Effect of Gamma Irradiation on Survival of Natural Microflora and Some Nutrients in Cereal Meals

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ABSTRACT

Wheat, corn, and oat meals with initial microbial contaminations of about 10^6 colony-forming units/g were irradiated (^{60}Co) with doses of 1, 10, and 25 kGy. The 1 kGy dose reduced the microbial load two log cycles, and the 10 kGy dose eliminated viable microorganisms in wheat and oat meals; about 1–10 colony-forming units/g of *Enterococcus* and *Clostridium* survived in corn meal after this dose. The dose of 10 kGy did not cause any measurable destruction of total amino acids. Thiamin was reduced 15–32% and riboflavin 10–16%. Irradiation did not cause significant increase in acid value, and also the increase in peroxide value, up to 50% in initial value, did not cause any adverse sensory effects.

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High-fiber meals have received widespread attention as a means of better nutrition and health protection (Vahouny 1982, Dubick 1983, Fleming et al 1983, Hetzel 1983). Cereals are recommended as a good source of fiber (Eggum and Beames 1984, Keeim and Kies 1979, Gordon et al 1983), and production and consumption of various meals based on whole cereals is increasing. Most often these foods are prepared from wheat, corn, and oats.

During harvesting and processing, and even during distribution, these products may become rather highly contaminated with various microorganisms and insects (Ingram and Farkas 1977). Some of these microorganisms may present a health hazard for humans, especially when it is considered that some cereal products, such as corn and oat flakes, may be consumed without additional heat treatment at home. For this reason, reduction of pathogenic microflora is highly desirable, and irradiation may be a method of choice, particularly considering the destruction of various nutrients by alternative heat decontamination or problems related to the use of pesticides (Maxcy 1982, Campbell et al 1986).

The irradiation doses required to eliminate insects are quite low and usually do not exceed 0.5 kGy. For reasonable reduction of microbial load, the necessary doses are usually higher than 1 kGy (Ingram and Farkas 1977). In this experiment we tested the elimination of natural microflora on ground wheat, corn, and oats by irradiation of 1–25 kGy and the effects of applied doses on fat, amino acids, and vitamins in these cereals.

MATERIALS AND METHODS

Samples of whole ground wheat, corn, and oat flakes (about 500 g) were sealed in polyethylene bags and irradiated with doses of 1.0, 10.0, and 25.0 kGy from a ^{60}Co source. The dose rate was about 1 kGy/hr with a max/min dose ratio of about 1.5. The samples for every applied dose were prepared in triplicate. Irradiation was performed in the temperature-controlled irradiation chamber at the Institute for Research, Use, and Production of Radioisotopes in Prague. The temperature during the irradiation was maintained at 21° C, and the controls, unirradiated samples, were kept sealed in polyethylene bags at the same temperature.

The bags were opened in a laminar flow chamber 48 hr after the treatment, and the cereals were tested for surviving microflora. Representative samples (10 g) were shaken in 30 ml of phosphate buffer, serially diluted, and aliquots were inoculated into media. Microbial enumeration was by standard plate count procedures (Marth 1978).

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Parallel samples of the investigated cereal meals were also incubated in broth at 35 and 22° C, and on Sabourad agar at 22° C for 30 days, to allow for regeneration of microbial cells. Growing colonies were stained according to Gram's method and further subcultivated on solid agar media, Endo agar, and blood agar for aerobic and anaerobic cultivations, respectively. Isolated microbial strains were then identified by usual biochemical and microscopy techniques. All media were purchased from USOL Inc. (Prague, Czechoslovakia).

Fat in the cereals was determined by Soxhlet extraction with *n*-hexane and diethylether; dried samples (5 g) were recycled with 200 ml of the solvents for 8 hr at 68° C. The acid values of fats were measured by titration with 0.1*N* KOH; peroxide values were determined by iodometric titration with 0.01*N* $Na_2S_2O_3$ (AOCS 1973, Davidek 1977).

Amino acids were determined after acid hydrolysis with 6*N* HCl, catalyzed with $SnCl_2$ for 10 hr at 120° C in glass ampoules by the ninhydrin method (Davidek 1977) on an amino acid analyzer AAA 339 (Mikrotechna, Prague, Czechoslovakia). Thiamin was determined after acid and enzymatic hydrolysis and oxidation with $K_3Fe(CN)_6$ as thiochrome by fluorescent spectrometry at 365.5 nm (Czechoslovak Normative Methods 1969). Riboflavin was determined after hydrolysis and extraction as lumiflavin by fluorescent spectroscopy at 435.5 nm (Czechslovak Normative Methods 1973).

RESULTS AND DISCUSSION

Initial microbial contamination of the tested cereal meals was about 10^6–10^7 colony-forming units (cfu)/g; irradiation with a dose of 1 kGy resulted in a decrease of the total viable count to about 10^4 cfu/g. Results of the microbiological examinations are summarized in Table I.

Wheat meal was initially contaminated with *Clostridium*, *Bacillus*, *Serratia*, *Enterococcus* and some mold species. Irradiation with the dose of 1 kGy presumably affected the survival competition pattern of the residual microflora (Thornley 1963, Ingram and Farkas 1977, Grecz et al 1983), so that *Micrococcus* were found only in the wheat meal samples treated with this dose. Their growth in the untreated samples was probably suppressed by the growth of predominant microflora (Grecz et al 1983). After irradiation with a dose of 1 kGy, the total microflora count was reduced, and the growth of *Micrococcus*, which is more radiation resistant (Thornley 1963), was facilitated.

The corn meal contaminated initially with *Clostridium*, *Bacillus*, *Escherichia*, *Serratia*, *Enterococcus*, and molds did not show any apparent change in this pattern after an irradiation dose of 1 kGy. *Enterococcus* and gram-positive *Clostridium* survived in this case even at the dose of 10 kGy, but the total viable count was then only about 1–10 cfu/g. No viable microorganisms were detected after the dose of 25 kGy.

Oat meal initially contained *Clostridium*, *Bacillus*, *Serratia*, *Pseudomonas* and molds. The application of the dose of 1 kGy eliminated *Pseudomonas* and the dose of 10 or 25 kGy prevented

growth of any microorganisms.

Irradiation did not increase greatly the acid value of cereal fats as may be seen in Table II; in wheat meal a small decrease was even observed. Peroxide values increased in direct relation to the radiation dose absorbed by the grains. The increase in the peroxide value was not accompanied by off-odors, which are usually observed at peroxide values over 100 μeq/g fat as was reported previously (Fullerton et al 1982, Hanis and Mnukova 1985).

Though lipids belong to the food constituents that are more sensitive to irradiation, published data (Delincee 1983) indicate that low-dose application of ionizing radiation, up to 10 kGy does not cause any significant changes in total lipids of cereals. Application of higher doses may lead to some changes, and their character is affected by a number of variables, such as type and composition of the lipid (Hanis and Mnukova 1985), presence of antioxidants, composition of irradiated food, irradiation conditions, etc. (Delincee 1983, Nawar 1983).

Amounts of thiamin, which is considered the most radiation sensitive of all of the water-soluble vitamins (Coates et al 1969, Delincee, *personal communication*) were decreased 24–58% when the cereals were treated with the dose of 25 kGy and 15–32% with a dose of 10 kGy (Table III). Riboflavin, which is claimed to be less radiation sensitive (Delincee 1981), showed a decrease of 21–42% after a dose of 25 kGy and 10–16% after a dose of 10 kGy.

The destruction of both vitamins in these cereals was significantly higher than the destruction usually observed in fortified diet mixtures, where the presence of various antioxidants, free radical scavengers, etc., plays an important protective role (Bason 1983). Coates et al (1969) reported no loss of thiamin in a chick diet irradiated up to 30 kGy, Iwado et al (1973) and Ito and Iizuka (1979) reported decreases in thiamin content by 3%, and Iizuka (1979) reported a 3% decrease in thiamin content after a 30-kGy dose and 12% after a 60-kGy dose. Van Kooij (1979) reported an average loss of about 18% in various diets irradiated up to 50 kGy, and Hanis et al (1985) reported losses of 6.7% and 22% in laboratory rat diets based on cereals irradiated with doses of 25 and 50 kGy, respectively.

Diehl (1975, 1979) and Delincee (*personal communication*) reported high destruction of thiamin, up to 36%, in cereals irradiated with low doses of 250–350 Gy. It is interesting that high destruction rates reported for thiamine were obtained with samples of cereals irradiated with the use of an electron accelerator, whereas the above-mentioned irradiation of feeds was performed with gamma sources. Usually we should expect lower losses of thiamine with the use of high-dose-rate machines than with low-dose-rate gamma sources (Diehl 1983).

Irradiation of these cereals up to a dose of 10 kGy did not cause any measurable decrease in the content of amino acids (Table IV). A dose of 25 kGy destroyed about 39% of methionine in wheat meal, 26% in corn meal, and 31% in oat meal. In addition, a loss of about 33% of cysteine was also observed in corn meal, but in the two other cereals cysteine did not show any measurable decrease. Data in the literature do not indicate much radiation destruction of amino acids in various foods and feeds up to doses of about 70 kGy (Ito and Iizuka 1979, van Kooij 1979, Ford 1979, Eggum 1979). The destruction observed in this experiment is again presumably related to less protective properties of pure cereals compared to the more complex systems of various mixtures.

We conclude that the overall average dose of 10 kGy, accepted by the Codex Alimentarius Commission, is very effective in microbial decontamination and does not adversely affect the sensory and nutritional quality of such foods.

TABLE I
Survival of Natural Microbial Contaminants in Some Cereal Meals After ^{60}Co Irradiation at 1.0, 10.0, or 25.0 kGy[a]

	Wheat Meal				Corn Meal				Oat Meal			
Contaminant	**0**	**1.0**	**10.0**	**25.0**	**0**	**1.0**	**10.0**	**25.0**	**0**	**1.0**	**10.0**	**25.0**
Escherichia	−−−	−−−	−−−	−−−	+++	+++	−−−	−−−	++−	+++	−−−	−−−
Bacillus	+++	+++	−−−	−−−	+++	+++	−−−	−−−	+++	+++	−−−	−−−
Serratia	+++	++−	−−−	−−−	+++	+−−	−−−	−−−	+++	++−	−−−	−−−
Enterococcus	+++	+++	−−−	−−−	++−	+−−	++−	−−−	−−−	−−−	−−−	−−−
Clostridium	+++	+++	−−−	−−−	+++	+++	+++	−−−	+++	+++	−−−	−−−
Pseudomonas	−−−	−−−	−−−	−−−	−−−	−−−	−−−	−−−	++−	−−−	−−−	−−−
Micrococcus	−−−	++−	−−−	−−−	−−−	−−−	−−−	−−−	−−−	−−−	−−−	−−−
Molds	+++	+++	−−−	−−−	+++	+++	−−−	−−−	+++	+++	−−−	−−−

[a]Each + or − symbol means positive or negative observation of microbial growth after 30 days of cultivation in one of triplicate examinations.

TABLE II
Changes in Acid and Peroxide Values of Fats of Irradiated (^{60}Co at 1, 10, or 25 kGy) Cereal Meals[a]

	Wheat Meal				Corn Meal				Oat Meal			
Value	**0**	**1.0**	**10.0**	**25.0**	**0**	**1.0**	**10.0**	**25.0**	**0**	**1.0**	**10.0**	**25.0**
Acid value, mg KOH/g of fat	27.62 +0.45	25.33 +0.68	23.97 +0.38	24.20 +0.26	52.45 +0.53	52.49 +0.46	51.00 +0.39	56.10 +0.51	47.39 +0.33	54.44 +0.42	53.80 +0.28	51.07 +0.44
Peroxide value, μeq/g fat	23.87 +1.18	33.23 +2.50	48.88 +2.83	52.93 +4.23	23.33 +1.82	32.84 +2.33	47.98 +2.85	56.72 +4.46	35.30 +3.26	33.09 +2.85	52.62 +2.85	58.19 +3.58

[a]Expressed as mean + SD of five analyses.

TABLE III
Changes in Thiamine and Riboflavin Content[a] of Cereal Meals After Irradiation (^{60}Co at 1, 10, or 25 kGy) (mg/kg dry matter)

	Wheat Meal				Corn Meal				Oat Meal			
Nutrient	**0**	**1.0**	**10.0**	**25.0**	**0**	**1.0**	**10.0**	**25.0**	**0**	**1.0**	**10.0**	**25.0**
Thiamine	4.20	4.00	3.20	2.10	2.00	1.40	1.35	0.85	6.17	6.00	5.23	4.68
Retention, %	100	95.2	76.2	50.0	100	70.0	67.5	42.5	100	97.2	84.8	75.8
Riboflavin	0.61	0.60	0.55	0.48	0.57	0.55	0.48	0.33	0.74	0.74	0.66	0.57
Retention, %	100	98.4	90.2	78.7	100	96.5	84.2	57.9	100	100	89.2	77.0

[a]Expressed as mean of three analyses; mean error 5%.

TABLE IV
Effect of ^{60}Co Irradiation on Content of Amino Acids, in Cereal Meals[a] (25 kGy)

Amino Acid	Initial Content in % of Total Amount[b]			Destruction in % of Initial Value		
	Wheat Meal	Corn Meal	Oat Meal	Wheat Meal	Corn Meal	Oat Meal
Aspartic acid	5.84 + 0.16	7.38 + 0.51	8.80 + 0.38	0	0	0
Threonine	2.93 + 0.05	3.45 + 0.15	3.40 + 0.11	0	0	0
Serine	4.64 + 0.04	4.62 + 0.11	5.07 + 0.25	0	0	0
Glutamic acid	30.62 + 1.06	22.06 + 0.45	23.72 + 0.33	0	0	0
Proline	11.00 + 0.34	9.01 + 0.42	6.32 + 0.17	0	0	0
Cysteine[c]	1.16 + 0.09	0.63 + 0.02	1.98 + 0.01	0	33.3 + 0.6	0
Glycine	4.07 + 0.06	3.43 + 0.05	5.09 + 0.15	0	0	0
Alanine	3.35 + 0.05	7.30 + 0.26	4.65 + 0.08	0	0	0
Valine	4.61 + 0.06	4.95 + 0.12	5.37 + 0.06	0	0	0
Methionine	1.69 + 0.03	1.40 + 0.03	1.12 + 0.02	39.4 + 0.7	26.4 + 0.7	30.8 + 0.6
Isoleucine	4.17 + 0.86	3.50 + 0.06	3.91 + 0.10	0	0	0
Leucine	7.10 + 0.37	12.49 + 0.35	7.83 + 0.22	0	0	0
Tyrosine	2.53 + 0.04	3.58 + 0.09	3.03 + 0.08	0	0	0
Phenylalanine	5.20 + 0.21	5.57 + 0.17	5.47 + 0.10	0	0	0
Histidine	3.63 + 0.03	4.51 + 0.13	3.47 + 0.11	0	0	0
Lysine	3.43 + 0.19	3.06 + 0.09	4.79 + 0.30	0	0	0
Arginine	4.93 + 0.15	4.01 + 0.10	7.07 + 0.75	0	0	0

[a]Expressed as mean + SD of eight analyses.
[b]Total amount of amino acids in mg/g of cereals: Wheat meal, 112.9 + 4.30; corn meal, 84.6 + 3.61; oat meal, 136.1 + 4.03.
[c]Cysteine = cystine/2.

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