

## Nutritional and other implications of irradiating meat

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Different methods have been developed to extend the shelf-life of meat and its products ranging from the traditional use of salt to canning, freezing and modified-atmosphere packaging. As well as these more conventional approaches to meat preservation, the use of ionizing radiation has also been extensively studied over many years.

The irradiation sources which are permitted for use with food are gamma photons from  $^{60}\text{Co}$  or  $^{137}\text{Cs}$ , high-energy electrons generated by machines, maximum energy 10 MeV and X-rays with a maximum energy of 5 MeV (Codex Alimentarius Commission, 1984).

At doses of about 25–50 kGy, irradiation can be used to achieve sterilization and in the 1960s shelf-stable radiation-sterilized meat products were developed to substitute for canned or frozen military rations. Currently, sterile meals are produced for immunocompromized patients using irradiation. With doses below 10 kGy, the process is effective in enhancing food safety through the inactivation of pathogenic micro-organisms such as *Salmonella* and *Campylobacter* and extending shelf-life by eliminating the micro-organisms responsible for normal spoilage.

Following the report of the Food and Agriculture Organization/International Atomic Energy Agency/World Health Organization Joint Expert Committee on the Wholesomeness of Irradiated Food (1981) which concluded that 'irradiation of food up to an overall average dose of 10 kGy produced no toxicological hazard and introduced no special nutritional or microbiological problems', there has been renewed interest in the use of lower doses of irradiation for the preservation of food. In 1991, the UK government introduced new regulations permitting the irradiation of seven categories of food, including chicken, under strictly controlled conditions (UK Government Regulations, 1990a). Currently, thirty-seven countries have approval for the irradiation treatment of a range of foods or food items and of these countries, twenty-six are using the process on a commercial scale (P. Loaharanu, personal communication).

### EFFECT OF IRRADIATION ON THE NUTRITIONAL VALUE OF MEAT

Much of the early work on irradiation examined foods treated with sterilizing doses but since recent applications often use doses well below 10 kGy, a realistic evaluation of the nutritional adequacy of irradiated meat should be based on results of experiments carried out with doses likely to be used in commercial practice. Nevertheless, when appropriate, reference is made to the effects observed at high doses.

#### *Proteins*

Proteins in meat are a source of energy and of essential amino acids. The early work of Johnson & Metta (1956) showed that the metabolizable energy of protein, lipid and carbohydrate is not adversely affected even by an irradiation dose of 30 kGy.

Extensive studies have been undertaken to elucidate the chemical changes occurring in irradiated proteins. Many different compounds have been identified and some of them, such as those containing S, which are formed from methionine and cysteine, contribute to the irradiation odour sometimes associated with treated meat (Marbach & Doty, 1956; R. L. S. Patterson and M. H. Stevenson, unpublished results). Nevertheless, the extent of chemical change is very small because of the protective qualities inherent in foods. Using chicken irradiated at 3 and 6 kGy and then cooked, it has been shown that the concentrations of component amino acids are not adversely affected by irradiation (de Groot *et al.* 1972). The protein efficiency ratio of irradiated and non-irradiated chicken stored at 5° for 4–7 d and then cooked also indicated that the 'nutritive value of the protein is not noticeably affected by the irradiation treatment of the chicken meat'. Moreover, in beef heated to inactivate enzymes and then sterilized with doses of 47–71 kGy, the concentrations of cystine, methionine and tryptophan, the three amino acids considered to be most sensitive to ionizing radiation, were not significantly affected by treatment even after storage for 15 months at room temperature (Josephson *et al.* 1978).

### *Lipids*

Many volatile compounds have been identified from pure triacylglycerols irradiated at high doses (60 kGy) but, as for proteins, the implications of these chemical changes on the nutritional value of the lipid component of meat are minimal. The effect of gamma irradiation (up to 10 kGy) at various temperatures on the fatty acid profiles of neutral and polar lipids from air- and vacuum-packed chicken muscle and skin have shown only minor changes (Maxwell & Rady, 1989). The skin lipids showed no significant alterations in composition while, as a class, the polar lipid fractions of muscle showed some slight changes. Nevertheless, irradiation caused insignificant effects even in the polyunsaturated fatty acids. These results are at variance with the significant reductions in polyunsaturated fatty acids reported for irradiated (4 kGy) herring oil stored for 3 months at –20° (Hammer & Wills, 1979). The different results obtained in these two experiments emphasize the importance of not extrapolating findings, obtained with a model system, to a real food. Fewer alterations to the polar lipids in chicken muscle occurred when samples were irradiated at –20° (Rady *et al.* 1988), thus suggesting that the lower irradiation temperature would be preferable for treatment of this product.

### *Vitamins*

Irradiation of vitamins in solution or in model systems results in considerable destruction of these compounds, but in irradiated foods the effects observed are generally not as marked (Diehl *et al.* 1991; Thayer *et al.* 1991). The losses are affected by the dose applied and they can be minimized by irradiation at freezing temperatures or by packaging the product in an inert atmosphere. It is important to point out that other food preservation methods, such as those involving heat, also destroy vitamins, so the effect of ionizing radiation on these minor constituents is not unique to the irradiation process. Most meats are cooked before consumption and they may also be stored, so the combined effects of irradiation, storage and cooking on the vitamin content are important.

Thiamin is the most radiation sensitive of the water-soluble vitamins. Measurements of the thiamin content of irradiated pork chops (Fox *et al.* 1989), chicken (Fox *et al.* 1989;

Hanis *et al.* 1989; M. H. Stevenson & W. D. Graham, unpublished results), turkey (Thomas & Calloway, 1957), minced beef (Wilson, 1959), beef liver (Williams *et al.* 1958) and bacon (Thayer *et al.* 1989) confirm that destruction of the vitamin reflects the dose applied and the conditions used during irradiation. Thiamin levels in chicken meat decrease with increasing dose but irradiation at freezing temperatures markedly reduces the effects observed (Fox *et al.* 1989; M. H. Stevenson & W. D. Graham, unpublished results). Similar results have also been reported for pork, where the calculated thiamin loss in raw pork irradiated at 3.34 kGy was 15% at  $-20^{\circ}$ , 35% at  $0^{\circ}$  and 47% at  $+20^{\circ}$  (Fox *et al.* 1989). The net effect of cooking and irradiation on thiamin retention has also been studied. Generally speaking, cooking irradiated bacon (Thayer *et al.* 1989), pork chops and chicken breasts (Fox *et al.* 1989) increases the rate of thiamin loss. According to Fox *et al.* (1989), the cooking-dose effect appears to involve chemical or physical changes in the tissues or vitamin which result in increased vitamin destruction or decreased extractability. Contrary to these observations, a protective effect has been identified for thiamin when bacon is cooked before irradiation (Thayer *et al.* 1989). Despite the fact that there are small reductions in the thiamin content of meats following irradiation, it should be noted that thiamin is even more sensitive to heat than to irradiation. Pork and beef sterilized by irradiation with doses of about 45 kGy retain approximately 85% of their thiamin compared with 20% for heat-sterilized pork and 65% for thermally processed beef (Josephson *et al.* 1978). The significance of the loss of thiamin in meats treated with low doses of irradiation, 3 kGy or below, must be considered in the light of the contribution which the meats make to the overall intake of the vitamin. It has been calculated that the net loss of thiamin in the American diet due to irradiation and cooking of pork chops and roasts would be 0.5–1.5% over the dose range 0.3–1.0 kGy which is the treatment level needed to control trichinosis. For irradiated cooked chicken, the dietary loss was estimated to be 0.01 and 0.08% at 1 and 3 kGy respectively, these doses being within the range needed to inactivate food-poisoning micro-organisms (Fox *et al.* 1989). Moreover, these calculations have assumed that all pork and chicken consumed will be irradiated and this is highly unlikely; thus, the quoted values represent the worst possible situation.

Riboflavin is the most stable vitamin to irradiation in almost all food substrates. In both raw pork chops and chicken breasts, riboflavin showed an initial increase with irradiation dose (Fox *et al.* 1989). Similar increases in riboflavin and other vitamins have also been reported previously in irradiated (6 kGy), cooked chicken (de Groot *et al.* 1972). These results suggest that irradiation alters the substrate to make riboflavin more extractable or measurable, possibly by freeing bound riboflavin. This observation may have implications for the nutritional value of the vitamin in irradiated foods.

Niacin in aqueous systems is more unstable than thiamin or riboflavin but it is stable in irradiated food. In pork chops irradiated at different temperatures with doses up to 5 kGy, no loss of niacin was observed while at a dose of 6.65 kGy and an irradiation temperature of  $0^{\circ}$ , the loss was 15% (Fox *et al.* 1989). Under the same irradiation processing conditions, the niacin concentrations in chicken breast meat showed no consistent effects.

The radiation resistance of pyridoxine is closer to that of riboflavin than to thiamin. No loss of pyridoxine was reported in cooked chicken which had been irradiated at a dose of 6 kGy (de Groot *et al.* 1972).

Cyanocobalamin is also stable to irradiation. Chicken meat pasteurized by exposure to

3 and 6 kGy showed no loss of cyanocobalamin (de Groot *et al.* 1972) and in pork chops irradiated with doses up to 6.65 kGy at temperatures between  $-20^{\circ}$  and  $+20^{\circ}$  no measurable changes in the concentration of this vitamin were noted (Fox *et al.* 1989). Even in radiation-sterilized chicken, the concentrations of cyanocobalamin were similar to those in frozen and in thermally sterilized samples (Thayer, 1990).

Studies on the sterilization of meat and chicken with high radiation doses indicated that losses of folic acid were zero or insignificant (Alexander *et al.* 1956; Thayer, 1990). Therefore, it is unlikely that any important effects would be observed at doses below 10 kGy.

Among the fat-soluble vitamins, vitamin E is the most radiation labile (Knapp & Tappel, 1961) and, therefore, the most sensitive indicator of the effects of irradiation on this class of vitamins. Relatively few studies have been reported on the effect of ionizing radiation on the low level of vitamin E present in meat (de Groot *et al.* 1972; Diehl, 1979; Lakritz & Thayer, 1992). Chicken meat, sealed in  $O_2$  permeable bags and irradiated at a temperature of  $4-6^{\circ}$  with doses between 1.0 and 10.0 kGy showed a linear decrease in  $\alpha$ - and  $\gamma$ -tocopherol with increasing dose levels. At 3 kGy, the dose which is likely to be used commercially, there was a 15% reduction in free  $\alpha$ -tocopherol and a 30% reduction for free  $\gamma$ -tocopherol (Lakritz & Thayer, 1992). However, it should be remembered that meats are not the main sources of vitamin E in the diet. As well as irradiation dose, the atmosphere surrounding a product during treatment has also been shown to have an effect on the retention of vitamin E. When a chick diet was sterilized with 50 kGy in vacuum, 90% of vitamin E was retained compared with approximately 50% when the irradiation was carried out in air (Kraybill, 1982).

Although vitamin A is sensitive to irradiation most of the foods that are important sources of this vitamin in the human diet, such as milk and butter, are unlikely to be commercially irradiated. Liver is a good source of vitamin A and after 1 and 2 weeks storage pork liver treated with 5 kGy at  $0^{\circ}$  contained 4 and 18% less respectively than the non-irradiated samples stored for the same periods. The corresponding losses for calf liver sausage under the same conditions were 10 and 12% respectively (Diehl, 1979). Studies by de Groot *et al.* (1972) showed that vitamin A was not affected in chicken meat irradiated at 3 or 6 kGy, stored for 4–7 d at  $5^{\circ}$ , cooked and then stored at  $-20^{\circ}$ .

Vitamin D in iso-octane was found to be more stable than vitamins E or A, while vitamin K in its pure form was the most stable of the fat-soluble vitamins (Knapp & Tappel, 1961). Recently, Thayer (1990) has reported no loss of vitamin D and about a 30% decrease in vitamin K in chicken meat sterilized (46–68 kGy) using irradiation. Effects of low-dose irradiation on the contents of these vitamins in meats have not been published.

On the whole, the effects of irradiation on the nutritive value of foods are minimal and these observations are substantiated by the results of the feeding studies which have been undertaken to establish the wholesomeness of irradiated food.

#### FEEDING STUDIES

In two studies, chicken meat irradiated at pasteurizing doses of 3 and 6 kGy was fed to albino rats in both a multigeneration study and also a 2-year chronic feeding study. In both cases, the chicken was included in the diet at 350 g/kg dry matter. The general appearance and behaviour, mortality, growth, food intake and constituents of blood and

urine were measured and no effects due to irradiation were observed (van Eekelen *et al.* 1971, 1972). More recently, Thayer *et al.* (1987) reported the results of nutritional, genetic, teratogenic and multigeneration feeding studies using frozen enzyme-inactivated, thermally sterilized, gamma-sterilized and electron beam-sterilized chicken meat. These studies required 135 405 kg chicken, enzyme-inactivated by heating to an internal temperature of 75–80°. The irradiated chicken received a dose of 45–68 kGy administered *in vacuo* at an internal temperature of –40°. Mice, hamsters, rats and rabbits were fed on diets containing 350 g chicken/kg dry matter except for the teratogenic studies where the inclusion level was 700 g/kg. Teratogenic and genetic toxicology studies showed no effects due to irradiation treatment. Chronic feeding and performance trials carried out with beagle dogs over a 40-month period and a 2-year chronic toxicity, oncogenicity and multigeneration reproductive study with mice also showed no detrimental effects due to ingestion of any of the diets. As well as these specific experiments, incidental evidence of the wholesomeness of irradiated foods is provided by the routine use of radiation-sterilized diets to feed laboratory animals. Many generations have been reared on these diets without showing any adverse affects (Swallow, 1991).

In 1986, a trial was carried out in China using twenty-one male and twenty-two female volunteers who consumed 62–71% of their total energy intake as irradiated foods for a period of 15 weeks (Brynjolfson, 1988). The diet included irradiated rice, meat products and fourteen different vegetables. Measurements of total energy intake, monthly biochemical and physical examinations and sensory evaluation of the food were carried out. The diet was well accepted and no adverse effects were noted.

#### DETECTION METHODS FOR IRRADIATED MEAT

Despite the fact that all the feeding studies carried out have confirmed that irradiated food is wholesome and that much more is known about the changes occurring in these treated foods than in those processed by more conventional methods, it is accepted that irradiated food must be labelled (UK Government Regulations, 1990*b*) so that the consumer has the option to select or reject irradiated food. In order to help enforce these legal requirements, methods to identify irradiated food have been developed.

In the case of meat and meat products, the methods which have been most researched and which have also been tested in collaborative trials are those based on the use of electron paramagnetic resonance (EPR) spectroscopy and on the formation of lipid degradation products, either long-chain hydrocarbons or 2-alkylcyclobutanones.

Free radicals are generated following irradiation but they survive for only less than 1 s in the meat and so are difficult to detect by conventional EPR spectroscopy, a tool used for studying species with unpaired electrons. On the other hand, if the radicals are trapped in hard, relatively dry environments, such as bone, their presence can be confirmed using this technique. The signals are very stable and can be detected for a number of years in samples given irradiation doses which are likely to be used in practice. It has been well documented that irradiated bone gives an EPR signal, the shape of which is independent of the origin of the bone and quite different from that observed in bone which has not been irradiated (Dodd *et al.* 1988; Goodman *et al.* 1989; Stevenson & Gray, 1989). The shape of the radiation-induced signal is specific for irradiation since none of the processing variables examined, such as storage (Stevenson & Gray, 1989) or

cooking (Gray & Stevenson, 1990), have generated a signal of similar shape. In addition, the signal was detectable under all conditions examined and even at a dose of 0.3 kGy which is well below that likely to be used commercially (Raffi, 1992). The value of the EPR technique for the identification of irradiated bone containing meat has been confirmed in collaborative trials (Scotter *et al.* 1990; Raffi, 1992).

Not all meat will contain bone so there are advantages in being able to use the meat itself for identification purposes. About 20 years ago, Nawar & Balboni (1970) suggested that long-chain hydrocarbons formed from fatty acids on irradiation could form the basis of a detection method. Recently, several research groups have concentrated on developing this approach for the routine control of irradiated meat (Biedermann *et al.* 1989; Sjöberg *et al.* 1992; Schreiber *et al.* 1993). The volatile hydrocarbons considered to be of most interest are those with one C less, or two C less and one double bond more than the fatty acid from which they are formed. Of the eight possible hydrocarbons formed from irradiation of the major fatty acids in meat, it has been suggested that the most appropriate markers are tetradecene, hexadecadiene and heptadecene (Nawar *et al.* 1990). These compounds are either absent or present in low concentrations in meats not irradiated. Two intercomparisons using volatile hydrocarbons to detect irradiated chicken, pork and beef (Schreiber *et al.* 1993) and chicken (M. H. Stevenson & W. Meier, unpublished results) have confirmed that the method can be used for qualitative identification of these irradiated meats.

The other group of compounds, 2-alkylcyclobutanones, which have been shown to be specific markers of irradiation treatment in meats have the same number of C atoms as their precursor fatty acids and the alkyl group is located in ring position 2. Both 2-dodecylcyclobutanone (DCB) and 2-tetradecylcyclobutanone which are formed from irradiated palmitic and stearic acid respectively have been detected in irradiated chicken, pork, lamb and beef (Boyd *et al.* 1991; Crone *et al.* 1992; Stevenson *et al.* 1993). No background levels of these compounds have been detected in samples not irradiated or in microbiologically-spoiled samples and they have always been present in irradiated samples even at doses well below those likely to be used commercially. Specificity of the compounds was further demonstrated when they were not produced by cooking or during storage (Crone *et al.* 1992). An intercomparison using irradiated chicken has confirmed that 2-DCB can be used to detect irradiated samples with an unknown processing history (M. H. Stevenson & W. Meier, unpublished results). At present, a more extensive trial is under way involving irradiated pork, chicken and liquid whole egg.

Following the success of these trials, standard protocols for the detection of irradiated meat containing bone, using EPR spectroscopy, and for chicken meat, using the hydrocarbon and cyclobutanone methods, have been published (Raffi *et al.* 1993) and recently submitted to the European Committee of Normalization.

#### CONCLUDING COMMENTS

Research has shown that irradiation introduces no special nutritional problems. The energy value of irradiated macronutrients in foods is not significantly affected by irradiation at doses up to 10 kGy and even beyond. Essential amino acids and fatty acids also suffer minimal changes but a few vitamins, especially thiamin, may be affected by the process. The losses incurred are generally no greater and are often smaller than those caused by other processes such as those involving heat and they can be minimized by

using appropriate conditions during irradiation and storage. Despite the body of evidence that confirms that irradiated food is wholesome, it must be labelled and so methods have been developed to detect irradiated food, the availability of which will help to enforce the labelling regulations and ensure that consumers' interests are protected and that the technology is not abused.

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