Some of the degradation mechanisms of starch during irradiation (Colonna et al., 1987) showing depolymerisation, transglucosidation (as recombinations), formation of free radicals and intramolecular rearrangements (with modifications)

Irradiation of starch also result in the formation of short chain oligosaccharides which are normally the hydrolytic products of starch digestion (Ananthaswamy, Vakil and Sreenivasan, 1970a) (Table 1). Maize starch irradiated at 10 kGy yields glucose, maltose, erythrose, ribose, mannose and other smaller molecular weight oxidative

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degradation products (Phillips, 1972; Berger, Agnel and Saint-Lebé, 1974; Murray, 1983) (Table 1).

Table 1 Radiolytic products of maize starch irradiated under oxygen and at 12-13% moisture content (Urbain, 1986)

Radiolytic product	Concentration (μg/g /10 kGy)
Formaldehyde	20
Acetaldehyde	40 (≤ 8 kGy)
Acetone	2.1 (> 20 kGy)
Malonaldehyde	2
Glycolaldehyde	9
Glyoxal	3.5
Glyceraldehyde and/or dihydroxyacetone	4.5
Hydroxymethylfurfural	1
Methylglyoxal	<0.25
Acetoin	<0.1
Furfural	<0.4
Formic acid	100
Acetic acid	<1.8
Glyoxylic acid	<0.5
Pyruvic acid	<0.2
Glycolic acid	<0.6
Malic acid	<1.3
Oxalic acid	<1.4
Methyl formate	traces
Ethyl alcohol	variable
Methyl alcohol	2.8
Glucose	5.8
Maltose	9.8
Mannose	0.1
Ribose	0.6
Xylose	0.4
Erythrose	1.2
H_2O_2	6.6 (1-4 kGy)

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2.3.1.3 Formation of short chain dextrins (oligosaccharides)

Ionising radiation causes greater fragmentation of the amylopectin starch fractions compared with amylose fraction (Raffi, Agnel, Boizot, Thiery and Vincent, 1985; Sokhey and Chinnaswamy, 1993). The short chain dextrins produced from debranched amylopectin chains are straight chain polymers of glucose of DP_n 2-20 and molecular weight of up to 3500 Da (Roberfroid, Gibson and Delzene, 1993).

Using HPLCSEC, Sokhey and Chinnaswamy (1993) found a short chain amylose fraction from an irradiated 100% amylopectin starch sample and that this amylose fraction increased with the irradiation dose implying that the amylopectin was being debranched in quantities proportional to the irradiation dose. Ananthaswamy et al. (1970a) irradiated wheat starch, wheat amylose and wheat amylopectin at 15% moisture content at 0, 0.2, 0.4, 0.6 and 2kGy. They observed greater increases in reducing sugar contents in high amylopectin starch than in the high-amylose starch samples. This may have significant nutritional consequences since short chain dextrins are known to be resistant to hydrolysis by α-amylase (Quigley, Hudson and Englyst, 1999) and to inhibit the α-amylases (Wursch and Del Vedovo, 1981).

2.3.1.4 Protected carbohydrates

Carbohydrates irradiated in the pure form are more susceptible to irradiation effects than in complex food systems (Urbain, 1986; Sokhey and Hanna, 1993). Radiolytic products formed from pure wheat starch at 5 kGy are equal to those produced in whole wheat flour irradiated at 50 kGy (Sabularse, Liuzzo, Rao and Grodner, 1992). Other food components like lipids, amino acids and proteins offer starch in the complex food environment provide some amount of protection (Diehl, Adams, Delincee and Jackubic, 1978). For example, addition of methionine or cystine to glucose in solution inhibited the formation of carbonyl compounds (Urbain, 1986). Therefore, caution must be exercised in extrapolating findings with pure substances, such as pure starch, to food systems (Josephson, Thomas and Calhoun, 1975).

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2.3.2 *Physical effect of irradiation on starch*

Irradiation causes chemical changes in starch which are not visible to the naked eye or even through the electron microscope (Sokhey and Chinnaswamy, 1992; Sokhey and Chinnaswamy, 1993). Polysaccharides degrade upon irradiation to small molecular weight compounds via the cleavage of glycosidic bonds and this leads to reduced viscosity in pastes made from starchy flours (Raffi et al., 1981b). Physical changes which are indicative of chemical changes are brought about through direct action of ionising irradiation (Faust and Massey, 1966) or through secondary effects in high moisture foods (Ehrenberg, Jaarma and Zimmer, 1957).

Adams (1983) reported that starch of irradiated wheat flour had decreased pasting viscosity, an increase in reducing power and an increase in amylopectin solubility. However, the physical changes that accrue from the chemical effects of irradiation can only be inferred from the behaviour of the starch when it is heated in suspension (Raffi et al., 1981a; b; c; d). Carbohydrates treated with gamma irradiation undergo depolymerisation which leads to breakdown of starch molecules, leading to reduced swelling power during gelatinisation (Michel, Raffi and Saint-Lebé, 1980; Raffi et al., 1981d). After irradiation, the viscosity of porridge (Michel et al., 1980; Martin, 1998) or sauces (Erasmus, 1996;) made from starch is decreased. Michel et al. (1980) reported an inverse relationship between irradiation dose and viscosity of cooked maize starch that was irradiated at ambient temperature. Starch paste when observed microscopically had appearance similar to overcooked starch (Erasmus, 1996). Similar reductions in viscosity have been reported for barley flour (Bhatty and MacGregor, 1988) and for rice flour (Sabularse, Liuzzo, Rao and Grodner, 1992). Reduced viscosity in starchy plant food sources due to irradiation probably leads to improved starch digestibility due to higher access by amylolytic enzymes into the starch molecules (Campbell, Classen and Balance, 1986; MacAllister, Rhode, Cheng and Forseberg, 1991). Measurement of viscosity has been proposed as method to detect irradiated starchy foods (Glidewell, Deighton, Goodman and Hillman, 1993; Hayashi, Todoriki and Kohyama, 1994).

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Using iodine staining, Martin (1998) observed that irradiated maize and sorghum starch stained lighter than the non-irradiated material and the higher the dose the lighter the staining. This has been explained by depolymerisation of the starch molecules resulting in short chain amylose molecules which stain lighter (Evers, 1979). Amylopectin chains are depolymerised more than amylose chains resulting in formation of short chain amylose molecules (Raffi et al., 1985; Sokhey and Chinnaswamy, 1993). This has been confirmed with size exclusion chromatography (HPLCSEC) (Sokhey and Chinnaswamy, 1993). The production of short chain amylose molecules leads to increased starch solubility. Kang, Byun, Yook, Lee and Chung (1997) found that glutinous (low amylose) starch irradiated at 10 kGy was three times more soluble than the untreated sample. Starch damage as determined by amylase susceptibility (Taylor, 1992), increased significantly from 10.4 to 16.8% for maize starch irradiated at 10 kGy (Martin, 1998). Starch damage has been defined as any structural change in starch, due to processing, that results in increased susceptibility to amylolytic enzymes, loss of granular integrity and increased water absorption (Chiang and Johnson, 1977; Colonna, Tayeb and Mercier, 1989).

Irradiation of wheat starch at 3 kGy has been reported to increase the crystallinity value from 104.5 to 112.4% (McArthur and D'Appolonia, 1984) and has also been been reported in rice starch irradiated at 10 kGy (Wootton, Djojonegoro and Driscoll, 1988). Rayas-Solis (1987) using differential scanning calorimetry of pure starch found that the gelatinisation temperature of pure bean starch increased from 65.4°C to 66.9°C when irradiated at 20 kGy. However, using scanning electron microscopy (SEM), no visible change was observed on maize starch granules irradiated at 10 kGy (Martin, 1998) and in waxy maize starch irradiated at 31 kGy (Sokhey and Hanna, 1993).

2.3.3 *Effect of irradiation on in vitro starch digestibility*

As with all other processing techniques, irradiation affects the nutritional value of foodstuffs (Diehl, 1981; Institute of Food Technologists, IFT, 1983; Khatak and Klopfenstein, 1988; Murano, 1995). Nutritional adequacy of foods treated with ionising

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radiation is of interest to consumers, legal authorities and processors alike (Voisine, Parent and Savoie, 1990). Therefore, the determination of nutrient bioavailability is an important aspect of assessing food irradiation as a processing technique (WHO, 1977; Council of Agricultural Science and Technology, CAST, 1986).

Factors that affect starch digestibility *in vitro* have been summarised as follows: gross food structure; cellular structure; starch granular structure (crystallinity hence degree of gelatinisation on cooking); viscosity and solubility; amylose-amylopectin ratio; starch-lipid complexes; starch-protein interactions; phytic acid, lectins and tannins; and amylase inhibitors (Holm, Asp and Bjorck, 1987; Wursch, 1989). Any of these factors that is affected by irradiation would lead to changes in starch digestibility.

Irradiation induces depolymerisation of starch molecules at doses about 2 kGy and this has been reported to improve starch digestibility by opening up the starch molecules to improve accessibility of α-amylase enzyme (Ananthaswamy et al., 1970a; b; MacArthur and D'Appolonia, 1984; Bhatty and MacGregor, 1992), by increased solubility and by reduced viscosity (Sokhey and Hanna, 1992; 1993; Rayas-Duarte and Rupnow, 1994). Corn starch solubility increased with irradiation dose between 0 and 20 kGy and then decreased (Sokhey and Hanna, 1992). Increased starch digestibility *in vitro* due to irradiation was reported for the larger molecular weight wheat starch amylopectin fractions than whole wheat starch or the isolated amylose fractions after irradiation (Ananthaswamy et al., 1970a; b).

Kume, Rahman and Ishigaki (1988) irradiated maize, cassava, wheat, sweet potato and potato starches at 10 and 25 kGy and hydrolysed these starches using fungal α-amylase. They found higher starch digestibility (2-3%) in the irradiated material compared to the control. However, the 2-3% increase in raw starch digestibility may not have been significant for starch intended as direct human food. Ito, Matsuyama and Sato (1977), using fungal α-amylase, also found increasing raw starch digestibility (0.3-0.6%) for maize and potato starch irradiated from 0 to 20 kGy.

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An overview of the methods used to determine the effects of processing on starch digestibility *in vitro* and *in vivo* are shown in Table 2.

Table 2 Overview of common methods used to evaluate starch digestibility (Dreher, Dreher and Berry, 1984)

In Vitro	Laboratory animals (*in vivo*)	Humans (*in vivo*)
Enzymes[1, 2, 3] Pancreatic alpha amylase	Animals[4, 5, 6] Male weanling rats (Holtzmann, Wistar or Sprague-Dawley), mice (Charles River) and chicks.	Subjects[7, 8, 9] Healthy, young adults. One or more subjects, double blind, cross over. (Except special medical cases)*
Conditions 0.5 – 2.0% starch solution in phosphate buffer, pH 5.9-7.0 at 37° C for 1-20 hours	Diet 35-55% starch plus basal ration (with complete nutritional requirements)	Diet (examples) Muffins 6-12/day or frozen pudding with 20% starch or starch blocker (16,666 units/day) or rice 75g/day, or baked potato 270 g/day.
Optional Pretreatment with 0.1N HCl and 0.75% pepsin for 20 min at 37°C.	Period 2-4 weeks of faeces collection	Period 1-10 days with stool collection daily
Calculation % hydrolysis = mg maltose/mg starch X 100	Calculation Apparent digestibility of dietary starch (%) = A- (B+C) X 100 A = Intake of starch B = Weight of starch in faeces C = $^1/_2$ X weight of post humous content of the gastrointestinal tract.	Calculation Apparent digestibility of dietary starch (%) = 100-(100% X marker food) X (% starch in faeces)/(marker faeces) X (% starch in food).
	Other measurements ^{14}C labelled substrate collected and $^{14}CO_2$ measured as labelled substrate, weight gain, necropsy of the pancreas or blood glucose level.	Measurements Blood glucose and insulin, breath hydrogen level
		Subject recall Indication of intestinal disorder or flatus.

NB. *Subjects with medical disorders, for example diabetics, may be used in special cases

1. Lee, Brooks, Kim, Hetlinger and Lebenthal (1985)
2. Soccoro et al. (1989)
3. Periago, Englyst and Hudson (1996)
4. Metta and Mitchell (1954)
5. Faulks, Southon and Livesey (1989)
6. Eggum, Juliano, Perez and Acedo (1993)
7. Nicklas et al. (1995)
8. Hamaker et al. (1995)
9. De Menezes, Lajolo, Seravalli, Vanucchi and Moreira (1996)

These methods have been used to evaluate starch digestibility objectively for the effects of other food processes such as extrusion and heating on starch digestibility (Metta and

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Mitchell, 1954; Fuchs, Gestanaduy and Suskind, 1992; Panlasiglui, Thompson, Juliano, Perez, Jenkins and Yiu, 1992; Hamaker, Rivera, Morales and Graham, 1995; Nicklas, Myers, Farris, Srinivasan and Berenson, 1995; De Schriver, Vanhoof and Ginste, 1999) both *in vitro* and *in vivo.*

Kume and Tamura (1987) irradiated maize, cassava, wheat, sweet potato and potato starches at 50 kGy and incubated them with fungal α-amylase at 40°C for 24 hours. Unlike other workers, they found reduced starch digestibility of the treated compared to the untreated (3-5% less). However, they incubated starch with the enzyme at 40°C for 24 hours under non-sterile conditions that could allow contamination by aerial microorganisms and this could have interfered with their results. However, in wheat flour and wheat starch extruded at 200°C, it was reported that the formation of β-bonded starch (2-4%) did not significantly affect the starch digestibility (Schweizer and Reimann, 1986; Siljestrom, Westerlund, Bjorck, Holm, Asp and Theander, 1986; Theander and Westerlund, 1987).

There are conflicting reports in the literature on the effects of irradiation on *in vivo* and *in vitro* starch digestibility. Read, Kraybill and Witt (1958) irradiated army rations at 28 and 56 kGy and fed this to male weanling rats *ad libitum* in such a way that irradiated material contributed 35% of the rat diet after treating it with antibiotics to avoid fermentation in the large intestines. By analysing the faeces for starch, they found reduced starch digestibility in food irradiated at 28 kGy and at 56 kGy, compared to the untreated control. However, Read, Kraybill, Worth, Thompson, Isaac and Witt (1961) found increased starch digestibility in plant foods irradiated at 50 kGy and fed to rats. MacAllister et al. (1991) used the same method and found reduced starch digestibility for maize and wheat flours irradiated at 15 kGy. Kashani and Valadon (1984) also reported reduced starch digestibility in Iranian pistachio irradiated at 10 kGy compared to the untreated control.

The feeding of male weanling rats *ad libitum* does not seem to bring out the effects of irradiation processing on starch digestibility clearly. Bhatty and MacGregor (1988) irradiated hull-less barley at 100 kGy, and by using α and β-amylases *in vitro,* found

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higher starch digestibility than the untreated control. However, *in vivo*, using male weanling rats fed *ad libitum*, they found slight reductions in starch digestibility (Bhatty and MacGregor, 1988). It is highly unlikely that foods irradiated at 50 kGy and above would have higher starch digestibility than the untreated control, given that Michel et al. (1980) had reported that irradiation of starch at 50 kGy was equivalent to pyrodextrinisation. Pyrodextrinisation usually results in reduced starch digestibility (Wurzburg, 1995).

From the above discussion, it appears as though there are conflicting reports on the effects of irradiation on starch digestibility in the literature. Effects of other processes such as extrusion, fermentation and germination, among others, on starch digestibility, are non-conflicting (Oste, 1991).

2.4 **Effect of irradiation on proteins**

2.4.1 *Effect of irradiation on chemical structure and properties of proteins*

Proteins in food serve as a source of energy and essential amino acids (Stevenson, 1992). They are built up of 20 different amino acids and of these 20, nine are not produced adequately in the human body and must be supplied in the diet (Giroux and Lacroix, 1998). Since all proteins consist of peptide-bonded chains of amino acids, the effect of irradiation on amino acids reflects the effects of irradiation on proteins (Wilkinson and Gould, 1998). Major reactions involve decarboxylation, oxidative deamination (in presence of oxygen) and reductive deamination (in absence of oxygen) (Liebster and Kopoldova, 1964). Gamma irradiation affects proteins by causing conformational changes, oxidation, rupture of covalent bonds, formation of protein free radicals, recombinations or polymerisation (Urbain, 1977; Cheftel, Cuq and Lorient, 1985). Depending on the nature a protein and irradiation dosage, the net result of irradiating proteins in the solid state could be crosslinking (aggregate formation) or molecular degradation (Dogbevi et al., 1999a). In Kidney beans proteins, for example, gamma irradiation caused increased deamidation, increased disulphide bond formation, decreased

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solubility and increased hydrophobicity (Dogbevi et al., 1999b). These changes are linked with changes in physico-chemical properties of proteins (Diehl, 1992).

In aqueous solution, if oxygen is absent, amino acids will undergo various reactions such as hydrogen abstraction, reductive deamination or decarboxylation during irradiation Oxygen blocks reductive deamination by removing e^-_{aq} and $^*H$ (Elias, 1987). The prevalence of ammonia and pyruvic acid and not carbon dioxide production in irradiated foods indicates that deamination and not decarboxylation reactions are dominant during irradiation (Diehl, 1991) (Figure 5). However, irradiation in the absence of water causes very few chemical changes in proteins (Diehl, 1990).

(5a) Hydrogen abstraction: $^*H + H_3N^+ - \underset{\displaystyle CH_3}{\overset{\displaystyle H}{C}} - COO^- \rightarrow NH_3^+ + {}^*\underset{\displaystyle CH_3}{C}\text{-}COO^- + H_2$

(5b) Reductive deamination: $e^-_{aq} + H_3N^+ - \underset{\displaystyle CH_3}{\overset{\displaystyle H}{C}} - COO^- \rightarrow {}^*\underset{\displaystyle CH_3}{\overset{\displaystyle H}{C}} - COO^- + NH_3$

(5c) Decarboxylation: $H_3N^+ - \underset{\displaystyle CH_3}{\overset{\displaystyle H}{C}} - COO^- \rightarrow H_3N^+ - {}^*\underset{\displaystyle CH_3}{\overset{\displaystyle H}{C}} + CO_2$

Figure 5 Irradiation induced breakdown mechanism for amino acids (Diehl, 1982)

Irradiation may split the peptide bonds in proteins to produce smaller peptides (Delincee, 1983; Hasselmann and Marchioni, 1991). Disulphide bonds may be broken with release of hydrogen sulphide in sulphur amino acids (methionine, cystine and cysteine) (Krumhar and Berry, 1990).

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The effects of irradiation on proteins are influenced by the binding forces of the proteins such as hydrogen bonds (Wong, 1989), hydrophobic bonds (Otte and Barz, 2000; Suh, Bae and Noh, 2000), ionic bonds and disulphide bonds (Simic, 1983; McHugh and Krochta, 1994). Proteins are, therefore, more resistant to irradiation than free amino acids (Davis and Delsignore, 1987).

Sulphur amino acids are important in food irradiation because they form radiolytic products with off-odours such as hydrogen sulphide, methylmercaptans and methyl sulphides (Wick, Yamanashi, Wertheimer, Hoff, Proctor and Goldblith, 1961; Merritt, Angelini, Wierbicki and Shults, 1975). The sulphur amino acids and aromatic amino acids react more readily with free radicals than aliphatic amino acids (Schaich, 1980; Franzini, Sellak, Hakim and Pasquier, 1994). Due to the rigid spatial structure of proteins, radicals formed as a result of irradiation are held together in position and have a high chance of recombination (Kempler and Haigler, 1982). The hydration state of the protein, temperature of irradiation, gas phase, dose of irradiation, and presence of other substances like lipids or carbohydrates all influence the radiation chemistry of the proteins (Delincee, 1983). There is evidence that protein breakdown products increase with increased irradiation dose (Merritt et al., 1978). However, off-odours are minimised when food is irradiated at subfrozen temperatures (Urbain, 1978).

It has been reported that proteins behave the same way under similar conditions of irradiation (Delincee, 1983; Diehl and Josephson, 1994; Hasselmann and Marchioni, 1991; Swallow, 1991b), hence, the radiation chemistry of proteins is predictable from the chemistry of their principle constituents (Thayer, 1994). Mild irradiation causes unfolding of the fibrous and globular proteins but intense irradiation may result in depolymerisation of fibrous proteins and aggregation of globular proteins (Simic, 1978; Elias, 1987).

Radiation induced denaturation of protein manifests itself as changes in viscosity of solutions, in solubility, in electrophoretic behaviour, in changes in absorption spectra, in enzyme susceptibility, exposure of SH groups, and in immunological changes (Ben-

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Hdech, Gallant, Bouchet, Gueguen and Melcion, 1991). Splitting of proteins into smaller molecules or aggregation into larger molecules have been observed singly or simultaneously (IAEA, 1982).

Maize and bean storage proteins are globular proteins (Shewry, 1995; Phillips, 1997; Marcone, 1999), hence, they are expected to undergo unfolding at low or mild irradiation doses and aggregation at high irradiation doses (Dogbevi, Vachon and Lacroix, 1999a). It is the unfolding of the protein molecules that results in increased digestibility at doses close to 2 kGy (Srinivas, Ananthaswamy, Vakil and Sreenivasan, 1972; Voisine, Parent and Savoie, 1990). However, irradiation at higher doses (above 2 kGy) may result in decreased protein digestibility. Mostafa (1987) reported that protein digestibility of peanuts increased with irradiation up to an optimum of 4 kGy beyond which it declined. Venugpal (1981), working with mackerel proteins, observed the start of precipitation of salt-soluble proteins at doses above 5 kGy and this may explain the reduction in protein digestibility due to the formation of crosslinkages.

2.4.1.1 Maillard reactions

Irradiation is known to cause intense Maillard reactions in food (CAST, 1986). It has been reported in Taiwanese rice of moisture content 11.5% irradiated at 3 kGy (Wang, Lee, Chang and Yet, 1983), in Australian rice of 16% moisture content and irradiated at 2.5 kGy (Wootton et al., 1988) and in Brazillian beans of 10% moisture content irradiated at 2 kGy (Cunha, Sgabieri and Damasio, 1993). Maillard reactions effects may reduce the protein digestibility of food on irradiation (Friedman, 1996c). Maillard reactions affect food protein quality in several ways: they reduce the availability of lysine (Franzini et al., 1994), render the protein indigestible through development of crosslinks (Lacroix and Outtara, 2000), produce enzyme inhibitors (Friedman, 1996a) and they also reduce the pH of proteins which leads to reduced digestibility (Pizzoferrato, Manzi, Vivanti, Nicoletti, Cordiani and Cogliandro, 1998). Loss in available lysine has a direct relationship with protein digestibility; the higher the loss in available lysine, the greater the loss in digestibility (Swaisgood and Catignani, 1991). Processing of foods rich in

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proteins and reducing sugars results in a chemical reaction between the carbonyl group of reducing sugars and amino acids especially the unbound ε-amino group of lysine present in the proteins (Erbersdobler, 1989; Hurrell, 1990; Friedman, 1996a) (Figure 6).

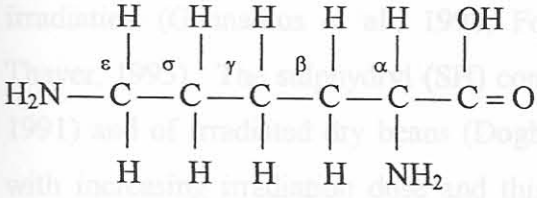

Figure 6 The chemical structure of lysine showing the α and ε amino groups (Holme and Peck, 1983)

2.4.1.2 Protein crosslinking

Irradiation of food at higher doses can result in the formation of protein crosslinks (Yamamoto, 1977; Josimovic, Radojcic and Milosavljevic, 1996) and these are usually observed as reduced solubility (Cheftel et al., 1985; Hayashi, Biagio, Saito, Todoriki and Tajima, 1991), increased viscosity (Giroux and Lacroix, 1998) increased hydrophobicity (Hayashi et al., 1991; Dogbevi et al., 1999b) and formation of higher molecular weight proteins (Hayashi et al., 1991; Gennadios, Rhim, Handa, Weller and Hanna, 1998).

Maillard reaction induced protein crosslinkages

Maillard reaction-induced crosslinks have been shown in irradiated proteins (Simic, 1983; Mezgheni et al., 1998). Maillard reaction induced protein crosslinking has been observed as increased molecular weight bands with electrophoresis (Dworschak, 1980; Krumhar and Berry, 1990) and as reduced protein solubility (Krumhar and Berry, 1990).

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Disulphide bond crosslinkages

Disulphide bonds are also formed during irradiation of food proteins (Davis and Delsignore, 1987). The sulphydryl groups in proteins are very sensitive to gamma irradiation (Gennadios et al., 1998; Fox, Lacritz, Hampton, Richardson, Ward and Thayer, 1995). The sulphydryl (SH) content of irradiated blood plasma (Hayashi et al., 1991) and of irradiated dry beans (Dogbevi et al., 1999b) has been shown to decrease with increasing irradiation dose and this indicates the formation of disulphide bonds. Protein digestibility is higher in raw sorghum flour than in cooked porridge (MacLean, de Romana, Placko and Graham, 1981) due to the formation of disulphide crosslinkages during cooking of sorghum porridge compared to the raw flour (Bookwalter, Kirleis and Mertz, 1987; Hamaker, Kirleis, Butler, Axtell and Mertz, 1987) and this may also be true in irradiated foods where these bonds are also formed (Dogbevi et al., 1999a; b).

Cystine derived crosslinks

Sulphur containing amino acids such as cystine, cysteine and methionine are very sensitive to irradiation at doses above 5 kGy (Partmann and Keskin, 1979). Above 5 kGy, desulphurisation occurs (Simic, 1983), and cystine, for example, forms a very reactive radical, dehydroalanyl (King, Mendelsohn, Gadbois and Bernstein, 1972). Dehydroalanyl reacts with lysine, for example, to form lysinoalanyl, a crosslinkage, in irradiated proteins (Venugpal, 1981). Cystine derived croslinkages increase with irradiation dose and they reduce the nutritive value of irradiated foods (van Kooij, 1977; Wang and Damodaran, 1990b). This may explain the crosslinkages reported in mackerel irradiated at 5 kGy by Venugpal (1981) as stated above.

Isopeptide crosslinkages

Isopeptide bonds can be formed in irradiated food proteins (Dogbevi et al., 1999a) and these result in formation of crosslinkage in proteins (Mezgheni, D'Aprano and Lacroix, 1998). Isopeptides are bonds formed between deamidated amino acids and lysine

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Deamidation is a process whereby an amino acid which is part of a protein molecule loses an ammonia molecule while it is still part of a protein (Metwalli and van Boekel, 1998). Asparagine and glutamine are the two amino acids that are able to lose ammonia molecules while part of a protein moiety, so as to deamidate (Stadtman, 1993; Santos, Tomasula and Kurantz, 1999).

2.4.1.3 Protected proteins

Like carbohydrates, proteins in foods are generally protected from radiolysis by other food components and changes produced by irradiation are practically negligible up to 10 kGy dose (Raica, Scott and Nielsen, 1972; Swallow, 1991a). Hanis, Mnukova, Jelen, Klir, Perez and Pesek (1988) reported no change in total amino acid content of wheat, maize and oat meals irradiated at 10 kGy. However, Khatak and Klopfenstein (1989) reported small but significant reductions in amino acid contents of wheat, maize, mungbean and chickpea after irradiation at 1.0, 2.5 and 5.0 kGy and in maize, soybean and wheat irradiated at 5, 7.5, 10 and 20 kGy (Hoosmand and Klopfenstein, 1995).

A large proportion of radiation energy goes into denaturation of proteins rather than into destruction of constituent amino acids and this destruction is even less in complex whole food systems (Hasselmann and Marchioni, 1991). Hence, radiation studies of isolated proteins in model studies may be useful to understand effects of irradiation on proteins but cannot be extrapolated to proteins in complex food systems because of protection by other food components (Bhusan and Kumta, 1977; Wills, 1980).

A summary of chemical changes caused by irradiation on proteins is given in Figure 7.

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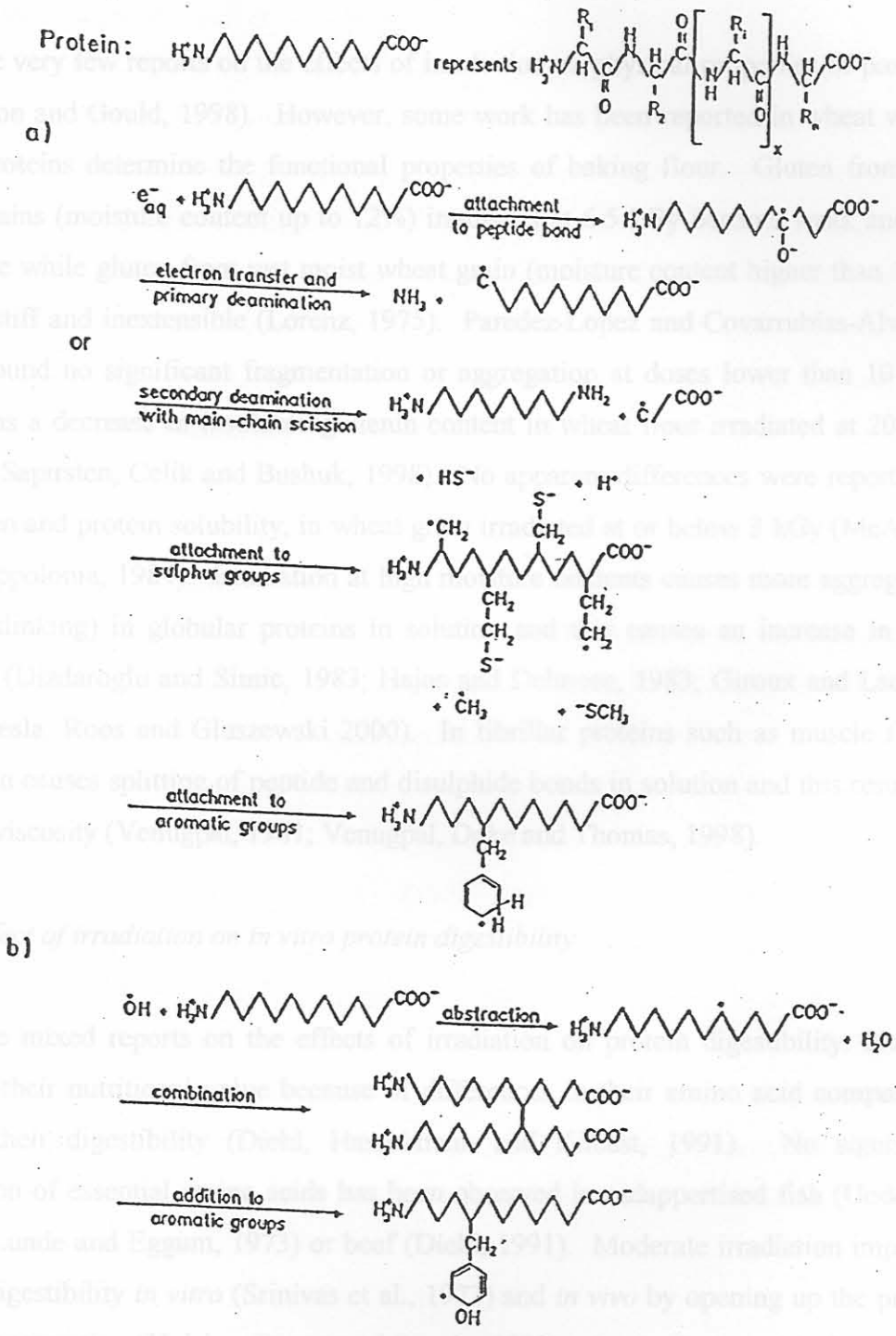

Figure 7 Changes in protein molecules caused by irradiation (Delincee, 1983)

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2.4.2 *Effects of irradiation on physical properties of proteins*

There are very few reports on the effects of irradiation on physical properties of proteins (Wilkinson and Gould, 1998). However, some work has been reported in wheat where gluten proteins determine the functional properties of baking flour. Gluten from dry wheat grains (moisture content up to 12%) irradiated at 6.5 kGy became weak and too extensible while gluten from wet moist wheat grain (moisture content higher than 12%) became stiff and inextensible (Lorenz, 1975). Paredez-Lopez and Covarrubias-Alvarez, (1984) found no significant fragmentation or aggregation at doses lower than 10 kGy There was a decrease in insoluble glutenin content in wheat flour irradiated at 20 kGy (Koskel, Sapirsten, Celik and Bushuk, 1998). No apparent differences were reported in wet gluten and protein solubility, in wheat grain irradiated at or below 3 kGy (McArthur and D'Appolonia, 1984). Irradiation at high moisture contents causes more aggregation (by crosslinking) in globular proteins in solution and this causes an increase in their viscosity (Dizdaroglu and Simic, 1983; Hajos and Delincee, 1983; Giroux and Lacroix, 1998; Ciesla, Roos and Gluszewski 2000). In fibrillar proteins such as muscle fibres, irradiation causes splitting of peptide and disulphide bonds in solution and this results in reduced viscosity (Venugpal, 1981; Venugpal, Doke and Thomas, 1998).

2.4.3 *Effect of irradiation on in vitro protein digestibility*

There are mixed reports on the effects of irradiation on protein digestibility. Proteins differ in their nutritional value because of differences in their amino acid composition and in their digestibility (Diehl, Hasselmann and Kilcast, 1991). No significant destruction of essential amino acids has been observed in radappertised fish (Underdal, Nordal, Lunde and Eggum, 1973) or beef (Diehl, 1991). Moderate irradiation improves protein digestibility *in vitro* (Srinivas et al., 1972) and *in vivo* by opening up the protein during denaturation (Voisine, Parent and Savoie, 1990). Access by proteolytic enzymes is increased by increased solubility and hydrophobicity in blood plasma (Hayashi et al, 1991) and in kidney beans (Dogbevi et al., 1999b) and through decreased viscosity in mackerel fish (Venugpal et al 1998). Indirectly, irradiation also improves digestibility

through inactivation of antinutritional factors (Abu-Tarboush, 1998), and by reduction of the food viscosity (Campbell et al., 1983).

An irradiation dose of 56 kGy was reported to have no effects on protein digestibility of nine food items (Read et al., 1961). Balance studies in human volunteers consuming a variety of foods irradiated with a dose of 28 kGy showed no effects on the protein digestibility as determined by balance studies (kraybill, 1958). Several reviews summarising the effects of irradiation on protein digestibility (Josephson et al., 1978; Kraybill, 1982; Murray, 1983; Diehl et al., 1991; Thayer et al, 1991). There is a general agreement that protein digestibility is unaffected by radiation doses up to 10 kGy (Diehl and Josephson, 1994) and even at higher doses (WHO, 1999).

Vakil, Aravindakshan, Srinivas, Chauhan and Sreenivasan (1973) found no significant changes in protein contents and amino acid profile and available lysine contents of wheat flours treated at 0.2 and 2.0 kGy. However, they reported an increase of 8% in the free amino acid content of wheat flours irradiated at 10 kGy. This led to the conclusion that the changes in physicochemical properties of wheat flour were of no major nutritional significance (Vakil et al., 1973). These findings are in agreement with those of other investigators who reported results of similar experiments on rice and buckwheat (Leonova and Sosedov, 1972), wheat (Metta and Johnson, 1959; Nair and Brownell, 1965; Doguchi, 1969; Pape, 1973), maize (Metta and Johnson, 1959), and on kidney beans (Metliski, Rogachev and Krushchev, 1968), potato (Jaarma and Henricson, 1964; Fujimaki, Makoto and Matsumoto, 1968. The protein digestibilities of irradiated mixed rat diets were not affected at doses up to 70 kGy (Ley, Bleby, Coates and Patterson, 1969; Eggum, 1977). This has convinced the Joint FAO/IAEA/WHO set up in 1999 to conclude that foods can be treated with irradiation doses higher than 10 kGy without compromising their protein digestibility (WHO, 1999).

In vitro determinations of protein digestibility of irradiated food products have been carried out with mixed results. Sheffner, Adachi and Spector (1957) irradiated milk powder, beef and turkey at 0 and 18.6 kGy and also heat processed similar samples in

cans at 116°C for 114 min. They compared the pepsin digestibility and pepsin followed by pancreatin digestibility of these products. They found reduced protein digestibility in thermally treated products but no change in the digestibility of the irradiated products compared to the untreated control. Joseph and Dickshit (1993) irradiated safflower meals at 7 Gy, 14 Gy, 28 Gy, 42 Gy and 10 kGy. There was no cause and effect relationship between irradiation and protein digestibility. However, Joseph and Dickshit (1993) reported some slight increase in protein digestibility, which could have been due to microbial contamination during the long incubation periods.

Mostafa (1987) irradiated peanut meal at 0, 1, 2, 3, 4, 5, 6, 7, 8 kGy and determined pepsin digestibility. He also heated the meal at 0, 100, 120, 140 and 160°C for 0-60 min. The highest protein digestibility was obtained in the peanut meal heated at 160°C for 1 hour while the irradiated peanut meal proteins had maximum pepsin digestibility at 3-4 kGy. There could have been an error in the method because it is known that heating affects the quality of proteins at temperatures as low as 121°C (Wu, Williams, Kunkel, Acton, Wardlaw, Huang and Grimes, 1994). The high protein digestibility obtained by this method in peanut meal heated at 160°C for one hour suggests that there could have been an error.

Bhatty and MacGregor (1988) using the multi-enzyme system of Hsu et al. (1977) found a higher protein digestibility for hull-less barley irradiated at 100 kGy compared to the control. The results showed that the pepsin method had better correlation between irradiation dose and protein digestibility than the multi-enzyme assay for barley. This study concluded that the multi-enzyme system is not effective in differentiating the effects of irradiation on protein digestibility since it excludes pepsin which is much more effective in bringing out differences in protein digestibility after processing (Bhatty and MacGregor, 1988). On the other hand, studies suggest in the more complex proteins as in legumes it is necessary to use multi-enzyme preparations for protein digestibility rather than a single enzyme (Lyons and Walsh, 1993).

Reddy et al. (1979), irradiated dry beans at 210 kGy and tested for protein digestibility using pepsin and multi-enzyme method of Hsu et al. (1977) and obtained higher protein digestibility compared to the pepsin method. The dose of irradiation used at 210 kGy was too high yet it still gave an improved protein digestibility using the multi-enzyme method. Badsha, Sattar and Bibi (1993) using the multi-enzyme method of Hsu et al. (1977) found higher protein digestibility for rapeseed irradiated at 1 kGy and then autoclaved at 121°C for 30 min compared to the control (untreated) and in samples irradiated at only at 1 kGy. The values they obtained were too close to bring out any significant effect of irradiation on protein digestibility. The method of preparation might also have caused the failure to detect the effects of irradiation on protein bioavailability. Delincee and Bognar (1993) irradiated beans, peas and lentils at 0, 10 and 50 kGy but decided to cook the treated materials for different time spans, *in vitro* and found no direct effect of irradiation on protein digestibility. This could be due to the differences introduced by varying the heating time.

The multi-enzyme method of determining protein digestibility works on the principle that during proteolysis, protons are released from the peptide bonds, resulting in a decrease in pH in a protein suspension (Boisen and Eggum, 1991). Therefore, the hydrolysis of a given mass of proteins is carried out from the pH. 8.0 and is monitored for 10 min at a 37° C (Hsu et al., 1977). The drop in pH after 10 min of digestion reflects the protein digestibility. However, pH drop may not accurately reflect the degree of hydrolysis (Swaisgood and Cattignani, 1991). In fact, a poor correlation was observed between the number of peptide bonds hydrolysed and the pH drop in the study of nine animal and plant proteins (Mozersky and Panettieri, 1983). Several factors can affect the rate of pH drop, including the buffering capacity of the protein food (Pedersen and Eggum, 1983; O'Hare, Curry and Allen, 1984). The pH-drop initial rate assay underestimates the digestibility of animal proteins such as egg, muscle and milk proteins (Pedersen and Eggum, 1983; Rasco,1994). Furthermore, it should be noted that, in the pH-drop assay, enzyme catalysis is not taking place at the same pH which could cause variations (Rasco, 1994). To overcome this obstacle, Pedersen and Eggum (1983) developed the pH-stat method where addition of 0.1M NaOH keeps the pH constant. The protein digestibility is

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then calculated from the amount of 0.1M NaOH that had to be added to keep the pH at 8.0 for 10 min of reaction (Boisen and Eggum, 1991).

Assay of the availability of amino acids by chemical methods has been proposed as a good determinant of protein digestibility by a number of authors such as Smith and Friedman (1984); Fidanza (1984); Chung, Swaisgood and Catignani (1986); Friedman and Finot (1990); Hambraeus (1991); Zakardas, Yu, Zakardas and Minero-Amador (1995). One such amino acid is lysine. Lysine apart from being easier to determine in its available and reactive form than other amino acids, is also reported to be the first essential amino acid to be rendered unavailable during thermal processing (Friedman, 1996b). Therefore, lysine availability is widely used to monitor processing damage (Narayan and Andreotti, 1989; Alonzo and Zapico, 1995; Fernandez-Artigas, Garcia-Villanova and Guerra-Hernandez, 1999).

On of the methods to determine protein digestibility is the fluorodinitrobenzene (FDNB) of Carpenter (1960). The method has very high coefficient of correlation with infant growth assays, hence it would eliminate other tedious and often unreliable *in vitro* methods (Swaisgood and Cattignani, 1991).

Farag (1999) irradiated sunflower meal and determined the protein digestibility by multi-enzyme method that was proposed by Hsu et al. (1977). He found that digestibility of irradiated meals autoclaved for 10 min was increased from 82% for 0 kGy, to 86.2% for the meal irradiated at 10 kGy and to 87.6% for the meal at 20 kGy. When he tested for lysine availability, he found that it decreased from 2.63% at 0 kGy to 2.53% for 10 kGy and to 2.43% for 20 kGy materials (unautoclaved). Some studies have shown that that effect of irradiation on protein digestibility was very clear. Protein digestibility rose from 0 to optimum of about 4 kGy and then decreased.

Table 3 gives a summary of some of the methods used in determining protein digestibility and/or quality.

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Table 3 A summary of methods used in the determination of both protein digestibility and quality (Hambraeus, 1991)

In vitro methods
Biochemical assays
Nitrogen analysis
Amino acid analysis
Analysis of available lysine (e.g. by fluorodinitrobenzene, FNB method)
Microbiological assays
Amino acid analysis
Digestion by proteolytic microorganisms
Streptococcus zymogenes
Tetrahymena pyriformis
Enzymatic assays
Enzyme preparations (e.g. pepsin assay, multi-enzyme assay, e.t.c.)
Proteolytic microorganisms
In vivo methods
Biological tests with experimental animals
Screening methods (e.g. Protein efficiency ratio)
Nitrogen balance studies (e.g. Biological value, BV)
Indirect nitrogen analysis
Toxicological studies
Human tests using adults or infants
Nitrogen balance studies (nitrogen excreted compared with nitrogen consumed)

Decrease in protein digestibility of foods irradiated at doses greater than 4 kGy was attributed to Maillard reactions, crosslinkages, racemisation and other reactions (Underdal et al., 1976; Urbain, 1977; Mostafa, 1988). Irradiation may open up the protein structure at lower doses thus increase digestibility (Yamamoto, 1977), while at higher doses it may cause other chemical reactions which may reduce protein digestibility (Institute of Food Technologists, IFT, 1983; Swallow, 1991a).

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2.5 Techniques used to study the effects of irradiation on molecular properties of starch in maize and bean flours that may cause changes in their starch digestibility

2.5.1 *Differential scanning calorimetry (DSC) of irradiated maize and bean flours*

Whenever a material undergoes a change in physical state (e.g. melting), or transforms from one form to another (e.g. crystalline to non-crystalline), or whenever it reacts chemically, heat is either absorbed (endothermic) or liberated (exothermic) (Noel and Ring, 1992). Change in the chemical state of a substance is accompanied by change in energy level that can manifest itself by the absorption of heat (endothermic enthalpy) or evolution of heat (exothermic enthalpy) and this can be determined using differential scanning calorimetry, DSC) (Harwalkar and Ma, 1996). The DSC technique has proved useful in determining the changes in thermal properties when starch is heated in water with indium or water as reference (Findlay and Barbut, 1990; Kalichevsky, Janoskiewwicz, Ablett, Blanshard and Lillford, 1992). It is used to determine thermal changes during gelatinisation (Fisher and Thompson, 1997) and retrogradation (Hwang, Heldman, Chao and Taylor, 1999). The thermal behaviour of gelatinising or retrograding starch is very specific to plant cultivar and to treatment of the food prior to gelatinisation or retrogradation (Stevens and Elton, 1972; Wang and Jane, 1994; Mua and Jackson, 1997).

Basically, DSC is a technique whereby the difference in energy input into a substance and a reference material is measured as a function of temperature while both materials are subjected to a programmed heating or cooling cycle (Karim, Norziah and Seouw, 2000). In the DSC, when a thermal transition occurs as the food is being heated, the energy absorbed by the sample is replenished by increased energy input to the sample to maintain the temperature balance. Since the energy input is precisely equivalent in magnitude to the energy absorbed in the transition, a recording of this balance energy yields a direct calorimetric measurement of the energy transition which is then recorded as a peak (Slade and Levine, 1988). The area under the peak is directly proportional to

the enthalpic change (ΔH) and its direction indicates whether the thermal event is endothermic or exothermic (Cooke and Gidley, 1992). The DSC also gives values for the onset of melting for either gelatinising fresh starchy foods or melting of retrograded amylose or amylopectin (T_o), the peak temperature value of the melting fresh starch or melting retrograded starch (T_p) and the final temperature of melting (T_c). All of the three parameters are specific to the starch source and treatments the food could have received, hence it can be used to determine the severity of processes that the food had undergone (Krueger, Knutson, Inglett and Walker, 1987; Russell, 1987; Knutson, 1990; Cooke and Gidley, 1992).

DSC was first used to measure gelatinisation and retrogradation of various starches by Stevens and Elton in 1972 (Stevens and Elton, 1972; Liu and Lelievre, 1992; Lelievre and Liu, 1994). Since then it has been used extensively to quantify crystallinity in both native and retrograded starches or processed food starches, to determine retrogradation kinetics and to study the effects of several factors that influence gelatinisation or retrogradation of starch or both (Lelievre, 1992). DSC has been used to determine changes in starch fraction of (a) wheat flour processed by extrusion (Asp and Bjorck, 1989; Colonna et al., 1989), (b) annealing (Jacobs and Delcour, 1998), (c) milling (Craig and Stark, 1984), (d) high temperature-low moisture content treatment of starch (Hoover and Manuel, 1996a; b), (e) pressure treatment (Onwulata and Elchediak, 2000). It is suspected that the changes in thermal properties of processed starch observed above might also occur during irradiation of maize and bean flours, hence, the proposal to study them using DSC. Any changes caused by irradiation which alter the crystallinity of amylopectin fraction of starch are shown by the DSC as changes in endothermic enthalpy (ΔH), onset temperature (T_o), peak gelatinisation temperature (T_p), and changes in final gelatinisation temperature (T_c) (Lelievre, 1992). Any change in the crystallinity of amylopectin molecules could lead to changes in the degree of gelatinisation (Krueger et al., 1987) and this could explain changes in starch digestibility caused by irradiation (Lund, 1984; Holm, Lundqvist, Bjorck, Eliasson and Asp, 1988) of porridges made from maize and bean flours irradiated at higher doses.

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

Irradiation of cereal and legumes flours at high doses may result in the formation of new bonds in their starches (Whistler and Ingle, 1965; Ghali et al., 1979). Ordinarily, starch is made up of two types of bonds, namely α (1-4) and α (1-6). However, on irradiation at 3-5 kGy, it has been reported that new bonds such as β (1-2), β(1-3) and β(1-4) and β(1-6) are formed in the starch molecules (Scherz, 1970; Raffi et al., 1981c) Gamma irradiation produces free radicals on starch molecules that can alter their size and structure (Grant and D'Appolonia, 1991; Sabulharse et al, 1991). The total β-glucan content of hull-less barley was reported to to be higher in those treated at 100 kGy compared to the untreated controls (Bhatty and MacGregor, 1988).

2.5.3 *Determination of molecular weight distribution of starch fractions by high performance liquid size exclusion chromatography (HPLCSEC)*

It has been shown that the molecular distribution of starch fractions is altered by irradiation (Roberts and Proctor, 1955; Faust and Massey, 1966). Amylopectin, the higher molecular weight fraction (MW 10^7- 10^9 Da) is degraded more than amylose (MW 10^6 Da) (Gallant, Bouchet, Buleon and Perez, 1992). The degraded amylopectin molecules lead to the formation of short chain amylose molecules of DP_n 15- 45 (Ghali, Ibrahim, Gabr and Aziz, 1979; Ghali, Gabr, Ibrahim and Aziz, 1979; Roushdi, Harras, El-Meligi and Bassim, 1981; Roushdi, Harras, El-Meligi and Bassim, 1983; Englyst, Kingman and Cummings, 1992; Sokhey and Hanna, 1993) and these are highly retrogradable (Siljestrom, Bjorck and Westerlund, 1989b). Changes in the amylopectin fraction of starch has been reported in foods processed by annealing (Cooke and Gidley, 1992; Krueger et al., 1987), extrusion (Theander and Westerlund, 1987) and may also occur during irradiation. Changes in the molecular weight of the starch fractions have been studied using HPLCSEC (Ghali, Ibrahim, Gabr and Aziz, 1979; Ghali, Gabr, Ibrahim and Aziz, 1979; Roushdi, Harras, El-Meligi and Bassim, 1981; Roushdi, Harras,

El-Meligi and Bassim, 1983; Siljestrom et al., 1989b; Englyst et al., 1992; Sokhey and Hanna, 1993).

Ordinary size exclusion chromatography by gel permeation is time consuming (Lund, 1984). It is faster to use HPLCSEC as described by Rounds and Nielsen (1994) and by Rounds and Gregory (1994). HPLCSEC separates the various starch fractions based on their molecular weights (Sokhey and Hanna, 1993) and the molecular weights are determined from pullulan standards (Rounds and Nielsen, 1994). The retention time of various molecular-weights of pullulan standards and of the starch fractions are inversely proportional to the $\log_{10}$ of their molecular weights (Rounds and Gregory, 1994).

2.6 The gaps in information on the effects of irradiation on starch and protein digestibility of maize and bean flours

The biggest gap is lack of consistent information on the effects of irradiation on starch and protein digestibility determined by *in vitro* methods. Most of the reports dealing with the effects of irradiation on nutrient bioavailability were based on animal feed studies, which were carried out in USA and Europe to test for irradiation as an additive (Brynjolsson, 1979; Diehl et al., 1991; United States Department of Agriculture, USDA, 1992; Food and Drugs Administration, FDA, 1994). Most of these tests were carried out for toxicity instead of than bioavailability. Irradiation is reported to increase starch digestibility in some plant foods while others report of reduced starch digestibility as discussed earlier. Therefore, there is need for objective determination of the effects of irradiation on starch bioavailability. Similarly, the effects of irradiation on protein digestibility are not clear. There is a need for quantifiable objective tests to determine the effects of irradiation on protein digestibility.

Effects of irradiation on the molecular properties of starch and proteins also need to be investigated. Changes at molecular levels for starch and proteins may determine how irradiation of foods can cause changes in the digestibility of starch and proteins and should be investigated.

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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

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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.

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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.

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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.

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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

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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:

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

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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).

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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).

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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).

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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.

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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.

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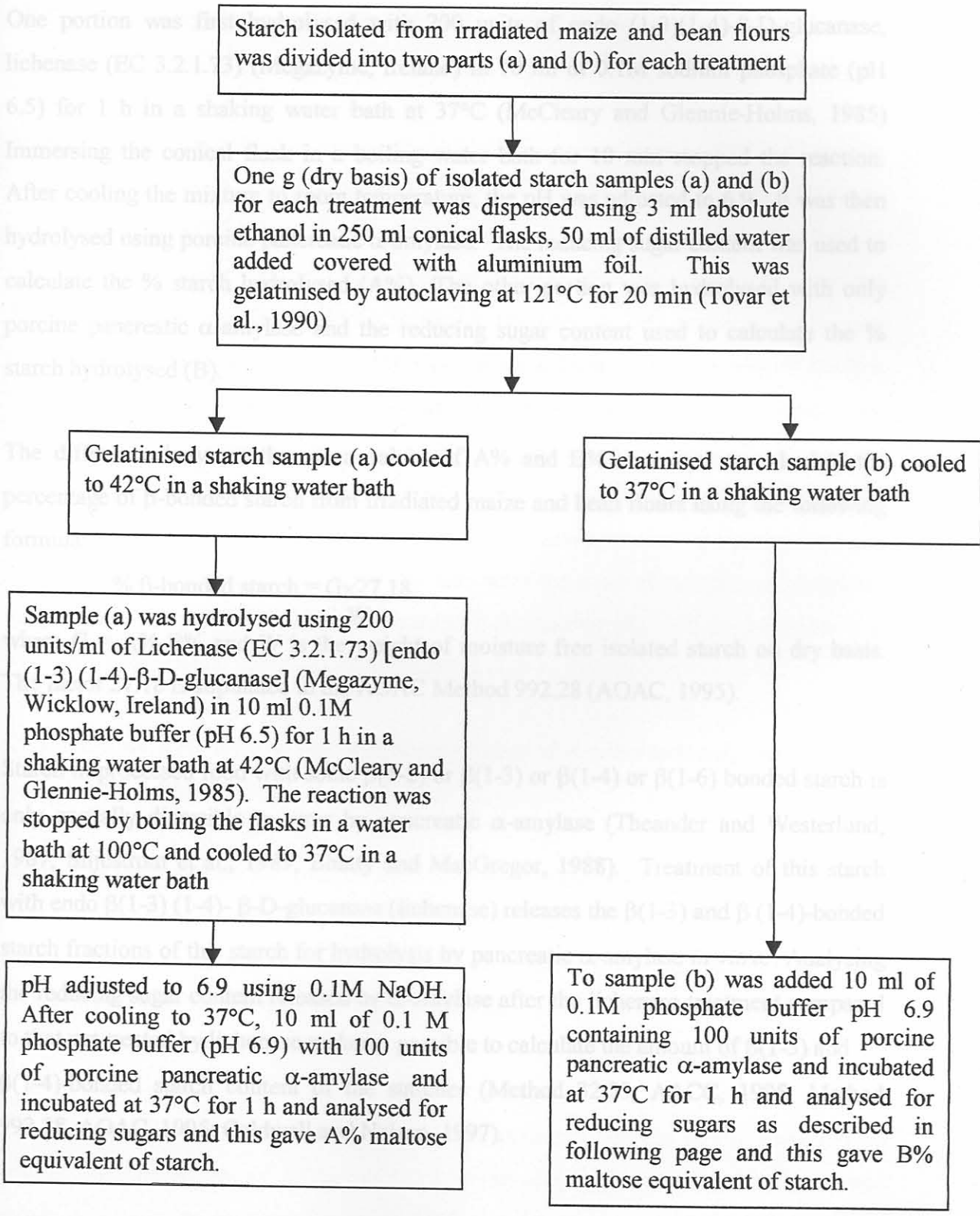

Figure 8 The analytical scheme for determining the β (1-3) and β (1-4) -bonded starch content of starches isolated from irradiated maize and bean flours

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).

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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-

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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

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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.

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CHAPTER 5

RESULTS

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

During Phase 1, various effects of irradiation on maize and bean flours were investigated. Firstly, the effects of irradiation on chemical composition of maize and bean flours were investigated. Secondly, the effects of irradiation on the viscosity of porridge made from maize flour, bean flour and their 70/30 maize:bean composite flours were determined. Thirdly, the effects of irradiation on *in vitro* starch digestibility of maize and bean flours were investigated. Fourthly, the effects of irradiation on *in vitro* protein digestibility of maize and bean flours were investigated. Fifthly, the effects of irradiation on the size and shape starch in whole maize and bean flours were investigated by scanning electron microscope.

5.1.1 *Effect of irradiation on the proximate chemical composition of maize and bean flours*

Irradiation had no significant ($p>0.05$) effect on the proximate chemical composition of maize flours (Table 4). Irradiation had no significant ($p>0.05$) on the proximate chemical composition of bean flours (Table 5).

5.1.2 *Effect of irradiation on the viscosity of porridge made from maize flour, bean flour and 70/30 maize:bean composite flour*

The pasting properties of porridges made from irradiated maize flour, bean flour and their 70/30 maize:bean composite flours were determined at 15, 20 and 25% total solids content for flours treated at 0, 2.5, 5, 7.5 and 10 kGy. The results are shown in Figures 9 to 11 and Tables 6 to 11.

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Table 4 Effect of irradiation on the proximate chemical composition of maize flour

	Irradiation dose (kGy)[1]		
	0	5	10
Moisture (% wet basis)	14.1±0.16a[2]	13.8±0.31a	13.9±0.22 a
Protein (% dry basis)	9.2±0.31a	9.3±0.42a	9.2±0.17a
Crude fat (% dry basis)	0.9±0.22a	0.8±0.16a	0.9±0.14a
Ash (% dry basis)	1.4±0.12a	1.4±0.25a	1.3±0.62a
Starch (% dry basis)	70.5±0.83a	70.3±0.95a	70.4±1.2a

[1] Values are means of three replicates determined six times ±standard deviation
[2] Mean values in a row with the same letters did not differ significantly at p>0.05

Table 5 Effect of irradiation on the proximate chemical composition of bean flour

	Irradiation dose (kGy)[1]		
	0	5	10
Moisture (% wet basis)	9.8±0.25a[2]	9.7±0.33a	9.8±0.16a
Protein (% dry basis)	24.8±0.42a	24.9±0.18a	24.8±0.41a
Crude fat (% dry basis)	0.9±0.21a	0.9±0.18a	0.8±0.33a
Ash (% dry basis)	2.7±0.31a	2.8±0.24a	2.7±0.28a
Starch (% dry basis)	54.2±0.30a	54.6±0.19a	55.2±0.47a

[1] Values are means of three replicates determined six times ±standard deviation
[2] Mean values with the same letter in a row did not differ significantly at p>0.05

Irradiation reduced the viscosity of porridges made from maize flour, bean flour and their 70/30 composite flour significantly ($p \leq 0.05$). Reductions in viscosity of the porridges were dose dependent (Figures 9-11, Tables 6-11).

The peak paste viscosity or the highest viscosity of the porridge during gelatinisation was reduced significantly ($p \leq 0.05$) by irradiation of maize flour, bean flour and their 70/30 composite flours (Tables 6, 7 and 8). The breakdown viscosity or the viscosity of porridges being stirred at a constant temperature was affected significantly ($p \leq 0.05$) by

irradiation (Tables 6, 7 and 8). During stirring at a constant highest temperature the porridge viscosity is reduced due to breakdown of hydrogen bonds between gelatinised starch molecules by the action of the stirrer. The setback viscosity is the viscosity of porridges on cooling from the gelatinisation temperature. It takes place due to formation of hydrogen bonds between amylose molecules. Porridges made from flours with high amylose contents develop higher setback viscosities. Setback viscosities of porridges made from irradiated maize flour, bean flour and their 70/30 composite flours were also affected significantly ($p \leq 0.05$) by irradiation (Tables 9, 10 and 11).

Figure 9a (not drawn to scale) shows that viscosity of the 15% total solids content porridges made from maize flour was reduced significantly ($p \leq 0.05$). Irradiation caused a reduction in peak paste viscosity of maize porridges by 48% at 2.5 kGy, 71% at 5.0 kGy, 84% at 7.5 kGy and 88% at 10 kGy for the 15% total solids content porridges (Table 6). Irradiation also caused a reduction in breakdown viscosity of 57% at 2.5 kGy, 76% at 5 kGy, 88% at 7.5 kGy and 92% at 10 kGy for the 15% total solids content maize porridges at 540°C (Table 6). Irradiation caused reductions in viscosity of the 15% total solids content maize porridges at setback temperature of 40°C by 31% at 2.5 kGy, by 53% at 5 kGy, by 69% at 7.5 kGy and by 77% at 10 kGy compared to the untreated control (Table 9).

Figure 9b (not drawn to scale) shows that irradiation had a significant ($p \leq 0.05$) reduction effect on the viscosity of a 20% total solids content porridges made from maize flour. Irradiation caused a significant ($p \leq 0.05$) reduction in the peak paste viscosity of a 20% total solids content maize porridge. The peak paste viscosity was reduced by 45% at 2.5 kGy, by 64% at 5.0 kGy, 80% at 7.5 kGy and by 86% at 10 kGy (Table 6). The breakdown viscosity of 20% total solids content maize porridges were also reduced significantly ($p \leq 0.05$) by irradiation. The breakdown viscosity of a 20% total solids content maize porridges was reduced by 41% at 2.5 kGy, 59% at 5.0 kGy, 75% at 7.5 kGy and by 82% at 10 kGy (Table 6). The viscosity of the 20% total solids content maize porridges at setback temperature of 40 °C was reduced by 30% at 2.5 kGy, by 52% at

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5 kGy, by 72% at 7.5 kGy and by 75% at 10 kGy compared to the untreated control (Figure 9b, not drawn to scale).

Table 6 Effect of irradiation of maize flour on the peak paste viscosity and breakdown viscosity of porridges at 15, 20 and 25% total solids content (w/v)

Irradiation dose (kGy)	Peak paste viscosity[1] (cP)			Breakdown viscosity[1] (cP)		
	15%[2]	20%	25%	15%[2]	20%	25%
0	2413±57e[3]	5050±24e	8300±72e	2060±54e	3610±48e	5910±42
2.5	1258±63d	2762±38d	5744±83d	887±63d	2146±41d	4769±25d
	(-48%)[4]	(-45%)	(-31%)	(-57%)	(-41%)	(-19%)
5.0	698±22c	1823±44c	3934±75c	490±36c	1476±53c	3510±27c
	(-71%)	(-64%)	(-53%)	(-76%)	(-59%)	(-41%)
7.5	396±41b	1025±28b	31741±48b	255±41b	892±55b	2329±62b
	(-84%)	(-80%)	(-62%)	(-88%)	(-75%)	(-61%)
10	292±31a	722±42a	1912±56a	171±31a	641±24a	1687±44a
	(-88%)	(-86%)	(-77%)	(-92%)	(-82%)	(-72%)

[1] Values are means of three replicates determined six times ± standard deviation
[2] Values are percentage total solids content (w/v)
[3] Values followed by different letters in the same column differ significantly at $p \leq 0.05$
[4] Values are reduction in viscosity as a percentage of the viscosity at 0 kGy

Figure 9c, (not drawn to scale), shows that irradiation reduced the viscosity of the 25% total solids content porridges made from maize flour irradiated at 0, 2.5, 5, 7.5 and 10 kGy significantly at $p \leq 0.05$. Irradiation also had significant effects on the peak paste viscosity. The peak paste viscosity of the 25% total solids content porridges were reduced by 31% at 2.5 kGy, 53% at 5.0 kGy, 62% at 7.5 kGy and by 77% at 10 kGy (Table 6). Irradiation also had significant ($p \leq 0.05$) effect on the breakdown viscosity of 25% total solids content maize porridges. Breakdown viscosity of the 25% total solids content maize porridge was reduced by 19% at 2.5 kGy, 41% at 5.0 kGy, 61% at 7.5 kGy and by 72% at 10 kGy (Table 6). At setback temperature of 30°C, irradiation caused decreases in viscosity of a 25% total solids content maize porridges by 31% at 2.5 kGy,

by 54% at 5 kGy, by 69% at 7.5 kGy and by 74% at 10 kGy compared to the untreated control (Figure 9c, not drawn to scale).

Figure 10a (not drawn to scale) shows that irradiation reduced the setback viscosity of the 15% total solids content bean porridges significantly ($p \leq 0.05$) at 30°C. Irradiation reduced the peak paste viscosity of the 15% total solids content bean porridges significantly ($p \leq 0.05$). The peak paste viscosity of the 15% total solids content bean porridges were reduced by 29% at 2.5 kGy, 47% at 5.0 kGy, 64% at 7.5 kGy and by 76% at 10 kGy (Table 7). Irradiation also reduced the breakdown viscosity significantly at ($p \leq 0.05$). The breakdown viscosity of the 15% total solids content porridges were reduced by 25% at 2.5 kGy, 42% at 5.0 kGy, 62% at 7.5 kGy and by 73% at 10 kGy compared to the untreated control (Table 7). Irradiation caused decreases in setback viscosity of the 15% total solids content bean porridges by 25% at 2.5 kGy, by 47% at 5kGy, by 58% at 7.5 kGy and by 67% at 10 kGy compared to the untreated control at 30° C (Figure 10a, not drawn to scale).

Figure 10b, (not drawn to scale), shows that irradiation reduced the viscosity of the 20% total solids porridges made from irradiated bean flour significantly ($p \leq 0.05$). Irradiation had significant ($p \leq 0.05$) effects on the peak paste viscosities of porridges made from bean flour at 20% total solids content. The peak paste viscosities of a 20% total solids content bean porridges were reduced by 16% at 2.5 kGy, 32% at 5.0 kGy, 50% at 7.5 kGy and by 60% at 10 kGy (Table 7). Irradiation also had significant effect ($p \leq 0.05$) on the breakdown viscosity of 20% total solids content porridges. The breakdown viscosity of 20% total solids content porridges was reduced by 20% at 2.5 kGy, 39% at 5.0 kGy, 62% at 7.5 kGy, and by 69% at 10 kGy (Table 7). Irradiation caused decreases in the setback viscosity of the 20 % total solids content bean porridges by 24% at 2.5 kGy, by 45% at 5 kGy, by 57% at 7.5 kGy and by 67% at 10 kGy compared to the untreated control at 30°C (Figure 10b, not drawn to scale).

Irradiation resulted in significant ($p \leq 0.05$) reductions in the setback viscosity of the 25% total solids content porridges prepared from bean flour (Figure 10c, not drawn to scale).

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Irradiation reduced the peak paste viscosity of the 25% total solids content bean porridges by 20% at 2.5 kGy, 43% at 5.0 kGy, 55% at 7.5 kGy and by 66% at 10 kGy (Table 7).

Table 7 Effect of irradiation of bean flour on the peak paste viscosity and breakdown viscosity of porridges at 15, 20 and 25% total solids content (w/v)

Irradiation dose (kGy)	Peak paste viscosity[1] (cP)			Breakdown viscosity[1] (cP)		
	15%[2]	20%	25%	15%[2]	20%	25%
0	1231±52e[3]	3209±64e	6118±96e	1121±51e	2311±58e	5106±83e
2.5	873±22d	2687±49d	4926±73d	836±41d	1849±62d	4445±88d
	(-29%)[4]	(-16%)	(-20%)	(-25%)	(-20%)	(-12%)
5.0	653±34c	2192±44c	3516±77c	644±31c	1418±56c	3687±49c
	(-47%)	(-32%)	(-43%)	(-42%)	(-39%)	(-28%)
7.5	445±50b	1613±71b	2736±22b	428±18b	971±27b	2880±39b
	(-64%)	(-50%)	(-55%)	(-62%)	(-58%)	(-44%)
10	301±12a	1286±42a	2106±44a	299±11a	723±42a	2323±28a
	(-76%)	(-60%)	(-66%)	(-73%)	(-69%)	(-54%)

[1] Values are means of three replicates determined six times ± standard deviation
[2] Values are percentage total solids content (w/v)
[3] Values followed by different letters in the same column differ significantly at $p \leq 0.05$
[4] Values are reduction in viscosity as a percentage of the viscosity at 0 kGy

The breakdown viscosity of the 25% total solids content porridges made from bean flours were also significantly reduced by irradiation. The breakdown viscosity of 25% total solids content porridges made from bean flours were reduced by 12% at 2.5 kGy, 28% at 5.0 kGy, 44% at 7.5 kGy and by 54% at 10 kGy compared to the untreated control (Table 7). Irradiation reduced the setback viscosity of the 25% total solids content bean porridges at setback temperature of 30°C by 23% at 2.5 kGy, by 44% at 5 kGy, by 56% at 7.5 kGy and by 66% at 10 kGy compared to the untreated control (Figure 10c, not drawn to scale).

Figure 11a (not drawn to scale) shows that irradiation reduced the setback viscosity of the 15% total solids content of the porridges made from 70/30 maize:bean composite flour significantly ($p \leq 0.05$).

Irradiation significantly ($p \leq 0.05$) reduced the peak paste viscosity of porridges prepared from the 70/30 maize:bean composite flours. The peak paste viscosity of the 15% total solids content porridges made from the 70/30 maize:bean composite flours were reduced by 52% at 2.5 kGy, 67% at 5.0 kGy, 80% at 7.5 kGy and by 86% at 10 kGy (Table 8). Irradiation also had a significant ($p \leq 0.05$) effect on the breakdown viscosity of 15% total solids content porridges prepared from 70/30 maize:bean composite flours (Table 8). Irradiation caused reductions in the viscosity of the 15% total solids content porridges prepared from the 70/30 maize:bean composite flour at setback temperature of 30°C by 29% at 2.5 kGy, by 51% at 5 kGy, by 66% at 7.5 kGy and by 74% at 10 kGy compared to the untreated control at setback temperature (Figure 11a, not drawn to scale).

Table 8 Effect of irradiation of 70/30 maize:bean composite flours on the peak paste viscosity and breakdown viscosity of porridges at 15, 20 and 25% total solids content (w/v)

Irradiation dose	Peak paste viscosity[1] (cP)			Breakdown viscosity (cP)		
(kGy)	15%[2]	20%	25%	15%[2]	20%	25%
0	2041±63e[3]	4495±77e	7611±82e	1772±36e[3]	3217±43e	5673±52e
2.5	972±41d	2274±53d	5497±48d	872±41d	2109±55d	4559±64
	(-52%)[4]	(-49%)	(-28%)	(-51%)	(-34%)	(-20%)d
5.0	684±16c	1934±28c	3807±55c	536±27c	1539±51c	3389±28c
	(-67%)	(-60%)	(-50%)	(-70%)	(-52%)	(-40%)
7.5	406±11b	1202±32b	2610±28b	308±19b	1014±45b	2273±26b
	(-80%)	(-73%)	(-66%)	(-83%)	(-69%)	(-60%)
10	293±10a	891±18a	1962±44a	192±13a	765±28a	1660±31a
	(-86%)	(-80%)	(-74%)	(-89%)	(-76%)	(-71%)

[1] Values are means of three replicates determined six times ± standard deviation
[2] Values are percentage total solids content (w/v)
[3] Values followed by different letters in the same column differ significantly at $p \leq 0.05$
[4] Values are reductions in viscosity as a percentage of the viscosity at 0 kGy

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Irradiation significantly ($p \leq 0.05$) reduced the peak paste viscosity of the 20% total solids content porridges prepared from 70/30 maize:bean composite flours. The peak paste viscosity of the 20 % total solids content 70/30 maize:bean composite flour porridges were reduced by 49% at 2.5 kGy, 60% at 5.0 kGy, 73% at 7.5 kGy and by 80% at 10 kGy (Table 8). Irradiation also reduced the breakdown viscosity of 20% total solids content porridges prepared from 70/30 maize:bean composite flours. The breakdown viscosities of porridges prepared from 70/30 maize:bean composite flours were reduced by 34% at 2.5 kGy, 52% at 5.0 kGy, 69% at 7.5 kGy and by 76% at 10 kGy compared to the untreated control (Table 8). Irradiation resulted in reductions in setback viscosity of the 20% total solids content porridges prepared from the 70/30 maize:bean composite flour at setback temperature of 30°C by 28% at 2.5 kGy, by 50% at 5 kGy, by 67% at 7.5 kGy and by 73% at 10 kGy compared to the untreated control (Figure 11b, not drawn to scale).

Irradiation reduced the peak paste viscosity of the 25% total solids content porridges prepared from 70/30 maize:bean composite flours by 28% at 2.5 kGy, 50% at 5.0 kGy, 66% at 7.5 kGy and by 74% at 10 kGy (Table 8). Irradiation reduced the breakdown viscosity of the 25% total solids content porridges prepared from 70/30 maize:bean composite flours by 20% at 2.5 kGy, 40% at 5.0 kGy, 60% at 7.5 kGy and by 71% at 10 kGy compared to the untreated control (Table 8). Irradiation resulted in significant ($p \leq 0.05$) reductions in setback viscosity of 25% total solids content porridges prepared from 70/30 maize:bean flour. The setback viscosity of 25% total solids content porridge made from 70/30 maize:bean flour was reduced by 29% at 2.5 kGy, by 51% at 5 kGy, by 65% at 7.5 kGy and by 71% at 10 kGy compared to the untreated control at the setback temperature of 30°C (Figure 11c, not drawn to scale).

Figures 10 and 11 and Tables 9 and 10 show that the reductions in viscosity at setback temperature of 30°C were greater in irradiated maize flour than in irradiated bean flour. However, the viscosity of the porridge made from their 70/30 composite flour was below that of the porridge made from irradiated maize flour but above that of the porridge made from irradiated bean flour (Figure 11 and Table 11).

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Table 9 Effect of irradiation of maize flour on the viscosity of porridges at 15, 20 and 25% total solids contents (w/v) at setback temperatures of 50, 40 and 30°C

Total solids content (% dry basis)	Irradiation dose (kGy)	Setback viscosity (cP)[1]		
		50°C	40°C	30°C
15	0	3619±27i	4774±188f	>6000
	2.5	1648±23f	2054±49d	1763±10d
	5.0	762±22c	1038±31c	1763±10c
	7.5	351±15b	553±20b	748±6b
	10	279±16a	344±18a	447±7a
20	0	>6000[3]	>6000	>6000
	2.5	5429±28k	>6000	>6000
	5	2911±35h	4171±16e	>6000
	7.5	1285±24e	2130±24d	5391±10f
	10	952±15d	1900±56d	3546±22e
25	0	>6000	>6000	>6000
	2.5	>6000	>6000	>6000
	5	>6000	>6000	>6000
	7.5	3853±19j	>6000	>6000
	10	2367±16g	5740±36g	>6000

[1]Values are mean of three replicates determined six times ± standard deviations
[2]Mean values followed by different letters in the same column differ significantly at $p \leq 0.05$
[3]The Rapid Visco Analyser only gives true viscosity readings up to 6000 cP

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With the 15% total solids content porridges, for example, the viscosity of porridges made from irradiated maize flour was reduced by 31% at 2.5 kGy, by 53% at 5 kGy, by 69% at 7.5 kGy and by 77% at 10 kGy compared to the untreated control at setback temperature of 30°C (Table 11). The setback viscosity of the 15% total solid content porridges made from irradiated bean flour was reduced by 24% at 2.5 kGy, by 47% at 5 kGy, by 58% at 7.5 kGy and by 67% at 10 kGy compared to the untreated control at 30°C.

Table 10 Effect of irradiation of bean flour on the viscosity of porridges at 15, 20 and 25% total solids contents (w/v) at setback temperatures of 50, 40 and 30°C

Total solids content (% dry basis)	Irradiation dose (kGy)	Setback viscosity (cP)[1]		
		50°C	40°C	30°C
15	0	1330±13c	1446±21e	1838±18e
	2.5	1251±25d	1427±7d	1631±19d
	5.0	850±16c	1118±11c	1514±49c
	7.5	539±12b	634±10b	837±19b
	10	459±17a	588±8a	735±13a
20	0	3748±85k	4578±19j	>6000[3]
	2.5	3248±26j	3457±29i	3931±18i
	5.0	2345±24h	2490±24h	2818±12h
	7.5	1744±23g	1787±13g	2434±17g
	10	1474±19f	1580±14f	2434±17f
25	0	>6000	>6000	>6000
	2.5	>6000	>6000	>6000
	5	>6000	>6000	>6000
	7.5	3826±23l	>6000	>6000
	10	2663±22i	4578±16j	5763±26j

[1]Values are mean of three replicates determined six times ± standard deviations

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[2]Mean values followed by different letters in the same column differ significantly at p≤0.05
[3]The Rapid Visco Analyser only gives true viscosity readings up to 6000 cP

The setback viscosity of the 15% total solids content porridges made from irradiated 70/30 maize:bean composite flour was reduced by 29% at 2.5 kGy, by 51% at 5 kGy, by 66% at 7.5 kGy and by 74% at 10 kGy compared to the untreated control at setback temperature of 30°C.

Table 11 Effect of irradiation of 70/30 miaize:bean composite flour on the viscosity of porridges at15, 20 and 25% total solids contents (w/v) at setback temperatures of 50, 40 and 30°C

Total solids content (% dry basis)	Irradiation dose (kGy)	Setback viscosity (cP)[1]		
		50°C	40°C	30°C
15	0	3992±67j	4330±18h	5955±40i
	2.5	2216±47f	2834±18f	3879±30f
	5.0	1327±21c	1852±36c	2451±33d
	7.5	742±16b	1156±27b	1408±16b
	10	464±17a	749±10a	903±5a
20	0	>6000[3]	>6000	>6000
	2.5	4457±16k	>6000	>6000
	5	2692±29g	3511±39g	4376±21g
	7.5	1745±12e	2495±15e	3759±21e
	10	1427±9d	1789±16d	2354±20c
25	0	>6000	>6000	>6000
	2.5	>6000	>6000	>6000
	5	>6000	>6000	>6000
	7.5	3950±20j	>6000	>6000
	10	2732±12h	5506±22i	5859±53h

[1] Values are mean of three replicates determined six times ± standard deviations
[2] Mean values followed by a different letter in the same column differ significantly at p≤0.05
[3] The Rapid Visco Analyser only gives true viscosity readings up to 6000 cP

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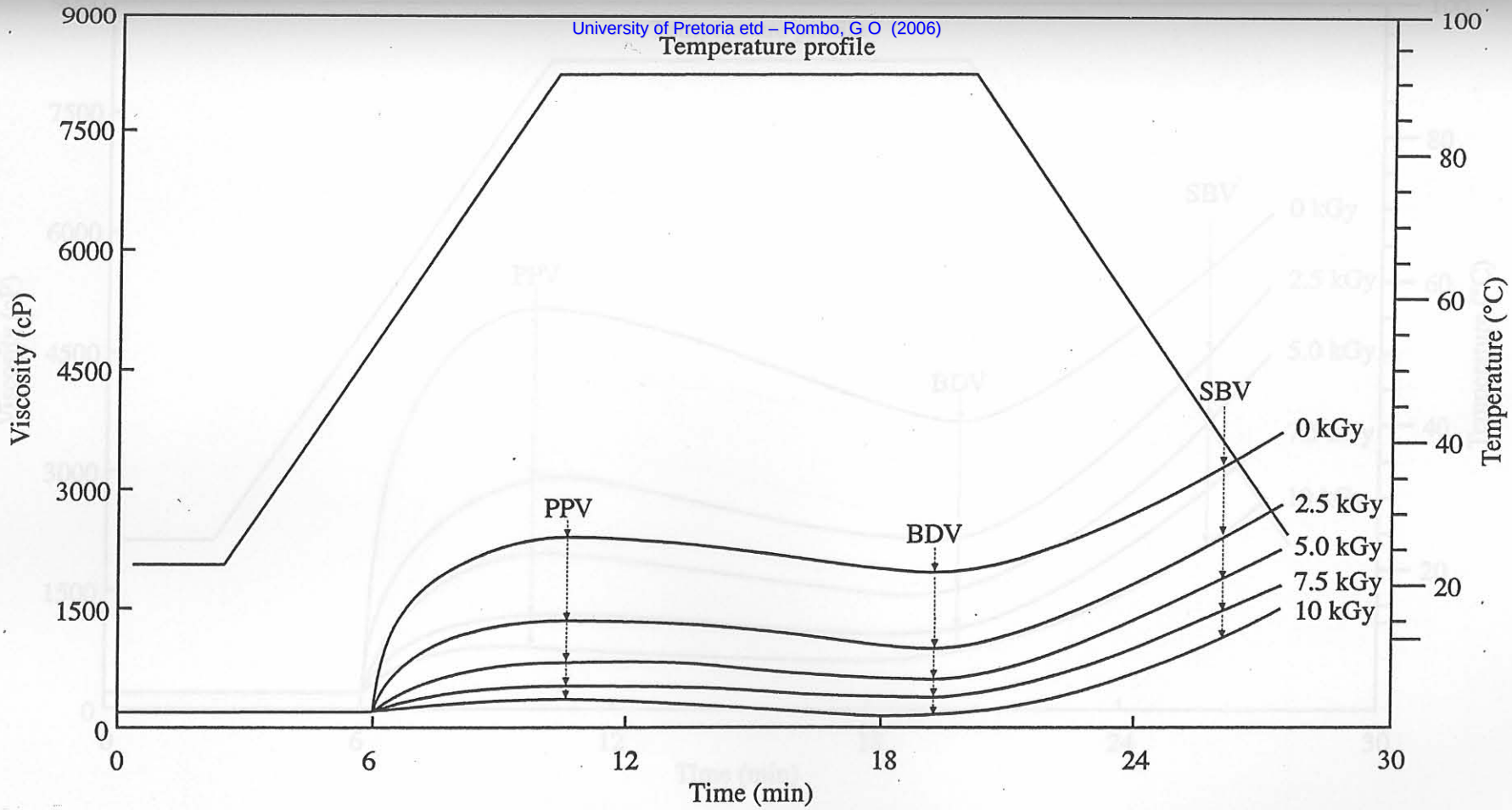

Figure 9 Schematic representation of the effects of irradiation on viscosity of porridges made from maize flour (a) at 15% total solids content (w/v), PPV: peak paste viscosity, BDV: breakdown viscosity, SBV: setback viscosity at 40°C (not drawn to scale)

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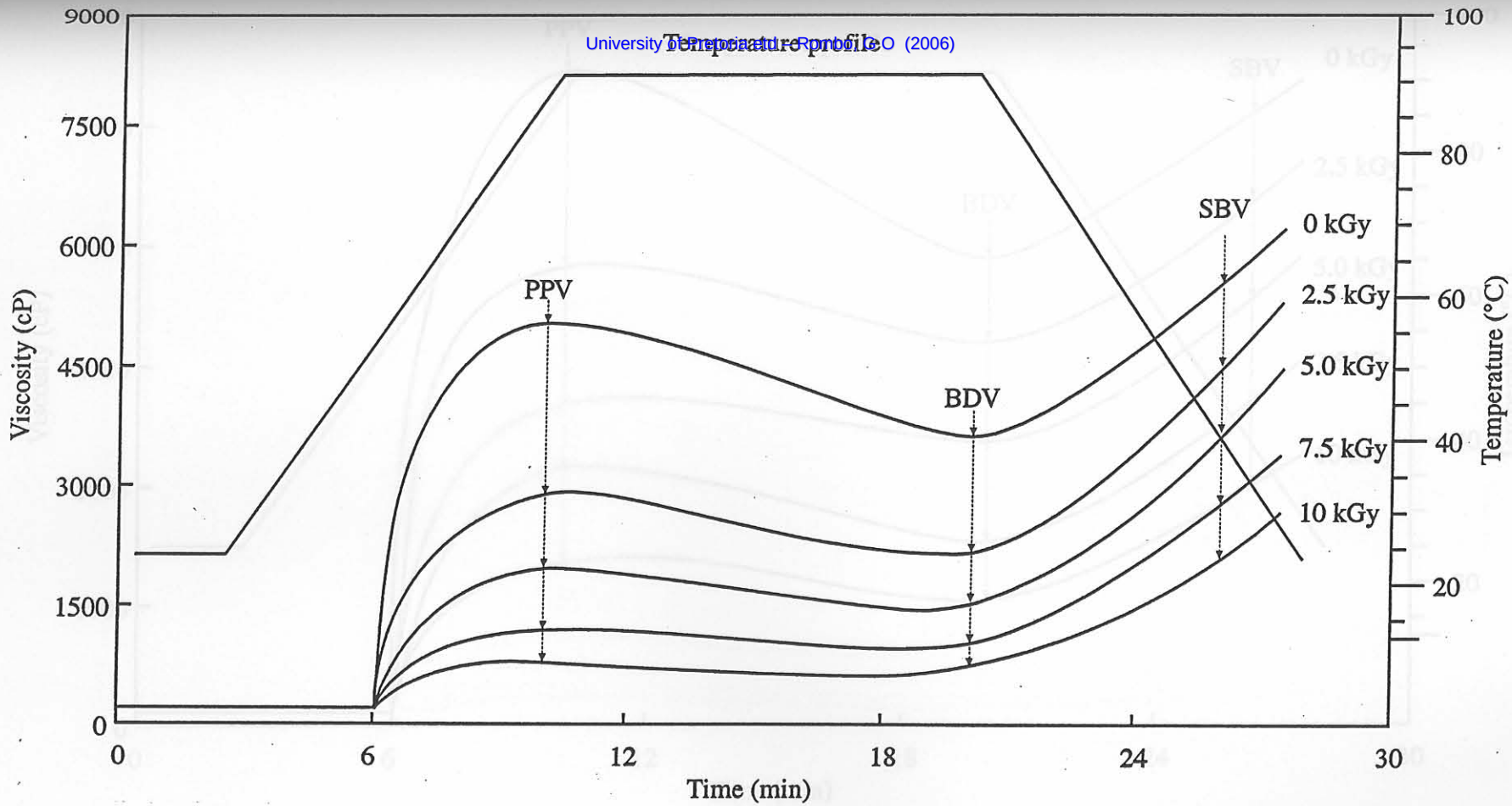

Figure 9 Schematic representation of the effects of irradiation on viscosity of porridges made from maize flour (b) at 20% total solids content (w/v), PPV: peak paste viscosity, BDV: breakdown viscosity, SBV: setback viscosity at 40°C (not drawn to scale)

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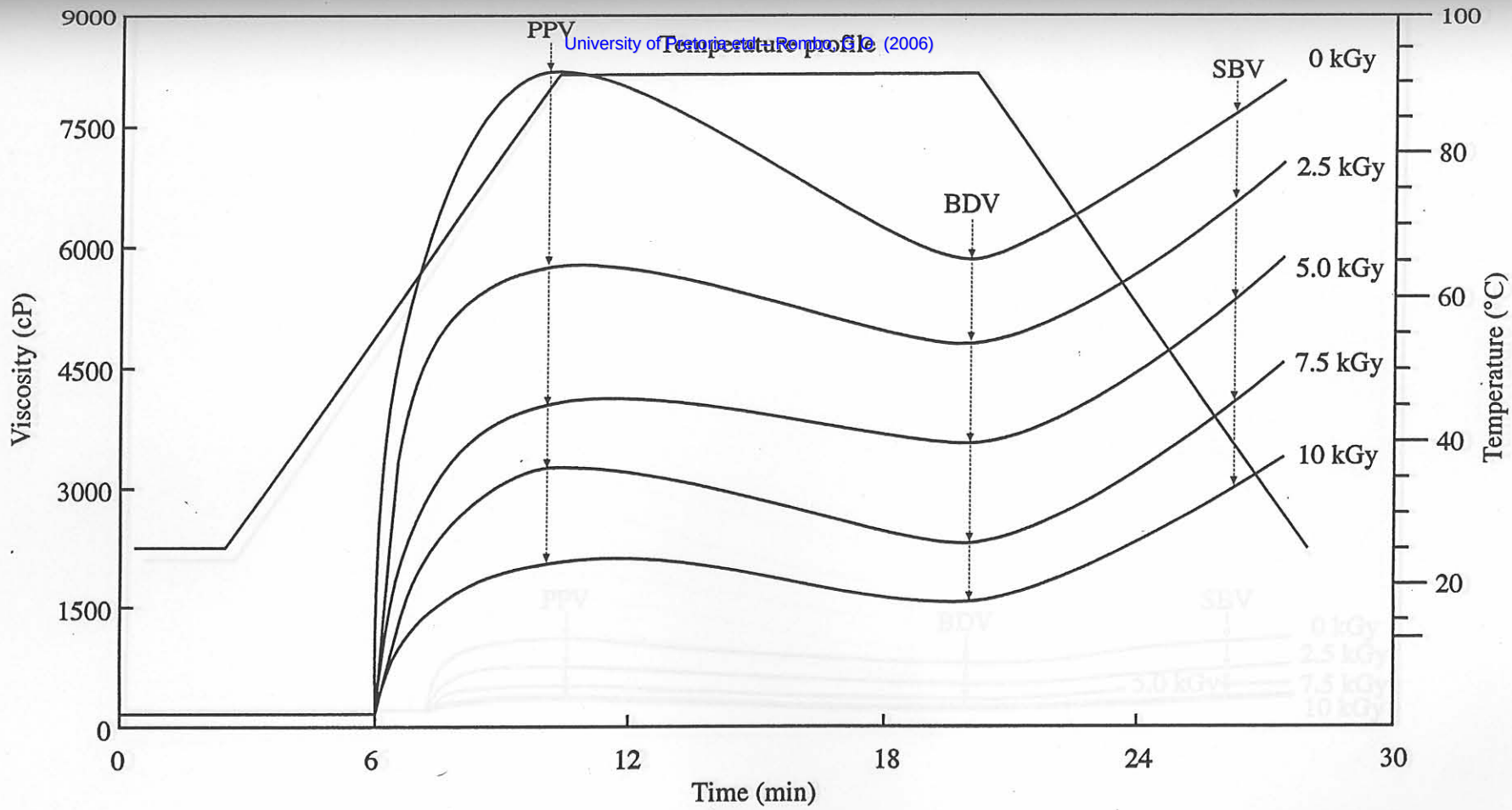

Figure 9 Schematic representation of the effects of irradiation on viscosity of porridges made from maize flour (c) at 25% total solids content (w/v), PPV: peak paste viscosity, BDV: breakdown viscosity, SBV: setback viscosity at 40°C (not drawn to scale)

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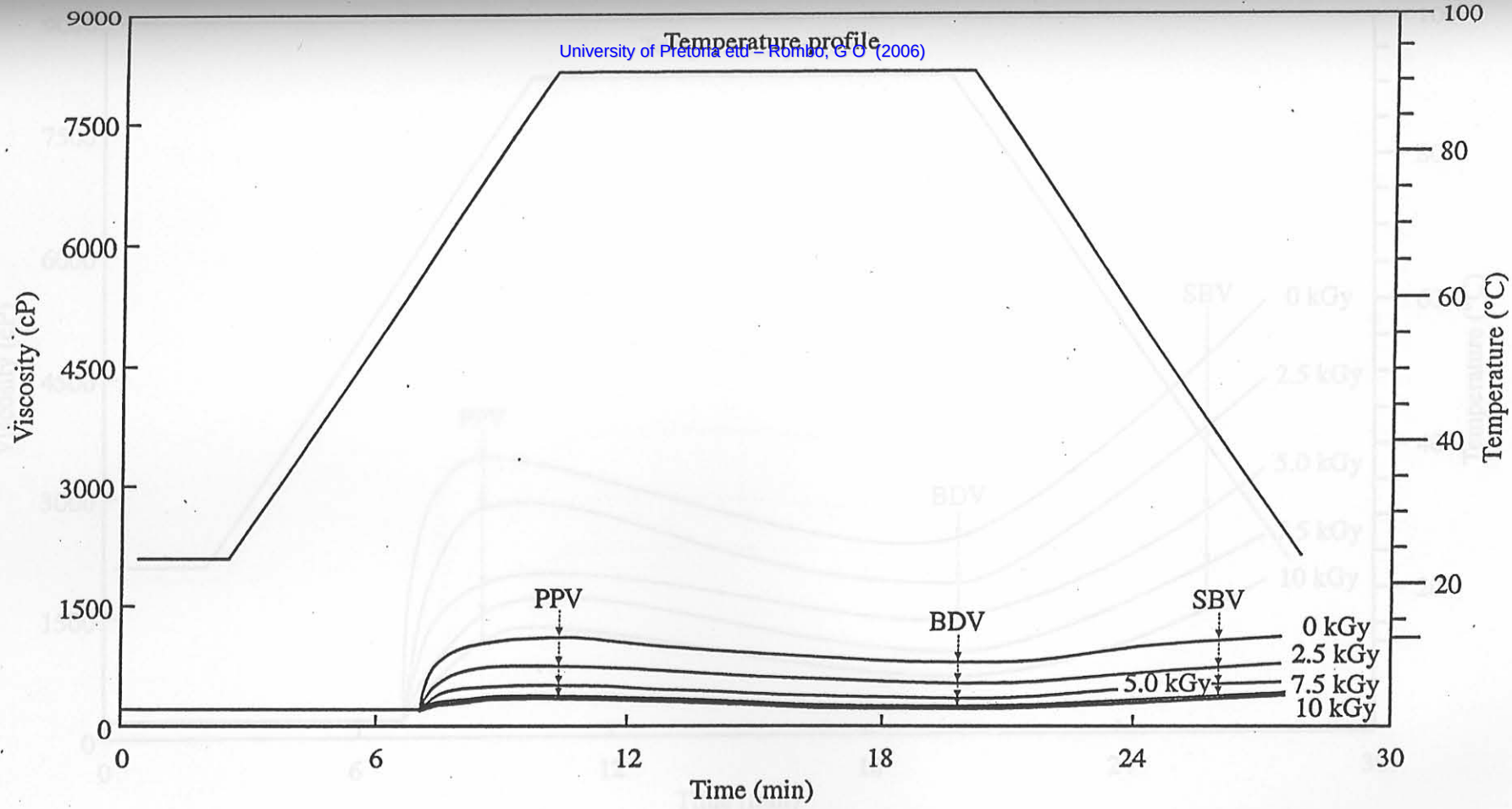

Figure 10 Schematic representation of the effects of irradiation on viscosity of porridges made from bean flour (a) at 15% total solids content (w/v), PPV: peak paste viscosity, BDV: breakdown viscosity, SBV: setback viscosity at 40°C (not drawn to scale)

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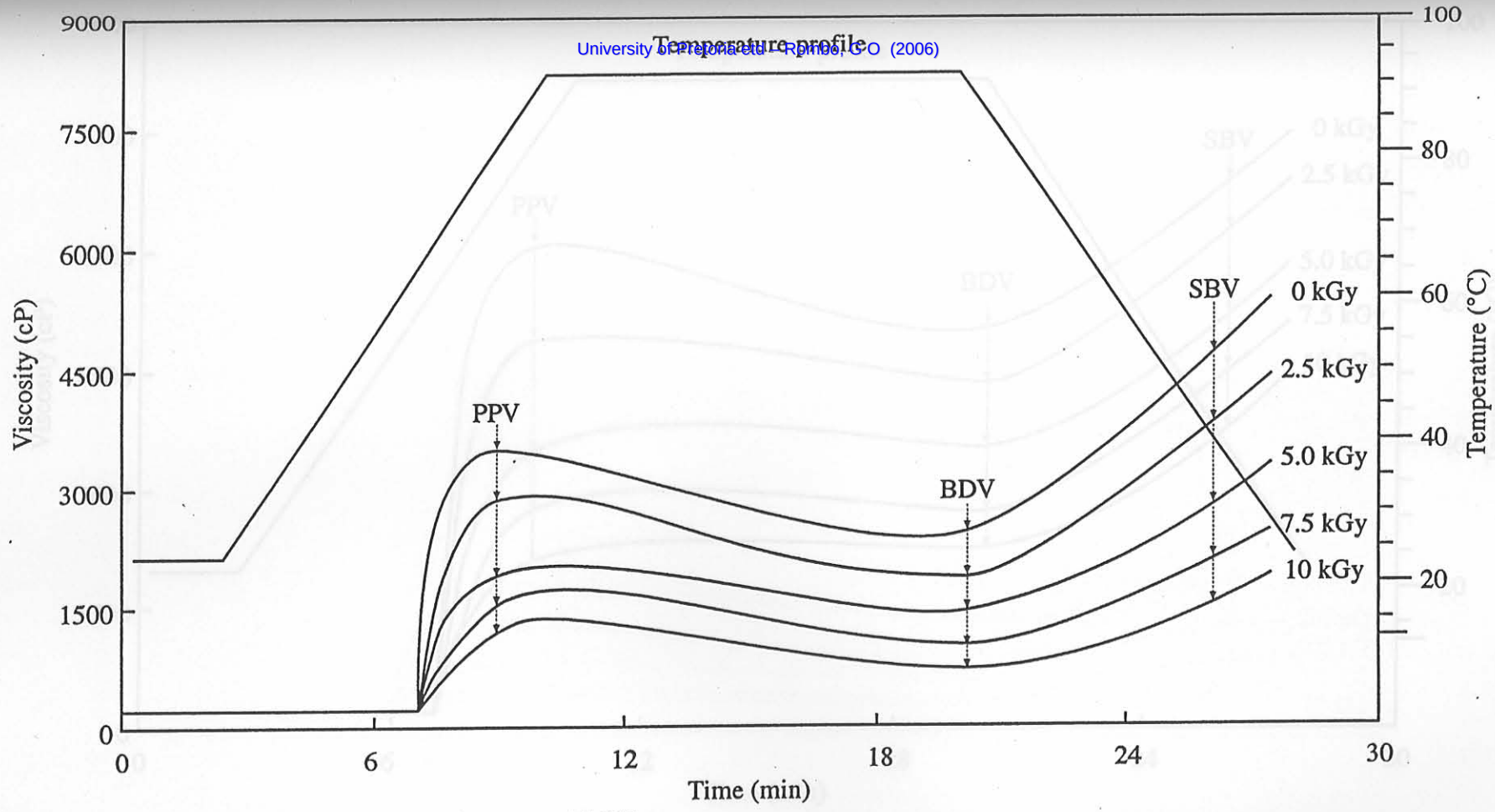

Figure 10 Schematic representation of the effects of irradiation on viscosity of porridges made from bean flour (b) at 20% total solids content (w/v), PPV: peak paste viscosity, BDV: breakdown viscosity, SBV: setback viscosity at 40°C (not drawn to scale)

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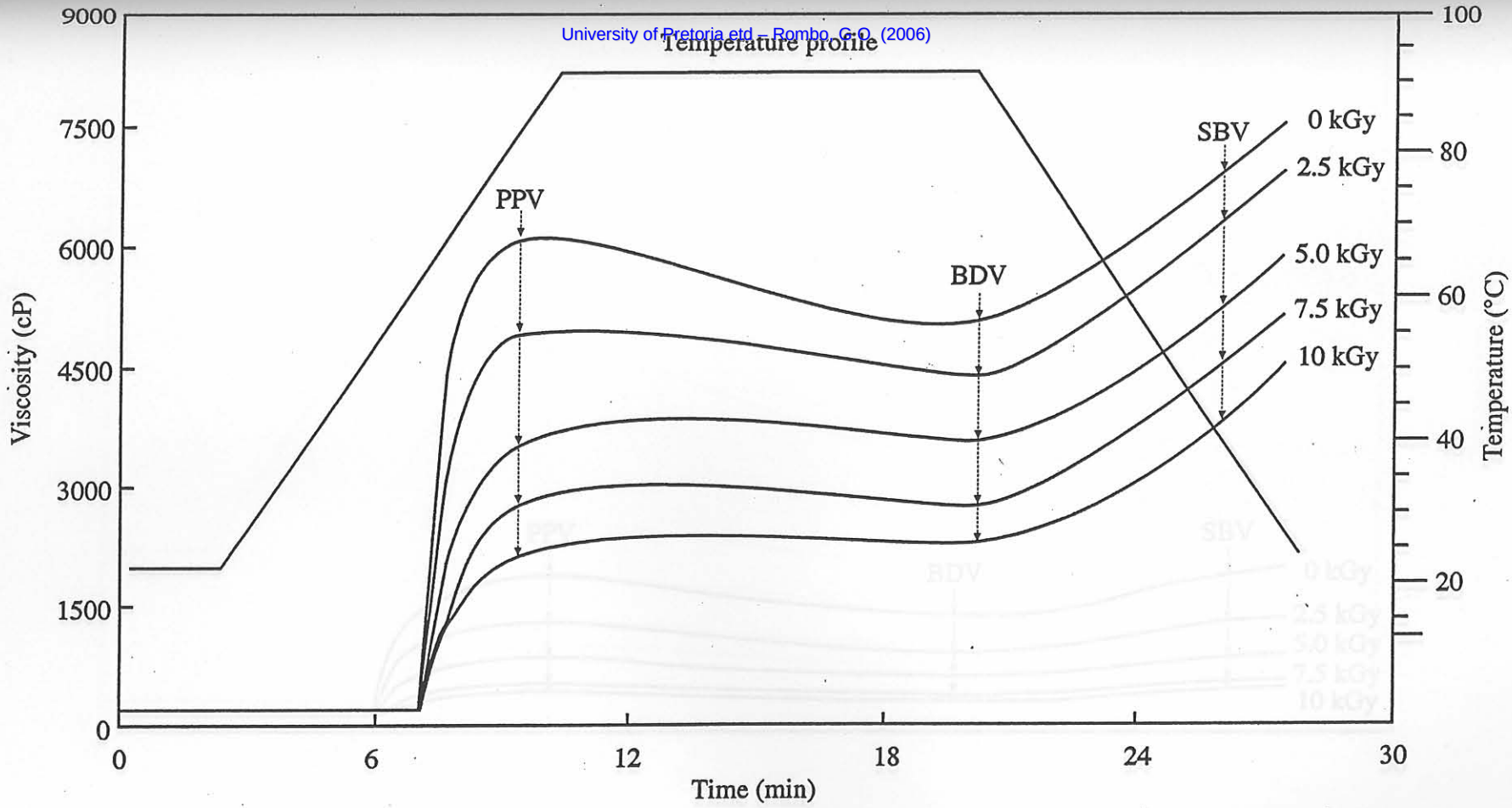

Figure 10 Schematic representation of the effects of irradiation on viscosity of porridges made from bean flour (c) at 25% total solids content (w/v), PPV: peak paste viscosity, BDV: breakdown viscosity, SBV: setback viscosity at 40°C (not drawn to scale)

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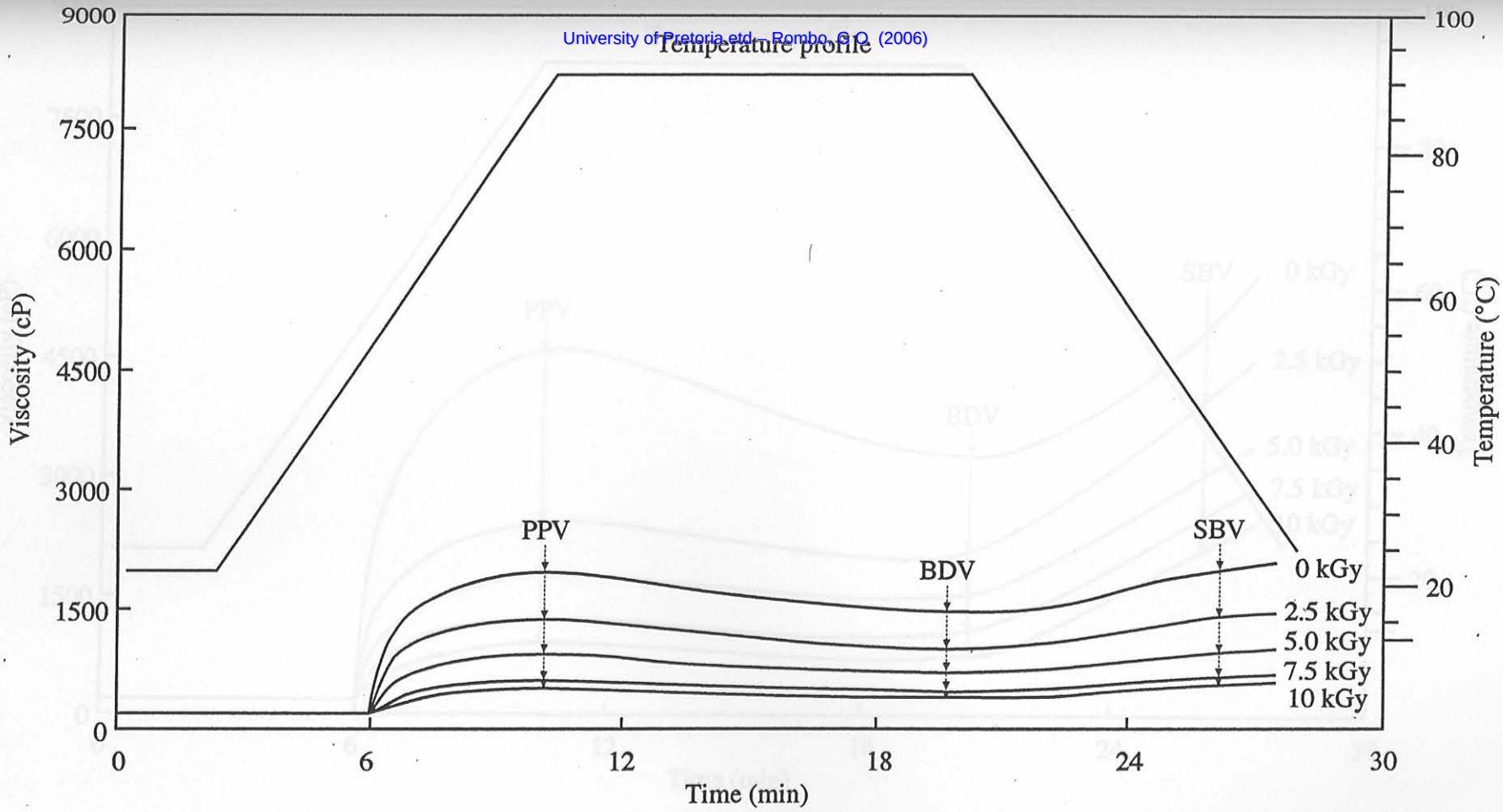

Figure 11 Schematic representation of the effects of irradiation on viscosity of porridges made from 70/30 maize:bean composite flour
(a) at 15% total solids content (w/v), PPV: peak paste viscosity, BDV: breakdown viscosity, SBV: setback viscosity at 40°C (not drawn to scale)

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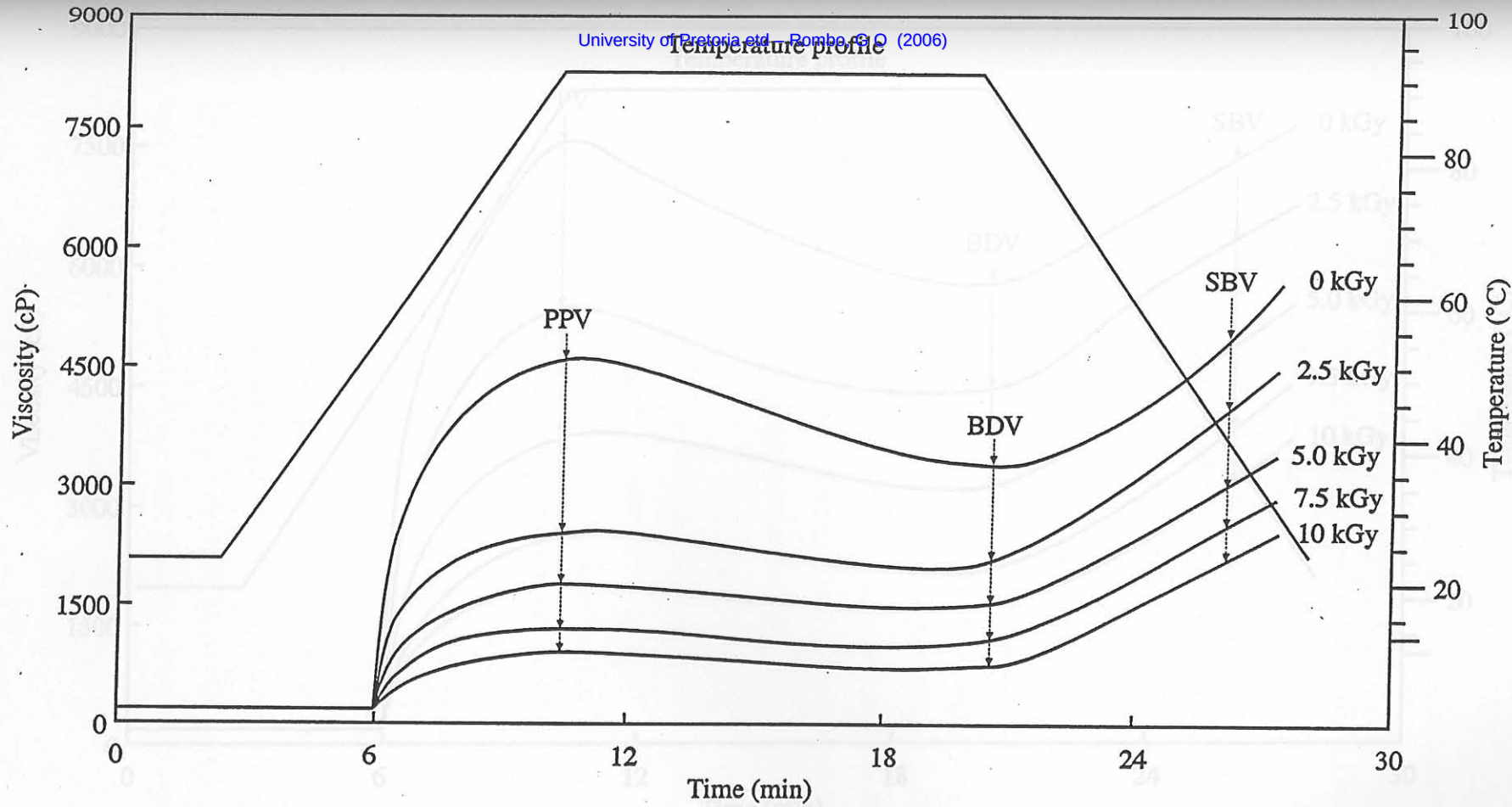

Figure 11 Schematic representation of the effects of irradiation on viscosity of porridges made from 70/30 maize:bean composite flour
(b) at 20% total solids content (w/v), PPV: peak paste viscosity, BDV: breakdown viscosity, SBV: setback viscosity at 40°C (not drawn to scale)

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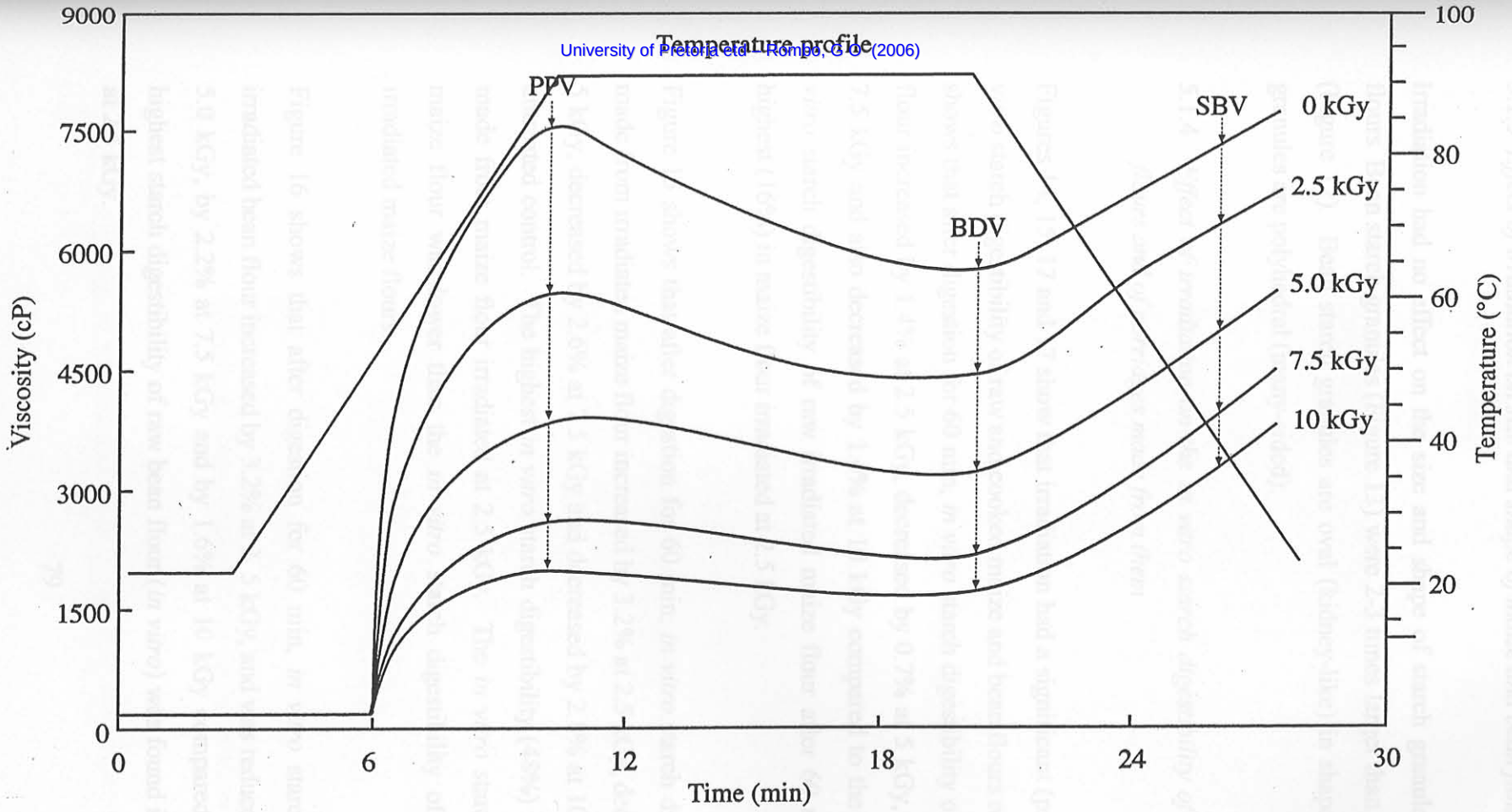

Figure 11 Schematic representation of the effects of irradiation on viscosity of porridges made from 70/30 maize:bean composite flour
(c) at 25% total solids content (w/v), PPV: peak paste viscosity, BDV: breakdown viscosity, SBV: setback viscosity at 40°C (not drawn to scale)

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5.1.3 *Effect of irradiation on size and shape of maize and bean flour starch granules*

Irradiation had no effect on the size and shape of starch granules in maize and bean flours. Bean starch granules (Figure 13) were 2-3 times larger than maize starch granules (Figure 12). Bean starch granules are oval (kidney-like) in shapes while maize starch granules are polyhedral (many-sided).

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

Figures 14, 15, 17 and 17 show that irradiation had a significant ($p \leq 0.05$) effect on the *in vitro* starch digestibility of raw and cooked maize and bean flours respectively. Figure 14 shows that after digestion for 60 min, *in vitro* starch digestibility of irradiated raw maize flour increased by 1.4% at 2.5 kGy, decreased by 0.7% at 5 kGy, decreased by 1.0% at 7.5 kGy and also decreased by 1.4% at 10 kGy compared to the untreated control. *In vitro* starch digestibility of raw irradiated maize flour after 60 min of digestion, was highest (16%) in maize flour irradiated at 2.5 kGy.

Figure 15 shows that after digestion for 60 min, *in vitro* starch digestibility of porridge made from irradiated maize flour increased by 3.2% at 2.5 kGy, decreased by 0.6% at 5 kGy, decreased by 2.6% at 7.5 kGy and decreased by 2.8% at 10 kGy compared to the untreated control. The highest *in vitro* starch digestibility (48%) was found in porridge made from maize flour irradiated at 2.5 kGy. The *in vitro* starch digestibility of raw maize flour was lower than the *in vitro* starch digestibility of porridge made from irradiated maize flours.

Figure 16 shows that after digestion for 60 min, *in vitro* starch digestibility of raw irradiated bean flour increased by 3.2% at 2. 5 kGy, and was reduced by 2.8% at 5.0 kGy, by 2.2% at 7.5 kGy and by 1.6% at 10 kGy compared to the control. The highest starch digestibility of raw bean flour (*in vitro*) was found in bean flour irradiated at 2.5 kGy.

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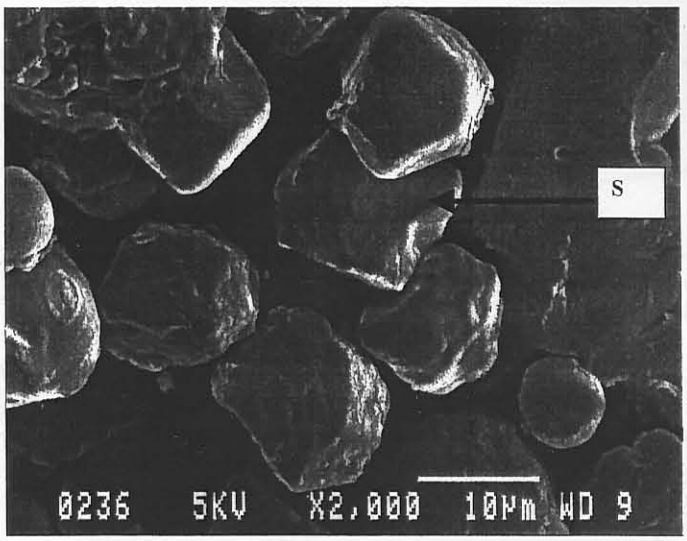

Figure 12 Size and shape of raw maize starch granule (S)

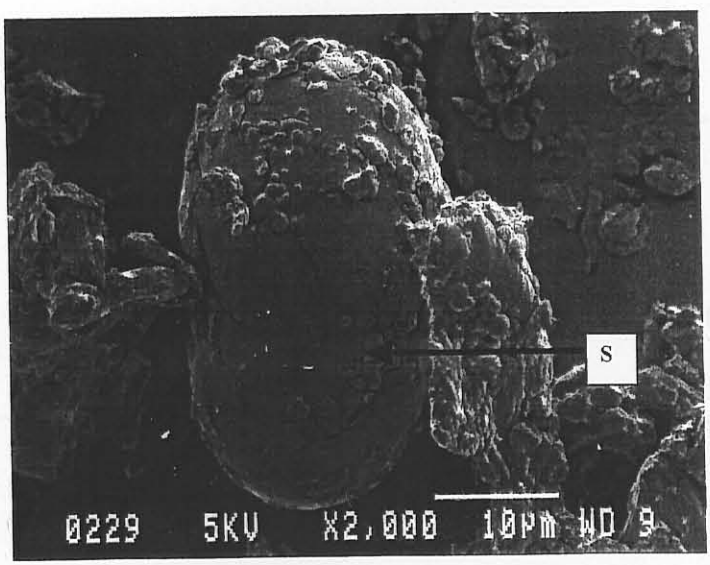

Figure 13 Size and shape of raw bean starch granule (S)

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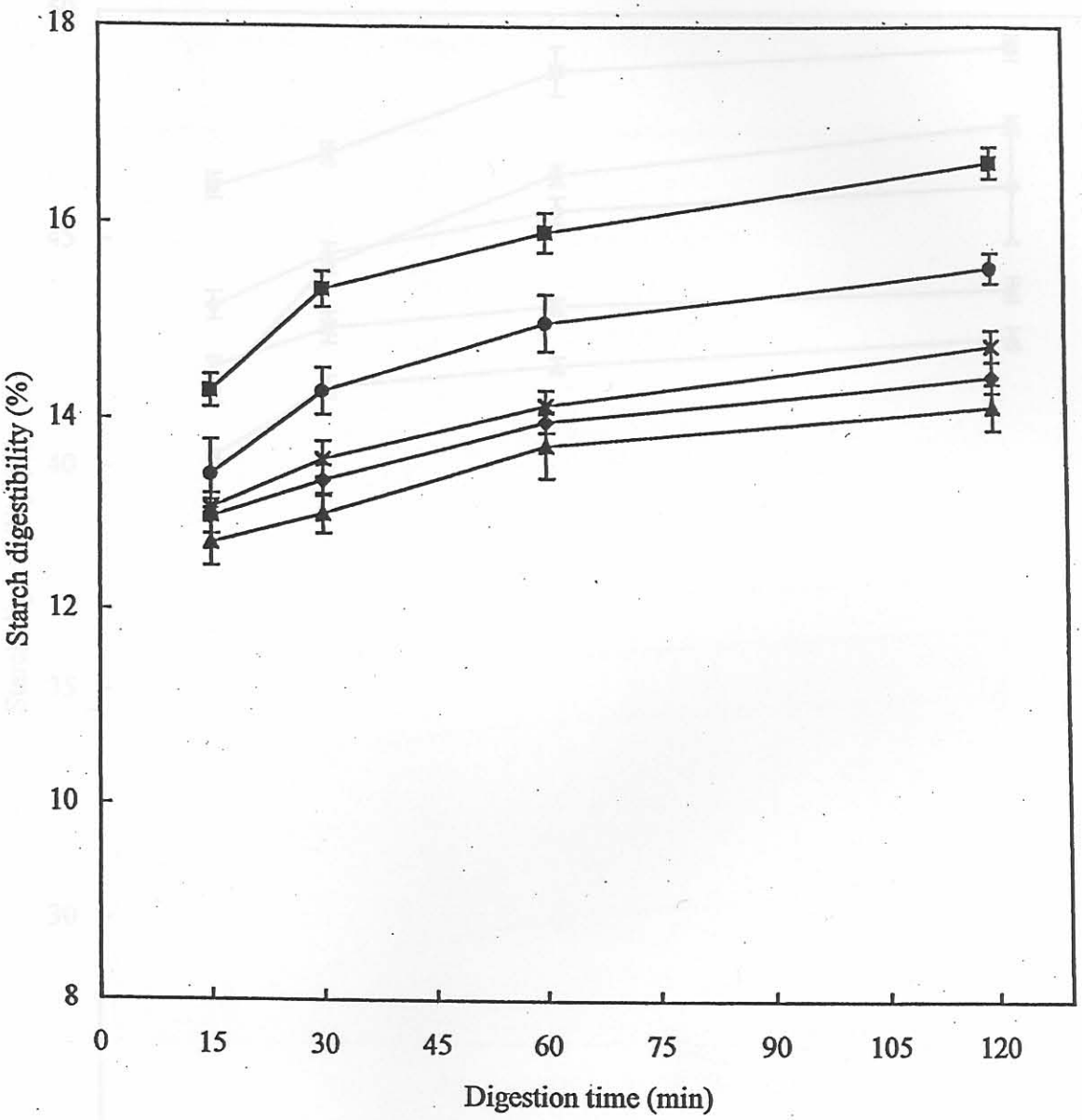

Figure 14 Effect of irradiation on *in vitro* starch digestibility of raw maize flours

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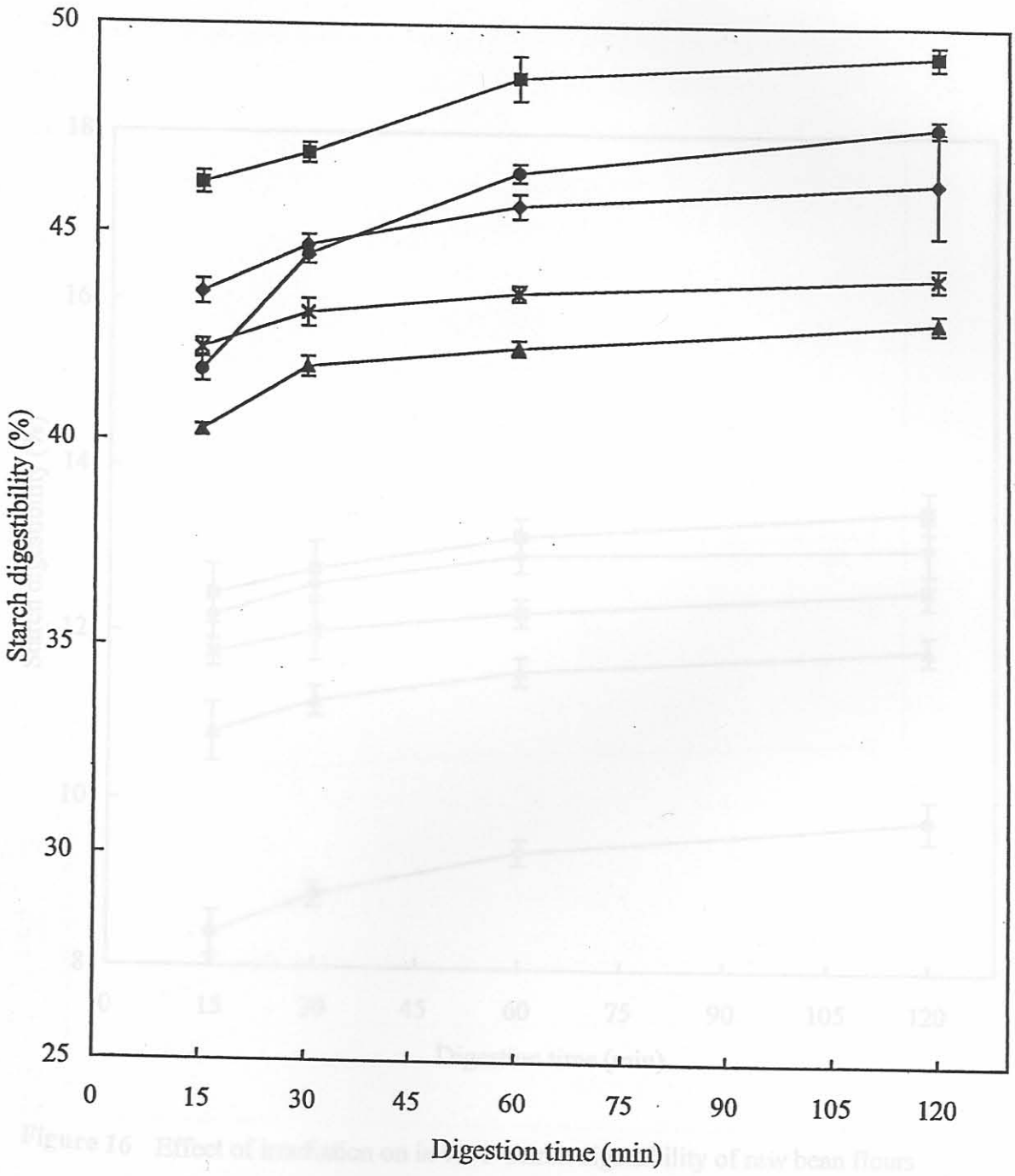

Figure 15 Effect of irradiation on *in vitro* starch digestibility of maize porridges

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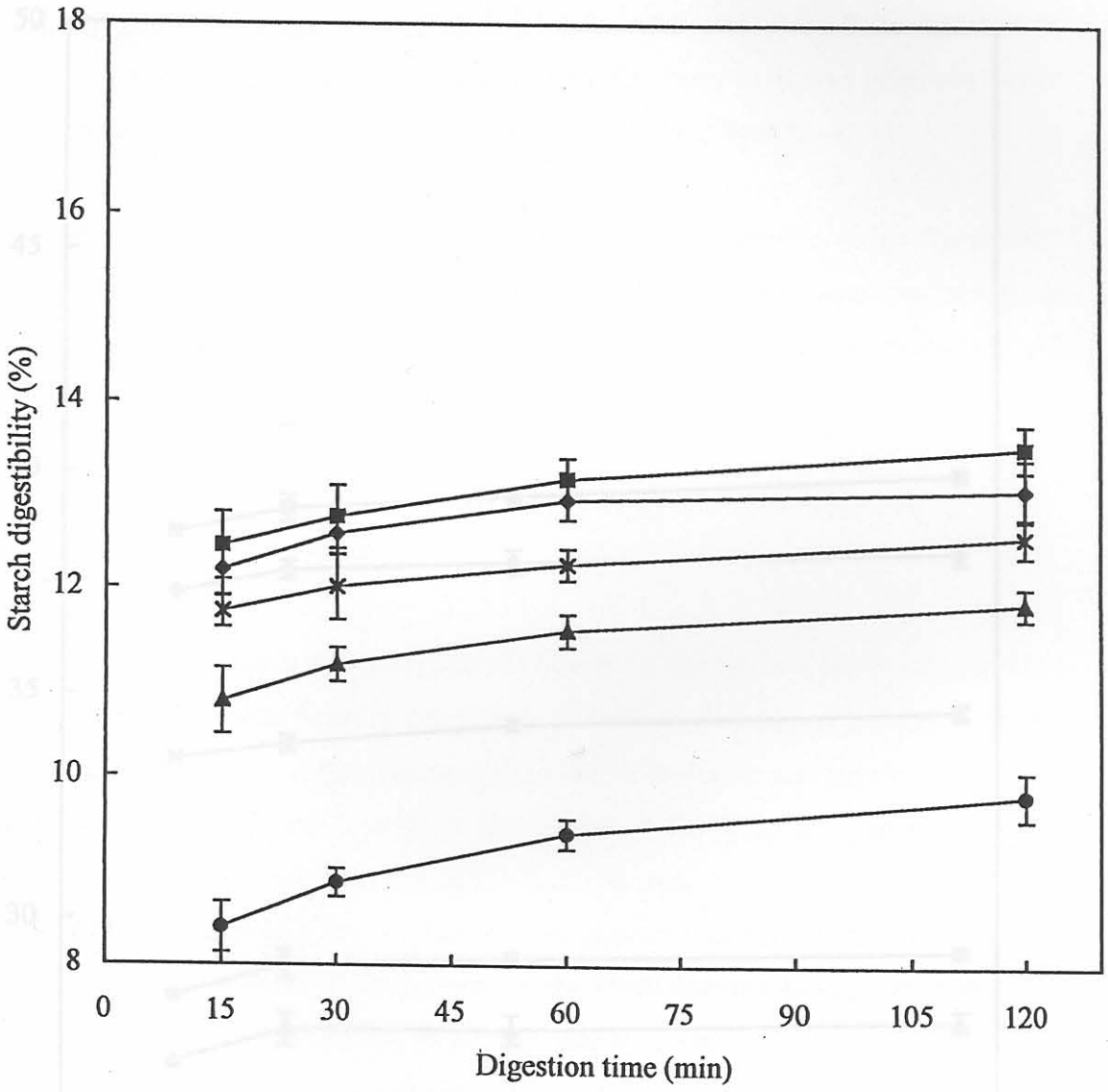

Figure 16 Effect of irradiation on *in vitro* starch digestibility of raw bean flours

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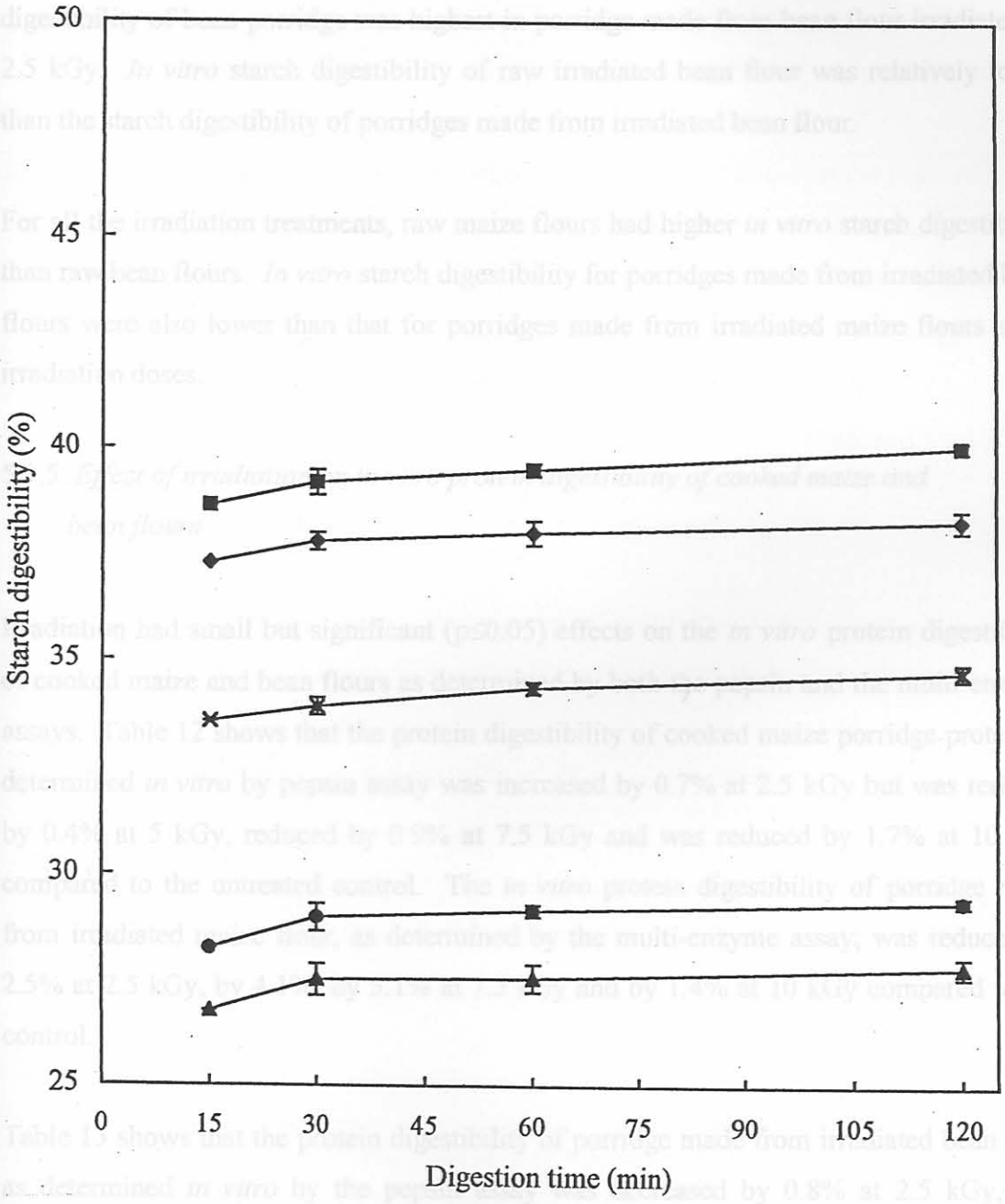

Figure 17 Effect of irradiation on *in vitro* starch digestibility of bean porridges

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Figure 17 shows that after digestion for 60 min, *in vitro* starch digestibility for porridges made from irradiated bean flour increased by 8.8% at 2.5 kGy, by 7.6% at 5 kGy, by 5% at 7.5 kGy and decreased by 2.1% at 10 kGy compared to the control. *In vitro* starch digestibility of bean porridge was highest in porridge made from bean flour irradiated at 2.5 kGy. *In vitro* starch digestibility of raw irradiated bean flour was relatively lower than the starch digestibility of porridges made from irradiated bean flour.

For all the irradiation treatments, raw maize flours had higher *in vitro* starch digestibility than raw bean flours. *In vitro* starch digestibility for porridges made from irradiated bean flours were also lower than that for porridges made from irradiated maize flours at all irradiation doses.

5.1.5 *Effect of irradiation on in vitro protein digestibility of cooked maize and bean flours*

Irradiation had small but significant ($p \leq 0.05$) effects on the *in vitro* protein digestibility of cooked maize and bean flours as determined by both the pepsin and the multi-enzyme assays. Table 12 shows that the protein digestibility of cooked maize porridge protein as determined *in vitro* by pepsin assay was increased by 0.7% at 2.5 kGy but was reduced by 0.4% at 5 kGy, reduced by 0.9% at 7.5 kGy and was reduced by 1.7% at 10 kGy compared to the untreated control. The *in vitro* protein digestibility of porridge made from irradiated maize flour, as determined by the multi-enzyme assay, was reduced by 2.5% at 2.5 kGy, by 4.1%, by 5.1% at 7.5 kGy and by 1.4% at 10 kGy compared to the control.

Table 13 shows that the protein digestibility of porridge made from irradiated bean flour as determined *in vitro* by the pepsin assay was decreased by 0.8% at 2.5 kGy, was increased by 0.1% at 5.0 kGy, but was reduced by 1% at 7.5 kGy and reduced by 1.9% at 10 kGy compared to the control. The protein digestibility as determined *in vitro* by the multi-enzyme method on cooked irradiated bean flour porridge was reduced by 2.6% at

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2.5 kGy, reduced by 3.6% at 5.0 kGy, reduced by 4.7% at 7.5 kGy and was reduced by 1.4% at 10 kGy compared to the control.

Table 12 Effect of irradiation on *in vitro* protein digestibility of porridge made from irradiated maize flours as determined by pepsin and multi-enzyme assays

Irradiation dose (kGy)	Pepsin method[1] (%)	Multi-enzyme method[1] (%)
0	68.5±0.47d[2]	81.8±0.37e[2]
2.5	69.2±0.10e	79.3±0.42c
5.0	68.1±0.29c	77.7±0.21b
7.5	67.6±0.21b	76.7±0.97a
10	66.8±0.18a	80.4±0.35d

[1]Values are means of three replicates determined six times ± standard deviation
[2]Mean values in a column with different letters differ significantly at $p \leq 0.05$

Table 13 Effect of irradiation *in vitro* on protein digestibility of porridge made from irradiated bean flours as determined by pepsin and multi-enzyme assays

Irradiation dose (kGy)	Pepsin method[1] (%)	Multi-enzyme method[1] (%)
0	66.1±0.66c[2]	81.8±0.37e[2]
2.5	65.3±0.11b	79.2±0.42c
5.0	66.2±0.05c	78.2±0.66b
7.5	65.1±0.78b	77.1±1.18a
10	64.2±0.70a	80.4±0.35d

[1]Values are means of three replicates determined six times ± standard deviation
[2]Mean values in a column with different letters differ significantly at $p \leq 0.05$

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5.2 Phase 2 Effect of irradiation on molecular properties of maize and bean flours and on their starches that may be responsible for decreases in their starch digestibility *in vitro* at doses higher than 2.5 kGy

In Phase 2, tests were performed to determine whether there were molecular changes in the irradiated maize and bean flours that may be responsible for the increase and decrease in *in vitro* starch digestibility. Colour measurements were performed on maize and bean flours after irradiation at 0, 5, 10, 20 and 40 kGy. Changes in the β-bonded starch content of maize and bean flours irradiated at 0, 5, 10, 20 and 40 kGy were also determined. The effect of irradiation on the degree of gelatinisation was determined using differential scanning calorimetry (DSC). The molecular weight distributions of starch molecules in irradiated maize and bean flours were determined using size exclusion high performance liquid chromatography (HPLCSEC).

5.2.1 *Effect of irradiation on the colour of maize and bean flours*

Irradiation caused significant ($p \leq 0.05$) changes in the L, a and b values of maize flours. The L values of maize flour were decreased by irradiation, while the a and b values were increased with the increasing dose of irradiation (Table 14).

Irradiation had significant ($p \leq 0.05$) effect on the L, a and b values of bean flour. As the irradiation dose increased the L values were decreased. However, the a and b values of bean flour were increased by the irradiation dose (Table 15).

Tables 14 and 15 show that there were slightly greater changes in the L values of maize flour than bean flour with increases in irradiation dose. However, there were slightly greater changes in a values of bean flour than maize flour with the increases in irradiation dose. The b values of both maize and bean flours were changed similarly by increases in irradiation dose.

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Table 14 Effect of irradiation on L[3], a[4] and b[5] values of maize flours

Irradiation dose (kGy)	L[1]	a[1]	b[1]
0	83.6±1.2d[2]	-0.3±0.5a	11.5±0.4a
5	80.5±0.1c	0.2±0.04b	11.8±0.1a
10	80.1±0.2c	0.4±0.1bc	12.6±0.1b
20	78.9±0.8b	0.6±0.04c	13.3±0.1c
40	77.4±0.6a	1.2±0.2d	14.8±0.6d

[1]Values are mean of three replicates determined six times ± standard deviation
[2]Values in the same column followed by different letters differ significantly at p≤0.05
[3]L values: Degree of lightness (White $^{+}100\leftarrow\rightarrow 0$ Black)
[4]a values: Degree of redness (red $^{+}100\leftarrow\rightarrow{}^{-}80$ green)
[5]b values: Degree of yellowness (yellow $^{+}100\leftarrow\rightarrow{}^{-}80$ blue)

Table 15 Effect of irradiation on the L[3], a[4] and b[5] values of bean flours

Irradiation dose (kGy)	L[1]	a[1]	b[1]
0	84.8±0.2e[2]	-1.1±0.1a	7.7±0.03a
5	83.7±0.2d	0.3±0.02b	8.7±0.1b
10	83.2±0.1c	0.4±0.03bc	9.8±0.1c
20	82.3±0.1b	0.6±0.02cd	10.5±0.1d
40	80.8±0.4a	0.7±0.02d	11.2±0.1e

[1]Values are mean of three replicates determined six times ± standard deviation
[2]Values in the same column followed by different letters differ significantly at p≤0.05
[3]L values: Degree of lightness (White $^{+}100\leftarrow\rightarrow 0$ Black)
[4]a values: Degree of redness (red $^{+}100\leftarrow\rightarrow{}^{-}80$ green)
[5]b values: Degree of yellowness (yellow $^{+}100\leftarrow\rightarrow{}^{-}80$ blue)

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5.2.2 *Effect of irradiation on the β(1-3) and β(1-4) -bonded starch contents of starches isolated from maize and bean flours*

Irradiation caused small but significant ($p \leq 0.05$) changes in the β-bonded starch content of starches isolated from irradiated maize and bean flours compared to the untreated controls. The β-bonded starch content in isolated starches increased with irradiation dose for both maize and bean flours. More β-bonded starch was formed in irradiated bean flour than from maize flour at any given irradiation dose (Figure 18).

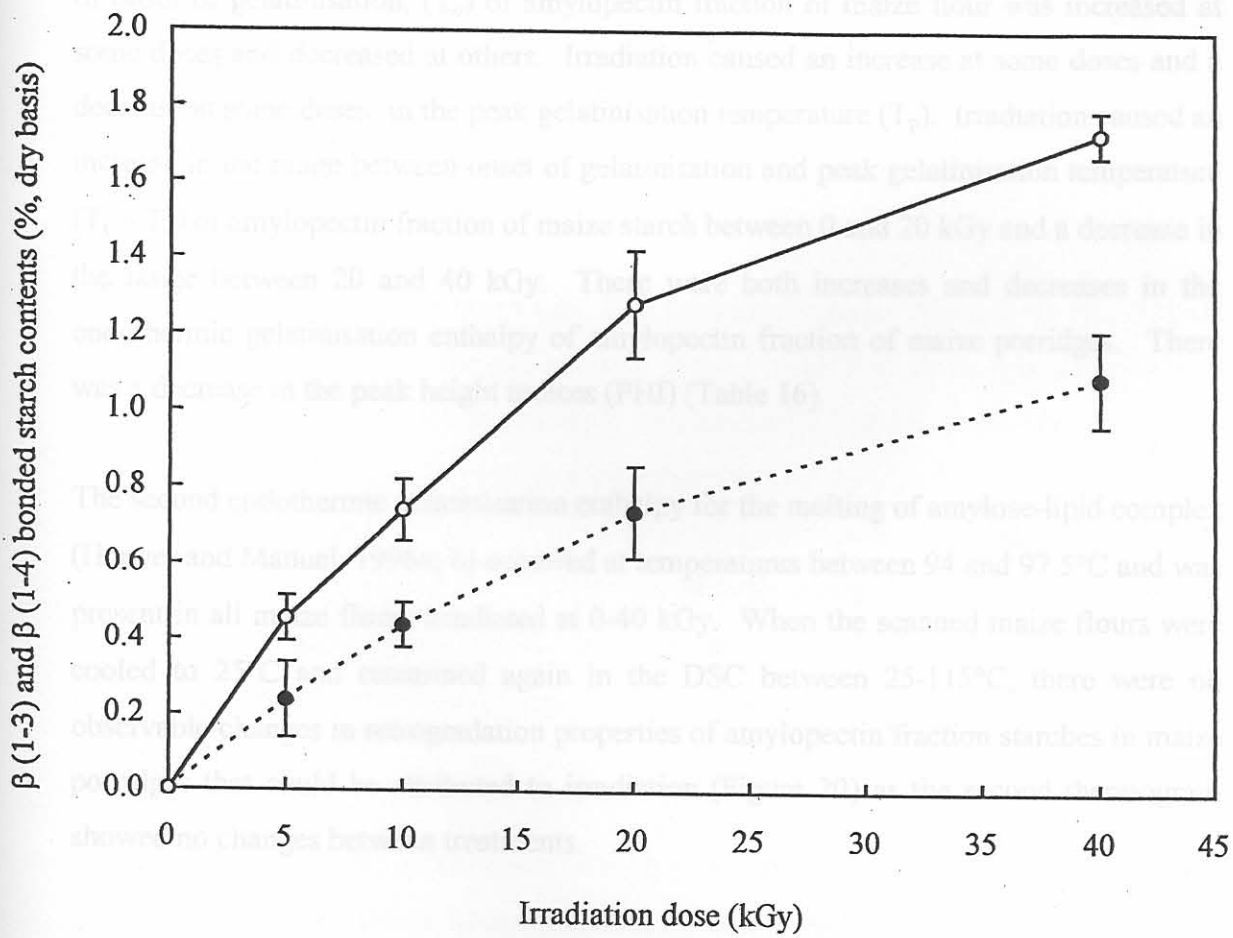

Figure 18 The β(1-3) and β(1-4)- bonded starch contents of starches isolated by wet milling from irradiated maize and bean flours

- - ●- - - The β (1-3) and β (1-4) bonded starch contents of irradiated maize flour starch
—○— The β (1-3) and β (1-4) bonded starch contents of irradiated bean flour starch

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5.2.3 *Effect of irradiation on thermal properties of amylopectin fraction of maize and bean flour starches*

Irradiation caused changes in the thermal properties of starches in maize and bean flours as determined by differential scanning calorimetry (DSC). The first scanning thermogram shows the effects of irradiation on the thermal properties of the amylopectin fraction of maize flour (Figure 19). As shown in Table 16 and Figure 19, the temperature of onset of gelatinisation, (T_o) of amylopectin fraction of maize flour was increased at some doses and decreased at others. Irradiation caused an increase at some doses and a decrease at some doses in the peak gelatinisation temperature (T_p). Irradiation caused an increase in the range between onset of gelatinisation and peak gelatinisation temperature ($T_p - T_o$) of amylopectin fraction of maize starch between 0 and 20 kGy and a decrease in the range between 20 and 40 kGy. There were both increases and decreases in the endothermic gelatinisation enthalpy of amylopectin fraction of maize porridges. There was a decrease in the peak height indices (PHI) (Table 16).

The second endothermic gelatinisation enthalpy for the melting of amylose-lipid complex (Hoover and Manuel, 1996a; b) occurred at temperatures between 94 and 97.5°C and was present in all maize flours irradiated at 0-40 kGy. When the scanned maize flours were cooled to 25°C and rescanned again in the DSC between 25-115°C, there were no observable changes in retrogradation properties of amylopectin fraction starches in maize porridges that could be attributed to irradiation (Figure 20) as the second thermogram showed no changes between treatments.

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Table 16 Effect of irradiation on thermal properties of amylopectin fractions of maize flour

Irradiation dose (kGy)	To[1] (°C)	Tp[2] (°C)	Tp– To (°C)	ΔH[3] (J/g)	PHI[4] (J/°C) ΔH/ Tp– To
0	63.5	71.8	8.3	10.21	1.23
5	63.9	72.4	8.5	10.29	1.21
10	63.2	72.1	8.9	9.28	1.04
20	62.8	72.4	9.6	10.58	1.10
40	62.83	72.1	9.3	9.20	0.99

[1] T_o is the onset temperature of endothermic gelatinisation enthalpy
[2] T_p is the peak temperature of the endothermic gelatinisation enthalpy
[3] ΔH is the endothermic gelatinisation enthalpy
[4] PHI is the Peak Height Index

Table 17 and Figure 21 show clearly that irradiation caused changes in the thermal properties of amylopectin fraction of bean flours. The first thermogram shows that irradiation caused an increase in the onset temperature (T_o) of gelatinisation. Irradiation also caused an increase in the peak gelatinisation temperature (T_p) (Table 17). Irradiation caused a reduction in the range between onset of gelatinisation and peak gelatinisation temperature ($T_p - T_o$) of amylopectin fraction of bean starch. Irradiation also caused an increase in the endothermic gelatinisation enthalpy of amylopectin fractions of bean flours. The peak height indices of amylopectin fraction of bean flours were increased consistently (Table 17).

The second endothermic gelatinisation enthalpy for the melting of amylose-lipid complex (Hoover and Manuel, 1996a; b) occurred at temperatures between 94 and 97.5°C and was present in all bean flours irradiated at 0-40 kGy. When the scanned bean flours were cooled to 25°C and rescanned again in the DSC between 25-1115°C, there were no observable changes in retrogradation properties of amylopectin fraction starches in bean

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porridges that could be attributed to irradiation (Figure 22) as the second thermogram showed no changes between treatments.

Table 17 Effect of irradiation on thermal properties of amylopectin fractions of bean flour

Irradiation dose (kGy)	To[1] (°C)	Tp[2] (°C)	Tp– To (°C)	ΔH[3] (J/g)	PHI[4] (J/°C) ΔH/Tp– To
0	67.0	75.5	8.5	4.60	0.54
5	67.5	75.5	8.0	5.65	0.71
10	68.0	75.9	7.9	8.71	1.10
20	69.4	76.2	6.8	9.50	1.40
40	71.2	76.5	5.3	9.00	1.70

[1] T_o is the onset temperature of gelatinisation,
[2] T_p is the peak temperature of the gelatinisation endotherm
[3] ΔH is the endothermic gelatinisation enthalpy
[4] PHI is the Peak Height Index

5.2.4 *Effect of irradiation on the molecular weight distribution of maize and bean flour starches*

Both maize and bean flours starches had four fractions as determined by high performance liquid size exclusion chromatography (HPLCSEC) using pullulan standards for the determination of molecular weight as shown in Figures 24 and 25. In maize the largest fraction had an average molecular weight of 4×10^6 Da, the second fraction had an average molecular weight of 4×10^5 Da. The third fraction had an average molecular weight of 2×10^5 Da. The fourth fraction had an average molecular weight of 2×10^3 Da (Figure 23).

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The amount of the first fraction of maize flour starch was reduced with increases in irradiation dose. The first fraction of maize starches decreased with increasing irradiation doses while the second, third and fourth fractions increased with irradiation doses.

Bean starch had four fractions as determined by HPLCSEC using pullulan standards for determination of the molecular weight. The first had an average molecular weight of 3×10^6 Da. The second fraction (normally referred to as intermediate molecular weight starch fraction) had an average molecular weight of 4×10^5 Da. The third fraction had an average molecular weight of 4×10^4 Da. The fourth fraction had an average molecular weight of 1×10^3 Da (Figure 24). A similar trend was observed for starch from the irradiated bean flour (Figure 24). However, the reductions in amount of the amylopectin fraction with irradiation dose were higher in bean flour starch compared to that of maize flour starch.

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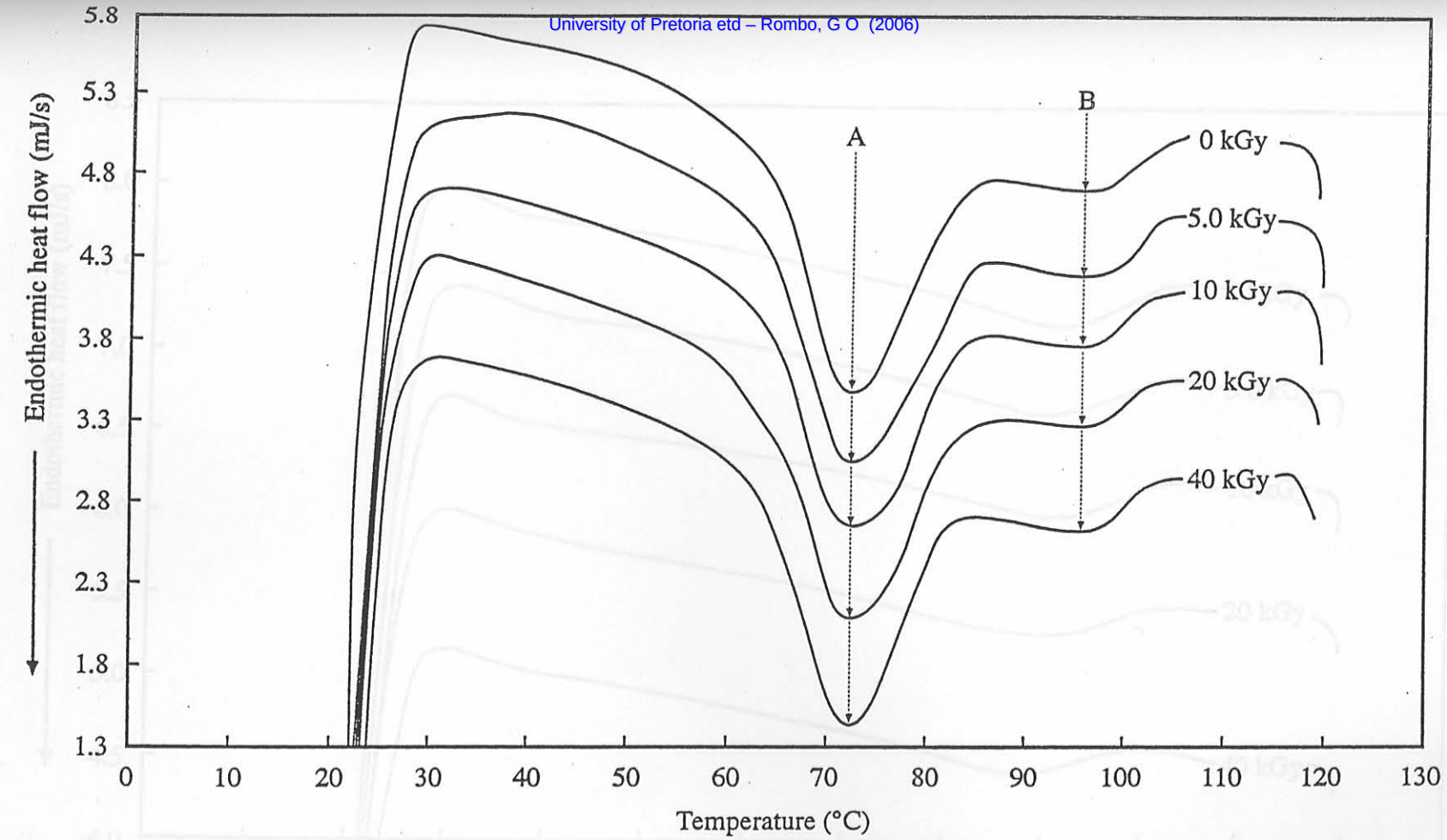

Figure 19 Effect of irradiation on gelatinisation properties of amylopectin in porridges made from maize flours
A: the endothermic gelatinisation enthalpy of amylopectin.
B: the endothermic melting enthalpy of amylose-lipid complex.

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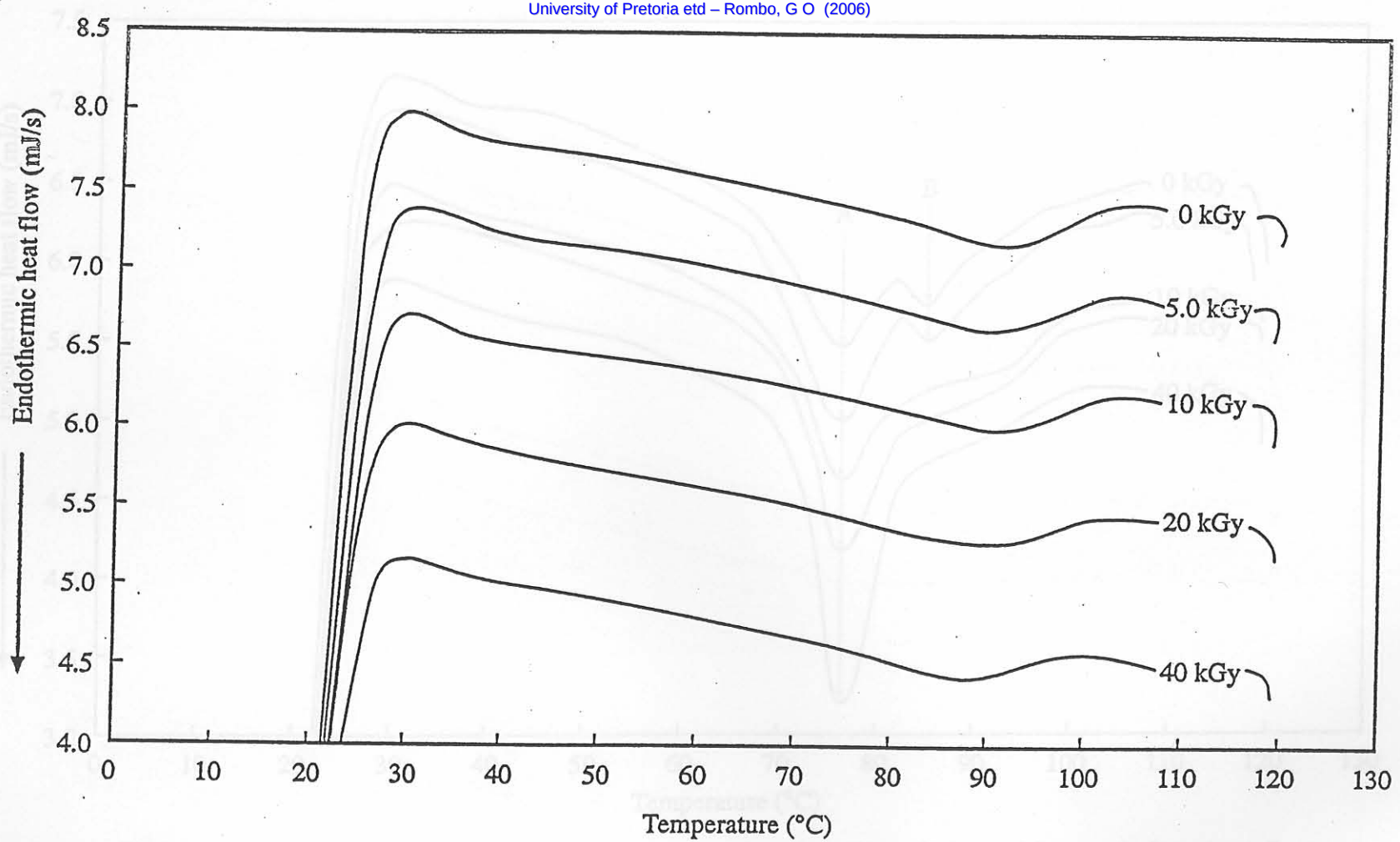

Figure 20 Effect of irradiation on retrogradation properties of amylopectin in porridges made from maize flours

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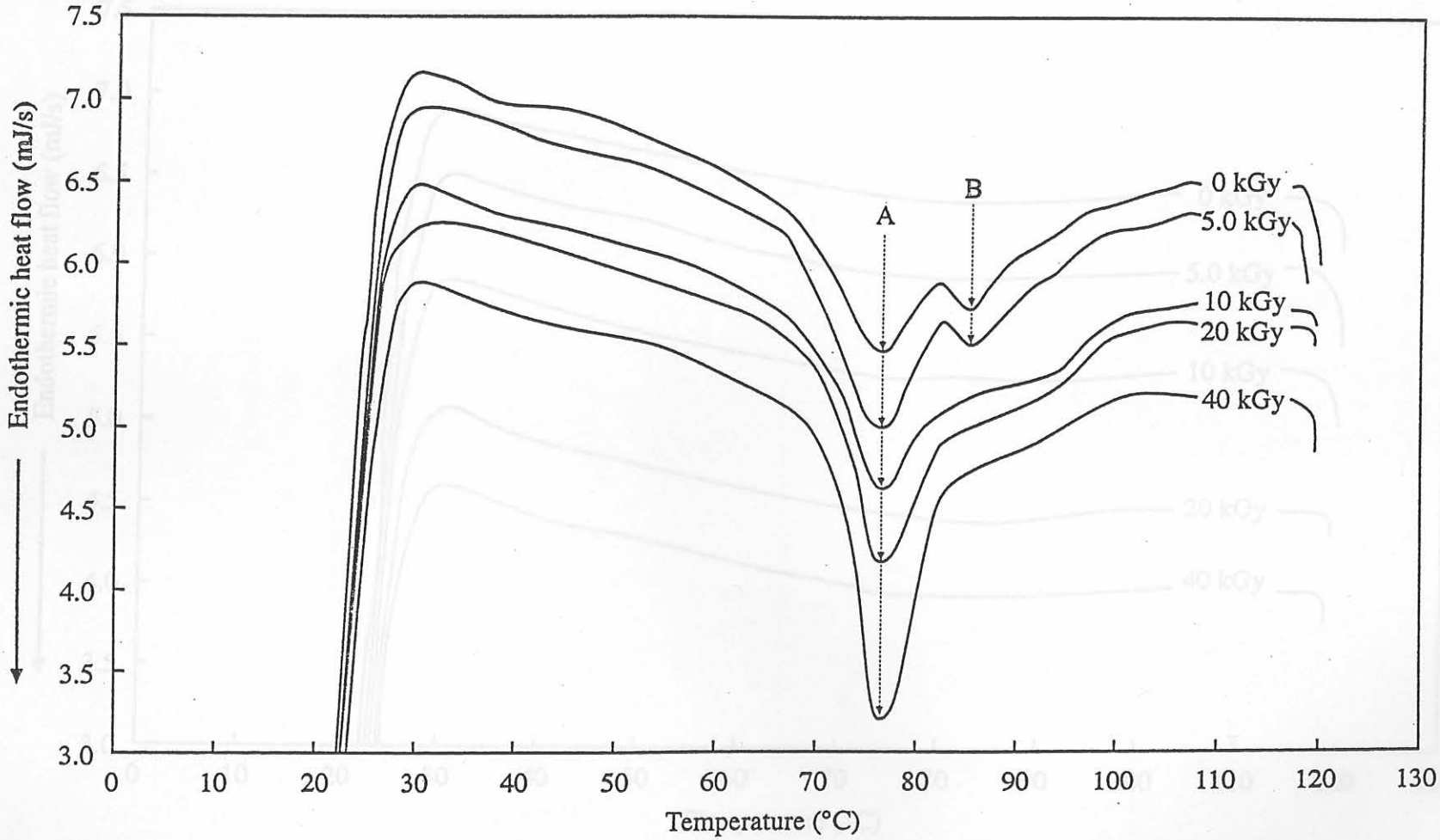

Figure 21 Effect of irradiation on gelatinisation properties of amylopectin in porridges made from bean flours
A: the endothermic gelatinisation enthalpy of amylopectin.
B: the endothermic melting enthalpy of amylose-lipid complex

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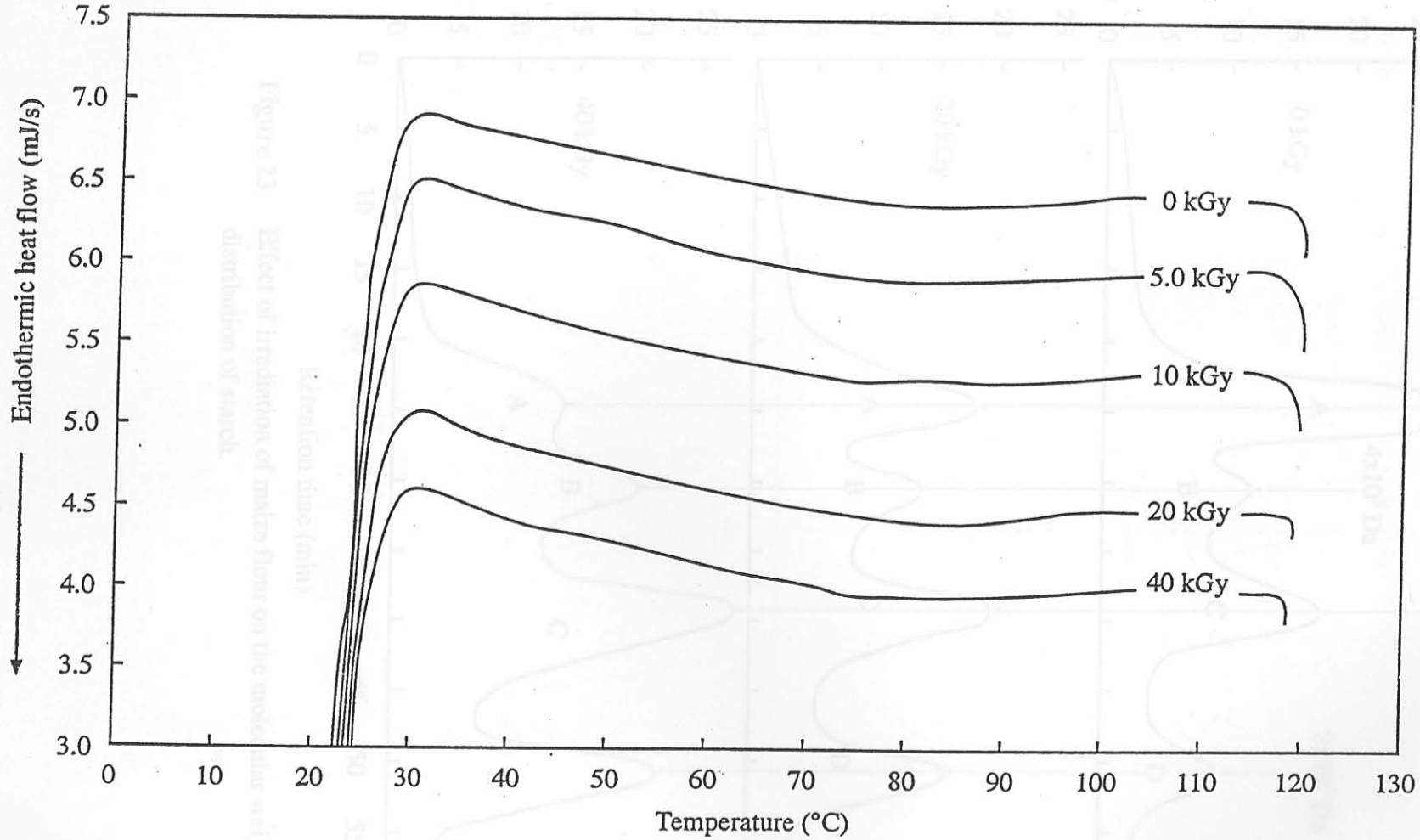

Figure 22 Effect of irradiation on retrogradation properties of amylopectin in porridges made from bean flours

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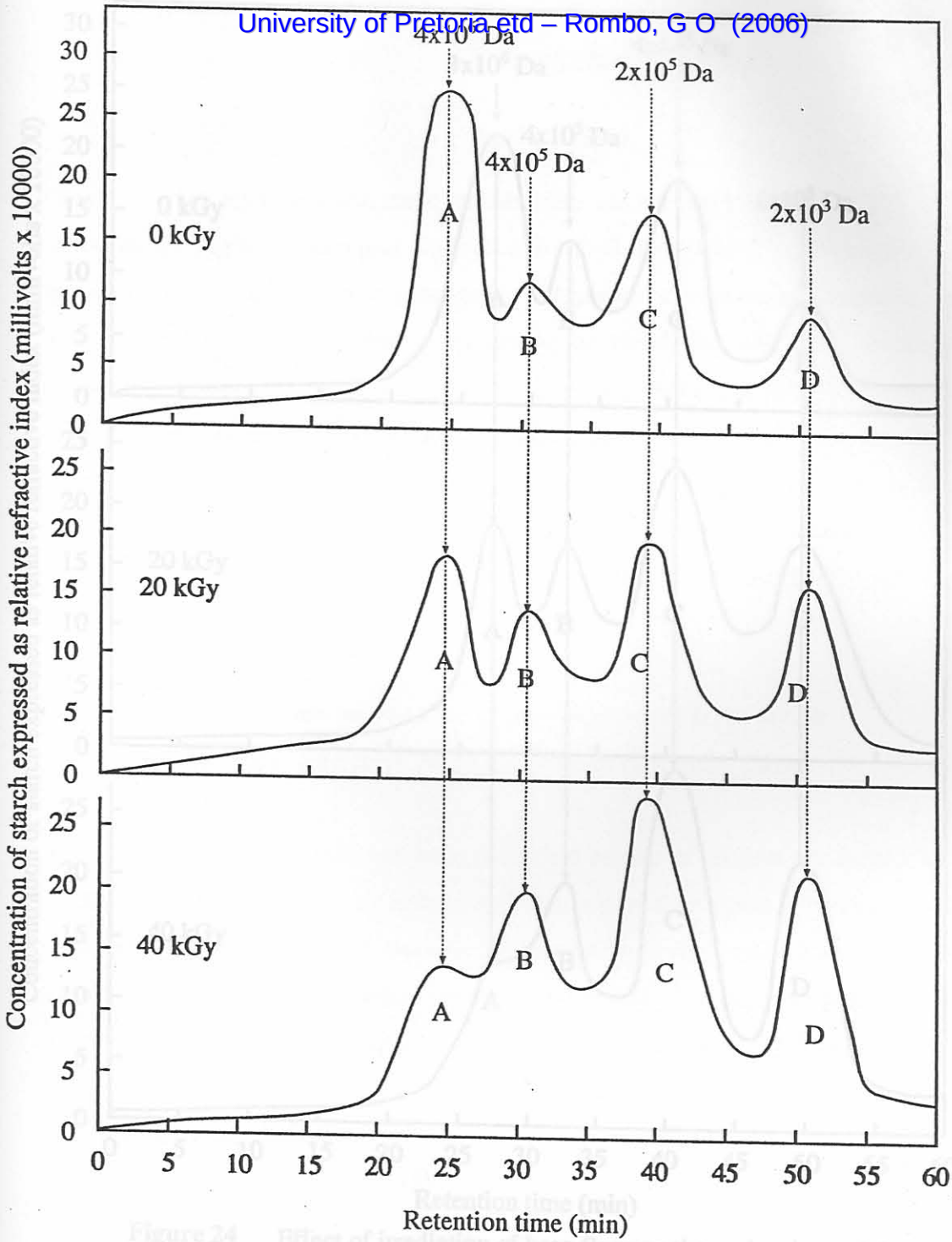

Figure 23 Effect of irradiation of maize flour on the molecular weight distribution of starch.

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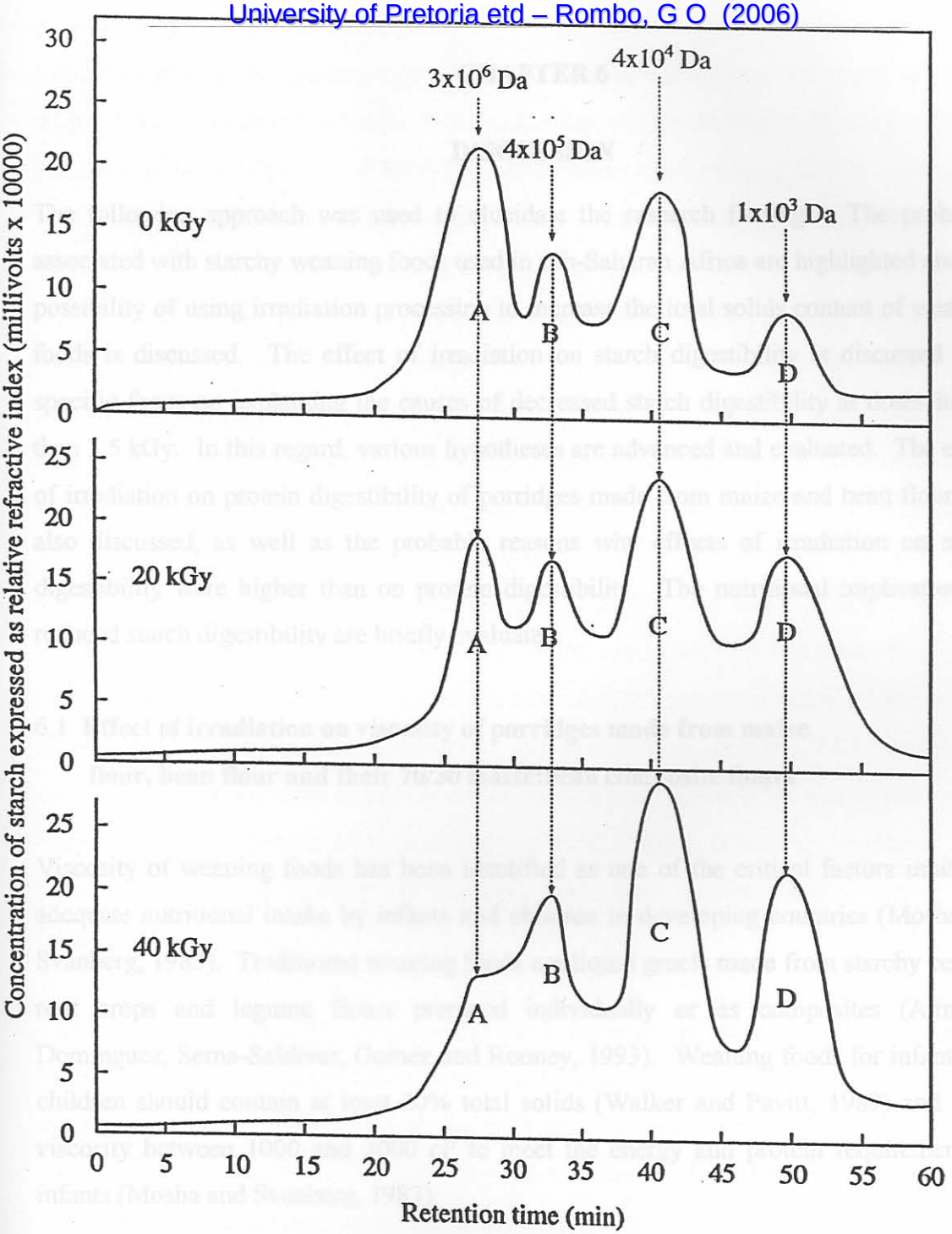

Figure 24 Effect of irradiation of bean flour on the molecular weight distribution of starch.

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CHAPTER 6

DISCUSSION

The following approach was used to elucidate the research findings. The problems associated with starchy weaning foods used in sub-Saharan Africa are highlighted and the possibility of using irradiation processing to increase the total solids content of weaning foods is discussed. The effect of irradiation on starch digestibility is discussed with specific focus on explaining the causes of decreased starch digestibility at doses higher than 2.5 kGy. In this regard, various hypotheses are advanced and evaluated. The effect of irradiation on protein digestibility of porridges made from maize and bean flours are also discussed, as well as the probable reasons why effects of irradiation on starch digestibility were higher than on protein digestibility. The nutritional implications of reduced starch digestibility are briefly evaluated.

6.1 Effect of irradiation on viscosity of porridges made from maize flour, bean flour and their 70/30 maize:bean composite flours

Viscosity of weaning foods has been identified as one of the critical factors inhibiting adequate nutritional intake by infants and children in developing countries (Mosha and Svanberg, 1983). Traditional weaning foods are liquid gruels made from starchy cereals, root crops and legume flours prepared individually or as composites (Almeida-Dominguez, Serna-Saldivar, Gomez and Rooney, 1993). Weaning foods for infants and children should contain at least 20% total solids (Walker and Pavitt, 1989) and be of viscosity between 1000 and 3000 cP to meet the energy and protein requirements of infants (Mosha and Svanberg, 1983).

The porridges or gruels used in developing countries usually contain as little as 5-10% total solids and this has very low energy density (only 900-1800 kJ/l) (Janssen et al., 1981; Harper and Janssen, 1985; Onofiok and Nnanyelugo, 1998; FAO, 2002). Milk from which the child is being weaned provides 2860 kJ/l while the weaning foods in developed countries supply 5980kJ/l (Walker and Pavitt, 1989).

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Irradiation resulted in significant ($p \leq 0.05$) reductions of viscosity of porridges made from maize flours, bean flours and their 70/30 maize:bean composite flours. It was possible to achieve a porridge viscosity of 1000-3000 cP at setback temperature of 50°C and a total solids content of at least 20% by using maize flour irradiated at 5 kGy. For maize porridge to be consumed as a weaning food at setback temperature of 40°C, the flour had to be irradiated at 7.5 kGy to meet the above concentration and consistency requirements. However, for consumption of porridges of at least 20% total solids content at 30°C, irradiation of maize flours up to 10 kGy did not meet the consistency requirements. The total solids content of porridges made from maize flour irradiated at 10 kGy could be increased to 20%. However, viscosity was less than 1000 cP at setback temperature of 50°C. Porridges made from bean flours irradiated at 5 kGy met the total solids and viscosity requirements at setback temperatures of 50, 40 and 30°C. For the porridges made from the 70/30 maize:bean composite flours, irradiation at 7.5 kGy was required to meet the consistency and 20% total solids content requirements at setback temperatures 50 and 40 °C. It was also established that maize, bean and the 70/30 maize:bean composite flours irradiated at 10 kGy could produce porridges of 25% total solids content and still meet the consistency requirements at 50°C. Martin (1998) has reported similar reductions in setback viscosity for porridges made from irradiated maize and sorghum flours.

The reductions in viscosity of porridges made from maize flour, bean flour and their 70/30 composite flour increased with irradiation dose. Chemical and physical effects of irradiation are dose dependent from the following equation given by Urbain (1978): $Y = 10^7 \times G \times D \times \rho$; where Y is the product yield, G is the number of molecules changed per 100 electron volts transferred to the food, D is the dose in Gray and ρ is the specific gravity of the food. Therefore, the degree of depolymerisation of starch is proportional to the dose of irradiation (Michel et al., 1980; Raffi et al., 1981a). 1 ev = 10^{-19} Joules (Urbain, 1986).

Any change in the structure of starch molecules is reflected in the changes in viscosity, solubility and swelling power of the starch granules (Sokhey and Hanna, 1993). The

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reduction in viscosity of starchy foods has been attributed to depolymerisation of starch through the breakage of glycosidic bonds (Michel et al., 1980). Starch molecules are composed of amylopectin, a highly branched polymer of glucose of higher molecular weight (Fraction I), and amylose, generally a straight chain polymer of glucose with a low molecular weight (Fraction II) (Chinnaswamy and Bhattacharya, 1986; Sokhey and Chinnaswamy, 1993). Ionising radiations cause more fragmentation in amylopectin molecules than in amylose molecules (Sokhey and Chinnaswamy, 1993). Debranching of starch in maize and bean flours during irradiation has been shown in this study by the HPLCSEC results. Researchers have shown that gamma irradiation increases the reducing sugar content of starches and this has been used to suggest that glycosidic bonds are being broken (Raffi et al., 1981a; b; c; d; Rayas-Solis, 1987; Lu et al., 1989; Sokhey and Chinnaswamy, 1993). Irradiation also depolymerises other high molecular weight polysaccharides that are present in the maize and bean flours such as cellulose by breaking the glycosidic bonds (Kertesz, Glegg, Boyle, Parsons and Massey, 1964; Merritt, 1972; Scherz, 1974). Broken glycosidic bonds in starch leads to increased solubility in both starch and cellulose (Merritt, 1972) and results in reduced swelling power of starchy foods (Rayas-Solis, 1987).

Porridges of consistency less than 1000 cP made from processed foods may either be too low in energy and protein content or may contain too much of the low molecular weight carbohydrates (Janssen et al., 1981; Harper and Janssen, 1985; Zhou and Kaplan, 1997). High concentrations of low molecular weight carbohydrates may exert high osmolarity and this may cause gastrointestinal problems such as diarrhoea (Zhou and Kaplan, 1997). High concentration of low molecular weight carbohydrates may also cause dehydration by osmotic pressure against the stomach and small intestines by limiting the amount of water available for hydration and could also lead to imbalances in insulin secretion in the infants and young children (Latham, 1990). Weaning foods of consistency above 3000 cP may be too thick to be consumed by infants since they may not be able to chew and digest it (Ngoddy et al., 1994).

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In the present study viscosity of porridges made from irradiated maize flour showed higher peak paste viscosity than porridges made from irradiated bean flours. This is probably because maize flour with higher amylopectin content swells more during gelatinisation than bean flours. Similarly, since the reductions in porridge viscosity is a function of the amylopectin content (Kertesz, Schulz, Fox and Gibson, 1959; Rayas-Solis, 1987; Tester and Morrison, 1990; Sokhey and Chinnaswamy, 1993; Thomas and Atwell, 1999), irradiation caused larger decreases in viscosity of porridges made from maize flour because of their high amylopectin contents compared to porridges made from bean flour. Normal maize starch contains only 25% amylose (Hoover and Manuel, 1996b; Thomas and Atwell, 1999), compared to bean flour with about 35% amylose (Hoover and Manuel, 1996a), and this may explain why porridges made from maize flour exhibited higher viscosity than porridges made from bean flour.

The pasting profile consists of three major phases (Walker, Ross, Wrigley and McMaster, 1988). The first phase is the swelling phase and consists of gelatinisation or swelling of amylopectin molecules and since the amylopectin is in the starch granule, the granules also swell and some amylose molecules are leached out (Batey, Curtin and Moore, 1997). This implies that the integrity of the starch granules is a major factor determining the rheological properties of a starch paste or gel (Tsai, Li and Lii, 1997). Any process, such as milling (Craig and Stark, 1984; Stark and Yin, 1986) or extrusion (Colonna et al., 1987), which reduces granular integrity of starch, reduces its swelling power. The viscosity increases during the swelling phase leading to the formation of peak paste viscosity. The swollen starch pastes consist of leached amylose, swollen granules, microgels and fractions of macromolecules (Dublier, Llamas and Le Meur, 1987).

The second phase is the breakdown phase and it refers to breakage of hydrogen bonds between water and amylopectin molecules by the shear action of the paddle (Schierbaum and Kettlitz, 1994; Yuan and Quail, 1999). The viscosities of the pastes are reduced during this phase. High shear rates cause extensive rupture of starch granules, release amylopectin and decrease the viscosity (Svegmark and Hermansson, 1990).

In the third phase, the setback phase (cooling phase), the paste is cooled from 95°C to 30°C and it consists of retrogradation or reformation of hydrogen bonds between amylose molecules (Atwell et al., 1988). On cooling, the viscosity of the maize and bean pastes was increased and this is called the setback viscosity, i.e. the viscosity that develops on cooling gelatinised starch (Atwell et al., 1988; Kim, Wiesenborn and Grant, 1997). Increase in viscosity occurs due to the formation of hydrogen bonds between the leached amylose molecules (Zeng, Morris, Batey and Wrigley, 1997).

In the present study, maize porridge with less amylose content had lower changes in viscosity during the setback phase than bean porridge. For example, increasing the total solids content of maize flour irradiated at 2.5 kGy from 15% to 20% led to an increase of 78% in setback viscosity at 50 °C while increasing the total solids content of bean porridge made from flour irradiated at 2.5 kGy from 15% to 20% resulted in an increase of over 100% because bean porridge has more amylose. Changes in setback viscosity depend on the amylose content of the porridge (Miles, Morris, Orford and Ring, 1985; Yuan, Thompson and Boyer, 1993). Retrogradation of amylopectin is a very slow process that requires several days or weeks (Bello-Perez and Paredes-Lopez, 1995). Another important consideration is that amylopectin at concentrations less than 35% total solids content in water would not reassociate in large quantities enough to show changes in viscosity due to retrogradation in less than one day (Orford, Ring, Carroll, Miles and Morris, 1987). A 30% total solids content paste made from waxy maize starch (99% amylopectin) showed some retrogradation endothermic melting after two days but did not develop maximum retrogradation endothermic melting until after 40 days of storage at 4°C (Bello-Perez and Paredes-Lopez, 1995; Liu and Thompson, 1998). Pure waxy starches do not develop setback viscosity (Yuan, Thompson and Boyer, 1993). Amylomaize VII (70% amylose content) did not develop set back viscosity because it does not gelatinise (Hoover and Manuel, 1996b).

From the HPLCSEC results of the present study with irradiated maize and bean flours, the low molecular weight starch fractions produced in starches isolated from irradiated maize and bean flours are most likely depolymerised or debranched amylopectin given

that during any physical or chemical treatment of starch amylopectin is preferentially debranched (Colonna et al., 1992). Examples of physical processes that lead to debranched amylopectin include milling (Craig and Stark, 1984; Stark and Yin, 1986), extrusion cooking (Colonna et al., 1989) and heating in dry state (Colonna et al., 1987). A study by Sokhey and Chinnaswamy (1993) on maize starch with 0 and 25% amylose (100 and 75% amylopectin) content showed a four fold decrease in amylopectin fraction compared to only two fold decrease in amylopectin fraction of a 50% amylose (50% amylopectin) content starch irradiated at 30 kGy. The higher the amylopectin content the higher the debranching. Even chemical processing such as lintnerisation causes more debranching in amylopectin fractions than in amylose (Whistler and Daniel, 1990; Noah, Guillon, Bouchet, Buleon, Molis, Gratas and Champ, 1998). Hence, larger decreases in peak paste viscosity of porridges made from irradiate maize flour than in the viscosity of porridges made from irradiated bean flours were observed.

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

This research work has shown that irradiation at 2.5 kGy caused a small but significant increase in the *in vitro* starch digestibility of raw maize flour or porridges made from maize and bean flours compared to the untreated control samples. Irradiation increased the *in vitro* starch digestibility of raw maize starch at 2.5 kGy compared to the untreated. Irradiation also increased the starch digestibility of raw bean starch at 2.5 kGy compared to the control. However, the maximum *in vitro* starch digestibility was obtained at 2.5 kGy in both maize and bean flours. Similar results were reported by Ananthaswamy et al. (1970a; b). It has been suggested that the higher *in vitro* starch digestibility for amylose, amylopectin and starch isolated from wheat and irradiated at 2 kGy was due to the opening up the starch molecules to greater access by amylase enzymes by depolymerisation of amylopectin which resulted in increased solubility of starch in the flours (Sreenivasan, 1974; Roushdi et al., 1983; Rayas-Solis, 1987; Sokhey and Chinnaswamy, 1993). Soluble starch is more accessible to enzymes than insoluble starch

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(Manners, 1979; MacGregor and MacGregor, 1985). This is probably what happened to starch in maize and bean flours irradiated at 2.5 kGy.

Raw maize flour had higher *in vitro* starch digestibility than raw bean flour. The bean starch granules in bean flour are about 2-3 times larger than maize starch granules in maize flour as shown by the electron micrographs. The smaller maize starch granules had higher *in vitro* starch digestibility in raw flour than raw bean flour starch. The large size reduces the availability of inner starch molecules to the α-amylase *in vitro*. Franco, Preto and Ciacco (1992) working with maize and cassava starches, found that the smaller starch granules were more digestible than the larger granules, even for the same plant, probably due to their higher surface area to volume ratio. The small starch granules expose larger surface area to hydrolytic enzymes than the large starch granules (Abbas, Scheerens and Berry, 1986; Socorro et al., 1989; Franco et al., 1992; Srisuma, Ruengsaklrach and Uebersax, 1994). MacGregor and MacGregor (1985) also reported that smaller barley starch granules were more digestible than large barley starch granules. Potato starch which are larger than maize starch and exhibit B-type crystallinity under x-ray diffraction are less susceptible to amylase enzymes than maize granules (Ring et al., 1988; Gerard, Colonna, Buleon and Planchot, 2001). Similar inference can be made for the large bean starch granules (2-3 larger) which exhibit C-crystalline form under x-ray diffraction compared to the smaller maize starch granules which exhibit A crystalline form (Hoover and Manuel, 1996a).

Maize starch granules have higher porosity than dry bean starch granules (Huber and BeMiller, 1997) and this may also explain the higher digestibility of raw maize starch compared to raw bean starch because enzymes need pores to gain access into starch granules. Pores have been shown by scanning electron microscopy of isolated starch granules from maize, sorghum and millet (all members of the Panicoideae subfamily of the Poaceae family) on the granule surfaces (Hauber and BeMiller, 1992; Gallant et al., 1992; Helbert, Schulein and Henrissat, 1996; Otto, Baik and Chuchajowska, 1997) Fannon,. Kanenaga, Harada and Harada (1990) pointed out that the maize starch granules, which contain pores, are more susceptible to digestion by amylases than potato

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or legume starches which do not have pores. Observing irradiated maize and bean flours using scanning electron microscopy did not show any pores in either maize or bean flours since the starch granules were not isolated. The protein matrices probably blocked any pores that could have been on any of the surfaces of the starch granules.

It is also known that raw dry bean flour has α-amylase inhibitors while maize flour has very little (Reddy, Sathe and Salunkhe, 1989; Wursch, 1989). So far, α-amylase inhibitory activity has been detected in most cereals, including wheat, barley, rye, sorghum, maize, oats, and others, though at levels much lower than those reported for legumes (Garcia-Olmedo, Salcedo, Sanchez-Monge, Gomez, Royo and Carbonero, 1987) and this may also explain why raw maize flour had higher starch digestibility than the raw bean flour. Proteinaceous inhibitors of α-amylase and proteases are widely distributed in cereals, legumes and other plants and serve as chemical mechanisms to avoid predation by animals (Silano, 1987). The difference between the starch digestibility of raw maize and dry bean flours by α-amylase *in vitro* could therefore also have been caused by the difference in the α-amylase inhibitor contents of their raw flours.

Low amylose content starches are hydrolysed faster than high amylose starch (Goddard, Young and Marcus, 1984; O'Dea and Holm, 1985; Berry, I'Anson, Miles, Morris and Russell, 1988; Englyst, Kingman and Cummings, 1992; Xue, Newman and Newman, 1996). It has been shown that waxy maize hydrolyses faster than amylomaize (Calvert, Newman, El-Negoum and Eslick, 1981; O'Dea and Holm, 1985; Hoover and Manuel, 1996b). Normal maize has 25% amylose (Thomas and Atwell, 1999) while dry beans has 35% amylose (Hoover and Manuel, 1996a). Unlike in maize starch, the digestibility of various raw legume starch is negatively correlated with amylose content (Dreher et al., 1984; Abbas et al., 1986; Wursch, 1989). This may explain why raw normal maize flour showed a higher *in vitro* starch digestibility compared to raw bean flour.

Starches of high amylose content are more resistant to hydrolysis by α-amylase even after gelatinisation by cooking. High amylose starch granules remain unchanged even after heating at 100°C for several hours due to their strong intermolecular hydrogen bonds

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(Behall, Schofield, Yuhaniak and Canary, 1986; Faulks et al., 1989). Under ordinary cooking the starch granules of bean flours do not complete their gelatinisation cycle due to rigid cotyledon cell wall matrix (Calvert, Newman, El-Negoumy and Eslick, 1981; Snow and O'Dea, 1981; Wursch et al., 1986). The incomplete gelatinisation of the high amylose starch fraction content of porridge made from bean flour may explain their lower *in vitro* starch digestibility compared to maize porridges.

Both cooked maize porridges and bean porridges had higher *in vitro* starch digestiblity compared to their raw forms. This is probably due to the high crystallinity of the raw starch granules of plant storage organs (Englyst and Cummings, 1985). The starch in raw flour granules is less accessible to amylase enzymes than in the cooked porridges. In most food systems, the starch granule has to be disintegrated by gelatinisation for digestion to take place (Alpers, 1987). In this study irradiation increased the starch digestibility probably by debranching amylopectin, which resulted in increased starch solubility, hence, increased digestibility. Bhatty and MacGregor (1988) presented evidence that irradiation at 100 kGy damaged barley starch and this led to a decreased peak paste viscosity, reduced molecular weight and increased solubility. Hence, the increases in digestibility of starches in raw maize and bean flour at or below 2.5 kGy. High crystallinity in raw maize and bean starch granules probably may limit access by amylase *in vitro* at doses higher than 2.5 kGy. Lower starch digestibility in raw starch was reported for wheat, maize, cassava, sweet potatoes irradiated at 10 kGy compared to the control (Kume and Tamura, 1987) probably due to increases in their crystallinity.

Porridges made from irradiated maize flours had higher *in vitro* starch digestibility than porridges made from irradiated bean flours. Cooked legume starches are known to be less digestible by amylase enzymes (Jenkins, Wolever, Taylor, Barker, Fielden, Baldwin, Bowling, Newman, Jenkins and Goff, 1981; Socorro et al., 1989; Foster-Powell and Miller, 1995; Bravo, Siddhuraju and Saura-Calisto, 1998). In general, legume starches differ from cereal starches both in their chemical composition (higher amylose content) and in their granular structure (Doublier, 1987; Orford et al., 1987; Eliasson, 1988), which restricts their swelling and solubilisation capacity during cooking hence lower

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starch digestibility for their porridges (Bogracheva, Davydova, Genin and Hedley, 1995). The cooked legume starches also have higher tendency towards retrogradation and syneresis which leads to development of more resistant starch after cooking (Schweizer, Anderson, Langkilde, Reimann and Torsdottir, 1990; Tovar and Velasco, 1995; Tovar and Melito, 1996; Skarabanja, Liljeberg, Hedley, Kreft and Bjorck, 1999). This is probably the reason why porridges prepared from irradiated maize maize flour had higher *in vitro* starch digestibility than those prepared from irradiated bean flours.

The changes in *in vitro* starch digestibility were higher in porridges made from irradiated bean flour compared to the porridges made from irradiated maize flour. This may be due to the greater effects of irradiation on the larger bean starch granules compared to maize starch granules. Irradiation causes more chemical changes in large particles compared to small ones (Diehl and Scherz, 1975; Diehl, 1982; McManus, 1982; CAST, 1986; Elias, 1987).

What happens to starch at doses higher than 2.5 kGy? Answering this question may explain the observed reductions in *in vitro* starch digestibility of raw maize and bean flours irradiated at doses higher than 2.5 kGy and in porridges made from them. Reducing sugars produced from irradiated barley endosperm increased with irradiation dose between 0 and 3 kGy then declined (Lorenz, 1975). Sucrose accumulation in sweet potatoes increased with irradiation dose up to 2 kGy and then decreased at higher doses (Hayashi and Kawashima, 1982a; b). Malt extracts from irradiated barley increased between 0 - 2.5 kGy and then declined at higher doses (Koskel, Celik and Ozkara, 1998). Starch in apples was converted to reducing sugars by irradiation up to 2 kGy but this conversion decreased at higher doses (Kovaks and Keresztes, 1989).

It has been observed by other researchers that the effects of irradiation on food are basically the same as those exerted by thermal treatment of food (Wick et al., 1961; Josephson et al., 1979; Diehl, 1982; Kraybill, 1982; Nawar, 1983; Institute of Food Technologists, IFT, 1983; 1986; Thomas, 1988; Thayer, 1990; Diehl et al., 1991; Griffith, 1992; Stevenson, 1992). In fact, several technical reports indicate that

irradiation and thermal processing have similar effects on some chemical properties of food (United Kingdom Working Party, 1964; United States Army Medical Research and Development Command, 1977; IFT, 1983; 1986; Brynjolfsson, 1979; Colonna, Buleon and Mercier, 1987; Thorne, 1991; Murano, 1995; Diehl, 1995; WHO, 1999). To understand the effects of irradiation on starches of maize and bean flours at doses higher than 2.5 kGy it is appropriate to look at the effect of thermal treatments of food at temperatures higher than 100°C on the properties of starch.

Increase in *in vitro* starch digestibility under mild thermal treatment and decrease of *in vitro* starch digestibility under severe processing conditions has been reported in extrusion cooking (Cheftel, 1986; Holm et al., 1988; Asp and Bjorck, 1989) and the following hypotheses were proposed and tested for the reductions in starch digestibility: (a) non-enzymatic browning (Maillard reactions) may lower starch digestibility because they result in the production of inhibitors of amylase enzymes (Lee, Chichester and Lee, 1977; Chichester and Lee, 1981; Friedman, Grosjean and Zahnley, 1986); (b) transglucosidation in autoclaved wheat flour because they result in the introduction of β-bonds between some of the starch molecules (Siljestrom, Eliasson and Bjorck, 1989b); (c) debranching of amylopectin produces resistant starch in foods (Ring, Gee, Whittam, Orford and Johnson, 1988; Wasserman and Timpa, 1991; Politz, Timpa and Wasserman, 1994) in the form of short chain amylose molecules which retrograde (Mercier and Felliet, 1977; Theander and Westerlund, 1987; Chinnaswamy and Hanna, 1990; Ring et al., 1988) and (d) changes in crystallinity of amylopectin fractions of starches that may cause reduced degree of gelatinisation of starch such as those developed during annealing of starch (Lund, 1984; Cooke and Gidley, 1992) and during pressure cooking (Onwulata and Elchediak, 2000).

The above hypotheses were proposed and tested *in vitro* to determine how resistant starch is produced during thermal processing. The present study adopted some of these hypotheses to test if similar changes were taking place in starch of irradiated maize and bean flours. Maize and bean flours were irradiated at 0, 5, 10 kGy and also at 20 and

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40 kGy to determine the effects of irradiation on starch molecular properties that may explain the changes in starch digestibility.

6.3 Hypotheses for the probable causes of decreases in *in vitro* starch digestibility with irradiation doses above 2.5 kGy

It was hypothesised that Maillard reactions may be taking place during irradiation of maize and bean flours which may result in the production of compounds which may inhibit amylase enzymes (Kato, 1987; Friedman, 1996a). In the determination of changes in L, a and b values of irradiated maize and bean flours, it was observed that non-enzymatic browning reactions (Maillard reactions) were taking place in irradiated maize and bean flours. The changes in colour of irradiated maize and bean flours were dose dependent. The flours became more brown as the irradiation dose increased. Maillard reaction produces melanoidins that adsorb on the hydrolytic enzymes thus inhibiting them (Horikoshi and Gomyo, 1976) and these include α-amylase (Oste, Sjodin, Jagerstad, Bjorck and Dahlqvist, 1985). Maillard reactions may result in pH changes in food systems, which may also inhibit digestive enzymes (Lee, Pintauro and Chichester, 1982; Yaylayan and Forage, 1992; Carbodevella, Hill, Armstrong, De Souza and Mitchell, 1994; O'Brien, 1995). If the brown colour changes observed above were due to the Maillard reactions, then the observed reductions in starch digestibility could in part be due to inhibition of α-amylase by compounds such as N-nitrosodiethylamine and N-nitrosopyrrolidine which have been shown to inhibit lactase, sucrase and maltase in rats fed brown egg albumin (Adrian, 1974; Finot, Aeschbacher, Hurrell and Liardon, 1990). Maillard reactions also produce amino reductones which may inhibit α-amylases (Kato, 1987). Maillard reactions also result in the formation of ε-lysylpyrrolaldehyde (LPA) which has been found to inhibit α-amylase (Ohmura, Shinohara and Murakami, 1983; Oste, Sjodin et al., 1985; Oste, Miller, Sjostrom and Noren, 1987).

The browning reactions observed in irradiated maize and bean flours were not as a result of caramelisation reactions since caramelisation requires much more drastic thermal treatments such as heating pure potato, wheat, rye and maize starch at 200°C (Palasinski,

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Tomasik and Wiejak, 1985; 1986; Baczkowicz, Tomasik and Wiejak, 1986; Ohkuma, Matsuda, Katta and Hanno, 1990; Wurzburg, 1995; Keyhani and Yaylayan, 1996; Ajandouz and Puigserver, 1999).

Byun, Kwon, Cho, Kim and Yang (1993) and Byun, Kang and Mori (1995) observed development of brown colours when whole soybean flours were irradiated at 0-20 kGy. However, there were no colour changes when this team of researchers irradiated isolated soybean starch at a similar dose range (Kang, Byun, Yook, Lee and Chung, 1997; Kang, Byun, Yook, Bae, Lee, Kwon and Chung, 1999). The browning changes in irradiated cereal and legume flours did not occur in isolated starch, which suggests that an extra compound or compounds not present in isolated starch was required for the brown colour to develop and this suggests Maillard reactions. Irradiation has been reported to cause Maillard reactions (non-enzymatic brownings) in other foods like casein glucose/agar mixture at 50 kGy (Harmuth-Hoene, 1976), maize at 50 kGy (Roushdi, Harras, El-Meligi and Bassim, 1981), in green gram, lentil, horsebean and bengal gram at 5 kGy (Rao and Vakil, 1985), rice at 2.5-3 kGy (Ahmad, Hussain and Haider, 1974; Wang et al., 1983; Wootton et al., 1988) in beans at 2 kGy (Cunha et al., 1993) in dairy products irradiated at 40 kGy (Hashisaka, Einstein, Rasco, Hungate and Dong, 1990) and in pepperoni irradiated at 3 kGy (Johnson, Sebranek, Olson and Wiegand, 2000). Maillard reactions during irradiation of maize and bean flours could have led to production of inhibitors of α-amylase enzymes *in vitro*.

It was hypothesised that irradiation of maize and bean flours at doses higher than 2.5 kGy may have resulted in the production of indigestible β-bonded starch through transglucosidation. Beta bonds could have been produced within (intra) and/or between different (inter) starch molecules. Beta bonded starch was found in starches isolated from irradiated maize and bean flours. The β(1-3) and β(1-4) bonded starch contents were higher in starches isolated from irradiated bean flours than in those from irradiated maize flours. This could be due to the fact that irradiation produces more chemical changes in the (2-3 times) larger bean starch granules than in maize starch granules.

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Beta glucan formation in processed wheat flour starches have also been reported for those processed at low moisture content/high temperature (130-220°C, 2-20 h) (Theander and Westerlund, 1987, Siljestrom et al., 1989a). However, when the total starch content was determined by enzymatic methods using amylase, there were no significant reductions (Schweizer and Reimann, 1986; Siljestrom, Westerlund, Bjorck, Holm, Asp and Theander, 1986). Using Nuclear Magnetic Resonance (NMR) Techniques, Theander and Westerlund (1987) and Siljestrom et al. (1989a) reported that the amount of beta bonded starch in extruded flours varied beween 4 and 7% for flours extruded at 150-250°C for 2-20 h. Using NMR techniques it is possible to identify all the types of β-bonds formed including β(1-2), β(1-3), β(1-4) and β(1-6) by transglucosidation of starch during extrusion of starchy foods (Theander and Westerlund, 1987; Siljestrom et al., 1989a).The β-bonds formed in wheat starch heated at 180°C were mainly β (1-3) and β (1-4) bonds as confirmed by nuclear magnetic resonance studies (Siljestrom et al., 1989a). The β-glucan content of the wheat flour heated at 180°C increased with heating time while the β-bonded starch content of extruded wheat flour and wheat starch increased with the extrusion temperature (Theander and Westerlund, 1987).

Similar reactions take place in starch of irradiated maize and bean flours. Transglucosidation has also been reported for maize and wheat starch irradiated at 3-5 kGy (Whistler and Ingle, 1965; Scherz, 1974; Roushdi et al., 1983) and in pentosans isolated from wheat and that were irradiated at 3 kGy (Grant and D'Appolonia, 1991).

It was hypothesised that irradiation altered the crystallinity of the amylopectin fractions of starch by debranching and depolymerisation to reduce their degree of gelatinisation and hence, the starch digestibility. The degree of gelatinisation of starch is a function of the amylopectin chain length (Krueger et al., 1987). Irradiation of maize and bean flours caused changes in the thermal properties of their starch as determined by DSC. This suggests changes in the crystallinity of amylopectin fraction, which could lead to higher enthalpy of gelatinisation. Higher endothermic enthalpy of gelatinisation, as was shown with DSC results for irradiated bean flours, implies reduced degree of gelatinisation at the same temperature and could lead to reduced starch digestibility (Lund, 1984; Holm et al.,

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1988; Shi and Seib, 1992). The may explain the reductions in starch digestibility of maize and bean flours irradiated at doses higher than 2.5 kGy and even the starch digestibility of porridges made from them.

Irradiation also increased the gelatinisation temperature by small but significant amounts in both maize and bean flours. This is contrary to the findings of Rao and Vakil (1985) who reported a decrease in gelatinisation temperature of irradiated green gram, lentil, horsegram and bengalgram flours as determined by Brabender amylograph. The sensitivity of the DSC to changes in gelatinisation temperature and that of Brabender amylograph may explain the differences. The gelatinisation temperature of waxy starches of the same botanical origin increases with the increase in the x-ray crystallinity of the starches as was found with six waxy rice starches (Tester and Morrison, 1990) and with two varieties of waxy starches each of rice, maize and barley (Shi and Seib, 1992).

From the DSC results maize flour, with higher amylopectin content, had greater increases in the gelatinisation temperature than the bean flour. Gelatinisation is a function of amylopectin content (Cooke and Gidley, 1992) and irradiation has more effect on the amylopectin fraction of starch than on the amylose fraction as shown by the HPLCSEC results (Sokhey and Hanna, 1993). Waxy starches are known to display larger changes in endothermic gelatinisation enthalpy during thermal processing compared to normal starches (Cooke and Gidley, 1992; Shi and Seib, 1992; Hoover and Manuel, 1996b). Starches with higher endothermic gelatinisation enthalpy require more energy to gelatinise fully during cooking (Johnson, Hardy, Baumel and White, 2001). Differences in the amylopectin contents of maize and bean flours may explain the observed reductions in the *in vitro* starch digestibility of their raw flours and of porridges made from these flours.

In physical treatments, Mercier and Felliet (1977), reported that starch may be solubilised without formation of maltodextrins during extrusion which implies that debranching of amylopectin is the main chemical change during extrusion as determined by size exclusion chromatography (Colonna et al., 1989; Lai and Kokini, 1991). There is a

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greater tendency to debranch amylopectin during irradiation than depolymerisation as shown by the difference in increases in reducing sugars compared to the decrease in the amylopectin content (Sokhey and Hanna, 1993). Other physical processes such as annealing, heat-moisture treatment or heating flour at low moisture content for several hours above gelatinisation temperature (Hoover and Manuel, 1996a; b), high pressure treatment (Hibi, Matsumoto and Hagiwara, 1993; Williams, 1994; Onwulata and Elchediak, 2000) and extrusion (Myllimaki, Eerikanen, Suorti, Forsell, Linko and Pourtanen, 1997) alter the crystallinity of amylopectin fraction of starch. This reduces the swelling power (viscosity) of pastes by breaking down the amylopectin fraction of starch. Starch with higher crystallinity behaves like RS_2 or raw starch. (Gerard et al., 2001). All these physical processes alter starch digestibility by their effects on amylopectin fractions. From the DSC results, it is probable that irradiation increased the crystallinity of amylopectin fractions of maize and bean flours and this could have led to the observed reductions in *in vitro* starch digestibility.

In the present study, it was found that irradiation caused an increase in gelatinisation onset temperature (T_o), increase in peak temperature (T_p) and a decrease in offset temperature (end of gelatinisation, T_c). The gelatinisation enthalpy, and the peak height index (ratio of gelatinisation enthalpy to the difference between T_o and T_p) were increased, especially for the bean flour. These changes may affect the degree of gelatinisation and this may lead to decreased starch digestibility. The DSC patterns given by the irradiated maize and bean flours between 25 and 115°C reflected changes in the integrity of amylopectin fractions in the starches of these flours and may explain the changes in starch digestibility with irradiation dose.

The first thermogram of irradiated bean flour starches showed increases in the gelatinisation temperature and decreases in the range between T_p and T_o with increases in irradiation dose. This type of change indicates a decrease in the degree of gelatinisation and could lead to decreased starch digestibility (Lund, 1984; Holm et al., 1988; Jane et al., 1999). However, the changes in gelatinisation properties of amylopectin fraction of starch in maize flour with the increase in irradiation dose were not as clear as changes in

thermal properties of bean amylopectin and may not be used to explain the decrease in digestibility with the increase in irradiation dose. Waxy maize starch (>95% amylopectin) has higher peak paste viscosity than amylomaize VII starch (30% amylopectin) under the same test conditions (Hoover and Manuel, 1996b).

The temperature of onset of gelatinisation and peak gelatinisation temperatures for irradiated maize flour showed both decreases and increases between treatments. The peak height indices (PHI) (the ratio of gelatinisation enthalpy to the difference between T_p and T_o) ($\Delta H/T_p-T_o$) (Krueger et al., 1987) for bean flour starches showed a more clear pattern than for maize. The PHI for bean flour starch increased with irradiation dose and this suggests reduced degree of gelatinisation but it is difficult to conclude the effects of irradiation on starch digestibility of maize flour based on the present study using DSC.

The observed reductions in starch digestibility of porridges made from maize and bean flours irradiated at above 2.5 kGy could also be due to increased crystallinity of amylopectin molecules due to their branches being shortened by irradiation. In starch granules, crystallinity is attributed to the ordered arrangements in adjacent double helices of amylopectin branches (Jane, Kasemsuwan, Leas, Zobel and Robyt, 1994) and is increased by the reduction in the average DP_n of the branch chains (Hizukuri, 1985; McPherson and Jane, 2000). Irradiation of maize and bean flours caused debranching of their amylopectin fractions as shown in the data from HPLCSEC. Debranching amylopectin leads to higher crystallinity as shown with DSC results. Higher crystallinity in starches of irradiated maize and bean flour leads to reduced degree of gelatinisation of starch thus to reduced starch digestibility (Lund, 1984; Holm et al., 1988).

Different baked products made from wheat, water and some sugar exhibit different degrees of gelatinisation due to a_w and this was observed as the amount of granule collapse in samples of these products when examined by scanning electron microscope (Hoseney, Atwell and Lineback, 1977). But in the present study, flours and porridges used were of same moisture content so the only varying factors, which might have caused

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reduction in starch digestibility, were those introduced by the different doses of irradiation.

Decrease in the degree of gelatinisation is shown on the DSC thermogram as a reduction in the peak size (Ndife, Sumnu and Bayindirh, 1998) and has been attributed to an increase in degree of amylopectin crystallinity by Ciesla et al. (1991a; 1992). Increased crystallinity as measured using x-ray diffraction techniques in amylopectin fraction of irradiated foodstuffs have been reported (McArthur and D'Appolonia, 1984; Wootton, Djojonegoro and Driscoll, 1988; Sokhey and Chinnaswamy, 1993; Ciesla, Gwardys and Mogilevski, 1991a; Ciesla, Zoltowski and Mogilevski, 1991b; Ciesla, Zoltowski and Diduszko, 1993; Thakur and Singh, 1993).

The second endothermic enthalpy found in the first heating thermogram for maize bean flours disappeared after treatment at 10 kGy and was higher for bean flour while in maize flour it was persistent for all the irradiation treatments. This could be due to the high intrinsic lipid content of maize flour starch compared to the intrinsic lipid content of bean flour starch (Thomas and Atwell, 1999). Whistler and BeMiller (1997) reported, that "only cereal starches contain significant amount of intrinsic lipids." Intrinsic lipid content of starch isolated from normal maize is about 0.75% (w/w) (Vasanthan and Hoover, 1992), while starch isolated from bean flour has only about 0.15% lipids (w/w) (Hoover and Manuel, 1996a).

The higher lipid content of maize starch could have protected the amylose/lipid complex in maize flour starch during irradiation, hence, the persistence of the second endogenous enthalpy from 0 to 40 kGy. It has been pointed out in the literature that in a complex food system consisting of protein, lipids and carbohydrates, these organic compounds tend to protect each other from damage by ionising radiation (Phillips, 1972; Diehl et al., 1978). This may explain why higher intrinsic lipid content of maize flour starch led to greater protection of amylose/lipid content of maize flour starch than bean flour starch amylose/lipid complexes. The complexes formed between amylose and lipids in starch during processing may reduce the starch digestibility (Holm, Bjorck, Ostrowska,

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Eliasson, Asp, Larsson and Lundqvist, 1983). Some of the reductions in the starch digestibility observed in irradiated maize flour could have been due the high intrinsic lipid content.

Normally the pregelatinised starch in maize and bean porridges should have shown endothermic melting enthalpy for the retrograded starch in the second thermogram. Retrograded amylopectin fraction of starch gives an endothermic melting enthalpy at 60-70°C (Tester and Morrison, 1990) while retrograded amylose shows endothermic melting enthalpy at 150-170°C (Sievert and Wursch, 1993). The endothermic melting enthalpy of retrograded amylopectin may not appear within one day of cooling (Shi and Seib, 1992; Yuan et al., 1993; Bello-Perez and Paredez-Lopez, 1995; Liu and Thompson, 1998) and reaches a maximum after about 10 days as was reported for maize starch by Fisher and Thompson (1997). This explains why there was no endothermic melting enthalpy in the second DSC thermograms of irradiated maize and bean porridges. The tests were performed in less than a day, during which amylopectin would not have retrograded and the temperature range used could not have shown an endothermic melting enthalpy. The difference between amylopectin and amylose is that retrograded amylopectin melts at 60-70°C while retrograded amylose only melt at 150°C or more (Miles, Morris, Orford and Ring, 1985; Westrate and van Amelsvoort, 1994). The temperature range used in this study reached a maximum of only 115°C thus it could not have determined the endothermic melting enthalpy of retrograded amylose.

It was hypothesised that irradiation may alter the molecular distribution by producing more of the indigestible short chain amylose molecules or oligosaccharides from debranched amylopectin. The molecular weight for the largest starch fraction in maize flour starch was 4×10^6 Da (amylopectin). This is comparable to 10-30 $\times$ 10^6 Da reported for maize starch in the literature (Jackson, Chotto-Owen, Waniska and Rooney, 1988; Jackson, Gomez, Waniska and Rooney, 1990; Wasserman and Timpa, 1991; Politz et al., 1994). The intermediate fraction had a molecular weight of 4×10^5 Da. The third fraction was 2×10^5 Da (presumed amylose). This is lower than the molecular weight of amylose reported by Takeda, Shitaozono and Hizukuri (1988) and by Takeda, Takeda and

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Hizukuri (1989) as 1.2-4.1×10^6 Da. The smallest fraction (presumed water-soluble oligosaccarides) had a molecular weight of 2×10^3 Da.

The largest fraction of bean starch had a molecular weight of 3×10^6 Da (amylopectin) which is lower than 10^7 Da reported in the literature (Banks, Greenwood and Muir, 1975; Lii and Chang, 1981; Vidal-Valverde and Frias, 1992; Frias, Fornal, Ring and Vidal-Valverde, 1998). The intermediate molecular weight fraction was 4×10^5 Da. The third fraction had a molecular weight of 4×10^4 Da (presumed amylose) and the smallest fraction was 1×10^3 Da (presumed water-soluble oligosaccharides). The HPLCSEC results indicated that maize and bean starches had similar molecular weight amylopectin fraction. The intermediate fractions of starch (second peak) were similar in molecular weight for maize and bean starches. The third fraction, presumably amylose, was slightly larger in maize starch than in bean starch. The fourth fraction (presumably short chain amylose fractions and water-soluble maltooligosaccharides) had a higher average molecular weight in maize than in bean starches. Short chain amylose molecules of molecular weight 10^3-10^6 Da were obtained by Sokhey and Chinnaswamy (1992) from waxy maize starch with 0% amylose content irradiated at 30 kGy which suggests that the amylopectin fraction of starch was being debranched.

The HPLCSEC results with starch fractions of irradiated maize and bean flours show the effects of irradiation on the amylopectin fractions and the effects of irradiation on the three lower molecular weight starch fractions as has been reported in other processing techniques on flours (Colonna et al., 1992; Sokhey and Chinnaswamy, 1992; Sokhey and Hanna, 1993). Lasekan and Lasekan (2000) also reported these four fractions when they extracted starch from popped sorghum and from malted sorghum by solubilisation using dimethyl sulphoxide. Amylopectin with an average molecular weight of 10^8-10^9 Da, is much larger than amylose fraction whose average molecular weight is only 5 x 10^6 Da (Whistler and BeMiller, 1997; Thomas and Atwell, 1999). More effect also takes place with extrusion in amylopectin than amylose (Wasserman and Timpa, 1991; Politz et al., 1994) due to its larger size.

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Depolymerisation or debranching of amylopectin fraction of starch from irradiated maize and bean flours increased with irradiation dose as indicated in the HPLCSEC results. Debranching of amylopectin has been reported for wheat starch extruded at temperatures lower than 80°C without any increase in the alcohol soluble oligosaccharides (formed from depolymerised starch) (Mercier and Felliet, 1977). Some food processing techniques such as grinding favour debranching instead of depolymerisation (Lund, 1984; Colonna et al., 1992). In the present study and HPLCSEC results indicate that both debranching which leads to increased crystallinity from DSC results and depolymerisation which leads to increased content of short chain amylose molecules took place from HPLCSEC results.

The oligosaccharides and short chain amylose molecules formed from debranched amylopectin fractions of irradiated maize and bean flour starches have been shown by other researchers to give endothermic enthalpy only after attaining the temperature higher than 125°C (Sievert and Pomeranz, 1991; Sievert and Wursch, 1993; Lin, Czuchajowska and Pomeranz, 1994; Westrate and van Amelsvoort, 1994). This has been shown to be the case for heat-treated starches or irradiated starches which depolymerise amylopectin fraction while producing more indigestible short chain water-soluble oligosaccharides (Rayas-Solis, 1987; Sokhey and Chinnaswamy, 1993). More depolymerisation of amylopectin fractions than the amylose fractions occur during irradiation of starchy foods (Roushdi et al., 1983; Urbain, 1986; Sokhey and Chinnaswamy, 1993).

The mechanism of resistant starch formation was proposed by Eerlingen, Jacobs, van Win and Delcour (1996), to be recrystallisation of straight chain amylose molecules by hydrogen bond formation, which produces micelles and lamellar structures by chain folding (retrogradation) and these lead to reduced accessibility by amylase enzymes. Debranched amylopectin fractions behave like straight chain amylose molecules and are resistant to digestion by α-amylase enzymes *in vivo* and *in vitro*. If irradiation of maize and bean flours caused debranching and depolymerisation of amylopectin fractions then the observed reductions in starch digestibility may partly be due to production of resistant

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starch at doses above 2.5 kGy. Some resistant starch may also have been produced from the β-bonded starch.

From the data presented on starch digestibility for maize and bean porridges respectively, it could be deduced that irradiation of maize and bean flours generated resistant starch. Resistant starch in infant foods may cause excessive diarrhoea because infants do not have sufficient microflora to ferment it (Siljestrom and Bjorck, 1990) and may result in growth retardation (Wursch, 1989). Resistant starch (RS_3), is mainly retrograded amylose and short chain oligosaccharides formed from debranched amylopectin molecules (DP_n 15-45) (Englyst et al., 1992; Botham et al., 1997). This may explain the decrease in starch digestibility in both raw and cooked maize and bean flours irradiated at doses above 2.5 kGy where more debranching of amylopectin molecules is likely to occur.

The most probable hypotheses to explain the reductions in raw and cooked starch digestibility of maize and bean flours irradiated above 2.5 kGy may be deduced from the effects observed by HPLCSEC, DSC thermograms and formation of β-bonded starch. The HPLCSEC indicates that there were large changes in contents of amylopectin fraction of both maize and bean starch. This confirms debranching and formation of highly retrogradable short chain amylose molecules from debranched amylopectin. DSC results indicate increased crystallinity in irradiated maize and bean flours. This probably led to reduced degree of gelatinisation that caused reduced starch digestibility *in vitro*. The formation of β-bonded starch in irradiated maize and bean flours could also have caused development of resistant starch since β-bonded starch is indigestible by α-amylase (Theander and Westerlund, 1987; Siljestrom et al., 1989a; Tsuji and Gordon, 1998). All these changes in molecular properties of starch occur in irradiated maize and bean flours simultaneously and may also be be interdependent.

If β-bonded starch in irradiated foods behaves like β-glucans of barley or rye flour then irradiation processing of weaning foods may have some undesirable effects on infants and children (Wood, 1992). The poor starch and protein digestibility of barley and rye

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for young chicks, for example, has been attributed to viscous non-starchy polysaccharides called β-glucans (Antoniou, Marquadt and Cansfield, 1981). The true metabolisable energy (TME) of cereals used as feed in non-ruminants is reduced by fibre (Wood, Weisz, Fedec and Burrows, 1989). Beta glucans have been reported to reduce the metabolisable energy (ME) in barley fed to pigs (monogastric mammals) compared to sorghum, maize and wheat at the same starch intake level (Klopfenstein, 1988) and may have similar impact on children fed porridges made from irradiated maize and bean flours. They impair ME uptake by increasing the viscosity of digesta and adsorption of hydrolytic enzymes (Klopfenstein, 1988) and may lead to reduced growth rate in young non-ruminants including infants and children (Wood, Anderson, Braaten, Cave, Scott and Vachon, 1989). However, at 10 kGy irradiation may lead to a reduction of only 3% in starch digestibility of porridge made from maize flour compared to an increase of 15% in the total solids content of the porridges due to reductions in viscosity as a result of irradiation.

6.4 Effects of irradiation on *in vitro* protein digestibility of porridges made from maize and bean flours

Irradiation between 0 and 10 kGy caused very small but statistically significant ($p \leq 0.05$) changes in protein digestibility of maize and bean porridges as determined by pepsin assay and multi-enzyme assay. The protein digestibility tended to fluctuate in an inconsistent manner that did not clearly give the effects of irradiation with a dose dependent trend in both assays. The values obtained from the pepsin assay were lower than those obtained from the multi-enzyme methods. Similar high and low values for protein digestibility of hull-less barley assayed by pepsin and multi-enzyme methods respectively, have been reported (Bhatty and MacGregor, 1988; Gomez, Waniska, Rooney and Lusas, 1988). It has been reported that there is more correlation between thermal processing and pepsin digestibility of sorghum protein (Gomez et al., 1988) and proteins of cooked barley (Bhatty and Whittaker, 1987) than with the multi-enzyme method.

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Cooked maize porridge proteins had higher digestibility by pepsin assay than cooked bean porridge proteins. Digestibility of dry bean proteins varies greatly (50-80%) but is always less than that of cereal proteins (70-90%) and animal proteins (80-85%) (Serna-Saldivar, Knabe, Rooney and Tanksley, 1987; FAO/WHO, 1990; Marero, Payumo, Aguinaldo, Matsumoto and Homma, 1991; Joseph and Swanson, 1993). Phaseolin, the major storage protein of dry beans, constituting about half the total seed protein, is resistant to digestion by proteolytic enzymes probably due to the compact nature of its structural characteristics (Nielsen, Deshpande, Hermodson and Scott, 1988; Moneam, 1990; Lanfer-Marquez and Lajolo, 1991).

Phaseolin is also reported to be resistant to proteolysis due to the stability of its tertiary structure imparted by an attached glycoprotein (Kohnhorst, Smith, Uebersax and Bennink, 1990; Ahn, Sen and Whittaker, 1991; Begbie and Ross, 1993). The values found in this study might be different from the reported maize and bean protein digestibility because some of those studies were done with whole maize and whole bean flours while the present study used degermed and dehulled maize flours and dehulled bean flours.

Dry beans contain considerable amounts of tannins (up to 0.93%) (Deshpande, Cheryan and Salunkhe, 1986). The beans used in this study were dehulled after soaking which might have allowed some water-soluble tannins to diffuse into the cotyledons. Tannins are known to bind either enzymes or substrate proteins and reduce protein digestibility (Aw and Swanson, 1993; Fish and Thompson, 1991). Dehulled maize flour has no tannins (Wursch, 1989) and the observed differences in protein digestibility of irradiated maize and bean porridge could be due to tannins. However, by the multi-enzyme assay, there were no significant differences in protein digestibility of porridges made from irradiated maize or bean flour. Higher protein digestibility *in vitro* was reported for beef, pork, turkey and evaporated milk irradiated at 18.6 kGy compared to thermal treatment at 116°C for 114 min by Shefner et al. (1957). Srinivas et al. (1975) and Nene, Vakil and Sreenivasan (1975) found increased increased protein digestibility in dehydrated shrimp

irradiated at 3 kGy and red gram beans irradiated at 10-30 kGy using *in vitro* pepsin assay.

Irradiation had very little effect on protein digestibility compared to starch digestibility of porridges prepared from maize and beans. This is because of the low molecular weight of maize and bean proteins compared to the molecular weight of amylopectin. The largest maize proteins have a molecular weight of 10^5 Da (Landry, Paulis and Fey, 1983; Essen, 1986; Shewry, 1995; Landry, 1997). The largest storage protein of dry beans, phaseolin, is made up of three fractions of molecular weight less than 10^5 Da (Ahn et al., 1991; Carbonaro, Marletta and Carnovalle, 1992; Marcone, 1999). Large molecules are chemically affected more than the small molecules by ionising radiations (MacManus, 1982; Elias, 1987). Soybean flour irradiated at 0-20 kGy showed no changes in protein patterns under SDS-PAGE electrophoresis (Byun, 1995). Chiou, Shyu and Tsai (1991) also found no change in the SDS-PAGE pattern of proteins in peanuts irradiated between 0-20 kGy. Pure solutions of animal proteins showed changes in SDS-PAGE patterns at doses as low as 5 kGy (Krumar and Berry, 1990).

The findings reported above suggest that in flours, the proteins are protected from the effects of irradiation by amylopectin. Working with isolated wheat gluten and gliadin, Srinivas, Ananthaswamy, Vakil and Sreenivasan (1972) found that irradiation at doses between 0 and 2 kGy increased their susceptibility to papain which suggests that the absence of protective high molecular weight amylopectin resulted in more exposure of hydrolytic sites in isolated wheat proteins.

Unchanged amino acid profiles were reported by Nene et al. (1975) for red grams irradiated at 30 kGy, and by Srinivas et al. (1975) for wheat and gluten irradiated at 10 kGy. However, Doguchi (1969) found a 6% decrease in amino acids in irradiated wheat gluten at 100 kGy. Destruction of amino acids in collagen was reported by Bowes and Moss (1962) at 500 kGy which is fifty times higher than the highest doses used in the present study with irradiated maize and bean flours.

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Ionising radiation can affect protein by promoting reactions such as deamination, cleavage of peptide and disulphide bonds and by addition to aromatic and heterocyclic residues (Urbain, 1977; Simic, 1978). However, all the reactions discussed above are influenced by pH, hydration state and temperature during irradiation (Simic, 1978; Taub, Robbins, Simic, Walker and Wierbicki, 1979). Masuda, Koseki, Yasumoto and Kitabatake (2000) found no change in the protein content of lyophilised egg albumin powder at irradiation doses up to 20 kGy though they reported greater changes at higher doses. Van Der Stichelen Rogier (1974) using SDS-PAGE electorphoresis even observed that there were more changes caused to egg proteins by freezing than by irradiation at 5-10 kGy. Some of the conventional techniques used to detect changes in proteins digestibility are not sensitive enough to bring out some subtle effects of irradiation on proteins. Ciesla, Roos and Gluszewski (2000) were able to detect changes caused in dry bovine γ-globulins, dry bovine α-globulins, globulins of egg white and bovine haemoglobin caused by irradiation at doses as low as 2.5 kGy using DSC. It is possible that the pepsin and multi-enzyme assays were not sensitive enough to show the effects of irradiation on maize and bean flour proteins at 0-10 kGy .

The report of a Joint FAO/WHO Expert Consultation on determination of protein digestibility (FAO/WHO, 1990) recommended the multi-enzyme assay procedure of Hsu et al. (1977). This method works on the principle that during proteolysis, protons are released from the cleaved peptide bonds and these reduces the pH of the food-enzyme mixture (Boisen and Eggum, 1991). However, the changes in pH that determine the protein digestibility may affect the enzyme activity. Enzymes perform best at an optimum pH and not over a whole range of pH (Pedersen and Eggum, 1983; Rasco, 1994). Some proteins have a high buffering capacity such as animal proteins and these may not respond well in the pH drop determinations (Moughan, Schrama, Skilton and Smith, 1989; Rasco, 1994). Using the multi-enzyme method, including corrections for proteins of high buffering capacity, Pedersen and Eggum (1981) estimated protein digestibility of 61 feed and food protein products and found a very high positive correlation with *in vivo* (rat) true protein digestibility ($r=0.9$, $p=0.001$). However, other reports indicate poor correlation between the multi-enzyme assay and digestibility in vivo

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(Wolzak, Bressani and Gomez-Brenez, 1981; Moughan et al., 1989; Rasco, 1994). Pepsin method might be the best for cereals but in the high fiber content legumes, it is recommended that multi-enzyme systems be used (Lyons and Walsh, 1993).

Minor differences in protein digestibility due to different processing conditions may be so small that pepsin and multi-enzyme assays may not be able to pick out the differences (Swaisgood and Cattignani, 1991). Wu, Williams, Kunkel, Acton, Wardlaw, Huang and Grimes (1994) determined the effects of autoclaving at 121°C for between 10 and 90 min on protein digestibility *in vitro* using the multi-enzyme technique of Hsu et al., (1977). The differences were so small that they ended up using the more sensitive available lysine method. A similar difficulty in determining protein digestibility of processed foods was reported by Srivastav, Das and Prasad (1990). They roasted bengal gram, maize and soybean at 0, 180, 215 and 250 °C for 1.5, 2.0 and 2.5 minutes, ground the food items into a powder and determined the protein digestibility *in vitro* using multi-enzyme method of Hsu et al. (1977).

Irradiation of maize and bean flours at doses up to 10 kGy may result in changes in protein digestibility which are too small to be detectable *in vitro* using either pepsin or multi-enzyme assays. It is a common practice in the food industry to use available lysine to monitor small changes in protein digestibility that may be caused by different process parameters such as heat, time, pressure, e.t.c. (Hurrell, 1984; Fernadez-Artigas et al., 1999; Morales et al., 2000).

Lysine availability and *in vitro* protein digestibility are used interchangeably in food industry to monitor effects of process on protein digestibility (Banga, Alonzo, Gallard and Perez-Martin, 1992; Evangelisti, Calcagno and Zunin, 1994). Lysine amino acid is the most reactive amino acid due its free amino group at the ε-amino side chain. There is a linear relationship between loss of available lysine and loss in digestibility (Sarwar, Peace and Botting, 1988; Chung et al., 1986; Eggum, Brunsgaard and Jensen, 1995; Emmert and Baker, 1995; Mohammed, Hill and Mitchell, 2000). The measurement of nutritionally available lysine is a reliable indicator of amino acid bioavailability (Sarwar

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et al., 1988) and protein digestibility (Chung et al., 1986) and has been used to assess the effects of extrusion cooking on the protein digestibility of extruded wheat flours (Asp and Bjorck, 1989). It may prove more usefull in future work to determine the effects of irradiation in the range 0 to 10 kGy by using available lysine method because it is more sensitive.

The potential of increasing the total solids content of weaning porridges made from starchy maize and beans to meet the energy and protein requirements of infants and children using irradiation technology has been confirmed in the present study. Protein fractions of maize and bean porridges are largely unaffected by irradiation at doses between 0-10 kGy. However, the small but statistically significant changes in starch digestibility of porridges must be considered in deciding appropriate doses for irradiating maize, beans and their 70/30 maize:bean composite flours.

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CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

Gamma irradiation significantly ($p \leq 0.05$) reduces the viscosity of porridges made from maize flour, bean flour and their 70/30 maize:bean composite flour. The viscosity reductions in the porridges are dose dependent with greater decreases at higher doses. This is probably due to depolymerisation and debranching of amylopectin molecules.

Maize flour requires an irradiation dose of at least 7.5 kGy to prepare porridge of total solids content of 20%, with a consistency of between 1000-3000 cP at a setback temperature of 40-50°C. For the porridge of the same total solids content, bean flour needs at least a minimum dose of 5 kGy to give a consistency of 1000-3000 cP at 30-50°C. For the porridge to have a viscosity of 1000-3000 cP and total solids content of 20% at 40-50°C the 70/30 maize:bean composite flour needs to be irradiated at 7.5 kGy. Porridge made from irradiated maize flour exhibits a higher viscosity than porridge made from irradiated bean flour at the same dose level. When flour is heated in water, the resistance to flow (viscosity) is caused by swelling of amylopectin molecules and maize flour with higher amylopectin content swells more than the bean flours.

When viewed by the scanning electron microscopy, there are no visible changes in the size and shape of both maize and bean flour starch granules due to irradiation up to 10 kGy. Bean starch granules are about two to three times as large as the maize starch granules and this may explain the differences observed in effects of irradiation on some of the molecular properties of maize and bean starch isolated from the treated flours.

While the effects of irradiation on porridge viscosity are rather clear its effects on starch digestibility are not. Irradiated maize and bean flours had a maximum *in vitro* starch digestibility at 2.5 kGy. At doses above 2.5 kGy, there were significant decreases in *in vitro* starch digestibility in both raw flours and porridges made from maize and bean flours compared with the control. However, in raw flours and porridges made from

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irradiated bean flours, the *in vitro* starch digestibility was higher than the control at 2.5, 5.0 and 7.5 kGy and lower than the control only at 10 kGy. Irradiation at 2.5 kGy caused an increase of 3.2% in starch digestibility of porridge made from maize flour compared to the control and a decrease of 2.8% at 10 kGy compared to the control. In porridge made from bean flour, irradiation caused an increase in starch digestibility of 8.8% at 2.5 kGy and a decrease of 2.1% at 10 kGy compared to the control.

Porridges made from irradiated bean flour have higher increases in starch digestibility than porridges made from irradiated maize flour at 2.5 kGy and this is due to differences in starch granule sizes. Overall, porridges made from irradiated maize flour have higher starch digestibility than the porridges made from irradiated bean flour and this is probably due to differences in amylose fraction content of starch of maize and bean flours.

At 2.5 kGy, irradiation results in increased starch digestibility in raw and cooked maize and bean flours due to the opening up of starch molecules by depolymerisation in general and debranching of amylopectin molecules in particular (Figure 26). Irradiation at the same dose has more effect on larger particles and bean starch granules are about 2-3 times larger than maize starch granules.

Models showing the possible effects of irradiation on properties of starch that may explain the changes observed in starch digestibility are summarised in Figures 25, 26 and 27.

Irradiation causes Maillard reactions in both maize and bean flours as indicated by colour changes and this may lead to reductions in starch digestibility due to the production of α-amylase inhibitors (Figure 25).

Irradiation results in the development of β-bonded starch possibly through transglucosidation and this increases with the dose of irradiation (Figure 27). It was observed that the reducing sugar contents of porridges made from irradiated flour

hydrolysed using endo (1-3) (1-4) β-D-glucanase (lichenase) enzyme in combination with α-amylase were higher compared to the reducing sugar content released by irradiated starch treated with α-amylase alone. More β-bonded starch is produced in bean flour (0.75%) than in maize flour (0.45%) at 10 kGy, probably due to the large size of bean starch granules compared to maize starch granules. Beta-bonded starch is only partially digestible by α-amylases and behaves like resistant starch.

Irradiation causes debranching of amylopectin fractions of starch molecules. Debranched amylopectin molecules exhibit increased crystallinity of starch in maize and bean flours. Increased crystallinity results in decreased degree of gelatinisation in porridges made from these flours which results in reduced starch digestibility (Figure 27). HPLCSEC data indicates that irradiation caused debranching of amylopectin (Figure 27). Debranched amylopectin also results in the production of short chain amylose molecules, which are resistant to hydrolysis by α-amylases *in vitro*.

The most likely causes of decreased starch digestibility of maize and bean flours *in vitro* are increased crystallinity of amylopectin caused by debranching. Increased crystallinity results in higher enthalpy of gelatinisation and higher temperature of gelatinisation, both of which results in reduced degree of gelatinisation and this may cause reductions in starch digestibility *in vitro*. Depolymerisation and/or debranching of amylopectin produces highly retrogradable short chain amylose molecules and this may also lead to decreased starch digestibility. Formation of β-bonded starch in flours of maize and beans during irradiation is another cause of decreased starch digestibility.

Irradiation at 0-10 kGy has very little effect on protein digestibility probably due to the protection by the large molecular weight amylopectin fractions in the starch. Larger amylopectyin fractions of starch are affected more by irradiation than the smaller molecular weight components of maize and bean flours.

Irradiation processing offers a high potential for use in increasing total solids content of porridges made from starchy cereals and legumes due to its effects of debranching and

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depolymerisation of starch in general and debranching of amylopectin in particular. There are some small but significant reductions in starch digestibility of porridges at 10 kGy (2.8% for maize and 2.1% for bean flours) but these can be outweighed by the ability to prepare porridges of higher total solids content with acceptable consistency.

Further research using other more advanced techniques such as X-ray diffraction, nuclear magnetic resonance, near infra-red spectroscopy and Raman spectroscopy may be used in future to elucidate the exact reactions that take place in cereal and legume flours during irradiation. Differential scanning calorimetry is reported as able to bring out very small changes in the molecular properties of proteins in irradiated foods. More work should be done using DSC to determine molecular changes in proteins during irradiation of different foods. The safety of irradiated foods for use as weaning foods may have to be studied further using weanling rats to clarify the effects of resistant starch formed during irradiation.

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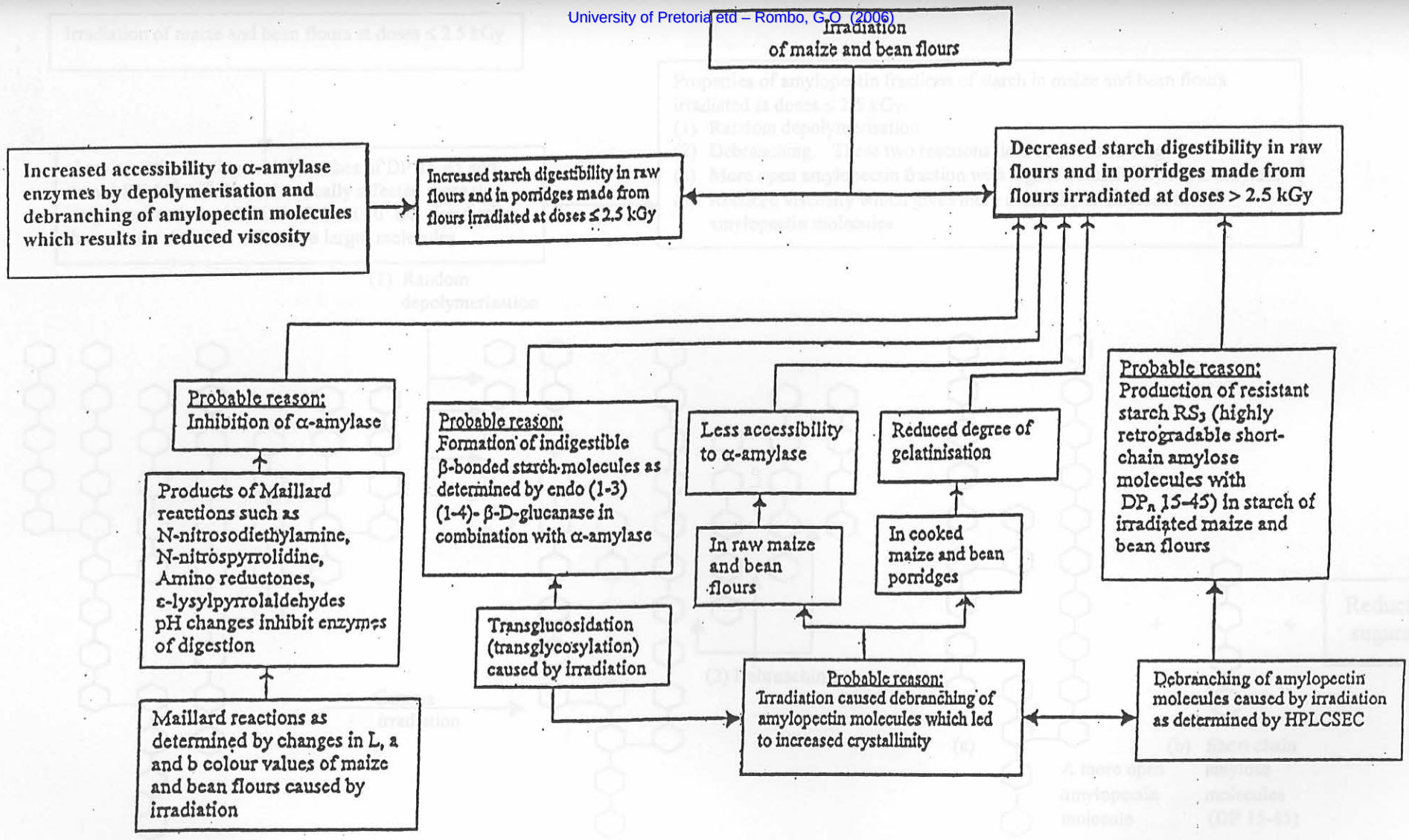

Figure 25 Proposed reasons for changes in *in vitro* starch digestibility of raw and cooked irradiated maize and bean flours

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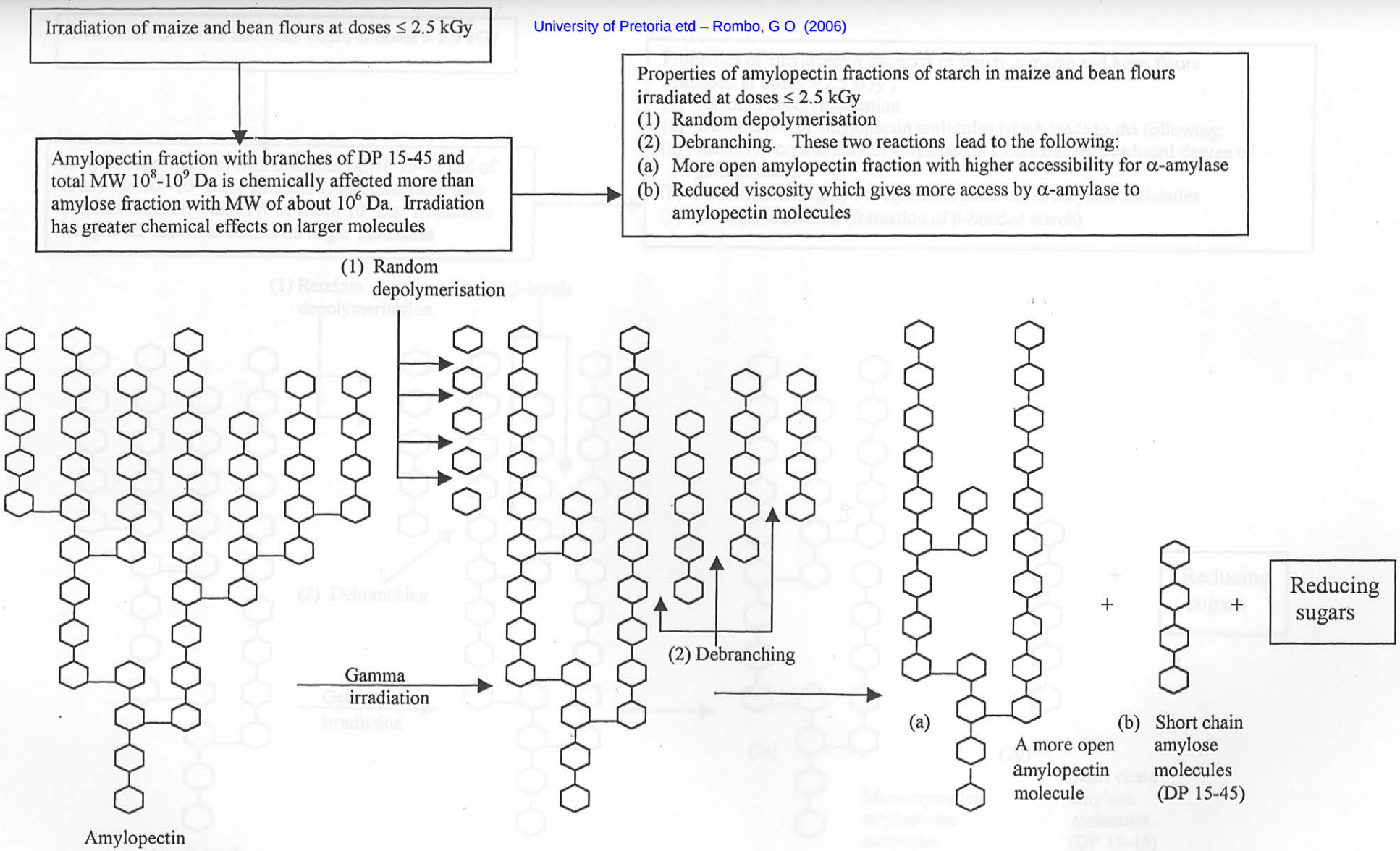

Figure 26 Probable effects of irradiation on amylopectin molecules of maize and bean flours at doses ≤ 2.5 kGy

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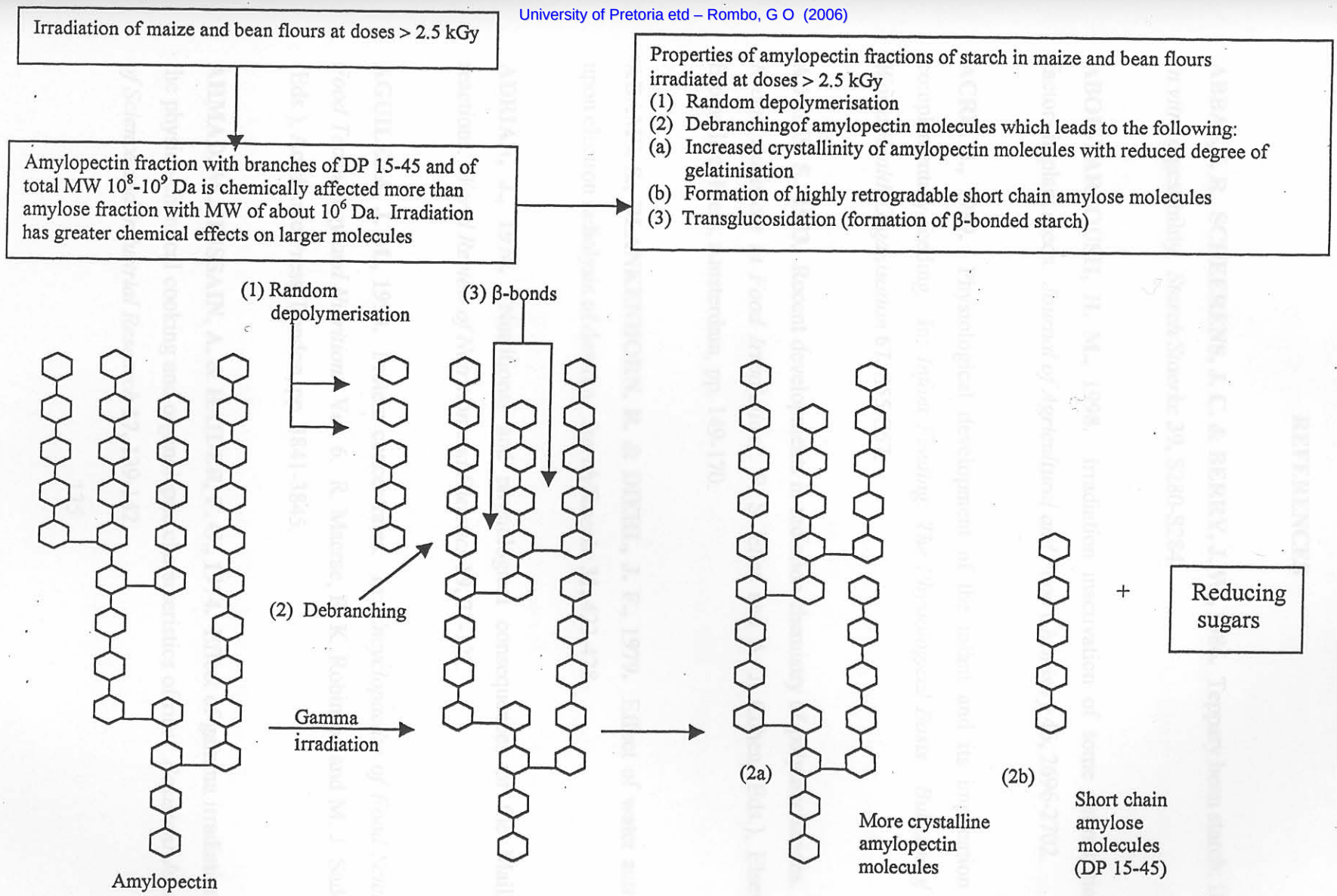

Figure 27 Probable effects of irradiation on amylopectin molecules of maize and bean flours at doses > 2.5 kGy

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CHAPTER 8

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