

Review

# The effect of ionising radiation on antinutritional factors and the nutritional value of plant materials with reference to human and animal food

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## Abstract

Although some plant materials contain substantial protein, carbohydrate and other nutrients, their bioavailability and utilisation by either humans or animals is relatively low, particularly in under-utilised/lesser-known/non-conventional crops. This is due to the presence of high proportions of various antinutrients. Moreover, the currently-adapted processing methods are relatively ineffective for complete inactivation/removal of such antinutrients. Ionising radiation treatment could serve as a possible additional processing method for inactivation or removal of certain antinutritional factors. This review therefore attempts to highlight the impact of gamma irradiation on the chemistry of various antinutrients, including non-starch polysaccharides (NSPs), and biological and nutritional qualities of foods and feeds. The potential effect of low irradiation dose levels of up to 10 kGy on the reduction of various antinutrients is also reviewed. This approach could open avenues for the possible utilisation of under-utilised and non-conventional crops and other agricultural residues as potential additional food and feed sources in the near future. © 2002 Elsevier Science Ltd. All rights reserved.

*Keywords:* Ionising radiation; Radiation chemistry of foods/feeds; Antinutritional factors; Non-starch polysaccharides; Nutritional evaluation

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## 1. Introduction

A world-wide supply of nutritionally balanced food is of fundamental importance. It is estimated that more than 800 million people in the world today are undernourished and/or malnourished. Most of the hungry are women and children (Conway & Toenniessen, 1999). Proteins, in particular, are of primary concern in this context. Legumes serve as the main source of dietary protein in many parts of the world. They complement cereals in terms of amino acid balance. On average, grain legumes contain 20–30% protein, and provide about 14 MJ of metabolisable energy  $\text{kg}^{-1}$  on a dry weight basis. In addition to containing substantial amounts of carbohydrates, legumes also serve as an important source of minerals such as calcium, magnesium, iron, zinc and potassium. Legume proteins are also much cheaper than protein from animal sources (Iyer, Salunkhe, Sathe, & Rockland, 1980a, 1980b).

Being a cheap source of dietary protein, legumes are now successfully used in child feeding programmes and food and feed formulations. Cereals and other crops also supply a substantial level of protein and high levels of carbohydrates. However, in some of these foodstuffs, the utilisation of available protein and carbohydrates is much less than that calculated from the chemical composition because of the presence of various antinutritional or antiphysiological substances, such as trypsin inhibitors, chymotrypsin inhibitors,  $\alpha$ -amylase inhibitors, lectins, phytates, goitrogens, glucosinolates, cyanogenic glucosides, oligosaccharides, polyphenols, toxic non-protein amino acids, antivitamin, allergens, saponins and alkaloids etc. (D'Mello, 1995; Liener, 1994a, 1994b; Makkar, Aderibige, & Becker, 1998; Makkar & Becker, 1998; Siddhuraju, Becker, & Makkar, 2000). In order to inactivate or reduce the above mentioned antinutrients, various conventional, simple processing methods have been used such as dry heating, roasting, boiling, soaking in water, alkali and acid, solvent extraction, germination and fermentation (Liener, 1994a; Sathe & Salunkhe, 1984; Siddhuraju & Becker 2001a, 2001b; van der Poel, 1989). However, none of these methods is able to completely remove all the

detected antinutrients that are present in seeds, grains or feed materials. A combination of processing methods is generally more effective than a single method. Advantages and disadvantages of various conventional processing methods have been discussed in several reviews. An additional technique is the application of ionising radiation, which has already been used for decontaminating food by killing bacteria, insects, other food born pathogens and to increase the shelf-life of fresh and dry food materials (Farkas, 1988; Molins, 2001; Thorne, 1991). The purpose of this review is to collate information on the effects of irradiation on antinutritional factors, nutritional characteristics and the biological value of food and feed materials. It is hoped that the information presented in this review will provide an impetus to further research in this area, enabling better utilisation of plant resources as sources of plant protein and carbohydrate for feeding tomorrow's world.

## 2. Physical and chemical aspects

### 2.1. Physics and chemistry of radiation processing

Food irradiation is a physical process involving an energy-input, that does not induce radioactivity in foods. The amount of energy input is called the radiation absorbed dose, and is measured in Grays ( $1 \text{ Gy} = 1 \text{ J kg}^{-1}$ ). It is similar in nature to the use of heat via either thermal (infrared) or microwave energies. In contrast to the gross and easily-detectable effects that conventional heat treatments have on foods, the radiation dose generates minute and mostly undetectable changes in chemical composition. This is due to the nature of the radiation and the selectivity and high efficiency with which it is transferred to the orbiting electrons in the atoms constituting food molecules or contaminants. When the activated orbiting electrons leave the atom, chemical changes occur in the atoms and molecules. This process is called ionisation, that is, the formation of positively charged atoms or molecules, known as cations (positive ions), formed by losing a negatively charged electron. The free electron is rapidly trapped by

surroundings atoms, forming negatively charged ions (anions). The ionisation process forms highly reactive atoms and molecules, called free radicals. Because of the selectivity of the energy-transfer mechanism, and the relatively small amount of energy needed to cause ionisation, the total amount of energy required to accomplish a desired technical effect in foods is greatly reduced to a level at which foods undergo no visible change. Most of the absorbed radiation energy (dose) is used in generating free radicals and in inducing chemical reactions between radicals or between radicals and other molecules. A minimal fraction of the absorbed energy is converted to thermal (heat) energy. Without heat or with only minimal heat, the freshness and the typical sensory and nutritional properties of foods are preserved.

Free radicals are also formed as by-products of normal and vital metabolic process in human physiology (e.g. oxidation and respiration), as well as in pathological processes leading to diseases (Pryor, 1984). In foods, identical free radicals are also formed by heat pasteurisation and by cooking (e.g. infrared, microwaves), boiling, baking, broiling, or frying of foods (Elias & Cohen, 1977; Lagunas-Solar, 1995). Therefore, free radicals, formed in the irradiation process, are not unique or different in nature or reactivity from those formed in biological or other common cooking processes. However, while pasteurisation requires heating to 50 °C for several minutes and cooking involves boiling, broiling, baking, or frying at temperature up to 300 °C for up to several hours, irradiating with a radiation dose of 10 kGy (1 Mrad) produces only a 2.4 °C temperature increase in 1 kg (2.2 lb) of food having the heat capacity of water (4.184 J/°C). This is about (3%) of the energy required to boil 1 l of water at 100 °C. Dry heating and conventional cooking result in the production of considerably higher amounts of free radicals than irradiation. Since no pattern or trends of toxicological significance have been established with these energetic conventional cooking processes, irradiated foods, as regulated by the Food and Drug Administration (FDA) and the international standards, are considered safe (FDA, 1986, 1991; WHO, 1977, 1981). Irradiation is also acknowledged to cause fewer overall physical and sensory changes than cooking, freezing, or canning (Josephson & Peterson, 1983; Molins, 2001; Urbain, 1986). As irradiation is a physical process, no external additives are involved. The irradiation process is, therefore, useful and desirable as an alternative in the preservation and processing of various fresh, perishable, and high-protein foods, with or without chemical additives or biological controls (Lochhead, 1989; Murray, 1990). The need for temperature and atmospheric controls can also be minimised, eliminated, or used in combination with packaging to delay food/feed spoilage (Lagunas-Solar, 1995).

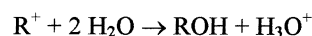
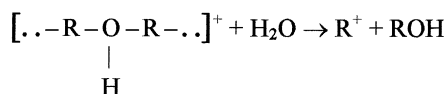
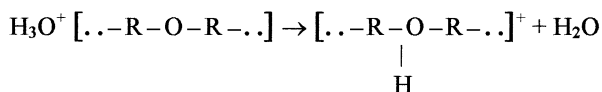
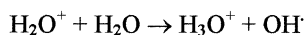
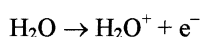
## 2.2. Radiation chemistry of food

Irradiation causes chemical changes in foods in amounts directly related to the radiation (treatment) dose. Based on extensive studies, several statements about these effects on foods can be made: (1) basic radiation chemistry kinetics and mechanisms allow prediction of the amount of radiolytic products formed, (2) the types of reaction induced by radiation are: (a) oxidation of metals and ions, (b) oxidation and reduction of carbonyls to and from hydroxyl derivatives, (c) elimination of double bonds, (d) decrease of aromaticity in aromatic and heterocyclic compounds, and (e) hydroxylation of aromatic and heterocyclic compounds. In addition, when oxygen is present, free radicals are oxidised and H<sub>2</sub>O<sub>2</sub>, peroxides, and hydroperoxides are formed. None of these reactions is unique to the radiation process. (3) no unique radiolytic product can be detected with current analytical methods in foods irradiated under the established guidelines and/or commercial conditions. In some frozen foods, dry foods and bone containing tissues, the radicals formed can be detected as they are trapped inside solid (crystalline) structures. (4) all radiolytic products formed as a result of radiation-induced chemistry are already known and are formed, in larger amounts in conventional food/feed processing methods. Reactions that do not take place in irradiated systems include the formation and condensation of aromatic rings and the formation of heterocyclic compounds. These reactions are known to take place at cooking temperatures yielding some toxic compounds such as carcinogens (Miller & Miller, 1986). In addition, all forms of conventional processing result in various degrees of vitamin and nutrient losses and in changes in the chemical composition of foods. According to the Institute of Food Technology (IFT, 1986) and others (Tomassi, 1988), some of these changes also result in the formation of toxic compounds. During an extensive review of the safety of irradiated foods, the FDA coined a new term: 'unique radiolytic products' or simply URPs (FDA, 1986), meaning new chemicals formed by irradiation. URPs have become one of the major toxicological concerns among uninformed consumers (Bruhn & Schutz, 1989; Bruhn, Schutz, & Sommer, 1988). Ionising radiations produce ions and other chemically excited molecules in the exposed medium. The activated molecules are responsible for the beneficial antimicrobial action of irradiation. However, they will also lead to some chemical/biochemical changes. The extent of these changes is dependent, among other factors, on the radiation dosage. The energy taken up by irradiated food is far less than that taken up by heated food, thus producing only very small amounts of reaction products. In various spices irradiated with a dose of 45 kGy, gas chromatographic studies revealed that radiation-induced compounds comprised less than 0.01% of

the total volatile constituents (Tjaberg, Underdal, & Lunde, 1972). Water forms primary OH radicals, H atoms, and hydration from the indirect action of radiation in foods, because these primary radicals react with food components to form a second set of radicals, thereby leading to final radiolysis products.

The formation, as well as the rate of decay, of free radicals is highly influenced by the moisture content of dry ingredients, as has been demonstrated with irradiated starches (Komiya & Nara, 1974). As pointed out earlier, free radical formation is not limited to irradiated foods. Although the generation and subsequent reaction of free radicals comprise the major route by which radiolytic products are formed, such reactions are also common during conventional food processing and storage operations. Electron spin resonance measurements have shown that the milling of grain and the heating of proteins in materials with a low water content also produce long-lived free radicals (Redmann, Axford, Elton, & Brivati, 1966; Uchiyama & Uchiyama, 1979).

Irradiation of high molecular weight carbohydrates in the solid state, as well as their aqueous solutions, causes the breaking of the external ether bridges. Two mechanisms can be assumed which take place simultaneously: (a) Direct action of radiation on the oxygen bridges in the solid state leads to the formation of an  $-O-$  radical; then the  $-O-C-$  linkage to the next hexose unit is split off producing a positive ion at the carbon atom and a glycosyl radical. In aqueous solutions  $H_3O^+$  ions are formed as the primary species with G-values<sup>1</sup> of  $\sim 2.8$  which means that they are sufficiently energetic to hydrolyse the glycosidic bond according to the mechanism of normal acid hydrolysis.

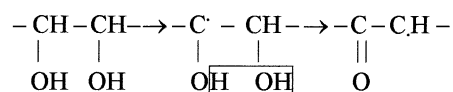


where R is the hexose unit.

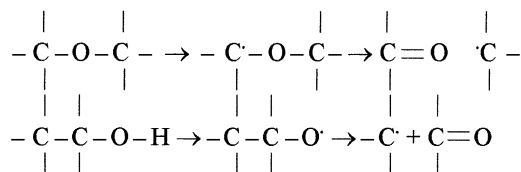
<sup>1</sup> The efficiency of a radiation-induced chemical transformation, i.e. the number of molecules reacting or produced by 100 electron volts, or per joule of absorbed energy from ionising radiation.

(b) Irradiation, directly or indirectly, causes alterations of some of the monosaccharide units in the polymer chain by radiation-induced dehydration (A) and  $\beta$ -splitting (B).

(A)



(B)



thereby forming deoxycarbonyl or deoxyacid groups in the chain (Scherz, 1974). Inulin, irradiated in the solid state, undergoes less degradation than it does in solution. In both cases similar radiolysis products such as deoxysugars, formaldehyde, organic acids and substances absorbing in ultra-violet are obtained, though their concentration is significantly lower in the case of inulin irradiated in the solid state. The presence of oxygen converts the first-order alcohol groups to aldehydes and acids. The ESR spectra obtained for inulin resemble those obtained for fructose, which implies that the radicals formed are similar in both cases (Bachman & Zegota, 1974).

Based on current knowledge of the chemistry of thermal and radiation processed foods, there is no scientific evidence that irradiation results in the production of toxic substances, because no new chemical compounds have ever been found that are not already present in the human diet. Trapped radical concentration was known to be greatly influenced by water content, oxygen levels and storage temperature, though no marked differences between rice and wheat and their varieties were found by Hayashi, Yano, and Namiki (1973). Ley (1963) concluded that no hazard exists from treatment of food with cobalt-60 in terms of induced radioactivity in the treated feed. The evidence from the Federal Register (1981, 1984) is that ionising radiations, of appropriate source energy levels, produce no detectable radioisotopes which are not already present in naturally-occurring foods. The maximum energy level of cobalt-60 is 1.33 MeV, which is insufficient to produce radioactivity. The Federal Register (1984) indicates that the difference between irradiated food and a comparable sample of non-irradiated food (measured in parts per million) is so small that there is no difference in terms of safety. Also,

according to Elias and Cohen (1977), most radiolytic compounds found in irradiated foods can also be found in non-irradiated foods. The carbonyls, peroxides and peroxide adducts are relatively harmless when ingested because they are so reactive. In fact, the reactive groups in food and in tissue cells, especially sulfhydryl groups, react readily and extremely rapidly with both peroxides and  $\alpha,\beta$ -unsaturated carbonyl compounds. Hence, it appears that it would take extremely large, chronic doses to produce harmful effects in mammals (Schubert, 1974).

### 3. The effect of irradiation on various antinutritional factors in foods of plant origin

#### 3.1. Protease inhibitors

Protease inhibitors in plants are a miscellaneous group of compounds that inhibit the digestion of proteins by animals, particularly monogastrics, and can depress their growth. Paradoxically, some of these compounds, e.g. trypsin inhibitors, may actually cause an increase in the secretion of digestive enzymes, including trypsin, chymotrypsin and elastase by inducing hypertrophy and hyperplasia of the pancreas. This led to the hypothesis that the growth depression caused by trypsin inhibitors was the consequence of an endogenous loss of amino acids in the form of enzymes being secreted by a hyperactive pancreas (Liener, 1994a, 1994b). Haider et al. (1981) have found significant changes in the trypsin inhibitor activity (TIA) of green gram at a radiation dose of 4 kGy. However, Sattar, Durrani, Mahmood, Ahmad, and Khan (1989) have reported considerable increases in protein values, and decreases in TIA during soaking and germination of irradiated green grams. Significant linear relationships ( $r = -0.960$  to  $-0.987$ ) have been reported in chick pea between the loss of TIA and soaking time of irradiated and unirradiated seeds and between the loss of TIA and increasing radiation dose (0.25–1.00 kGy) with little or no effect on protein content (Table 1) (Sattar, Atta, & Akhtar, 1990). The loss of TIA was found to be 54.5% when soybeans were subjected to 10 kGy (Frag, 1989). The very wide variation in the reduction of protease inhibitor activity in soybean, when subjected to irradiation, may be due to different moisture levels or the different varieties of beans used.  $\gamma$ CIA and TIA activities in *Pongamia glabra* oil cake were reduced by 78 and 84%, respectively, on exposure to 50 kGy which was a significantly greater reduction than that achieved with conventional processing methods such as fermentation and various solvent extraction methods (Rattansi & Dikshit, 1997).

The degradation of protease inhibitors, on exposure to  $\gamma$ -radiation, was directly proportional to the radia-

tion dose. In soybeans the level of inactivation of TIA increased linearly with increase in radiation dose (41.8, 56.3, 62.7 and 72.5% loss of TIA activity at radiation levels of 5, 15, 30 and 60 kGy, respectively; Farag, 1998). The author suggested that effective deactivation of the antinutritional factors present in soybeans could be safely accomplished through irradiation processing at dose levels up to 60 kGy. Irradiation and germination of dry field bean (*Vicia faba*) seed samples caused inactivation of 5.6 and 10.4% of the total TIA at dose levels of 1 and 2 kGy, respectively, compared to the unirradiated samples. At irradiation doses of 3 kGy, no further reduction in the total trypsin inhibitor activity of dry seeds was observed; however, irradiation at the higher doses of 4 and 5 kGy caused 38 and 45.1% losses, respectively (El-Morsi, Abdel-Salam, Ismail, Aboul-Fetouh, & Fawzay, 1992). Inactivation of trypsin inhibitor in irradiated samples could be attributed to the destruction of disulphide (–S–S–) groups. Lee (1962) observed that sulfhydryl (–SH) and disulphide (–S–S–) groups in proteins are apparently highly susceptible to irradiation. Khattak and Klopfenstein (1989) also showed that sulphur containing amino acids were liable to become damaged by radiation, particularly in the legumes. Iyer et al. (1980b) reported that  $\gamma$ -irradiation (1–5 kGy) of dehydrated (10–12% final moisture level) samples, followed by cooking was effective in reducing TIA and CIA in three bean varieties (*Phaseolus vulgaris* L.: great Northern, red kidney, and pinto). Lynn and Raoult (1976) showed that different sites on the two fractions of lima bean protease inhibitor responded differently to different radicals: i.e. the antichymotryptic site of lima bean protease inhibitor-IV was deactivated at comparable rates by the three major radical species involved i.e.,  $e_{aq}^-$ , OH and  $O_2^-$ , while the antitryptic sites of lima bean protease inhibitor-IV and III were mostly affected by OH. As the proteins are of nearly identical primary composition, the differences in response to radiolysis suggest that conformational changes alone can play a significant role in modifying

Table 1  
Effect of  $\gamma$ -irradiation and subsequent soaking in tap water on trypsin inhibitor activity (TIU/g) of chickpea (Sattar, Atta, & Akhtar, 1990)<sup>a</sup>

Soaking periods (h)	Irradiation dose (kGy)					Mean
	Control	0.25	0.5	0.75	1	
Control	330	329	328	320	316	325a
3	318	310	304	298	284	303b
6	310	302	292	283	277	293c
9	303	295	283	276	246	281d
12	276	269	245	238	229	251e
Mean	307a	301b	290c	283d	270e	
CV	6.6	7.3	10.4	10.7	12.6	

<sup>a</sup> For column and row means, the values sharing common letter are not significantly different ( $P < 0.05$ ).

irradiation damage (Lynn, 1973). Preferential radiolytic attacks were at the –S–S– bridges by  $e_{aq-}$ , OH and this occurred in a manner that altered the –S–S– bonds forming the disulphide loops which bear the active antitryptic and anti-chymotryptic sites. Effects at those sites could conceivably occur with little apparent change in the primary amino acid composition of the lima bean protease inhibitor (Lynn & Raoult, 1976). A dose of 10 kGy caused decreases in trypsin (by 35%) and chymotrypsin (by 71%) inhibitor activities in soybean defatted flour, whilst its in vitro digestibility increased from 80 to 84% (Abu-Tarboush, 1998; Table 2). An improvement in the in vitro digestibility of safflower oilcake, as a result of a decline in proteinase inhibitor activity brought about by irradiation, was reported by Joseph and Dikshit (1993). Irradiation of pure crystalline soybean trypsin inhibitor with a dose of 100 kGy caused no change in its activity (Hafez, Mohamed, Singh, & Hewedy, 1985); however, 98.7% inactivation of the same trypsin inhibitor was achieved in aqueous solution. The same authors found no change in CIA after soaking soybeans (15–31% moisture), while the TIA decreased under the same conditions. Joseph and Dikshit (1993) studied the effects of irradiation on trypsin and chymotrypsin inhibitor activities in safflower oilcake, and they found that TIA was inactivated at a dose of 42 Gy, whereas the chymotrypsin inhibitor activity was more resistant to irradiation in safflower oilcake than in soybean flour, possibly due to variations in the structure of the inhibitor itself. El-Morsi et al. (1992) found that irradiation of faba bean (*Vicia faba* L.), accompanied by germination, caused inactivation of 6 and 10% of total TIA at dose levels of 1 and 2 kGy, respectively. Loss of 45% of TIA activity was achieved by the use of 5 kGy in the same study. Hafez et al. (1985) found a small decrease in TIA in soybeans (7.4% moisture) at low dose levels but an increased radiation dose of 100 kGy caused a decrease in the trypsin inhibitor activity of 25%. A similar reduction (25%) was observed by the same authors for soybean when the moisture content was increased and the radiation dose was low. No significant change in CIA was found after

soaking soybeans (15.3, 22.5, and 30.5% moisture) when subjected to similar radiation doses. In general, CIA levels in soybeans did not depend on moisture content when subjected to radiation. Nene, Vakil, and Sreenivasn (1975a) found that irradiation of red gram samples (5–30 kGy) did not have any effect on TIA levels. Ghazy (1990) reported no change in TIA due to irradiation up to 5 kGy in dry kidney bean (*Phaseolus vulgaris* L.) seeds, whereas irradiation at 5 kGy reduced TIA by 5.2–16% of its original activity when the seeds were germinating for 24–96 h compared to non-irradiated germinating seeds.

El-Shibawi (1984) reported that the decrease in TIA in Faba bean matched the breakage of disulphide bonds as the irradiation dose rose. Farag (1989) reported that the detoxification dose needed for complete inactivation of all the antinutritional factors naturally present in full-fat soybeans seemed to be higher than the maximal dose level of 10 kGy recommended by the JECFI in 1980 (WHO, 1981). This was attributed to the low water content (7.3%) of soybeans, which does not favour the production of enough radiolytic products for the denaturation of all antinutritional factors.

### 3.2. $\alpha$ -Amylase inhibitors

In addition to trypsin and chymotrypsin inhibitors,  $\alpha$ -amylase inhibitors have been reported to be responsible for underutilisation of plant starch, leading to a lower metabolisable energy value for some carbohydrates (Marshall, 1975; Marshall & Lauda, 1975). The  $\alpha$ -amylase inhibitor activity in the defatted seed flour of *Moringa peregrina* was decreased by 44 and 48% upon treatment with 7.0 and 10.0 kGy, respectively. Although 10.0 kGy caused more destruction of  $\alpha$ -amylase inhibitor compared to 7.0 kGy, the difference was not significant ( $P > 0.05$ ) (Abu-Tarboush, 1998). Irradiation treatment of *M. peregrina* defatted flour with 7.0 kGy is as effective as heat treatment at 70 °C for 120 min (Al-Kahtani, 1995). However, no information is available regarding the impact of radiation on  $\alpha$ -amylase inhibitor in legumes, cereals and other plant food/feed sources.

Table 2

Effect of irradiation on trypsin and chymotrypsin inhibitor activities and in vitro protein digestibility of soybean defatted flour (Abu-Tarboush, 1998)<sup>a</sup>

Dose (kGy)	Trypsin inhibitor (units/mg)	Destruction (%)	Chymotrypsin inhibitor (units/mg)	Destruction (%)	In vitro protein digestibility (%)
0.0	76.8a		9.8a		79.8d
1.0	73.3b	4.6	8.6b	12.2	81.2c
3.0	68.2c	11.2	7.0c	28.6	81.8bc
5.0	65.8d	14.3	4.2d	57.1	82.3bc
7.0	56.0e	27.1	4.1d	58.2	83.1ab
10.0	50.0f	34.9	2.8e	71.4	84.2a

<sup>a</sup> Means in column with different letters differ significantly ( $P < 0.05$ ).

### 3.3. Phytohaemagglutinins

Phytohaemagglutinins (lectins) constitute one of the main physiologically-active components of foods of plant origin. Since some are antinutrients, the consumption of foods containing certain lectins can have serious consequences on growth and health (Pusztai, 1991). Pusztai, Clarke, Grant, and King (1981) proposed that the toxic effect of lectins, when administered orally, might be related to their ability to bind to some specific receptor sites on the surface of the epithelial cells lining the intestine, thus leading to non-specific interference with the absorption or transport of nutrients across the intestinal wall. When soybean was subjected to a radiation dose of 10 kGy, the phytohaemagglutinating activity was reduced by 50% (Farang, 1989) which is a significantly higher reduction than with normal processing techniques such as germination, soaking and dehulling (Liener, 1994a). The lectin activity remaining after irradiation treatment is quite similar to that remaining after dry heat treatment (De Muelenaere, 1964). In soybeans processed at 15, 30, and 60 kGy, the phytohaemagglutinating activity against rabbit red blood cells was reduced by 50, 75, and 94%, respectively (Farang, 1998). Similar results for soybean were also reported by Mahrous (1992). Radiolytic modifications have been shown to decrease the number of carbohydrate binding sites in Con A lectin that are, responsible for its biological properties (precipitation capacity of glycogen and aggregation of blood erythrocytes) without significantly changing the nature of the carbohydrate–protein interaction (Moore & Mudher, 1978).

### 3.4. Oligosaccharides

Even though legumes constitute an important and cheap source of protein in the developing world, their consumption is limited because they are often difficult to cook and contain oligosaccharides that cause flatulence (Thakur & Singh, 1994). These oligosaccharides escape digestion due to a lack of  $\alpha$ -1–6 galactosidase in the mammalian intestinal mucosa and are therefore not absorbed into the blood, but are metabolised by the microflora of the lower intestinal tract. This results in the production of large amounts of CO<sub>2</sub>, H<sub>2</sub>, and small quantities of methane (Rackis, 1975; Wanger, Carson, Becker, Gumbmann, & Danhof, 1977). The removal of  $\alpha$ -galactosides, using normal processing methods (soaking and boiling) appears to be relatively ineffective (Liener, 1994a) but Rao and Vakil (1983) reported that irradiation of green grams at 2.5 kGy reduced the level of oligosaccharides by 20%, including a 50% reduction of stachyose and raffinose, the two most gas-forming sugars. Furthermore, oligosaccharides disappeared completely during germination (48 h) of seeds, irra-

diated at 2.5–10 kGy, though  $\alpha$ -galactosidase activity was not changed. Irradiation after 24 h of exposure to water reduced the stachyose and raffinose content of navy beans below the level obtainable by germination alone for 72 h but not as low as that obtainable after 96 h of germination. However, irradiation greatly reduced the size of the rootlets, an effect of possible technological significance (Snauwaert & Markakis, 1976). The key flatulence-producing raffinose-type oligosaccharides in mung beans (*Vigna radiata*) were degraded by irradiation at the onset of the germination (0–2 days) compared to controls where degradation occurred much later (>4 days). However, the levels of the reducing sugars, mainly glucose, fructose and galactose, which are metabolised easily, were higher in the irradiated samples. At low doses (0.25 kGy), irradiation had no effect on germination and sprout length, indicating that the irradiated beans are suitable for use as bean sprouts (Machaiyah, Pednekar, & Thomas, 1999). These observations clearly indicate that  $\gamma$ -irradiation, at dose levels suitable for insect disinfection, improved the digestibility and nutritional quality of mung beans by reducing the content of oligosaccharides responsible for intestinal gas production. Kidney beans, irradiated at 3 kGy and germinated for 48 h, lost nearly 57, 70 and 32% of their raffinose, stachyose and verbascose contents, respectively. The decrease in oligosaccharide content was accompanied by an increase in the concentration of total soluble sugars (Ghazy, 1990). However, oligosaccharides disappeared from samples irradiated at 3 kGy after germination for 96 h.

Hasegawa and Moy (1973) found that germination in soybean was completely suppressed by irradiation at 5 kGy while treatment with 2.5 kGy resulted in only 30–40% of the beans having measurable rootlets. Analysis after 96 h of incubation (irradiated at 2.5 kGy; after 24 h of germination) showed that raffinose and stachyose had been reduced by 76 and 82%, respectively, with increases in the concentration of monosaccharides compared to a non-irradiated control. The texture of the cooked, air-dried, irradiated sample was softer than the control, as measured by shear press, suggesting a possible reduction in cooking time of about 34%. The average degree of polymerisation of starch is significantly reduced on irradiation and, consequently, there is a reduction in viscosity, a phenomenon that forms the basis of a detection test for irradiated foods (Molins, 2001). Kertesz, Schulz, Fox, and Gibson (1959) demonstrated that the relative viscosities of solutions of 2% maize starch that had received radiation doses of 1 and 5 kGy were 41.7 and 4.6%, respectively. These values measured for untreated controls and samples heated at 140 °C for 30 min were 54.1 and 30.7%, respectively. It has also been observed that the structure of irradiated starch undergoes a change in sensitivity to the action of the enzymes  $\alpha$ - and  $\beta$ -amylase (Ananthaswamy,

Vakil, & Sreenivasan, 1970a, 1970b) leading to an increase in maltose, maltotriose and maltotetrose production when compared with nonirradiated starch. Formic acid, acetaldehyde, and formaldehyde are the major radiolytic products of carbohydrate molecules. However, product distribution may differ with different water activities, e.g. in starch the formation of malondialdehyde and formic acid was reported to increase upon reducing the water content, while other reactions, i.e. the formation of glucose and formaldehyde, were practically independent of the water content. Moreover, the produced compounds are found in many natural products and in conventionally treated foods. Although some of these compounds are toxic to unicellular organisms, they are not toxic to mammals in the concentrations produced by irradiation (Farkas, 1988). According to Raffi, Agnel, Thiery, Frejaville, and Saint-Lébe (1981), the radiolytic end products of starch are the same, irrespective of whether the source is maize, potato, bread, wheat, or rice. However, more research studies on *in vitro* and *in vivo* starch digestibility and glycaemic responses of irradiated food materials are required.

### 3.5. Other antinutritional factors

Farag (1999) reported that a combination of autoclaving for 10 min plus irradiation up to 20 kGy reduced the level of chlorogenic acid in sunflower meal by 87% more than other processing methods. Deshpande and Aguliar (1975) found that, in two varieties of coffee beans, roasting caused reductions in the concentrations of chlorogenic acid, caffeic acid and carbohydrates, whereas radiation treatment increased the concentrations of carbohydrates without affecting the concentrations of chlorogenic and caffeic acid.

According to AFMA (1972), soybean meal for use in all types of livestock feed should be processed to remove urease until the activity of the enzyme causes a pH rise of not more than 0.2 pH units. Farag (1998) reported that radiation doses of 5, 15, 30 and 60 kGy reduced urease activity in soybeans so that the residual enzyme

caused pH to fall by 1.45, 0.35, 0.27 and 0.24 pH units, respectively.

Antinutritional factors, such as phytic acid in cereals and nitrates in leafy vegetables, are of major concern. Phytic acid chelates mineral cations and proteins, forming insoluble complexes, which leads to reduced bioavailability of trace minerals and reduced digestibility of proteins (Reyden & Selvendran, 1993). Cooking did not decrease phytic acid in sorghum porridge, but cooking and irradiation caused a significant decrease (40%) (Duodu, Minnaar, & Taylor, 1999). Similarly, treatment of soybean seeds with irradiation, alone or in combination with soaking, reduces the level of phytate compared to controls (Table 3) (Sattar, Neelofar, & Akhtar, 1990a). This reduction is probably due to chemical degradation of phytate to the lower inositol phosphates and inositol by the action of free radicals produced by the radiation. (De Boland, Garner, & O'Dell, 1975). Another possible mode of phytate loss during irradiation could have been through cleavage of the phytate ring itself. Sattar, Neelofar, and Athar (1990a) observed that phytate values significantly decreased with increasing germination period (24–120 h) and irradiation dose (0.05–0.20 kGy) ( $P < 0.01$ ). The maximum reduction in phytate levels (90 and 76% reduction) that occurred during germination of samples dosed at 0.20 kGy (Table 4) was greater than that produced by the germination of unirradiated soybean seeds (74 and 55% reduction) for 120 h in distilled and tap water. Reduction in both total and free gossypol by 14 and 7.9%, respectively by irradiation at a dose level of 10.0 kGy has been observed in cotton seed (Abu-Tarboush, 1998). But irradiation was not effective in reducing gossypol to the permissible level of 0.06%.

Doses of 10.0 and 7.0 kGy significantly reduced the tannin content of Shahlla sorghum variety from 0.35 to 0.26 mg of catechin equiv/100 g but not that of the Hemaira variety (Abu-Tarboush, 1998). Moreover, the effects of fermentation and other soaking treatments on the tannin content of sorghum grain were greater than those of irradiation. Similar observations have been made regarding the reduction of gossypol in cotton seeds.

Table 3  
Effect of irradiation and soaking at ambient conditions on phytate content of soybean(mg/100 g) (Sattar et al., 1990a)<sup>a</sup>

Soaking time (h)	Unirradiated (control)	Radiation doses (kGy)				Mean
		0.25	0.50	0.75	1.0	
0	212	183	135	110	102	148
3	171 (145)	114 (140)	108 (119)	103 (110)	93.5 (97.5)	118 (122)
6	139 (100)	108 (90.0)	101.5 (85.0)	91.0 (83.0)	71.5 (72.5)	101.1 (86.1)
9	121.7 (90.0)	101 (83.0)	86.2 (79.7)	81.0 (70.2)	64.5 (63.2)	90.7 (77.2)
12	110 (78.9)	81.0 (76.7)	77.5 (70.0)	61.9 (60.5)	60.0 (56.5)	78.0 (68.5)
Mean	151 (125)	117 (114)	102 (97.7)	89.3 (86.6)	78.2 (78.3)	

<sup>a</sup> Soaking in tap water. Values in parentheses represent soaking in distilled water.



#### 4. Biological and nutritional evaluation

Comparative biological evaluations, using *in vivo* and *in vitro* systems, have been widely applied in order to assess the overall nutritional quality of irradiated food samples. Irradiation of certain dry vegetables seems even to have some positive nutritional consequences. It is known that starch is mainly responsible for the textural quality of, cereals and legumes, and especially for changes during cooking (Osman, 1967). The cooking time of  $\gamma$ -irradiated broad beans (*Vicia faba*) was reduced by about 18% at an irradiation dose of 5 kGy and by 30% at 10 kGy. Irradiation also caused textural improvements, which are desirable in the development of high quality products (Ismail, 1976). The above experiments also showed that the maximum viscosity of starch tended to decrease, depending on the irradiation dose used. The maximum viscosity was 880, 530 and 310 B. U. for control, 5 and 10 kGy irradiated samples, respectively. These results provide adequate evidence that irradiation of high polymer molecules, such as starch, cellulose and pectin, causes alterations in some of their physical properties, namely viscosity, mechanical strength, swelling and solubility. Neither 0.5 nor 10 kGy doses had any significant effect on total available carbohydrates, total free sugars, total starches or dextrans or on the eight individual free sugars identified in pistachio kernels (Kashani & Valadon, 1984). Recently Rombo, Taylorm, and Minnaar (2001) reported that irradiated (0–10 kGy) maize, kidney bean and their 70:30 composite flours markedly reduced the viscosities of porridges in a dose-dependent manner. However, in raw maize, cooked maize and cooked bean flours maximum starch digestibility was observed at a dose of 2.5 kGy, followed by a small but significant decrease in starch digestibility at higher doses (5.0, 7.5 and 10 kGy), more in maize than in bean flours. This was probably due to the depolymerisation of starch in the flours by  $\gamma$ -irradiation (Rayas-Duarte & Rupnow, 1993). The

experiments conducted by Jumel, Harding, and Mitchell (1996), on determination of absolute-average molecular weights and conformational parameters for a homologous series of irradiated guar gum samples, showed that the molar masses and viscosities of the irradiated samples decreased with increasing irradiation dose. However, these decreases were not linear and there was no significant change in the gross conformation due to irradiation.

A chick bioassay test showed that radiation treatment (180 kGy) improved the nutritive value and metabolisability of lentils (Daghir, Sell, & Mateos, 1983). Irradiation at 10 kGy reduces the cooking time of red gram (*Cajanus cajan*) whilst preserving many of the B vitamins (Sreenivasan, 1974). However, slight degradation of proteins also occurs, which increases the susceptibility of this product to proteolytic action *in vitro*.

Radiation processing of diets for specific stocks of laboratory animals (specific pathogen and germ-free populations) has long been a commercial practice in several countries, because irradiated feeds have clear-cut advantages over heat treated or fumigated feeds with regard to palatability, nutrient value, and microbiological safety of the diet (Ley, Bledy, Coates, & Paterson, 1969). Farag (1989) reported that soybeans irradiated at a dose level of 10 kGy, retained their normal levels of moisture, crude protein, fat and ash. This dose level does not result in the denaturation of protein, and does not affect the nitrogen containing components of the food materials. It has been postulated that protein and crude fat in the complex matrix of food stuffs are more radiation resistant than when in a pure state (Diehl & Scherz, 1975). In general, losses of essential amino acids as a result of irradiation treatment were greater than those of non-essential amino acids and the most limiting amino acids in soybean were not affected by irradiation treatment (Farag, 1989). No differences were noted in the total protein, total amino acid, free amino acid, fat or fatty acid compositions

Table 4  
Effect of  $\gamma$ -irradiation and subsequent germination on phytate content (mg/100 g) of soybean<sup>a</sup> (Sattar et al., 1990b)<sup>b</sup>

Germination period (h)	Unirradiated control	Irradiation doses (kGy)				Mean
		0.05	0.10	0.15	0.2	
Control	212	205	200	195	190	200a
24	131	125	106	95.3	80.2	107b
48	101	97.3	81.2	73.8	60	82.7c
72	80.6	75	62.5	57.6	52.5	65.6d
96	69.5	62.5	51.9	45.0	43.8	54.5e
120	55.0	47.5	41.3	30.5	20.5	39.0f
Mean	108a	102b	90.5c	82.8d	74.5	
CV	53.0	56.2	64.5	71.5	80.4	

<sup>a</sup> Germination in distilled water.

<sup>b</sup> Column and row means values sharing common letters are not significantly different ( $P < 0.05$ ).

of lentil, mung bean and two variety of wheat when subjected to three dose levels of  $\gamma$ -irradiation (0.5, 1.0 and 3.0 kGy) (Hossain, Talima, Suzuki, & Todoriki, 1988). In the same study, only an insignificant increase in the amount of free glucose, accompanied by a corresponding decrease in the amount of starch, were noted with increasing radiation dosage in lentil and mung bean. Ismail and Osman (1976) found that irradiation at dose levels of 5–10 kGy caused no significant change in the amino acid composition of broad beans. The losses of some amino acids in irradiated soybean mentioned earlier might have been due to the radiation induced formation of free radicals, leading to the splitting of peptide bonds and the subsequent deamination-decarboxylation of some of the amino acid linkages (Elias & Cohen, 1977). Nene, Vakili, and Sreenivasan (1975a) demonstrated that the irradiation of red gram protein increased its in vitro digestibility. The observed enhancement in proteolytic digestion was attributable either to degradation of proteins into small fragments making them more susceptible to enzymes, or to partial destruction of trypsin inhibitors. Shih and Kalmar (1987) showed that the deamidation of oilseed proteins resulted in an increase of some functional properties such as solubility, the ability to form emulsions and whippability. Rahma and Mostafa (1988) studied the effect of irradiation on the functional properties of peanut flours and their results show that irradiation increased both water (solubility) and fat absorption (hydrophobicity) of treated flours. However, the increase in fat absorption was higher than that for water. Dissociation and denaturation are known to cause increase in the fat and water absorptions of treated protein compared to native protein. Deamidation is important for evaluating the nutritive value of proteins, since hydrolyses of end-groups and peptide bonds have been reported to directly influence digestibility (Lacroix, Amiot, & Brisson, 1983). Dario and Salgado (1994) showed that cowpea bean flours irradiated at 0.2 kGy had better digestibility than non-irradiated flours, and the irradiated flours even when cooked in a microwave oven, were superior to those cooked using more conventional methods. The combination of autoclaving for 10 min plus irradiation up to 20 kGy has a beneficial effect on the protein digestibility of sunflower meal with little effect on its

content of soluble protein and available lysine (Farak, 1999).

Samples of full-fat soybean were tested for their nutritional value after being irradiated at dose levels of 5, 15, 30, and 60 kGy in a study by Farag (1998). The gross compositions, such as dry matter, moisture, ash, crude protein, crude fat and crude fibre, of raw and irradiated beans were found to be similar. However, available lysine was reduced by 3, 4 and 6% when seeds were irradiated at 15, 30, and 60 kGy, respectively, but these changes were statistically not significant. Irradiation of broad beans (*Vicia faba*), at levels of 2.5, 5, 10 and 20 kGy, did not induce any significant change in their chemical composition (El-Niely, 1996). It could be concluded that the amount of water (70 g kg<sup>-1</sup>) in raw soybeans does not favour the production of enough radiolytic products and water-free radicals to induce significant change in the gross composition of soybean. Total protein efficiency (TPE) of raw soybeans in broiler chicks was found to be 0.967, whereas the TPE values (Table 5) were significantly increased to 1.191, 1.368, 1.688 and 1.892 when the beans were irradiated at doses of 5, 15, 30 and 60 kGy respectively (Farag, 1998). Similarly, Nene et al. (1975b) reported that irradiation of wheat, at dose levels of 0.4 and 5 kGy, increased the net protein utilisation of this dietary component in chicks. Reddy, Publos, and McGinnis (1979) reported that, based on chick growth, the nutritional value of all the varieties of beans they tested was significantly improved by irradiation treatment. Cornwell, Greenwood, Crook, and Burson (1969) concluded that there was no evidence of loss of nutritional adequacy or induced toxicity of cereal grains irradiated at 195 kGy, when such products were included at 755 days in the diet of mature laying hens. Hayakawa, Suzuki, Hayashi, and Kawashima (1985) determined the nutritive value of poultry diets irradiated at 25 kGy by feeding them to rats. The treatment had no effect on gross live performance, on net nitrogen utilisation or on important liver enzyme parameters that indicate changes in protein quality. Performance characteristics were compared with those seen in rats fed diets autoclaved at 120 °C for 30 min where the heat treatment resulted in decreased growth rate and increased liver fat deposition.

Table 5

Effect of processing on total protein efficiency (TPE) of soybeans through chick feeding trials (Farag, 1998)<sup>a</sup>

Dose (kGy)	Group starting weight (g)	Group finishing weight (g)	Weight gain (g)	Feed intake (g)	Crude protein intake (g)	TPE
0	342±5.08	522±6.63	180±7.98	1006±39.67	186±7.34	0.968c±0.01
5	341±4.97	614±1.87	273±4.04	1185±53.23	219±9.85	1.23d±0.06
15	342±4.46	684±3.67	342±3.17	1354±25.72	251±4.76	1.37c±0.03
30	340±5.26	701±4.05	361±3.22	1156±22.07	214±4.08	1.68b±0.02
60	342±5.45	716±3.63	374±2.58	1069±15.90	198±2.94	1.89a±0.02

<sup>a</sup> Mean ± standard deviation. Values followed by the same letters in a column are significantly different ( $P < 0.05$ ).

In sunflower meal, autoclaving plus irradiation up to 20 kGy markedly improved the *in vitro* protein digestibility by about 90% (Farag, 1999). The study of El-Niely (1996) showed that the *in vitro* protein digestibility of broad beans irradiated at 2.5, 5, 10 and 20 kGy, was improved by 4.5, 10, 16 and 20%, respectively. Al-Masri and Zarkawi (1999) reported that  $\gamma$ -irradiation (100–350 kGy) increased the *in vitro* organic matter digestibility (IVOMD) and *in vitro* digestible energy (IVDE) of wood sawdust and broiler litter while significantly decreasing the values of crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) ( $P > 0.05$ ). The digestible energy of wood sawdust and broiler litter increased by 1030, 1040, 1330 and 1510 kJ/kg DM at doses of 100, 150, 250 and 350 kGy, respectively, compared with controls. They also observed that the dose of 350 kGy had the highest effect, resulting in a 33% reduction in both CF and ADL, 30% in NDF and 24% in ADF but there was no significant ( $P > 0.05$ ) effect of irradiation on gross energy (GE) or total nitrogen. Al-Masri (1998) also reported no effect on gross energy values when laying-hens' excreta were irradiated at doses of 10–150 kGy. Moreover, there was no significant ( $P > 0.05$ ) effect of irradiation on digestible energy when the irradiation dose was 50 kGy. Gamma-irradiation (50–200 kGy) has been used to decrease the cell-wall constituents in some agricultural residues rich in lignocellulosic materials (Al-Masri, 1999; Al-Masri & Zakawi, 1994a, 1994b). Fibre levels may be reduced in direct proportion to the level of irradiation due to depolymerisation and delignification (Sandev & Karavanov, 1977). It appears that radiation results in random depolymerisation and decomposition of cellulose (Jerome, Millett, & Lawton, 1952), and seriously weakens the cellulosic fibre (Gilfillan & Linden, 1955). Hence, irradiation treatment has the potential to increase the nutritive value of broiler litter used as feedstuffs for ruminants. Neither *in vivo* nor *in vitro* tests conducted on rats after short-term feeding studies with irradiated bean diets revealed any mutagenic activity.

Some physicochemical changes in macronutrients are observed in irradiated foods. The effects of  $\gamma$ -radiation (1–10 kGy) on selected functional properties were studied in four legumes: green gram, lentil, horse-bean and Bengal gram. The water absorption capacity of irradiated legume samples increased, although pasting temperature was not appreciably changed. Maximum gelatinisation viscosity decreased progressively in all samples with increasing radiation dose. Irradiation at 2.5–10 kGy, of all legume samples, caused a significant reduction in cooking time compared to controls. The force required to compress cooked samples was reduced when they were irradiated. Samples irradiated at 2.5 and 5 kGy were rated as softer than a control sample by

members of a taste panel. Sensory evaluation of cooked unirradiated and irradiated (5 kGy) samples revealed no significant differences in acceptability (Rao & Vakil, 1985). Metta and Johnson (1959), also using rats, found no difference in the biological value or protein digestibility of wheat gluten or corn protein irradiated with either 28 or 93 kGy. They also found that the concentrations of lysine and arginine in these proteins were unaltered.  $\gamma$ The irradiation treatment of beans tended to increase nitrogen retention by chicks and decrease uric acid nitrogen excretion in relation to nitrogen intake. Irradiation tended to improve nitrogen retention by chicks fed lentils and significantly increased the metabolisable energy value of Red Chief Lentils (Daghir et al., 1983).

Gamma irradiation has been reported to increase the food value of rye (Campbell, Classen, Reichert, & Campbell, 1983) and hull-less barley (HLB) (Classen, Campbell, Rossnagel, Bhatti, & Reichert, 1985) for chicks. The improvement noted for rye and hull-less barley is thought to be due to depolymerisation of soluble pentosans (Campbell et al., 1983) and  $\beta$ -glucan (Classen et al., 1985), respectively. These carbohydrate polymers cause the intestinal contents to become viscous and interfere with nutrient assimilation and the general well-being of the chick. Irradiation of rye and hull-less barley, fed to chicks, improves the apparent absorption of fat, amino acids and starch (Campbell et al., 1983; Classen et al., 1985). Irradiation significantly improves the weight gain to feed-ratio of chicks fed either hulled oats or hull-less oats. Apparent fat retention and tibia ash were higher in chicks fed irradiated hull-less oats than in those fed untreated hull-less oats (Campbell, Classen, & Ballance, 1986). Chick body weight, apparent amino acid retention, fat retention, tibia ash, and weight gain to feed ratios were lower in chicks fed with autoclaved (121 °C for 20 min) barley than in those fed with untreated barley. However, irradiation (60 kGy), subsequent to autoclaving barley samples, eliminated these effects (Campbell et al., 1986). Irradiation appears to benefit cereals containing soluble or mucilaginous fibre types, as typified by the  $\beta$ -glucan of barley and oats. These fibres appear prone to irradiation-induced depolymerization, as indicated by the increased  $\beta$ -glucan solubility and reduced extract viscosity of irradiated barley and oat samples (Campbell et al., 1986; Campbell, Sosulski, Classen, & Ballam, 1987). It appears that irradiation of wheat at 1 kGy is useful for the control of insect infestation without adversely affecting the quality of the grain (Köksel, Celik, & Tuncer, 1996). However, above 1 kGy, irradiated samples exhibited significantly lower scores for stickiness, firmness, and bulkiness when compared with control samples, probably because of deterioration in both starch and gluten.

Dario and Salgado (1994) compared the effects of different thermal treatments and irradiation (cooking at

low pressure, microwaving and autoclaving) on cowpea (*Vigna unguiculata*) seed flours. The protein contents of all irradiated (0.2 kGy) samples significantly increased and the carbohydrate contents decreased compared with non-irradiated ones. The same authors reported that the digestibility of cowpea bean flours irradiated at 0.2 kGy was superior to that of non-irradiated ones and that the flours cooked in a microwave oven were nutritionally superior to those cooked under low pressure, autoclaved or even raw. The *in vitro* enzymatic digestibility of red gram protein was increased by irradiation (Nene et al., 1975b). Similarly, Srinivas, Ananthaswamy, Vakil, and Sreenivasan (1972) reported an increase in the liberation of free amino acids from irradiated wheat subjected to autolysis or proteolytic digestion. However, radiation processing of wheat (0.2 kGy) did not adversely affect the protein efficiency ratio (PER) in rats (Vakil, Aravindakshan, Srinivas, Chauhan, & Sreenivasan, 1973). Increases in the net protein utilisation of chicks fed irradiated (4 or 50 kGy) wheat have also been observed (Moran, Summers, & Blayey, 1968). Hickman, Mclean, and Ley (1964) reported no harmful effects from feeding wheat that had been  $\gamma$ -irradiated at levels of 0.2–2 kGy. Rats fed the irradiated grain for four successive generations failed to show any adverse or beneficial effects on growth, reproductive performance or progeny health. Similarly, there were no effects on the physical and milling characteristics of wheat treated with 0.1, 0.25, 0.50, 1.25 and 1.75 kGy (Fifield, Golumbia, & Pearson,

1967). In the studies with chicks, Moran et al. (1968), showed that  $\gamma$ -irradiation (5–50 kGy) of wheat bran significantly increased protein and phosphorus utilisation. Body weight, food utilisation, fat retention, and bone ash of chicks were substantially improved after three weeks by feeding irradiated rye (0–100 kGy) and these values were maximal at 60 kGy. Irradiation of the entire diet resulted in no further improvement over irradiating the rye fraction alone. The improved performance coincided with radiation-induced damage to rye polysaccharides, as indicated by reduced viscosity and increased concentrations of reducing sugars (Campbell et al., 1983). Antoniou and Marquardt (1981) reported that both insoluble and soluble pentosans contributed to the deleterious effects of feeding rye. Like the soluble pentosans, the insoluble fraction was reported to be very hygroscopic, and in a hydrated form could contribute to the viscosity of the intestinal contents. Brown (1979) speculated that fibre suspensions in general may impede nutrient diffusion, thereby influencing the digestion and absorption processes. Gamma-irradiation, may alleviate this effect and improve the digestion and retention of most nutrients by depolymerisation and consequent disruption of the gel network.

Lawton, Bellamy, Hungate, Bryant, and Hall (1951) suggested that irradiation could be used to increase the availability of wood carbohydrates to rumen microorganisms. Using *in situ* techniques, McMannus, Manta, McFarlane, and Gray (1972a, 1972b, 1972c)

Table 6  
Crude protein (%  $\times$  6.25)<sup>a</sup> and growth of chicks at two weeks fed irradiated beans<sup>b</sup> (Reddy et al., 1979)

Beans	Crude protein		Body weights (g)	
	Control	Irradiated	Control	Irradiated
<i>Pinto</i>				
6R-577	21.1	20.5	120d,e,f	156c
6R-113	20.7	21.2	128d	158c
6R-129	21.8	21.6	116d,e,f,g	178b
<i>Red Mexican beans</i>				
RS-43	21.7	21.5	84j,k	113e,f,g,h
RS-59	22.4	22.6	70k	105g,h,I
Commercial	22.6	22.5	80j,k	124d,e
<i>Pink beans</i>				
6R-122-SR	21.1	20.7	109f,g,h	147c
<i>White pea beans</i>				
W-122-16-1	23.5	23.8	91i,j	125d,e
W-122-16-4	24.2	24	88j	119d,e,f
W-122-23-2	23.6	23.4	92i,j	123d,e,f
5R-310	23.2	22.9	94i,j	122d,e,f
Duty	22.5	23	85j	102h,i
UI-76	22.2	22.3	93i,j	122d,e,f
Soybean meal	48		233a	
CV			6%	

<sup>a</sup> Average of duplicate determinations. Irradiated samples had 210 kGy and all samples were autoclaved for 10 min.

<sup>b</sup> Means without a common letter are significantly different ( $P < 0.05$ ).

found that irradiation of rice straw and cotton fibre increased the rate of dry matter disappearance in the rumen. Leonhardt, Henning, Nehring, Baer, Flachowsky, and Wolf (1983) showed that the digestibility of wheat, oat, barley and rye straws can be increased by up to 80% by treatment with  $\gamma$ -rays or accelerated electrons, as both types of radiation depolymerise cellulose and hemicellulose. Many authors (Hennig, Leonhardt, Wolf, Flachowsky, & Bär, 1982; Pritchard, Pidgen, & Minsin, 1962) have reported that the size of the irradiation dose greatly affects fibre digestibility. NaOH treatment alone did not influence the chemical composition of wheat and triticale straws, but irradiation, after alkaline treatment, significantly reduced the level of crude fibre (CF), neutral detergent fibre (NDF) and acid detergent fibre (ADF) proportionally to the dose. In addition, the levels of nitrogen free extractives (NFE) and  $\alpha$ -linked glucose polymers were markedly increased in the irradiated straws. NaOH treatment raised the potential rumen degradability of the DM of both straws but irradiation increased not only their potential but also their effective degradability (Tables 6–8) (Gralak, Mahmood, & Barej, 1994). Irradiation at 10 or 50 kGy did not improve the nutritive value of rice bran fed to broiler chickens (Wang, Marquardt, Guenter, Zhang, &

Han, 1997). This lack of response was probably not caused by a failure to break the rice bran polysaccharides as the dosage was of sufficient energy to bring this about (Lesson & Marcotte, 1993). However, the irradiation may have been ineffective due to the absence of any viscous carbohydrates in rice bran. Sandev and Karaivanov (1979) reported that decreases in the crude content of fibre in alfalfa hay, grain straw, corn cobs and wheat bran were directly proportional to the increasing dose of radiation. However, they also observed that the digestibility of the individual nutritive substances in  $\gamma$ -irradiated roughages was the same as that of substances subjected to steam pelletising. The results of a biological evaluation of the protein in piglets (Van Kooij, 1979) showed that the protein quality of sow feed was not noticeably affected by either treatment, while the protein quality of creep and hog feed was more adversely effected by heat than by irradiation. Neither the proximate constituents or total amino acid profiles of irradiated wheat were changed sufficiently to affect nutritional quality (Vakil et al., 1973). However, molecular fragmentation of starch and proteins took place, thus influencing the rheological properties and baking qualities of irradiated wheat. No significant variations in the PER of rats fed irradiated (0.2 and 2 kGy)

Table 7

The effect of irradiation and NaOH treatment on composition (%) of wheat straw (WS) and triticale straw (TS) ( $n=6-9$ ) (Gralak et al., 1994)<sup>a</sup>

Variation	Dry matter	% in dry matter								
		Crude protein	Crude fibre	Crude lipid	Ash	NFE	NDF	ADF	$\alpha$ -Linked glucose	
Straw	WS	95.2	5.6	27.6	1.6a	5.8	59.3	56.0	34	12.8
	TS	96.1	6.2	28.6	1.4b	5.6	58.4	52.9	34.4	15.7
NaOH (%)	0	96.9	5.5	28.4	1.4	5.5	59.2	54.6	33.6	15.7
	2	94.4	6.0	27.2	1.5	5.6a,b	59.8	54.5	34.6	13.6
	4	94.8	6.2	28.7	1.4	6.0b	57.6	52.2	34.4	13.5
Radiation (kGy)	0	95.4	5.2	38.8a	1.4	5.7	48.9a	62.6a	37.6a	12.1a
	300	94.5	6.3	26.2b	1.5	5.5	60.5b	54.0b	33.8a,b	14.2a,b
	600	95.2	6.1	19.3c	1.5	5.9	67.2c	46.8c	31.2b	16.5b

<sup>a</sup> Means followed by different letters within column of the same variation differ at  $P < 0.05$ .

Table 8

The effect of irradiation and NaOH treatment on the rumen DM and CF (potential and effective) degradability of wheat straw (WS) and triticale straw (TS) ( $x \pm S.E.M.$ ,  $n=6-9$ ) (Gralak et al., 1994)<sup>a</sup>

Variation		Potential degradability (%)		Effective degradability (%)	
		DM	CF	DM	CF
Straw	WS	70.4 $\pm$ 2.3	76.5 $\pm$ 3.7	48.2 $\pm$ 1.2	44.9 $\pm$ 1.3
	TS	70.4 $\pm$ 4.3	66.8 $\pm$ 9.8	45.1 $\pm$ 2.5	40.4 $\pm$ 2.3
NaOH (%)	0	66.3 $\pm$ 3.6a	83.5 $\pm$ 11.5	46.1 $\pm$ 2.4	42.2 $\pm$ 2.7
	2	67.0 $\pm$ 2.8a	58.9 $\pm$ 4.8	46.0 $\pm$ 1.8	39.7 $\pm$ 1.3
	4	77.9 $\pm$ 4.6b	72.6 $\pm$ 7.7	47.7 $\pm$ 3.2	46.0 $\pm$ 2.7
Radiation (kGy)	0	61.5 $\pm$ 2.7a	76.8 $\pm$ 10.8	41.1 $\pm$ 1.6a	42.6 $\pm$ 3.0
	300	72.6 $\pm$ 4.4b	68.7 $\pm$ 7.6	47.0 $\pm$ 1.7b	40.9 $\pm$ 2.0
	600	77.0 $\pm$ 2.5b	69.4 $\pm$ 9.7	51.6 $\pm$ 1.7b	44.3 $\pm$ 2.5

<sup>a</sup> DM, dry matter; CF, crude fibre. Means followed by different letters within column of the same variation differ at  $P < 0.05$ .

or non-irradiated wheat-legume diet were observed by Vakil et al. (1973).

### 5. Advantages and disadvantages of irradiation over conventional techniques

Heat treatment of sunflower meal affects its amino acid composition and nutritional quality. Alexander and Hill (1952) observed a decrease in the lysine content of sunflower meal after dry heating at 121 °C. Autoclaving sunflower meal at 103.5 kPa significantly decreased the performance of chicks (Morrison, Clandinin, & Robble, 1953) and rats (Evans & Bandemer, 1967). Moreover, Provansal, Cug and Cheftel (1975) observed that heat treatment led to the formation of unusual amino acids, such as lysinoalanine, alloisoleucine and ornithine. Whereas the protein quality and amino acid composition of laboratory animal diets were not significantly affected by radiation doses as high as 70 kGy, the protein quality of an autoclaved diet (102 °C for 5 min) was significantly affected (Eggum, 1979). Farag (1999) suggested that the combination of autoclaving for 10 min plus irradiation at levels up to 20 kGy has a beneficial effect on the protein quality of sunflower meal. There is little effect on the content of soluble protein or, available lysine, and chlorogenic acid is reduced by 87% more than by other processing methods.

Irradiation of soaked and dehydrated beans at 5 kGy caused about a 50% reduction in cooking time (Iyer et al., 1980a). The processing technique with  $\gamma$ -irradiation did not adversely affect protein quality. The results reported by Joseph and Dikshit (1993) with safflower oilcake suggest that, even a low dose of irradiation (4.2 kGy), which is well below the advocated safe level of 10 kGy, is effective in reducing protease inhibitors and improving *in vitro* protein digestibility. Irradiation has the advantage over other methods that few steps are involved compared with, for example, solvent extraction. Also, the chance of degrading nutrients is less at this low-dose exposure than it is with heat-treatment or the use of strong solvents, such as HCl/NaOH. Free radical ingestion does not create any toxicological or other harmful effects. This has been confirmed by a long-term laboratory study in which animals were fed a very dry milk powder irradiated at 45 kGy. No mutagenic effects were noted and no tumours were formed and no toxic effects were apparent in the animals over nine successive generations (Schubert, 1969). Toasted bread (unirradiated), actually contains more free radicals than very dry foods that have been irradiated (Merritt, 1989). Vitamin losses are generally less if oxygen is excluded during irradiation and if the temperature is low. Under optimal conditions, vitamin losses in foods irradiated at doses up to 1 kGy are considered to be insignificant. At

higher doses the effect of irradiation will depend on the specific vitamin, temperature, dose, food, and packaging. Depending on the food, thiamine levels may be reduced more by storage and cooking than by irradiation, especially if the food has been exposed to air during storage, but not necessarily if it has been packaged without oxygen.

Irradiation causes changes in food, although all of them have been found to be benign. More than 40 years of multispecies and, multigenerational animal studies have shown that there are no toxic effects from eating irradiated foods. Additionally, human volunteers consuming diets consisting of up to 100% irradiated food have shown no ill effect (Diehl, 1995). Recently, a Joint FAO/IAEA/WHO Study Group on High-Dose Irradiation reviewed data relating to the toxicological, nutritional, radiation chemical and physical aspects of food irradiated to doses above 10 kGy and concluded that foods treated with doses greater than 10 kGy can be considered safe and nutritionally adequate when produced under established Good Manufacturing Practice (WHO, 1999). Irradiation produces so little chemical change in food that it is difficult to design a test to determine whether a food has been irradiated (Stevenson, 1994). Small numbers of new compounds are formed when food is irradiated, just as new compounds are formed when food is exposed to heat. Early research described these new compounds as 'unique radiolytic products' because they were identified after food was irradiated (Diehl, 1995) but subsequent investigations showed that the free radicals and other compounds produced during irradiation are identical to those formed during cooking, steaming, roasting, pasteurisation, freezing, and other forms of food preparation. All reliable scientific evidence, based on animal feeding tests and consumption by human volunteers, indicates that these products pose no unique risk to human beings. In fact, people requiring the safest food, i.e. hospital patients receiving bone marrow transplants, are routinely given irradiated foods. Thus, as with pasteurisation, the evidence suggests that food irradiation can do nothing except improve the quality of a food supply.

The physico-chemical properties of the non-starch polysaccharides (NSPs) are responsible for their anti-nutritive activities in broiler chicken and other animals. In particular, soluble, viscous NSPs depress the digestibility of proteins, starch and fat (Smits & Annison, 1996). The viscosities of NSPs in food materials can be reduced by irradiation rather than by using heat-processing techniques. Irradiation is more effective in the reduction of protease inhibitors, oligosaccharides and phytic acid in legumes and cereals than other domestic and industrial processing techniques. From the above results we may conclude that radiation techniques should serve as additional processing methods for both conventional and non-conventional crops in the future.

## 6. Conclusions and future areas of research

Currently-employed conventional processing methods are relatively ineffective for the reduction or removal of antinutritional factors, particularly for the hydrolysis of non-starch polysaccharides (NSPs). In this context, irradiation is one possible alternative and additional processing technique for reducing both heat-stable and heat-labile antinutrients. In particular, irradiation levels up to 10 kGy, which are admissible for the irradiation of foods, seem to be effective in inactivating antinutrients such as protease inhibitors, lectin, phytic acid, non-starch polysaccharides and oligosaccharides without altering the nutritional quality of the food. This method is therefore one of the most promising techniques. Higher levels of irradiation, up to 600 kGy, can be used to improve the rumen degradability of the dry matter and crude fibre of various plant residues and cereal straws. Further extensive research on the processing of agricultural residues by irradiation is still required but, if successful, irradiation will enhance the utilisation, not only of conventional foods but also of un-conventional food materials for humans and animals, especially in the developing world.

Only limited information is available on the impact of irradiation on many of the antinutrients in food materials, such as oligosaccharides, protease inhibitors, lectins,  $\alpha$ -amylase inhibitors, phytic acid, polyphenols, glucosinolates and various toxic non-protein amino acids, which can be present in quite large amounts, not only in conventional but also in non-conventional and little-known plant food materials (D'Mello, 1995; Grant et al., 1995; Liener, 1994a, 1994b; Siddhuraju, Vijayakumari, & Janardhanan, 1997; Makkar, Becker, Sporer, & Wink, 1996); further investigations in this direction are also required. Moreover, the radiolytic products of such antinutrients should be evaluated using appropriate analytical techniques both in their purified forms and when they are mixed with the complex matrices of food materials. Further in vivo and in vitro studies and toxicological analyses are needed to assess the efficiency with which irradiation inactivates antinutrients and to quantify its effects on nutritional quality, particularly the impact of doses higher than 10 kGy.

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## References

Abu-Tarboush, H. M. (1998). Irradiation inactivation of some antinutritional factors in plant seeds. *Journal of Agricultural and Food Chemistry*, *46*, 2698–2702.

- Alexander, J. C., & Hill, D. C. (1952). The effect of heat on the lysine and methionine in sunflower seed oil meal. *Journal of Nutrition*, *48*, 149–151.
- Al-Kahtani, H. A. (1995). Some antinutritional factors in *Moringa peregriana* (Al-Yassar or Al-ban) and soybean products. *Journal of Food Science*, *60*, 395–398.
- Al-Masri, M. R. (1998). Changes in contents and *in-vitro* digestibility of laying-hens excreta used as feeds due to drying and gamma irradiation. *Applied Radiation and Isotopes*, *49*, 767–771.
- Al-Masri, M. R. (1999). *In vitro* digestible energy of some agricultural residues as influenced by gamma irradiation and sodium hydroxide. *Applied Radiation and Isotopes*, *50*, 295–301.
- Al-Masri, M. R., & Zarkawi, M. (1994a). Effects of gamma irradiation on cell wall constituents of some agricultural residues. *Radiation Physics and Chemistry*, *44*, 661–663.
- Al-Masri, M. R., & Zarkawi, M. (1994b). Effects of gamma irradiation on chemical compositions of some agricultural residues. *Radiation Physics and Chemistry*, *43*, 257–263.
- Al-Masri, M. R., & Zarkawi, M. (1999). Digestibility and composition of broiler litter, as affected by gamma irradiation. *Bioresource Technology*, *69*, 129–132.
- American Feed Manufacturers Association. (1972). *Dehulled soybean meal. AFMA feed ingredient guide*. Arlington, VA: American Feed Manufacturers Association.
- Ananthaswamy, H. N., Vakili, U. K., & Sreenivasan, A. (1970a). Effect of gamma radiation on wheat starch and its components. *Journal of Food Science*, *35*, 795–798.
- Ananthaswamy, H. N., Vakili, U. K., & Sreenivasan, A. (1970b). Susceptibility to amylolysis of gamma irradiated wheat. *Journal of Food Science*, *35*, 792–794.
- Antoniou, T., & Marquardt, R. R. (1981). Influence of rye pentosans on the growth of chicks. *Poultry Science*, *60*, 1898–1904.
- Bachman, S., & Zegota, H. (1974). Physico-chemical changes in irradiated ( $\gamma$ Co 60) inulin. In: *Improvement of food quality by irradiation* (pp. 61–75). International Atomic Energy Agency: Vienna, Austria.
- Brown, W. (1979). Interactions of small molecules with hydrated polymer networks. In G. E. Inglett, & S. I. Falkehog (Eds.), *Chemistry and nutrition* (pp. 1–13). New York: Academic Press.
- Bruhn, C. M., & Schutz, H. (1989). Consumer awareness and outlook for acceptance of food irradiation. *Food Technology*, *43*, 93–97.
- Bruhn, C. M., Schutz, H., & Sommer, R. (1988). Food irradiation and consumer values. *Ecology of Food and Nutrition*, *21*, 219–223.
- Campbell, G. L., Classen, H. L., & Ballance, G. M. (1986). Gamma irradiation treatment of cereal grains for chick diets. *Journal of Nutrition*, *116*, 560–569.
- Campbell, G. L., Classen, H. L., Reichert, R. D., & Campbell, L. D. (1983). Improvement of the nutritive value of rye for broiler chickens by gamma irradiation-induced viscosity reduction. *British Poultry Science*, *24*, 205–212.
- Campbell, G. L., Sosulski, F. W., Classen, H. L., & Ballam, G. M. (1987). Nutritive value of irradiated and  $\beta$ -glucanase treated wild oat groats (*Avena fatua* L.) for broiler chickens. *Animal Feed Science and Technology*, *16*, 243–252.
- Classen, H. L., Campbell, G. L., Rossnagel, B. G., Bhatti, R., & Reichert, R. D. (1985). Studies on the use of hull-less barley in chick diets. Deleterious effects and methods of alleviation. *Canadian Journal of Animal Science*, *65*, 725–733.
- Conway, G., & Toenniessen, G. (1999). Feeding the world in the twenty-first century. *Nature*, *402*(Suppl.), C55–C58.
- Cornwell, B. P., Greenwood, T., Crook, L. T., & Burson, D. M. (1969). Poultry feeding trial with irradiated grain. Wantage, Berkshire, UK: Wantage Research Laboratory.
- D'Mello, J. P. F. (1995). Antinutritional substances in legume seeds. In J. P. F. D'Mello, & C. Devendra (Eds.), *Tropical legumes in animal nutrition* (pp. 135–172). UK: CAB International.
- Daghir, N. J., Sell, J. L., & Mateos, G. G. (1983). Effect of gamma irradiation on nutritional value of lentils (*Lens culinaris*) for chicks. *Nutritional Reports International*, *27*, 1087–1093.

- Dario, A. C., & Salgado, J. M. (1994). Effect of thermal treatments on the chemical and biological value of irradiated and non-irradiated cowpea bean (*Vigna unguiculata* L. Walp) flour. *Plant Foods for Human Nutrition*, 46, 181–186.
- De Boland, A. R., Garner, G. B., & O'Dell, B. L. (1975). Identification and properties of 'phytate' in cereal grains and oilseed products. *Journal of Agricultural and Food Chemistry*, 23, 1186–1189.
- De Muelenaere, H. J. H. (1964). Effect of heat treatment on the hemagglutinating activity of legumes. *Nature*, 201, 1029.
- Deshpande, S. N., & Aguilar, A. A. (1975). Effects of roasting temperatures and gamma irradiation on the content of chlorogenic acid, caffeic acid and soluble carbohydrates of coffee. *International Journal of Applied Radiation and Isotopes*, 26, 656–661.
- Diehl, J. F. (1995). *Safety of irradiated foods*. New York: Marcel Dekker.
- Diehl, J. F., & Scherz, H. (1975). Estimation of radiolytic products as a basis for evaluation the wholesomeness of irradiated foods. *International Journal of Applied Radiation and Isotopes*, 26, 499–507.
- Duodu, K. G., Minnaar, A., & Taylor, J. R. N. (1999). Effect of cooking and irradiation on the labile vitamins and antinutrient content of a traditional african sorghum porridge and spinach relish. *Food Chemistry*, 66, 21–27.
- Eggum, B. O. (1979). Effect of radiation treatment on protein quality and vitamin content of animal feed. In: *Decontamination of animal feeds by irradiation*, (pp. 55–67). Vienna, Austria: International Atomic Energy Agency.
- Elias, P. S., & Cohen, A. J. (1977). *Radiation chemistry of major food components*. Amsterdam: Elsevier Scientific.
- El-Morsi, E. A., Abdel-Salam, H. S., Ismail, H. A., Aboul-Fetouh, S. A., & Fawzay, A. M. (1992). Effect of gamma irradiation on trypsin inhibitor and subunit structure of proteins in field bean (*Vicia faba*) seeds during germination. *Minia Journal of Agricultural Research and Development*, 14, 849–871.
- El-Niely, H. F. G. (1996). *Chemical constituents and digestibility of broad beans subjected to irradiation treatment*. MSc thesis, Women's College, Ain Shams University, Cairo, Egypt.
- El-Shibawi, K. M. S. (1984). *Biochemical changes in protein of some food stuffs by ionising radiation*. MSc thesis, Biochemistry Department Faculty of Agriculture, Cairo University, Giza.
- Evans, R. J., & Bandemer, S. L. (1967). Nutritive value of some oil-seed proteins. *Cereal Chemistry*, 44, 417–426.
- Farag, M. D. E. H. (1989). Radiation deactivation of antinutritional factors: trypsin inhibitor and hemagglutinin in soybeans. *Egyptian Journal of Radiation Sciences and Applications*, 6, 207–215.
- Farag, M. D. E. H. (1998). The nutritive value for chicks of full-fat soybeans irradiated at up to 60 kGy. *Animal Feed Science and Technology*, 73, 319–328.
- Farag, M. D. E. H. (1999). Effect of radiation and other processing methods on protein quality of sunflower meal. *Journal of the Science of Food and Agriculture*, 79, 1565–1570.
- Farkas, J. (1988). *Irradiation of dry food ingredients*. Boca Raton, FL: CRC Press.
- FDA. (1986). Irradiation in the production and handling of food. *Federal Register*, 51, 1337.
- FDA. (1991). Irradiation in the production, processing and handling of food. 21 code of Federal Regulations, Section 179 (p. 26).
- Federal Register. (1981). Document registration of the government of the United States. *Federal Register*, 46, 18992–18993.
- Federal Register. (1984). Document registration of the government of the United States. *Federal Register*, 49, 5714–5722.
- Fifield, C. C., Columbia, C., & Pearson, J. L. (1967). Effects of  $\gamma$ -irradiation on the biochemical storage and bread making properties of wheat. *Cereal Science (Today)*, 12, 253–257.
- Ghazy, M. A. (1990). Effect of  $\gamma$ -irradiation on some antinutritional factors in kidney bean (*Phaseolus vulgaris* L.) seeds. *Minia Journal of Agricultural Research and Development*, 12, 1965–1980.
- Gilfillan, E. S., & Linden, L. (1955). Effects of nuclear radiation on the strength of yarns. *Textile Research Journal*, 25, 773–777.
- Gralak, M. A., Mahmood, S., & Barej, W. (1994). Rumen degradability of dry matter and crude fibre of irradiated and sodium hydroxide treated straws. *Archives of Animal Nutrition*, 47, 63–74.
- Grant, G., More, L. J., McKenzie, N. H., Dorward, P. M., Buchan, W. C., Telek, L., & Pusztai, A. (1995). Nutritional and haemagglutination properties of several tropical seeds. *Journal of Agricultural Science (Cambridge)*, 124, 437–445.
- Hafez, Y. S., Mohamed, A. I., Singh, G., & Hewedy, F. M. (1985). Effect of  $\gamma$ -irradiation on protein and fatty acids of soybean. *Journal of Food Science*, 50, 1271–1274.
- Haider, F. (1981). Inactivation studies on the trypsin inhibitor activity of green gram cultivars. *Nutritional Reports International*, 23, 1167–1171.
- Hasegawa, Y., Moy, J. H. (1973). Reducing oligosaccharides in soybeans by gamma-radiation controlled germination. In *International Symposium on Radiation Preservation of Food, Proceedings-Symposium on Preservation of Food. IAEA-SM-166/39* (pp. 89–103). Vienna, Austria: International Atomic Energy Agency.
- Hayakawa, S., Suzuki, H., Hayashi, T., & Kawashima, K. (1985). Effect of gamma-irradiation on the nutritive value of domestic animal feeds. *Reports of Natural Food Research Institute*, 46, 109–113.
- Hayashi, T., Yano, M., & Namiki, M. (1973). Formation and Disappearance of the Radiation-induced Radicals in Foods. Part 2, rice and wheat. *Food Irradiation*, 8, 95–99.
- Hennig, A., Leonhardt, J., Wolf, I., Flachowsky, G., & Bär, M. (1982). The proof of straw treatment with  $\gamma$ -rays *in vitro*. *Archives in Animal Nutrition*, 32, 789–795.
- Hickman, J. R., Mclean, D. L. A., & Ley, F. J. (1964). Rat feeding studies on wheat treated with gamma irradiation. I. Reproduction. *Food and Cosmetics Toxicology*, 2, 15–20.
- Hossain, M. M., Talima, M., Suzuki, T., & Todoriki, S. (1988). Effects of  $\gamma$ -irradiation on the chemical composition of lentil, mung bean and wheats. *Food Irradiation*, 23, 66–71.
- IFT. (1986). Effect of food processing on nutritive values: a scientific status summary by the Institute of Food Technologists expert panel on food safety. *Food Technology (Chicago)*, 40, 109–116.
- Ismail, F. A. (1976). Effect of irradiation on broad bean (*Vicia faba*) Textural qualities. *Lebensmitte- Wissenschaft und-Technologie*, 9, 18–20.
- Ismail, F. A., & Osman, A. Z. (1976). Improvement in digestibility of broad bean (*Vicia faba*) by gamma-irradiation. *Isotope Radiation Research*, 8, 17–22.
- Iyer, V., Salunkhe, D. K., Sathe, S. K., & Rockland, L. B. (1980a). Quick-cooking beans (*Phaseolus vulgaris* L.): I. investigation on quality. *Qualitas Plantarum—Plant Foods for Human Nutrition*, 30, 27–43.
- Iyer, V., Salunkhe, D. K., Sathe, S. K., & Rockland, L. B. (1980b). Quick-cooking beans (*Phaseolus vulgaris* L.): II. phytates, oligosaccharides and antienzymes. *Qualitas Plantarum—Plant Foods for Human Nutrition*, 30, 45–52.
- Jerome, F. S., Millett, M. A., & Lawton, E. (1952). Effect of high energy cathode rays on cellulose. *Industrial and Engineering Chemistry*, 44, 2848–2852.
- Joseph, A., & Dikshit, M. (1993). Effect of irradiation on the proteinase inhibitor activity and digestibility (*in vitro*) of safflower oilcake. *Journal of American Oil Chemists' Society*, 70, 935–937.
- Josephson, E. S., & Peterson, M. S. (1983). *Preservation of foods by ionization radiation* (3 vols). Boca Raton, Florida: CRC Press.
- Jumel, K., Harding, S. E., & Mitchell, J. R. (1996). Effect of gamma irradiation on the macromolecular integrity of guar gum. *Carbohydrates Research*, 282, 223–236.
- Kashani, G. G., & Valadon, L. R. G. (1984). Effects of gamma irradiation on the lipids, carbohydrates and proteins of Iranian pistachio kernels. *Journal of Food Technology*, 19, 631–638.
- Kertez, Z. I., Schulz, E. R., Fox, G., & Gibson, M. (1959). Effects of ionising radiation on plant tissues. 4. Some effects of gamma radiation on starch and starch fractions. *Food Research*, 24, 609–617.



- Khattak, A. B., & Kloppenstein, C. F. (1989). Effects of gamma irradiation on the nutritional quality of grain and legume. *Cereal Chemistry*, 66, 170–171.
- Köksel, H., Celik, S., & Tuncer, T. (1996). Effects of gamma irradiation on durum wheats and spaghetti quality. *Cereal Chemistry*, 73, 507–509.
- Komiya, T., & Nara, S. (1974). The effects of moisture contents on the formation of radicals in various starches by  $\gamma$ -irradiation. II. On the changes of radical contents of irradiated various starches in absorption and desorption of moisture. *Food irradiation in Japan*, 9, 62.
- Lacroix, M., Amiot, J., & Brisson, G. J. (1983). Hydrolysis and ultrafiltration treatment to improve the nutritive value of rapeseed proteins. *Journal of Food Science*, 48, 1644–1645.
- Lagunas-Solar, M. C. (1995). Radiation processing of foods. An overview of scientific principles and current status. *Journal of Food Protection*, 58, 186–192.
- Lawton, E. J., Bellamy, W. D., Hungate, R. E., Bryant, M. P., & Hall, E. (1951). Some effects of high velocity electrons on wood. *Science*, 113, 380–382.
- Lee, C. C. (1962). Electron paramagnetic resonance (EPR) and packing studies on  $\gamma$ -irradiation flour. *Cereal Chemistry*, 39, 147–155.
- Leonhardt, J. W., Henning, A., Nehring, K., Baer, M., Flachowsky, G., & Wolf, I. (1983). *Proceeding for nuclear techniques for assessing and improving ruminant feeds*. Vienna, Austria: FAO/IAEA.
- Lesson, S., & Marcotte, M. (1993). Irradiation of poultry feed II. Effect on nutrient composition. *World's Poultry Science Journal*, 49, 120–131.
- Ley, F. J. (1963). Technological aspects of food irradiation with particular reference to Salmonella elimination. In *Radiation control of salmonella in food and feed products*. Technical Service Report 22. Vienna, Austria: International Atomic Energy Agency.
- Ley, F. S., Bledy, J., Coates, M. E., & Paterson, J. S. (1969). Sterilisation of laboratory animal diets using  $\gamma$ -radiation. *Laboratory Animals*, 3, 221.
- Liener, I. E. (1994a). Antinutritional factors related to proteins and amino acids. In Y. H. Hui, J. R. Gorham, K. D. Murrel, & D. O. Cliver (Eds.), *Foodborne disease handbook* (vol. 3) (pp. 261–309). New York: Marcel Dekker Inc.
- Liener, I. E. (1994b). Implications of antinutritional components in soybean foods. *Critical Reviews in Food Science and Nutrition*, 34, 31–67.
- Lochhead, C. (1989). The high-tech food process foes find hard to swallow. *Food Technology*, 43, 56–59.
- Lynn, K. R. (1973). Some effects of  $\gamma$ -irradiation on dilute solution of chymotrypsinogen. *International Journal of Radiation Biology*, 23, 227–233.
- Lynn, K. R., & Raoult, A. P. D. (1976). Irradiation of lima bean protease inhibitor in dilute aqueous solutions. *Radiation Research*, 65, 41–49.
- Machaiiah, J. P., Pednekar, M. D., & Thomas, P. (1999). Reduction in flatulence factors in mung beans (*Vigna radiata*) using low-dose  $\gamma$ -irradiation. *Journal of the Science of Food and Agriculture*, 79, 648–652.
- Mahrous, S. R. (1992). *Effect of some treatments on some toxic compounds present in foods*. PhD thesis, Faculty of Agriculture, Cairo University, Egypt.
- Makkar, H. P. S., Aderibige, A. O., & Becker, K. (1998). Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. *Food Chemistry*, 62, 207–215.
- Makkar, H. P. S., Becker, K. (1998). Plant toxins and detoxification methods to improve feed quality of tropical seeds. Review. In *Pre-conference symposium II on management of feed resources and animal waste for sustainable animal production in Asia-Pacific Region beyond 2000; 8th Conference on Animal Production* (pp. 467–480). June, Seoul National University, Seoul, Korea.
- Makkar, H. P. S., Becker, K., Sporer, F., & Wink, M. (1997). Studies on nutritive potential and toxic constituents of different provinces of *Jatropha curcas*. *Journal of Agricultural and Food Chemistry*, 45, 3152–3157.
- Marshall, J. J. (1975).  $\alpha$ -amylase inhibitors from plants. In A. Jeanes, & J. Hodge (Eds.), *Physiological effects of food carbohydrates*. ACP Symposium 15. Washington, DC: American Chemical Society.
- Marshall, J. J., & Lauda, C. M. (1975). Assay of  $\alpha$ -amylase inhibitor activity in legumes. *Die Starke*, 27, 274–278.
- McMannus, W. R., Manta, L., McFarlane, J. D., & Gray, A. C. (1972a). The effect of diet supplementation and  $\gamma$ -irradiation on dissimilation of low-quality roughages by ruminants. I. Studies on the terylene-bag technique and effects of supplementation of base ration. *Journal of Agricultural Science (Cambridge)*, 79, 27–40.
- McMannus, W. R., Manta, L., McFarlane, J. D., & Gray, A. C. (1972b). The effect of diet supplementation and  $\gamma$ -irradiation on dissimilation of low-quality roughages by ruminants. II. Effects of gamma irradiation and urea supplementation on dissimilation in the rumen. *Journal of Agricultural Science (Cambridge)*, 79, 41–53.
- McMannus, W. R., Manta, L., McFarlane, J. D., & Gray, A. C. (1972c). The effect of diet supplementation and  $\gamma$ -irradiation on dissimilation of low-quality roughages by ruminants. III. Effects of feeding gamma-irradiated base diets of wheat straw and rice straw to sheep. *Journal of Agricultural Science (Cambridge)*, 79, 55–66.
- Merritt, C. (1989). Radiolytic products. Are they safe? In *Safety factors influencing the acceptance of food irradiation technology*, TECDOC-490. Vienna, Austria: International Atomic Energy Agency.
- Metta, V. C., & Johnson, B. C. (1959). Biological value of gamma irradiated corn protein and wheat gluten. *Journal of Agricultural and Food Chemistry*, 7, 131–133.
- Miller, E. C., & Miller, J. A. (1986). Carcinogens and mutants that may occur in foods. *Cancer*, 58, 1795–1803.
- Molins, R. A. (2001). *Food irradiation: principles and applications*. New York: John Wiley & Sons.
- Moore, J. H., & Mudher, S. (1978). Implication of tryptophan in concanavalin A-erythrocytes interactions: a radiolytic study. *International Journal of Radiation Biology*, 34, 475–479.
- Moran, E. T., Summers, J. D., & Blayley, H. S. (1968). Effect of cobalt 60 gamma irradiation on the utilisation of energy, protein, and phosphorus from wheat bran by the chicken. *Cereal Chemistry*, 45, 469–479.
- Morrison, A. B., Clandinin, D. R., & Robble, A. R. (1953). Effect of processing variables on the nutritive value of sunflower seed oil meal. *Poultry Science*, 32, 492–495.
- Murray, D. R. (1990). *Biology of food irradiations*. New York: John Wiley & Sons.
- Nene, S. P., Vakil, U. K., & Sreenivasan, A. (1975a). Effect of gamma radiation on physico-chemical characteristics of red gram (*Cajanus cajan*) starch. *Journal of Food Science*, 40, 943–947.
- Nene, S. P., Vakil, U. K., & Sreenivasan, A. (1975b). Effect of gamma radiation on red gram (*Cajanus cajan*) proteins. *Journal of Food Science*, 40, 815–819.
- Osman, E. N. (1967). Starch in food industry. In R. L. Whistler, & E. F. Paschall (Eds.), *Starch: chemistry and technology* (vol. 2). London: Academic Press.
- Pritchard, G. I., Pidgen, W. J., & Minsin, D. J. (1962). Effect of gamma radiation on the utilisation of wheat straw by rumen microorganisms. *Canadian Journal of Animal Science*, 42, 215–217.
- Provansal, M. M. P., Cug, J. A., & Cheftel, J. (1975). Chemical and nutritional modifications of sunflower proteins due to alkaline processing. Formation of amino acid cross-links and isomerization of lysine residues. *Journal of Agricultural and Food Chemistry*, 23, 938–943.
- Pryor, W. A. (1984). *Free radicals in biology*. London: Academic Press.
- Pusztai, A. (1991). Plant lectins. In J. D. Phillipson, D. C. Ayres, & H. Baxter (Eds.), *Chemistry and pharmacology of natural products* (pp. 105–199). Cambridge, UK: Cambridge University Press.

- Pusztai, A., Clarke, E. M. N., Grant, G., & King, T. P. (1981). The toxicity of *Phaseolus vulgaris*, lectin. Nitrogen balance and immunochemical studies. *Journal of the Science of Food and Agriculture*, 32, 1037–1046.
- Rackis, J. J. (1975). Oligosaccharides of food legumes.  $\alpha$ -galactosidase activity and flatulence problem. In A. Jeans, & J. Hodge (Eds.), *Physiological effects of food carbohydrates*. Washington, DC: American Chemical Society.
- Raffi, J. J., Agnel, J.-P. L., Thiery, C. J., Frejaville, C. M., & Saint-Lébe, L. R. (1981). Study of  $\gamma$ -irradiated starches derived from different foodstuffs: a way for extrapolating wholesomeness data. *Journal of Agricultural and Food Chemistry*, 29, 1227–1232.
- Rahma, E. H., & Mostafa, M. M. (1988). Functional properties of peanut flour as affected by different heat treatments. *Journal of Food Science and Technology*, 25, 11–15.
- Rao, V. S., & Vakil, U. K. (1983). Effects of gamma-irradiation on flatulence-causing oligosaccharides in green gram (*Phaseolus aureus*). *Journal of Food Science*, 48, 1791–1795.
- Rao, V. S., & Vakil, U. K. (1985). Effects of gamma radiation on cooking quality and sensory attributes of four legumes. *Journal of Food Science*, 50, 372–375.
- Rattansi, R., & Dikshit, M. (1997). Protease inhibitors and *in vitro* digestibility of karanja (*Pongamia glabra*) oil seed residue. A comparative study of various treatments. *Journal of American Oil Chemists' Society*, 74, 1161–1164.
- Rayas-Duarte, P., & Rupnow, J. H. (1993). Gamma-irradiation affects some physical properties of dry bean (*Phaseolus vulgaris*) starch. *Journal of Food Science*, 58, 389–394.
- Reddy, S. J., Publos, M. H., & McGinnis, J. (1979). Effect of gamma irradiation on nutritional value of dry field beans (*Phaseolus vulgaris*) for chicks. *Journal of Nutrition*, 109, 1307–1312.
- Redmann, D. G., Axford, D. W. E., Elton, G. A. H., & Brivati, J. A. (1966). Mechanically produced radicals in flour. *Chemistry and Industry (London)*, 1298.
- Reyden, P., & Selvendran, R. R. (1993). Phytic acid: properties and determination. In R. Macrae, R. K. Robinson, & M. J. Sadler (Eds.), *Encyclopedia of food science, food technology and nutrition* (pp. 3582–3587). London: Academic Press.
- Rombo, G. O., Taylor, J. R. N., & Minnaar, A. (2001). Effect of irradiation, with and without cooking of maize and kidney bean flours, on porridge viscosity and *in vitro* starch digestibility. *Journal of the Science of Food and Agriculture*, 81, 497–502.
- Sandev, S., & Karaivanov, I. (1977). The composition and digestibility of irradiated roughage treatment with gamma irradiation. *Tierernahrung und Fuetterung*, 10, 238–242.
- Sandev, S., & Karaivanov, I. (1979). The composition and digestibility of irradiated roughage. In *Decontamination of animal feeds by irradiation* (pp. 83–87). Vienna, Austria: International Atomic Energy Agency.
- Sathe, S. K., & Salunkhe, D. K. (1984). Technology of removal of unwanted components of dry beans. *Critical Reviews in Food Science and Nutrition*, 21, 263–287.
- Sattar, A., Atta, S., & Akhtar, M. A. (1990). Effect of radiation and soaking on trypsin inhibitor and protein content of chickpea (*Cicer arietinum* L.). *Nahrung*, 34, 509–514.
- Sattar, A., Durrani, S. K., Mahmood, F., Ahmad, A., & Khan, I. (1989). Effect of soaking and germination temperatures on selected nutrients and antinutrients of mungbean. *Food Chemistry*, 34, 111–120.
- Sattar, A., Neelofar, X., & Akhtar, M. A. (1990a). Effect of radiation and soaking on phytate content of soybean. *Acta Alimentaria*, 19, 331–336.
- Sattar, A., Neelofar, X., & Akhtar, M. A. (1990b). Irradiation and germination effects on phytate, protein and amino acids of soybean. *Plant Foods for Human Nutrition*, 40, 185–194.
- Scherz, H. (1974). Some theoretical considerations on the chemical mechanism of the radiation-induced depolymerization of high molecular carbohydrates. In *Improvement of food quality by irradiation* (pp. 39–50). Vienna, Austria: International Atomic Energy Agency.
- Schubert, J. (1969). Mutagenicity and cytotoxicity of irradiated food components. *Bulletin of World Health Organisation*, 41, 873–875.
- Schubert, J. (1974). Irradiation of food and food constituents. Chemical and hygienic consequences. In: *Improvement of food quality by irradiation* (pp. 1–38). Vienna, Austria: International Atomic Energy Agency.
- Siddhuraju, P., & Becker, K. (2001a). Effect of various indigenous processing methods on alpha-galactoside, mono- and disaccharide content of an Indian tribal pulse, *Mucuna pruriens* var. *utilis*. *Journal of the Science of Food and Agriculture*, 81, 718–725.
- Siddhuraju, P., & Becker, K. (2001b). Effect of various domestic processing methods on antinutrients and *in vitro* protein and starch digestibility of two indigenous varieties of Indian tribal pulse, *Mucuna pruriens* var. *utilis*. *Journal of Agricultural and Food Chemistry*, 49, 3058–3067.
- Siddhuraju, P., Becker, K., & Makkar, H. P. S. (2000). Studies on the nutritional composition and antinutritional factors in three different germplasm seed materials of an under-utilised tropical legume, *Mucuna pruriens* var. *utilis*. *Journal of Agricultural and Food Chemistry*, 48, 6048–6060.
- Siddhuraju, P., Vijayakumari, K., & Janardhanan, K. (1996). Chemical composition and protein quality of the little-known legume, velvet bean (*Mucuna pruriens* (L.) DC.). *Journal of Agricultural and Food Chemistry*, 44, 2636–2641.
- Shih, F. F., & Kalmar, A. D. (1987). SDS-catalysed deamidation of oilseed proteins. *Journal of Agricultural and Food Chemistry*, 35, 672–675.
- Smits, C. H. M., & Annison, G. (1996). Non-starch plant polysaccharides in broiler nutrition-towards a physiologically valid approach to their determination. *World's Poultry Science Journal*, 52, 203–221.
- Snauwaert, F., & Markakis, P. (1976). Effect of germination and gamma irradiation on the oligosaccharides of navy beans (*Phaseolus vulgaris* L.). *Lebensmittel-Wissenschaft und-Technologie*, 9, 93–95.
- Sreenivasan, A. (1974). Compositional and quality changes in some irradiated foods. In: *Improvement of food quality by irradiation*, (pp. 129–155). Vienna, Austria: International Atomic Energy Agency.
- Srinivas, H., Ananthaswamy, H. N., Vakil, U. K., & Sreenivasan, A. (1972). Effect of  $\gamma$ -irradiation on wheat proteins. *Journal of Food Science*, 37, 715–718.
- Stevenson, M. H. (1994). Identification of Irradiated foods. *Food Technology*, 48, 141–144.
- Thakur, B. R., & Singh, R. K. (1994). Food irradiation: chemistry and applications. *Food Reviews International*, 10, 437–473.
- Thorne, S. (1991). *Food irradiation*. London: Elsevier Applied Science.
- Tjaberg, T. B., Underdal, B., & Lunde, G. (1972). The effect of ionising radiation on the microbiological content and volatile constituents of spices. *Journal of Applied Bacteriology*, 35, 473–478.
- Tomassi, G. (1988). Nutritional and safety aspects of natural antioxidants. In R. Walker, & E. Quattuci (Eds.), *Nutritional and toxicological aspects of food processing* (pp. 1–14). New York: Taylor and Francis.
- Uchiyama, S., & Uchiyama, M. (1979). Free radical production in protein-rich food. *Journal of Food Science*, 44, 1217–1220.
- Urbain, W. M. (1986). *Food irradiation*. Orlando, FL: Academic Press.
- Vakil, K. U., Aravindakshan, M., Srinivas, H., Chauhan, P. S., Sreenivasan, A. (1973). Nutritional and wholesomeness studies with irradiated foods. India's program. In *Radiation preservation of food* (pp. 673–702). Vienna, Austria: International Atomic Energy Agency.
- Van der Poel, A. F. B. (1989). Effects of Processing on antinutritional factors (ANF) and nutritional value of legume seeds for non-ruminant feeding. In J. Huisman, A. F. B. van der Poel, & I. E. Liener (Eds.), *Recent advances of research in antinutritional factors in legume seeds* (pp. 213–229). Wageningen, The Netherlands: Pudoc.

- Van Kooij, J. G. (1979). Chemical and biological evaluation of the nutritive value of heat-sterilised and radappertized feed mixtures. In *Decontamination of animal feeds by irradiation*, (pp. 89–110). Vienna, Austria: International Atomic Energy Agency.
- Wang, G. J., Marquardt, R. R., Guenter, W., Zhang, Z., & Han, Z. (1997). Effects of enzyme supplementation and irradiation of rice bran on the performance of growing Leghorn and broiler chickens. *Animal Feed Science and Technology*, *66*, 47–61.
- Wanger, J. R., Carson, J. F., Becker, R., Gumbmann, M. R., & Danhof, I. E. (1977). Comparative flatulence activity of beans and bean fractions for the man and rat. *Journal of Nutrition*, *107*, 680–689.
- WHO. (1977). *Wholesomeness of irradiated foods. Report on Joint FAO/IAEA/WHO Expert Committee, technical report series no. 604*. Geneva, Switzerland: World Health Organization.
- WHO. (1981). *Wholesomeness of irradiated foods. Report on Joint FAO/IAEA/WHO Expert Committee, Technical report series no. 659*. Geneva, Switzerland: World Health Organization.
- WHO. (1999). *High-dose irradiation: wholesomeness of food irradiated with doses above 10 kGy. Report of a Joint FAO/IAEA/WHO Study Group, technical report series no. 890*. Geneva, Switzerland: World Health Organization.