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).

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 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).

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

(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 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

(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 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

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, 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.

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., 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 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 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, 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

Hizukuri (1989) as 1.2-4.1$\times10^6$ 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.

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 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 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.

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.

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

(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 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.