VITAMINS AND ENZYMES IN MILK

Effect of Gamma-Radiation on Activity

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Interest in the use of radiations from fission products includes consideration of sterilizing biological products to accomplish stabilization. Tests were undertaken to measure vitamin and enzyme retention in milk and related products coincident with irradiation from cobalt-60. Vitamin A, tocopherols, and ascorbic acid were highly sensitive to destruction within the time period required for sterilization (at relatively low intensities). Carotenoids and riboflavin were moderately sensitive. The enzyme phosphatase, however, which is very sensitive to heat inactivation, was only slightly changed during identical exposures. Tests with raw whole milk, pasteurized whole milk, evaporated milk, cream, margarine, Cheddar cheese, and cream cheese demonstrate that irradiation at 80,000 roentgens per hour induces severe losses in nutritive quality before sterilization is accomplished. Changes in enzyme activity, however, are sharply reversed in comparison with best effects on natural products. Protein denaturization apparently can be avoided, relatively, while accomplishing this type of sterilization.

Food sterilization by gamma-radiation has been investigated in these laboratories. A part of the project was a study of the associated changes in nutritive value of foods exposed to high energy radiations. This paper presents the results of irradiating fresh and evaporated milk, cream, cheese, butter, and margarine by γ-rays from a cobalt-60 source, at doses ranging from 80,000 to 1,920,000 roentgens. There was considerable destruction of vitamins, but relatively little inactivation of the enzyme phosphatase. The nutrients studied chiefly were vitamin A, carotenes, vitamin E, ascorbic acid, and riboflavin.

Although the many studies of processing of foods by radiations from fission products have indicated the possibility of applying this technique, the problems of radiation sterilization cannot be considered as solved unless there is a clear picture of the associated changes induced by the high energy radiations. Vitamins and enzymes are sensitive to radiations, especially to the ionizing radiations such as x-rays, γ-rays, and cathode rays. A few papers have been published recently concerning the effect of such radiations on vitamins in pure solution and in certain foods (*6*, *9*, *10*).

Experimental Procedures

Milk samples were irradiated in previously sterilized borosilicate glass test tubes for 1, 3, 6, or 12 hours in a cobalt-60 radiation source as described by Manowitz (*8*). Samples were introduced into the cylindrical source chamber by remote manipulation (*5*). The polyvinyl chloride film technique of Henley and Miller (*7*) was used to measure the radiation intensity in the sample container. Over the period of these experiments the average dose rate was found by this technique to be 80,000 roentgens per hour ($\pm 5\%$).

The controls that accompanied the samples were kept under the same conditions as those irradiated, except that they received no gamma-radiation. Whole, unpasteurized milk was obtained from a milk-processing plant less than 2 hours before the experiments. Immediately following irradiation the samples and controls were analyzed for vitamins and enzyme activity.

Vitamin A and carotenes were determined colorimetrically (*2*, *3*); a fluorometric analysis was carried out for riboflavin (*1*, *2*). Reduced ascorbic acid was determined by visual titration with 2,6-dichlorophenolindophenol (*2*). Tocopherols were assayed colorimetrically, based on a modified Emmerie and Engel method (*4*, *11*). Milk phosphatase activity was measured by the official method of the Association of Official Agricultural Chemists (*1*).

The procedures for irradiation and analysis of market samples of evaporated milk were similar to those used for fresh milk. Samples of butter, cheese, and margarine, all obtained from the market, were irradiated in polyethylene bags for 1, 3, 6, 12, or 24 hours. The controls in polyethylene bags were again kept under the same conditions as the irradiated samples, except that they received no gamma-radiation. Samples of cream

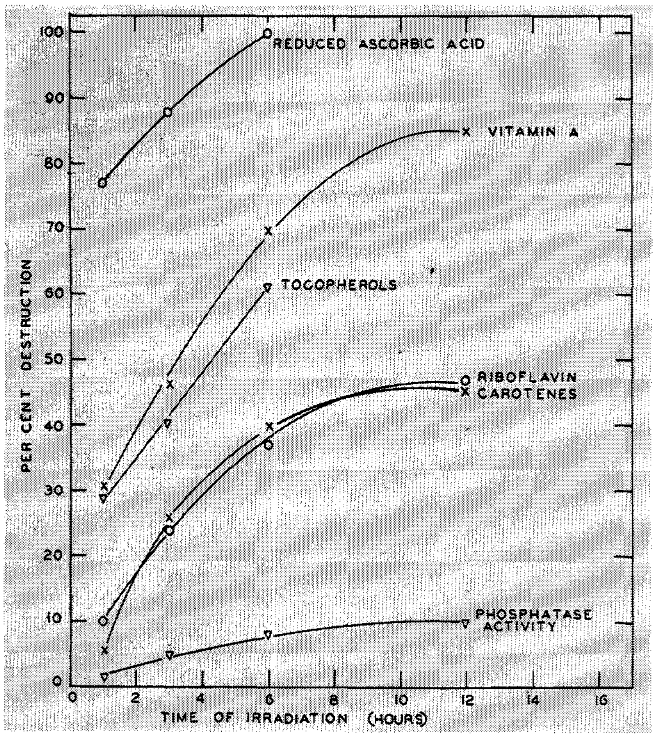


Figure 1. Effect of gamma-rays on vitamins and phosphatase activity in raw whole milk

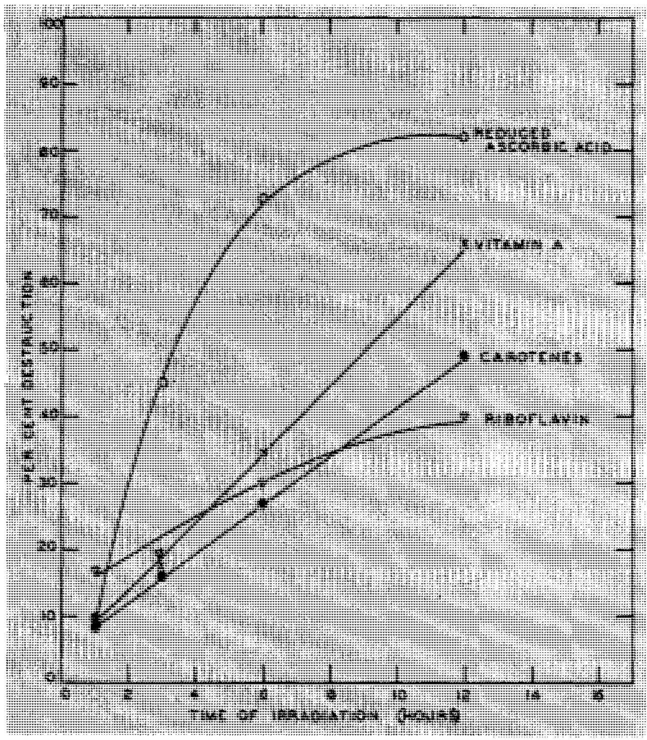


Figure 2. Effect of gamma-rays on vitamins in evaporated milk

were treated in the same manner as milk samples.

Results

The analytical results for vitamin A, carotenes, tocopherols, reduced ascorbic acid, riboflavin, and phosphatase activity in irradiated milk are summarized in Table I. The percentages of destruction of vitamins and phosphatase activity are plotted against the time of irradiation (in hours) in Figure 1. The energy absorbed by individual milk samples during irradiation was not measured directly; the doses reported in this paper are the product of the measured dose rate in the source chamber and radiation time.

The effects of γ-rays on vitamin A,

Figure 3. Relation of vitamin concentration and irradiation time in evaporated milk

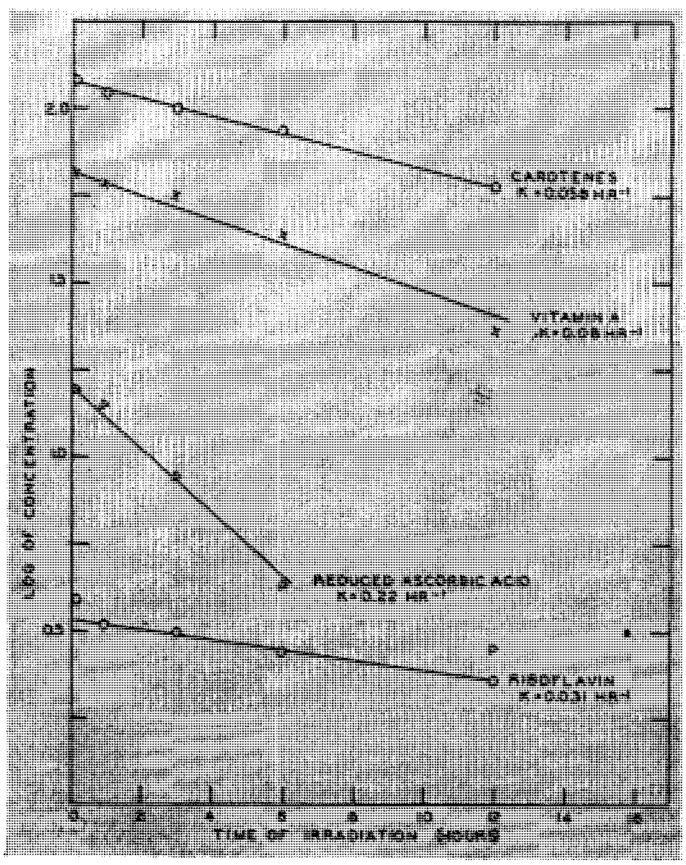
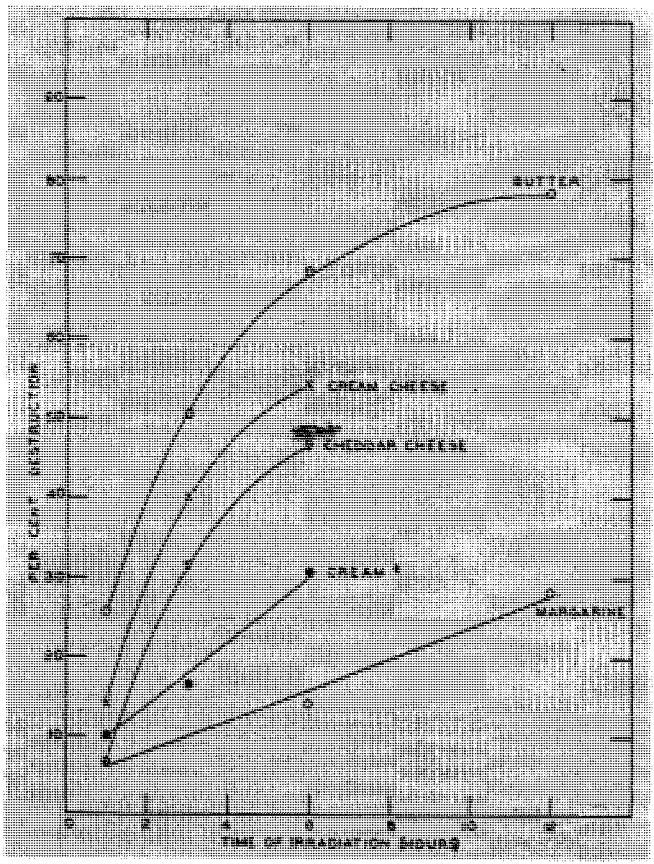


Figure 4. Effect of gamma-rays on vitamin A in butter, cheese, cream, and margarine



carotenes, riboflavin, and reduced ascorbic acid in evaporated milk are presented in Table II and Figure 2. The average temperatures during irradiation were −2.2° (milk, vitamin A, and carotene determinations), 0.0° (riboflavin), and 5.6° C. (reduced ascorbic acid). The linear relationship between the logarithm of concentrations of vitamins in irradiated evaporated milk and irradiation time, as shown in Figure 3, indicates that the destruction reactions follow the law of first-order reactions within the range of these experiments. The values of the velocity constant, k, for vitamin A, carotenes, riboflavin, and reduced ascorbic acid are given under their corresponding curves in Figure 3. The inactivation dose and specific inactivation dose of some of the vitamins investigated are presented in Table III.

While the intensity of radiation permitted by the cobalt-60 source was too low for satisfactory use in food processing, the data made available are nevertheless of value as an index of relative stabilities. The total dosage was within the approximate range (1,000,000 to 1,500,000 roentgens) required to accomplish sterilization of mixed bacterial cultures. Comparable data at higher intensities will be reported later.

The percentage of destruction of vitamin A at dosages up to 0.96 × 10^6 roentgens in irradiated butter, cheese, cream, and margarine are presented in Table IV. The average temperatures during irradiation were 1.7°, 11°, 18°, and 5.0° C. for butter, cheese, cream, and margarine, respectively. The per cent destruction of vitamin A in butter, cheese, cream, and margarine is plotted against the time of irradiation in Figure 4.

Table I. Effect of γ-Rays on Vitamins and Phosphatase Activity in Raw Whole Milk

Time of irradiation[a], hours	*Control*	*1*	*3*	*6*	*12*
Vitamin A					
γ per 100 ml. of milk	65.4	45.1	35.4	19.9	9.9
% destruction	...	31	46	70	85
Total carotenoids					
γ per 100 ml. of milk	26.2	24.8	19.5	15.7	14.3
% destruction	...	5.3	26	40	45
Riboflavin					
γ per ml. of milk	1.35	1.21	1.03	0.85	0.72
% destruction	...	10	24	37	47
Tocopherols					
γ per 100 ml. of milk[b]	119	85	71	47	...
% destruction	...	29	40	61	...
Reduced ascorbic acid					
γ per ml. of milk	2.6	0.60	0.26	0.00	0.00
% destruction	...	77	88	100	100
Phosphatase activity					
Phenol unit/0.5 ml. of milk	732	720	696	678	660
% destruction	...	1.6	4.9	7.4	9.8

[a] Dose rate, 80,000 roentgens per hour.
[b] Pasteurized milk.

Table II. Effect of γ-Rays on Vitamins in Evaporated Milk

Time of irradiation[a], hours	*Control*	*1*	*3*	*6*	*12*
Vitamin A					
γ per 100 ml. of milk	66.6	60.5	56.3	43.6	23.0
% destruction	...	9.3	16	35	66
Total carotenoids					
γ per 100 ml. of milk	119	110	100	87	60
% destruction	...	8.0	17	27	49
Riboflavin					
γ per ml. of milk	3.95	3.30	3.19	2.77	2.37
% destruction	...	17	19	30	40
Reduced ascorbic acid					
γ per ml. of milk	15.7	14.2	8.7	4.3	2.9
% destruction	...	9.0	45	73	82

[a] Dose rate, 80,000 roentgens per hour.

Table III. Inactivation Dose (D_0) and Specific Inactivation Dose (D_0/C)

	Vitamin A in Raw Milk	*Vitamin A in Evap. Milk*	*Tocopherols in Past. Milk*	*Ascorbic Acid in Evap. Milk*
D_0, roentgens	416,000	912,000	504,000	376,000
D_0/C, r./g./ml.	6.3 × 10^{11}	1.5 × 10^{12}	4.2 × 10^{11}	2.4 × 10^{10}

Table IV. Effect of γ-Rays on Vitamin A in Butter, Cheese, Cream, and Margarine

Time of Irradiation[a], hours	*Control*	*1*	*3*	*6*	*12*
Butter					
γ per gram of butter	4.47	3.29	2.21	1.43	0.97
% destruction	...	26	51	68	78
Cream cheese					
γ per gram of cheese	2.40	2.06	1.44	1.11	...
% destruction	...	14	40	54	...
Cheese (Cheddar)					
γ per gram of cheese	2.58	2.40	1.75	1.37	...
% destruction	...	7	32	47	...
Cream					
γ per ml. of cream	1.78	1.60	1.47	1.23	...
% destruction	...	10	17	31	...
Margarine					
γ per gram of margarine	11.71	10.89	...	10.10	8.42
% destruction	...	7	...	14	28

[a] Dose rate, 80,000 roentgens per hour.
Destruction of vitamin A in butter after 24 hours irradiation was 94%.

Acknowledgment

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Literature Cited

(1) Assoc. Offic. Agr. Chemists, "Methods of Analysis," 1950.
(2) Association of Vitamin Chemists, Inc., "Methods of Vitamin Assay," New York and London, Interscience Publishers, 1951.
(3) Boyer, P. D., Spitzer, R., and Jensen, C., *Ind. Eng. Chem., Anal. Ed.*, **16**, 101 (1944).
(4) Emmerie, A., and Engel, C., *Rec. trav. chim.*, **57**, 1351 (1938).
(5) Gaden, E. L., Jr., Quarterly Progress Report, Food Irradiation and Associated Studies," U. S. Atomic Energy Commission, *Rept.* **NYO-3317** (Dec. 1, 1951).
(6) Gaden, E. L., Jr., Henley, E. J., and Collins, V. P., *Food Technol.*, **5**, 506 (1951).
(7) Henley, E. J., and Miller, A., *Nucleonics*, **9** (6), 62 (1951).
(8) Manowitz, B., *Ibid.*, **9** (2), 10 (1951).
(9) Proctor, B. E., and Goldblith, S. A., *Ibid.*, **3** (2), 32 (1948); **5** (3), 56 (1949).
(10) Proctor, B. E., and O'Meara, J. P., *Ind. Eng. Chem.*, **43**, 718 (1951).
(11) Quaife, M. L., *J. Biol. Chem.*, **169**, 513 (1947).

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