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.

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

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).

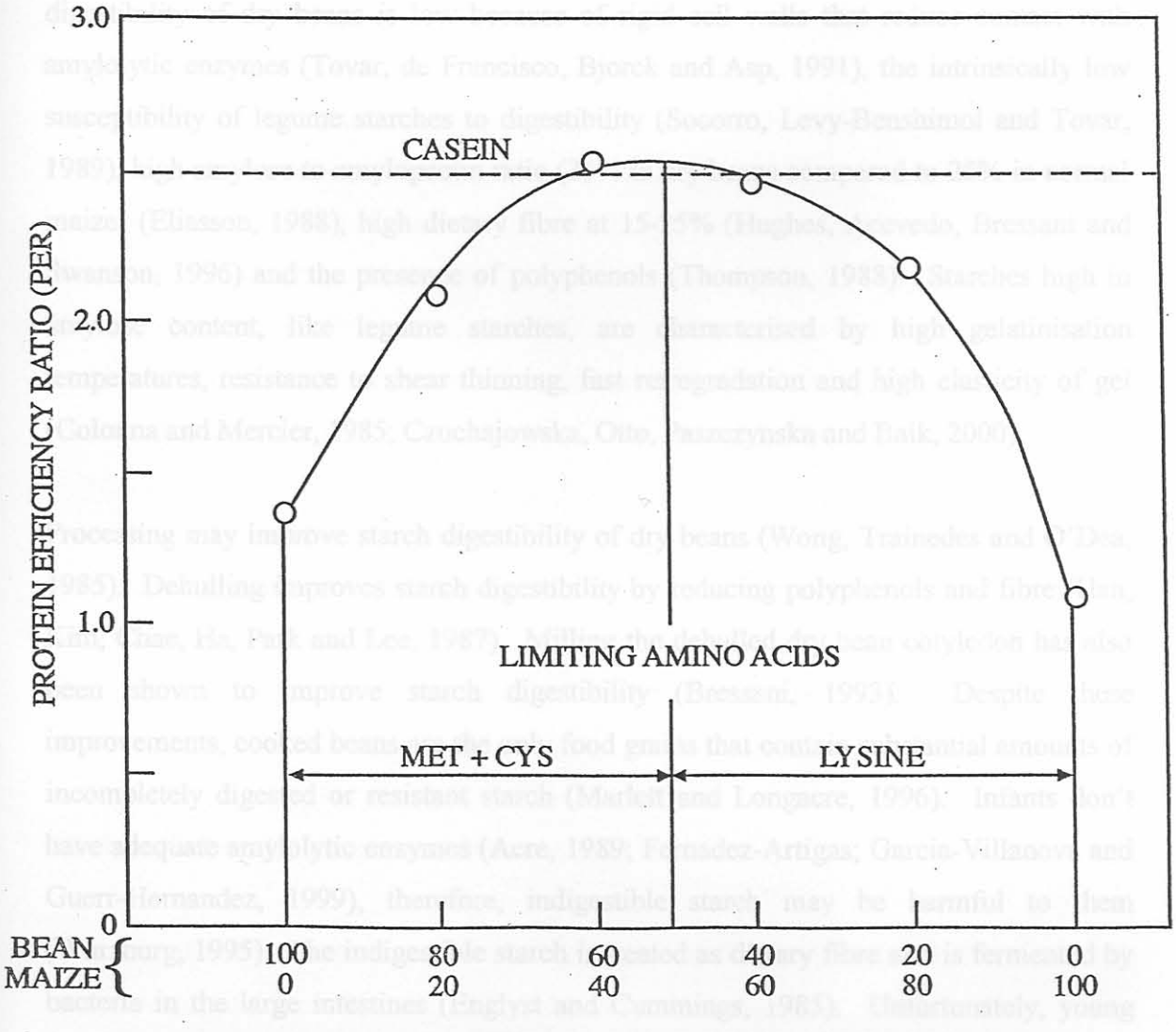

Figure 1 Complementary nutritional effects produced by mixtures of maize and navy bean at protein level of 8.3% (Yadav and Liener, 1978)

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

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

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:

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

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).

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

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,

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

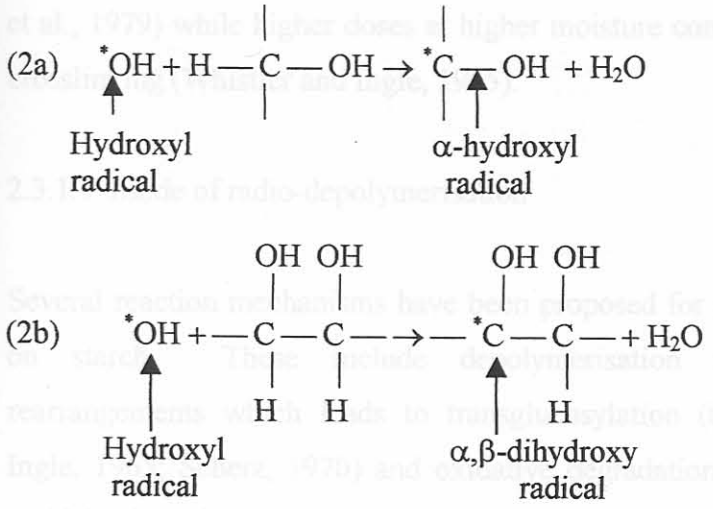

Figure 2 Action of hydroxyl radical on carbohydrates (Hasselmann and Marchioni, 1991)

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).

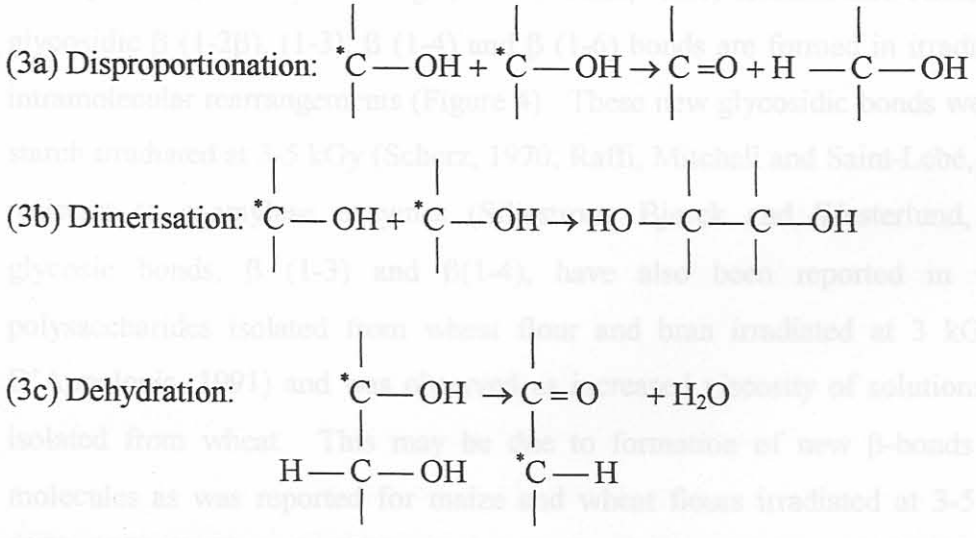

Figure 3 Pathways of carbohydrate degradation on irradiation (Hasselmann and Marchioni, 1991).

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

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

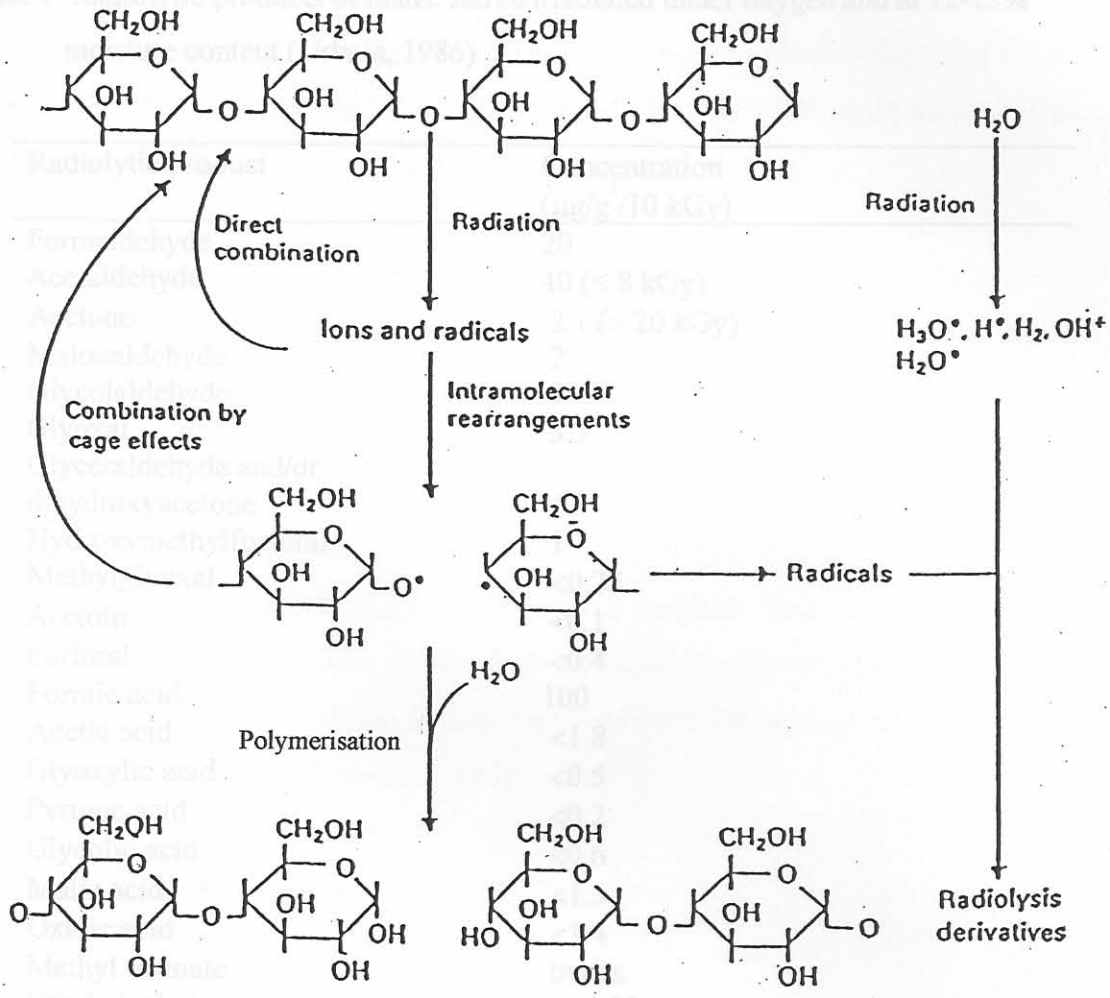

Figure 4 Some of the degradation mechanisms of starch during irradiation (Colonna et al., 1987) showing depolymerisation, transglucosidation (as recombinations), formation of free radicals and intramolecular rearrangements (with modifications)

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

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)

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).

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).

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

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.

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

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

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

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).

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-

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

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).

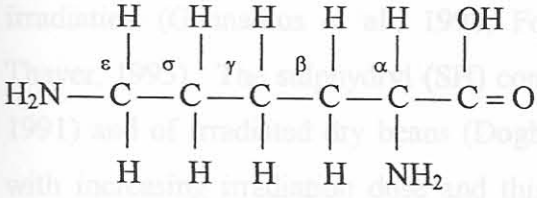

Figure 6 The chemical structure of lysine showing the α and ε amino groups (Holme and Peck, 1983)

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).

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

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.

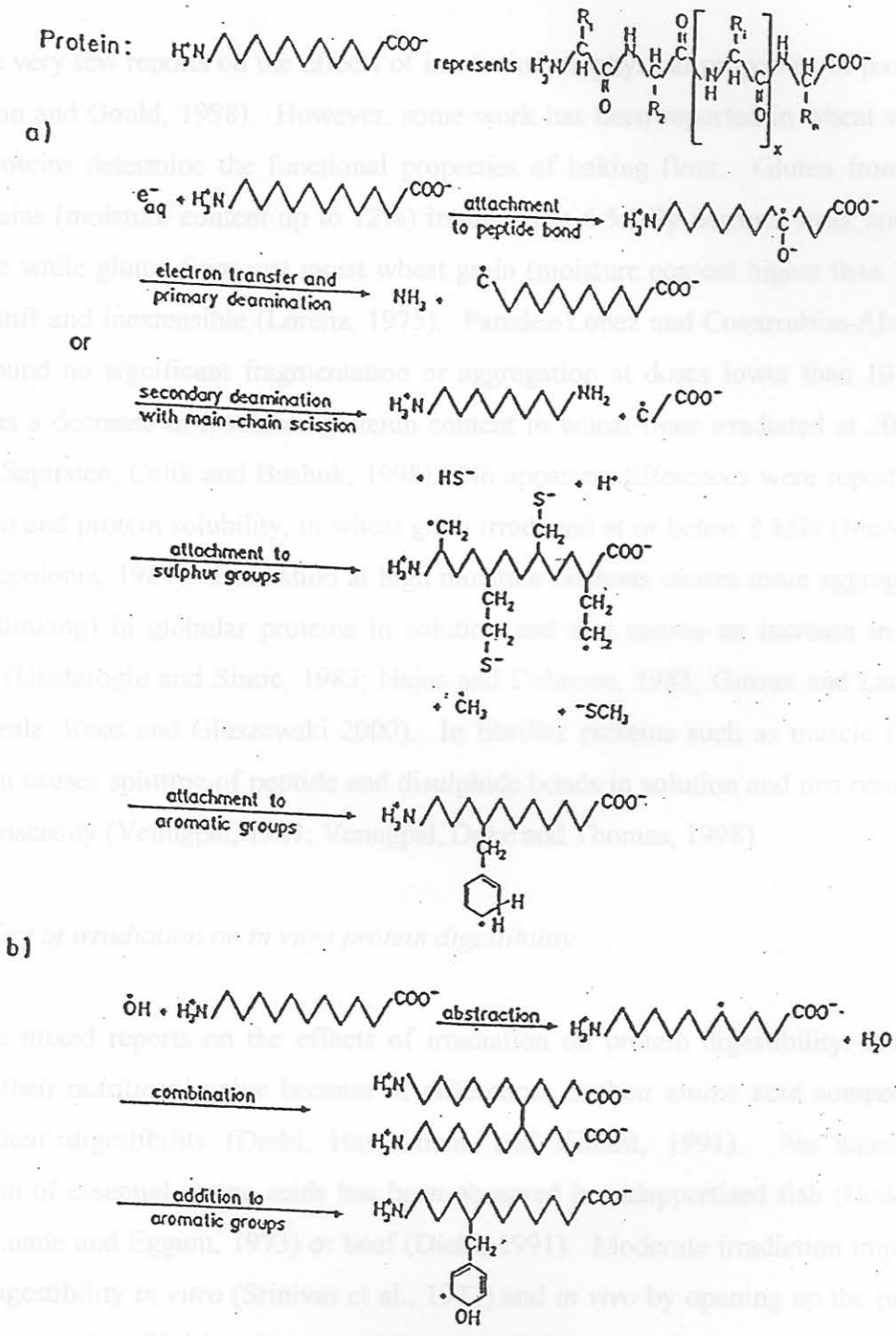

Figure 7 Changes in protein molecules caused by irradiation (Delincee, 1983)

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

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.

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).

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.

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.