

Review Article

Food Irradiation—A Means of Controlling Pathogenic Microorganisms in Food

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Salmonella enteritidis, S. typhimurium, Campylobacter jejuni and Listeria monocytogenes frequently cause foodborne infections. Food of animal origin, especially poultry, poultry products, eggs and egg products are main sources of contamination. To ensure hygienic quality of these products, it is necessary to take protective measures—not only of a hygienic nature. Food irradiation is one of the protective methods available. Comprehensive data concerning sensory, physiological and toxicological characteristics of irradiated food exist. However, to determine eventual sources of risk associated with the inappropriate use of radiation, it is necessary to generate additional comprehensive microbiological and engineering data for all foods concerned. Finally, predictive modelling will contribute considerably to the solution of the overall problem.

Introduction

Biological contamination of food plays an important role in the transmission of diseases to humans. Up to 70% of the 1.5×10^9 foodborne diseases worldwide in children younger than 5 years, a number reported by the World Health Organization (WHO), are caused by microbially contaminated food. This applies also to developing countries in particular where food shortage and unhygienic practices are frequent. In these countries typhoid and paratyphoid diseases prevail, while in industrialized countries a growing incidence of gastroenteric infections has been observed.

In both developing and industrialized countries, however, the actual incidence of foodborne disease, according to a WHO estimate, exceeds the statistics by a factor of about 100 (1,2). To improve the situation, firstly the deplorable hygienic conditions which lead to food contamination must be changed. Processing of food with ionizing radiation, besides other measures, may help to overcome residual risks.

To evaluate the wholesomeness of this preservation method, irradiated food has been investigated over decades for eventual chemical changes, toxicity, changes in the microbial flora and nutritional quality. From 1970 to 1982 these studies were coordinated by an international project (IFIP) with seat in Karlsruhe. A joint expert committee of WHO, Food and Agriculture Organization (FAO) of the United Nations, and the International Atomic Energy Agency (IAEA) concluded from these data in 1980 that 'the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard; hence, toxicological testing of foods so treated is no longer required'. As far as nutritional quality and microbiological status are concerned, 'the Committee considered that the ir-

radiation of food up to an overall average dose of 10 kGy introduces no special nutritional or microbiological problems. However, the Committee emphasized that attention should be given to the significance of any changes in relation to each particular irradiated food and to its role in the diet' (3). This expert judgement was reason to establish a Codex Alimentarius Standard concerning the safety of irradiated food and measures of control and documentation of food irradiation (4,5). A new joint expert committee has completely confirmed this judgement on the basis of all data published meanwhile in May 1992 (6).

Following this Codex Standard, irradiated food was legally permitted in several countries, but not in the Federal Republic of Germany (7). In those industrialized countries where food irradiation is permitted, clearances are confined to food, the hygienic safety of which is not guaranteed by conventional preservation methods. Such food presently includes spices, dried vegetables, poultry, mechanically deboned poultry meat, and deep-frozen shrimps.

Poultry and poultry products, eggs and products containing fresh eggs belong to the risk food for which no efficient protective measures exist so far. Besides the health risk for consumers, contaminated food causes considerable economic damage which amounts to 240 million DM per year according to a report on the incidence of Salmonella in Germany from 1977 to 1982 (8). It is to be examined, therefore, to what extent food irradiation as a protective measure may be applied to poultry, eggs and egg products.

Foodborne Infections and Sources of Contamination

Since 1980 the WHO Surveillance Programme on Foodborne Infections and Intoxications in Europe has

been collecting data on individual cases and epidemic outbreaks of foodborne infections from 24 national registration systems. A comparative evaluation of these data is difficult as the nature of the national reports is *not internationally compatible*. Even more, foodborne diseases are differently defined in different countries. As only a few individual registration systems were changed since 1980, the general increase in the number of actual cases is assumed to reflect a real increase. Accordingly, gastrointestinal diseases have primarily been caused by *Salmonella* and *Campylobacter* besides *Shigella* and *Yersinia* (9). *Listeria monocytogenes* has frequently been observed as well (10).

Salmonella enteritidis

In North and South America and in Europe, the number of infections caused by *Salmonella enteritidis* has increased strongly since 1985. In 1979, according to WHO, *S. enteritidis* was the most frequently found *Salmonella* serovar in two out of 21 countries, in 1987 the number had increased to nine, of which eight were European countries. The causes of this heavy increase are unknown. Epidemiological data from U.S.A., Hungary, Spain, France, Norway and Great Britain always indicate eggs, egg-containing food or poultry as sources of infection. The incidence of *Salmonella typhimurium*, however, a widespread *Salmonella* serovar as well, has been found to decrease from 67% to 38% (11).

In a 1990 study of the German Federal Health Office to evaluate the epidemiological situation in Germany (12), *S. enteritidis* isolates from institutions of human and veterinary medicine were differentiated using methods of molecular biology. As in Great Britain first and later also in other countries of Central Europe, phage type 4 (PT4) has been found to be the most frequent representative of *S. enteritidis* in 79.6% of human isolates, 78.0% of food and 66.7% of poultry isolates. 86.6% of all strains investigated, and 95.5% of PT4 contained solely a serovar-specific plasmid which is one of the reasons for the virulence of these bacteria.

Analyses of membrane proteins which reveal a great uniformity of the isolates suggest that this bacterial strain has perfectly adapted to its environmental conditions (12). The reason for the Europe-wide high dominance of PT4 has not been explored. There is some evidence of increased invasivity in young chicken, however (13). In Germany, PT4 has not been found resistant against antimicrobial agents in 1990, while at this time multiple-resistant strains were isolated in Great Britain and Norway (12). In 1991, however, 4.9% of the *S. enteritidis* strains isolated in Germany were resistant to antibiotics; this applied especially to isolates from food and poultry, according to studies reported by the German Federal Health Office. PT4 was detected in 67% of all isolates and in 33.9% of poultry isolates. 79.4% of all isolates contained solely the virulence plasmid which, however, had already been detected in samples of 1925 and 1937/38 (14).

A comprehensive analysis of the data of veterinary samples collected in Germany from 1984 to 1991 has

shown that the incidence of *S. enteritidis* in livestock and environmental samples is proportional to the incidence of salmonellosis in humans. In livestock, *S. enteritidis* has mainly been found in isolates of chickens, ducks and geese, and in eggs and egg products (15).

Listeria monocytogenes

Various epidemic listerioses have been found to be caused by contaminated food including pasteurized milk and cheese (16–18) but also coleslaw (19) and mushrooms (20).

According to a study on the occurrence of *L. monocytogenes* in salads and vegetables (21), only 2.7% of all samples, and 6% of all prepared salads were contaminated. The risk associated with vegetable food hence is relatively low; it emerges from storage of ready-to-serve salads and freshly squeezed vegetable juices, as *Listeria* is capable of growing also at low temperature. Listerioses after consumption of poultry and meat products have not been recorded so far, even though poultry meat is very strongly contaminated by *Listeria*. Studies from slaughtering to marketing of poultry (22,23) have shown 40.6% of chicken and 32.2% of turkey from local supermarkets in the U.S.A. to be contaminated by *Listeria sp.* The proportion of *L. monocytogenes* was 13.1 and 15%, respectively. In samples taken at slaughterhouses of neck skin, whole livers after chilling, cecum and large intestine content, and in samples of feathers and from hot-water tanks *Listeria* were not detected, neither in the investigations of chicken nor of turkeys. However, up to 38% of samples from service water tanks and circulation systems and from mechanically deboned meat were contaminated; the extent of contamination has been found to grow with the number of processing operations. At the end of the chicken processing line 33.3% of packaged livers, 36.7% and 70%, respectively, of skin samples of drumsticks and wings were contaminated by *L. monocytogenes*.

Campylobacter jejuni

In the aforementioned WHO Surveillance Programme on Foodborne Infections and Intoxications in Europe (9) *Campylobacter jejuni* is listed as one of the most frequent bacterial causes of intestinal disease. In Great Britain, the incidence of *Salmonella*-caused diseases is comparable to that of *Campylobacter*-caused infections. According to research coordinated by the FLAIR/COST Programme 'Prevention and control of potentially pathogenic microorganisms in poultry and poultry meat processing' *Campylobacter* has been found everywhere in the chain from livestock to the product offered for sale (24–27).

Improvement of the Hygienic Quality of Food

Mainly food of animal origin, especially poultry, poultry products, eggs and egg products have been found to be associated with outbreaks of foodborne infections and intoxications (9,11–15). Measures to

improve hygienic conditions in livestock management are advised in the EC directive concerning zoonosis (28). New concepts of improved slaughtering hygiene, chilling and deep-freezing are being developed and tested. For the risk sectors mentioned before, additional protective measures should be taken. A possibility of protecting the product is by radiation processing.

Hygienic measures

Hygienic measures must be taken in all sectors involved, starting from animal breeding. As *S. enteritidis* PT4 in chicken seems to spread through infestation of the ovaria (11), the question arises whether *Salmonella*-free flocks can be achieved at all. Studies in Sweden have shown that contamination by *Salmonella* cannot be safely excluded despite control and cost intensive breeding and fattening conditions (Wierup, pers. comm.). A 1989–1991 government programme in the Netherlands to eliminate *S. enteritidis* from breeding flocks and production farms had the effect that during the first year of the programme only 1% of breeding flocks, but as much as 7.5–10% of commercial flocks were still infected (29).

Infection reducing measures taken in the animal include artificial colonization of the intestine with a protective microflora, and immune prophylaxis (30,31). Feeds, especially those containing animal proteins, are frequently sources of contamination. Conventional decontamination methods include pelletizing, exposure to ethylene oxide and addition of organic acids. Food additives such as probiotic compounds and antibiotics, *Bacillus subtilis* and *Bacillus licheniformis* as protective cultures, and polysaccharides which compete with *Salmonella* for intestinal adhesion have been tried as well. Complete elimination of *Salmonella* has not been achieved by any of these methods (32,33). Feed irradiation has been investigated as an alternative method (32,34).

Other starting points of infection reducing measures are livestock management conditions, slaughtering and subsequent processing steps (22,23,35,36). As all these are cost intensive (8), government incentives and stricter controls to guarantee compliance with new legal regulations (28,29) will be inevitable.

Ionizing rays and combined methods

On the microbiology of irradiated poultry products comprehensive product-specific data are available (37). Recent papers describe the destruction kinetics of the main causes of foodborne infections in the past years, *S. enteritidis*, *S. typhimurium*, *L. monocytogenes* and *C. jejuni*.

D_{10} values for *S. typhimurium*, after irradiation with up to 2.5 kGy at 10°C under different atmospheres, are 0.436–0.622 kGy (38). For *L. monocytogenes*, after irradiation of inoculated minced chicken meat with doses up to 2.5 and 1.0–2.0 kGy, respectively, at 12° and 2–4°C D_{10} values were 0.417–0.553 kGy (39) and 0.59–1.03 kGy (40). Irradiation of whole chickens with 2.5 kGy at 4°C reduced the *Listeria* contamination, but

failed to completely eliminate the pathogen (41). *Campylobacter* and *Yersinia* are much more sensitive than *Salmonella* and *Listeria* (42).

It is difficult, however, to calculate from the wealth of data the required minimum radiation dose to kill the pathogens, as the antimicrobial effect of irradiation depends on many factors. Ionizing radiation causes DNA breaks and enzyme damage (43,44). Free radicals form which convert cell components into reactive hydroperoxides and lead to cell damage. Sensitivity of various microorganism species to radiation varies strongly; *Moraxella osloensis*, with a D_{10} value of 5–10 kGy, has been found most resistant (45). Among the most sensitive microorganisms are gram-negative rods, followed by gram-positive cocci and rods, yeasts, molds, fungal spores, aerobic and anaerobic spore formers (37,43). Spores and dried cultures of low water activity are more resistant than vegetative cultures, in which free radicals from intracellular water increase the extent of cell destruction.

Sensitivity of a vegetative culture depends also on the physiological cell age; it is highest at the end of the logarithmic growth phase. Higher resistance in the lag phase is explained by the high enzyme content of cells which lowers intracellular oxygen levels. Anaerobic conditions during irradiation lead to higher D_{10} values (44).

In the dosage range considered, ionizing radiation damages microorganisms on a sublethal level; it need not necessarily result in a loss of the ability to divide or destruction of cells. Potential resistance to irradiation therefore depends highly on the substrate in which the microorganisms are irradiated (44,46). In chemically defined model solutions sensitivity is higher than in so complex a medium like food. Food contains agents with sulphhydryl groups which react preferably with free radicals and so protect microorganisms. Microorganisms adhering to surfaces are more resistant to external stress than those in suspension (47). For *Salmonella panama*, after irradiation with 2.5 kGy at 5°C and –18°C, respectively, D_{10} values were 0.67 and 1.29 kGy. In the presence of an average *Salmonella* contamination of less than 100 cfu per cm² chicken skin, irradiation at 2.5 kGy is expected to yield a *Salmonella*-free product. However, *Salmonella*-positive samples were still detected under these conditions (44). Hence data of experiments in bacterial suspensions in model solutions are just as unreliably transferred to food as are data from meat to meat pieces plus skin.

Finally, processing parameters like temperature, oxygen concentration and composition of the atmosphere, respectively, as well as sample packaging influence the destruction kinetics of pathogens.

A loss of sensory quality at higher irradiation doses is a limit to the use of irradiation. According to literature data, sensory attributes of poultry meat, eggs and egg products are not affected by doses of about 3 kGy (48,49). The spore former *Clostridium botulinum* with a relatively high D_{10} value of 2–3.5 kGy is not eliminated safely by a maximum irradiation dose of 3 kGy advised for preservation of the sensory quality of

poultry and poultry products. Lactic acid bacteria, spoilage flora in the product concerned, have shown high resistance to radiation of up to 1.4 kGy for *Enterococcus faecalis* (50,51). According to the US Food and Drug Administration (FDA) an irradiation dose of 3 kGy ensures that enough spoilage organisms survive to spoil the irradiated product and warn consumers against its contamination before the spores of *C. botulinum* germinate (10).

When maximum doses limited by sensory aspects fail to safely destruct pathogenic microorganisms, a combination of methods may be applied, e.g. irradiation at elevated temperatures and at reduced relative humidity. Maximum synergistic effect is obtained when the destruction rates of each of the individual methods are about the same (44). With oxygen excluded, changes in sensory quality appear only at higher irradiation doses.

Conclusion

It should not be overlooked that processing by ionizing radiation cannot replace good manufacturing practices and is ineffective in disguising good production hygiene. Processing of food with ionizing radiation then is a technically feasible measure to improve food safety. Comprehensive data are available on the destruction kinetics of pathogenic microorganisms by processing with ionizing radiation. These data were usually generated in separate investigations under different conditions; hence comparison or even transfer of data to matrices other than the original is not yet possible.

In view of the many potential influencing factors, the minimum radiation dose to eliminate pathogens reliably can be determined only on the basis of food-related material data for specific pathogens obtained by defined processing parameters. If used for predictive modelling, such data should also allow to predict the microbiological consequences of a change in the defined operating conditions.

Varying sensitivities to irradiation of individual species or serovars of the relevant pathogens require profound knowledge of the distribution of these pathogens in poultry breeding stations, slaughterhouses and in products to be marketed.

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