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Recent Progress of Food Irradiation Researches in Japan with Special Reference to Wholesomeness Aspects and Detection Methods

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1. Introduction

In recent years, some cooperative efforts at the international level have been required for commercial applications of food irradiation, e.g. (1) wholesomeness studies conducing to clearing up debates on irradiated foods and to reduction of consumer fear, (2) process and administrative controls for manufacturing and dealing irradiated products and (3) development of detection methods for irradiated foods.

These trends may greatly stimulate the commercial applications of food irradiation. Commercialization could not confine the food irradiation to individual countries, but will bring about the international trade in irradiated products and materials to be irradiated. In this context, international harmonization of regulatory control and technological cooperation among different countries are desirable for further progress in commercialization. In 1988, an International Conference on the Acceptance, Control of and Trade in Irradiatied Food jointly organized by FAO, WHO, IAEA, ITC-UNCTAD / GATT was held in Geneva, Switzerland (Geneva FI Conf. 1988) and these problems associated with commercialization and control of food irradiation were discussed. An international document adopted by the consensus in the Geneva FI conf. 1988 describes general concepts arising from the enforcement of practical application of food irradiation.

On the one side, the possible role of food irradiation research to contribute to control and regulatory aspects of food irradiation has become well realized as time of its comercialization is coming along. One of researches of this kind is the development of detection methods of irradiated foods. On the other side, rapid and remarkable progress in food safety studies being made year by year might produce some new problem or parameter to be examined in wholesomeness aspects for irradiated foods. Considering importance of consumer acceptance, studies based on the latest methodology and highly developed tools would be requested to solve moot points, if circumstances require.

This review paper is concerned with recent progress in two research subjects, i.e. (1) some highlights of wholesomeness aspects and (2) detection methods for irradiated foods. In addition, the conception concerning approach toward the phase of commercialization documented at the Geneva FI Conf. 1988 will be briefly introduced in connection with the recent results of studies.

2. Recent progress in wholesomeness studies and related debates

Although the conclusion of the FAO / IAEA / WHO Expert committee in 1980 on toxicological acceptability of foods irradiated with 10 kGy or less was announced, further wholesomeness studies would be still requested at least by two reasons, i. e. to eliminate remaining public concern for irradiated foods and to respond to remarkable progress in sciences associated with safety aspects. Some fraction of wholesomeness data will also contribute to establish the control systems for manufacturing and distribution processes and be incorporated into the good manufacturing practice(GMP). Some examples of studies and debates will be described.

2.1. Some results obtained in recent wholesomeness studies in Japan

A committee on food irradiation research was organized in the Japan Radioisotope Association in 1986 and continued to study for 6 years mainly on research subjects directly or indirectly associated with wholesomeness evaluation and partly on the feasibility of food irradiation in comparison with other existing food processing methods.[1] Some of research subjects investigated in the Committee are introduced here. In connection with international debates on irradiation of sugar containing fruits, mutagenicity induction in glucose solutions irradiated with gamma rays was studied under different conditions.[2,3,4]

The results obtained are summarised as follows:

(1) In bacterial reverse mutation tests in Salmonella typhimurium TA98, TA100 and TA102 according to the standard method of Ames et al.[5] (preincubation, for 20 minutes at 37℃), glucose aqueous solutions (mostly 1-3%) irradiated by 10 kGy or less with and without oxygen bubbling afforded the negative results.

(2) In the reversion assay by the Niemand method (preincubation, for 3.5-5.0 hours at 37℃), the results obtained with glucose solutions irradiated without oxygen bubbling were negative, while those irradiated with oxygen bubbling were negative, while those irradiated with oxygen bubbling were positive.

(3) Clastogenic activity of glucose solutions irradiated with oxygen bubbling was demonstrated in chromosomal aberration tests with cultured Chinese hamster lung cells(CHL) in the absence of S9 mix.

(4) Both mutagenic (in bacterial cells) and clastogenic (in CHL) activities in vitro of irradiated glucose solutions were inhibited by addition of S9 mix.

(5) No significant induction of micronuclei was found in the peripheral blood of mice that were administered with the glucose solutions irradiated with oxygen bubbling.

(6) From these results (1)-(5), it is suggested that the mechanisms for inactivating the mutagenic activity of irradiated glucose solutions that is found in vitro only by the Niemand method, may exert in vivo.

(7) Mutagenicity in bacterial cells(TA104) of glucose solutions irradiated in the presence of oxygen was also effectively suppressed by adding some vegetable and fruit juices, especially those of myoga and radish.[4] The clastogenic activity of the irradiated glucose solutions in cultured CHL cells was completely inhibited by mango fruit juice.[2] From these findings and the fact that no mutagenic activity was observed with the mango fruit flesh irradiated by 10 kGy, it was plausible that sugary fruits themselves may contain some factor to prevent formation or manifestation of mutagenic activity resulting from irradiation. And thus, it can be concluded that irradiation of fruits for disinfestation(up to 1 kGy) does not induce mutagenic activity inside them.

Another research subject which has caused international issues for a long time is the possible induction of polyploid cells by intake of freshly irradiated wheat. A study group working at National Institute of Nutrition(NIN), India, presented a series of papers insisting on the induction of polyploid cells or dominant lethals by intake of freshly irradiated wheat in experiments using several different species of animals and a few malnourished children.[6~9] Also, Renner[10] reported the increased proportion of polyploid cells in Chinese hamsters following ingestion of the radiation sterilized diet (45 kGy), although the results were not interpreted as a mutagenic effect of the irradiated diet. On the other hand, the observations of the NIN group were not reproduced in experiments conducted by Tesh et al.[11] and Chauhan et al.[12] However, these reports from NIN have been widely exploited by opponents to commercial applications and deeply affected the consumer acceptance of irradiated food, resulting in considerable confusion and chronic distrust on wholesomeness of irradiated foods.

The cytogenetic effects of ingestion of a freshly irradiated wheat flour diet were studied in terms of incidences of polyploids and micronuclei in rats and Chinese hamsters by Tanaka and his coworkers.[13] Rats were fed with a diet containing wheat flour freshly irradiated by 0.75 kGy or unirradiated during 6 and 12 weeks Chinese hamsters with a diet containing wheat flour freshly irradiated by doses up to 30 kGy during 72 hours of an unirradiated control diet. Micronucleus formation analysis was performed according to the method of Hayashi et al.[14] Which did not require any special skill and gave reliable results of scoring micronuclei. As the results, no significant difference in the incidences of polyploids in bone marrow cells and micronuclei of the reticulocytes in the peripheral blood was observed between groups fed with an irradiated or unirradiated wheat flour diet with both rats and Chinese hamsters. Thus, the findings reported by the NIN group and Renner were not reproduced in the recent study conducted in Japan,[13] which simultaneously examined both parameters of polyploid and micronucleus inductions, similarly to other repeated experiments.[11,12] For reference, it was observed that cholchicine applied as a mitotic poison did not induce polyploid in the bone marrow cells of Chinese hamster, but did micronuclei induction in peripheral blood of the same animal. In this study, the control incidence levels, variance of observed values and number of cells examined in one group were acceptable. Such duplex analyses of incidences are considered to increase the reliability of the above conclusion.

2.2 Brief discussion on criticisms against food irradiation

Some allegations against practical applications of food irradiation appeared in scientific publications. Although they do not contain the experimental data obtained by the authors themselves, it would be instructive to discuss on them from the viewpoint of scientific evaluation and implication of wholesomeness of irradiated foods.

Piccioni[15] claimed that food irradiation exposes the consumer to a whole new range of carcinogens. The experimental data cited were concerned with in vitro mutagenicity of irradiated sugar solutions in S. typhimurium, induction of choromosome aberrations by irradiated nucleic acid in cultured CHO cells, mutation induction in Drosophila melanogaster, polyploid formation by freshly irradiated wheat and so on. They are related only to mutagenicity and polyploidy, but not to carcinogenicity.

Tritsch[16] similarly claimed that there is abundant evidence that irradiated food is potentially dangerous, in that readily detectable amounts of mutagens and carcinogens are produced during irradiation. The evidences quoted were as follows :(1) formation of mutagenic formaldehyde from sugar irradiation, (2) peroxidation of unsaturated fatty acids, followed by production of carcinogen from benzo[a]pyrene contained in a model food system and (3) polyploid induction in peripheral lymphocytes in children by intake of freshly irradiated wheat.

With these two allegations, the following items are pointed out ;

(1) Piccioni and Tritsh did make no mention of a numerous experimental data supporting the safety of irradiated foods.

(2) The quality of data is very important for reliability, which depends upon the design of experiment, methods and materials as well as statistical analysis. In this meaning, the data from the NIN concerning polyploid induction can not be adopted, as already analyzed.

(3) With respect to radiation-chemical aspects, effects of irradiation of food on its components should be analyzed with the complex mixture system of actual food, not only with dilute and single solutions of the component. The yield of radiolysis products is different between these two systems.

(4) As mentioned above, the facts that the results of mutagenicity tests with metabolic activation and micronuclei tests in mice for irradiated glucose solutions were all negative, may strongly suggest that sugar irradiation under practical conditions does not exert mutagenic effects in mammals.

(5) The results obtained with animal feeding tests should be reviewed, especially with carcinogenicity tests. It would be also helpful as a general reference for evaluation of the safety of irradiated foods to examine the successful practice of irradiating diets of laboratory animals during these about 30 years.

(6) With respect to the allegation of Piccioni, the safety studies on radiotoxin formation by potato irradiation provided the negative result.[17,18,19] In conclusion, based on the results of tests on irradiated sugar solutions, polyploid induction, radiotoxin formation and others, it can be considered that the allegation of Piccioni have expired.

(7) As for peroxide formation and oxidation of benzo[a]pyrene to its active carcinogenic form, peroxide values of irradiated meats were found not to increase, and no evidence for toxic effects from its long-term ingestion was obtained.[20] In the peroxidation study, Gower and Wills[21] employed too high concentration of benzo[a]pyrene (BP), 10 mg / kg, as compared with that in actual food(1 μg / kg or less). BP itself exhibits mutagenic activity by metabolic activation without irradiation. Accordingly, the content of BP in food to be irradiated should be controlled in the GMP.

(8) With respect to the allegation of Tritsch, based on the results on sugar irradiation relating to formaldehyde production, radiation-induced peroxidation of unsaturated fatty acids and a repeated experiment for polyploid induction, it is also concluded that no definite evidence for toxic effect on human consumption was found.(Note)

On this occasion, it would be relevant for food safety evaluation that the mutagenicity has been observed more or less by recent investigations with various foods or food processing regardless irradiation treatment, e.g. such traditional foods as spices and pickles, cooked(by frying, baking or boiling) meats and their products and Maillard reaction products.

3. Studies on detection methods for irradiated foods.

3.1 General aspects

Needs for detection methods to identify irradiated foods is recognized in process control of food irradiation, administrative control of irradiated foods and their international trade. These control procedures are executed not only by inspection, dosimetry and documentation, but also by analytical identification.

The international document on food irradiation of the Geneva FI conf. 1988 stated that governments should encourage research into methods of detection of irradiated food so that administrative control of irradiated food once it leave the facility can be supplemented by an additional means of enforcement, thus facilitating international trade and reinforcing consumer confidence in the overall control system.

In practice, the unirradiated control sample may not be available, the change by irradiation to be determined should be sufficiently large and preferably specific to irradiation treatment. Detection methods are required with some general criteria as follows:

(1) Measured parameters are independent of irradiation, storage and cultivation conditions

(2) Effective thronughout the shelflife period of the foods to be determined

(3) sensitive to practical dose treatments.

(4) Confident, reliable and reproducible method

(5) To be practical method (simple, rapid, low-cost, no use of any complicated instrument, small sample size, etc.)

(6) Basically specific to irradiation treatment

(7) Dose dependence(feasible for estimation of the dose used)

In the early 1980s, almost no available detection method was presented. However, during course of the last decade, anticipation of developments of detection methods of irradiated foods has been enhanced. Several international efforts of studies on detection methods such as programmes of FAO / IAEA(ADMIT) and EC (BCR) are now in progress.[22,23]

At present, the following methods have been developed in ADMIT, BCR and other research projects:

(1) Physical methods

Electrical impedance (potatoes, fish)
ESR (spices, herbs, fruits, vegetables, meats, poultry, fish, shellfish, mashroom)
Luminescence (spices, herbs, potatoes, onions, cereals, fruits, vegetables, fish, shellfish)
NIR (spices, herbs)
Viscosity (spices, herbs, cereals)
Adhesion of epidermis (onions)
Rheology of cell wall (fruits, vegetables)
MW of protein (meats poultry, fish, shellfish)
DSC (cereals)

(2) Chemical methods

Gas (H_2 & CO) formation (spices, herbs, meats, poultry, fish, shellfish)
Cell wall change (potatoes)
Nucleic acide (meats, poultry)
o-Tyrosine (meats, poultry)
Lipid derivatives (meats, poultry)
Carbonyl compounds (fruits, vegetables)

(3) Biological methods

DEFT-APC (spices, herbs)
PAL, chlorogenic acid, wound healing (potatoes)
Rooting (onions)
Germination, rooting (cereals)
Germination, rooting of seeds (fruits, vegetables)
peroxidase, protease (meats, poultry)
Microflora (fruits, vegetables, fish, shellish)
Mycelium formation, pigment formation (mashroom)

3.2 Studies on detection method conducted in Japan

Several detection methods have been developed also in Japan. Hayashi et al.[24,25] studied electrical impedance measuring technique as a detection method for irradiated. potatoes, which are the only commercial product of food irradiation This technique has been reported by several authors as promising one for the identification of irradiated potatoes.[26-28] Hayashi and his coworkers[24,25] investigatied experimental conditions in details to establish the optimum determination condition. The magnitude, resistance and reactance of electrical impedance of potato tubers were significantly influenced by irradiation. It was noted that the ratios of the impedance parameters at 5 kHz to 50 kHz are not affected by different types of electrodes to be used. The ratio of the impedance magnitude at 5 kHz to 50 kHz(z_{5k} / z_{50k}) afforded the larger differnece between unirradiated and irradiated(100 Gy) potatoes than those of resistance, and the z_{5k} / z_{50k} value was dependent upon the radiation dose. Difference in z_{5k} / z_{50k} between unirradiated and irradiated potatoes was found dependent upon the measuring temperature and the region of the tuber punctured with electrodes. The storage condition of potato tubers prior to impedance measurement and determination temperature considerably influenced the reliability of the detection. Based on these detailed examinations, Hayashi and his coworkers recommended as follows: the impedance ratio z_{5k} / z_{50k} determined at 22℃ at an apical region of the potato tuber which was preincubated at 22℃ for 3 days or longer gives the best detection of radiation treatment of potato tubers. This value of impedance ratio increases with increasing radiation doses and varied with potato cultivar. If the potato cultivar is known, irradiated potato tubers can be detected by determination of impedance ratio. The "Danshaku" cultivar of potatoes irradiated at the Shihoro potato irradiation Center could be differentiated from unirradiated potatoes of the same cultivar. The impedance ratio of unirradiated potatoes was lower than 2.75 and that of irradiated(about 100 Gy) ones higher than 2.75.

As one biological detection methods, the germination tests have been proposed. Kawamura et al.[29,30] investigated an embryo cultivation method for identifying irradiated citrus fruits. Seeds collected from grapefruit, orange and lemon fruits were removed their outer and inner coats and 10 selected embryos taken out from inside were placed on the filter paper moistened with distilled water in a petri dish to subject to the germination test. After incubation at 35℃ for 3 to 4 days, germination rates were determined by shooting. The germination rates more than 50% were regarded as an indication of unirradiated fruits. The detection limit of the method was 0.15 kGy. Since the practical dose range of citrus fruit irradiation for disinfestation is 0.30-1.0 kGy, the germination test could be applied to identification of irradiated citrus fruits, regardless their harvest time and storage conditions. Germination test was also tried to apply to identification of gamma irradiated wheat by Kawamura et al.[31] and Zhu et al.[32] Root and shoot lengths are more sensitive to gamma irradiation than the germination percentages.[31] The minimum dose range required for inhibition of root elongation varied, 0.15-0.5 kGy with imported cultivars and 0.3-1.0 kGy with domestic cultivars. Germination percentage decreased with doses more than 5.0 kGy. Storage periods of grains up to 12 months revealed little effect on root length. It is worthy of note that inhibition of root growth by fumigation with methyl bromide can be distinguished from the radiation inhibition by germination pattern. The germination test for wheat is still effective 12 months after gamma irradiation.

Identification by electron spin resonance(ESR) spectrometry was studied by Uchiyama and his coworker.[33,34] They reported that ESR spectra of allspice and cinamon irradiated with practical doses had a principal signal with a g-value of 2.0043 and a minor signal at 30 G lower field from the principal signal. The minor signal was generated only by gamma irradiation, little influenced by humidity and stable for considerably long time. The ESR method by determination of a minor signal is applicable to the detection of allspice and cinamon irradiated by 5 kGy or more for up to 6 months or one year after irradiation. Detection of irradiated spices by viscosity measurement was investigated by Hayashi et al.[35] Viscosities of black and white peppers, ginger and turmeric decreased with increasing doses when they were determined after heat gelatinization at pH 12. A normalized viscosity parameter, viscosity / starch content, resulted in almost constant values for un-irradiated black and white peppers, regardless the harvested districts. Thus, irradiated black and white peppers can be detected by viscosity measurement without any information on the harvested district.

Whole eggs irradiated with 2 kGy were detected by a immunochemical method using anti-albumin antibody.[36] This sensitive and specific method was effective for detection of egg albumin even after heating and in the processed foods.

Amounts of radiolytic H_2 and CO gases retained in black and white pepper seeds, cereals and frozen foods after irradiationwere found dose-dependent and they were determined, using a ceramic vibrating mill for pulverization, to identify the irradiation treatment.[37]

4. Approach from research toward commercialization of food irradiation

Food processing is considered to be essential for supplying sufficient amount of wholesome foods to consumers in any individual countries as well as for improvement of global circumstances of food supply. Research and development of food irradiation have been conducted since 1940s as one of novel food processing technologies. During the course of the long period of the development, two major advantages of this radiation processing technology have been demonstrated, i.e. (1) to extend the shelflife of foods and reduce post-harvest food losses, especially in tropical areas, and (2) to improve food hygienic aspects by killing foodborne pathogens / parasites of inactivating toxigenic microbes. On the other hand, the novel technology of food processing provoked public fear and concern relative to consumption of irradiated foods. Such psychological / emotional background against food irradiated, which was enhanced by active opponents, may be due to lack of sufficient knowledge of irradiation effects to differentiate them from nuclear events and of proper public relations. In progress of research and development, it has become clear that food irradiation is feasible as a unique food processing technology under specified conditions and also within the limitation, similarly to other conventional food processing technologies. Under these recent circumstances, the regulatory authority of each country urged to control and regulate the commercial applications of food irradiation with the specified regulation at the national level for consumer protection. Recently, as the demands for trade of irradiated foods increase, needs for international harmonization in control and regulation of the manufacturing, labelling and marketing of irradiated foods have been rapidly enhanced. In the Geneva FI Conf. 1988, various problems relative to commercialization were discussed with special regard to the international trade of irradiated foods, e.g. on wholesomeness aspects, process control, regulatory control, labelling, personnel training and inspection of facilities, consumer acceptance and choice and so on.

The control in food irradiation involves at least two aspects of process control including quality control and administrative control of irradiated products by the regulatory authority concerned. For these controls, the Geneva FI Conf. 1988[38] recognized the Codex General Standard for Irradiated Foods(1984) and the Recommended International Code of Practice for the Operation of Radiation Facilities Used for the Treatment of Foods(1984). However, the contents of these codices are concerned only with the general principle of the control for manufacturing process and radiation facility. It would be preferable to supplement with the more detailed GMPs of guidelines for individual food items to avoid any technological troubles. Recent concepts of GMP and hazard analysis-critical control point(HACCP) system for food safety principles also facilitate the control of manufacturing process and irradiated foods. These may be achieved by the results of studies on relating research subjects. For examples, foods contaminated by Cl. botulinum type E should be stored in the cold even after irradiation to avoid lisk of outgrowth and toxin production of surviving type E spores, based on their appreciable radiation resistance and, since some physiological enhancement of aflatoxin production by Asperqillus spp. was observed at very low doses (about 0.05kGy), it is recomended to irradiate spices with doses greater than 0.4 kGy. The results of examining microbial contamination by a direct epifluorescent filter technique(DEFT) give relevant information on the hygienic condition of food to be treated and facilities to be used. Those data are also to be considered in the GMP. with radicidation, for example chicken meat irradiation, the HACCP system is used to assure the safety of irradiated food products. The HACCOP program can be properly designed based on the data obtained with relating wholesomeness studies.

Needs of detection methods for irradiated foods for regulatory control and consumer choice were already described. Several methods developed are being accepted by the international programs.

With respect to labelling, Codex General Standard for irradiated Foods(1984) and a report of the Codex Committee on Food Labelling(1989) are available for reference.

5. Concluding remarks

Recent remarkable advances in food safety studies are providing more evidences and deeper understanding of wholesomeness aspects of irradiated foods. Furthermore, their detection method which is requested for commercial applications of food irradiation is now being successfully developed and some methods will be accepted as the official analytical identification tool. However, such progress appears to be not yet well understood among consumers and industries. Hereafter, it would become more important for achievement of successful commercialization to arouse interest of consumers and food industries in this novel food processing technology.

[Note] In a study on irradiated synthetic diets containing polyunsaturated fats and benzo[a] pyrene(BP),[21] it was observed ;

(1) Peroxidation of unsaturated fatty acids took place during storage of both irradiated and unirradiated samples at the same rate and the oxidation of BP also increased at the same rate rate in both samples except the first 2 days when oxydation product formation was more rapid in irradiated sample.
(2) In the sample irradiated in vacuo, lipid peroxidation and the rate or BP oxidation was greatly reduced. Thus, irradiation of vacuum-packed foods is unlikely to initiate lipid peroxide formation of BP oxidation to carcinogen.
(3) Antioxidants inhibited both lipid peroxidation and BP oxidation in irradiated sample.

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