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DESTRUCTION RATES OF MS 102 IN MILK USING IRRADIATION AND IRRADIATION PLUS HEAT

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MILK IS A PERISHABLE COMMODITY that must be preserved as it moves in the marketing channels. The development of sources of ionizing radiations stimulated interest in their use to preserve milk, thereby reducing or eliminating the need for pasteurization and refrigeration.

The general field of radiation preservation of milk has been recently reviewed by Goldblith and Proctor[1] and Bierman, Proctor, and Goldblith.[2] The flavor problems accompanying irradiation is the chief deterrent to progress in this area. The threshold doses at which an off-flavor is induced by high voltage cathode rays, ranged from about 7,000 to 25,000 rep. (Roentgen equivalent physical). The acceptability of the irradiated products decreased with increased dosage.

Since the low doses cited above produce an off-flavor but are too low for preservation, a combination of irradiation plus a heat treatment might have advantages. Kempe reported that spores of *C. botulinum* are more easily killed by heat after irradiation.[3]

This study was instigated to determine the effect of irradiation and irradiation plus heat on the destruction characteristics of vegetative micro-organisms; specifically, vegetative cells of the heat resistant organism Micrococcus species 102.

[1] Goldblith, S. A., and B. E. Proctor (1956). Radiation preservation of milk and milk products. I. Background and problems. Jour. Dairy Sci. 39 (4): 374-379.

[2] Bierman, G. W.; B. E. Proctor, and S. A. Goldblith (1956). Radiation preservation of milk and milk products. II. Off-flavors in milk and cream induced by ionizing radiations as judged by organoleptic tests. Jour. Dairy Sci. 39(4): 379-391.

[3] Kempe, L. L. (1955). Combined effects of heat and radiation in food sterilization. Applied Microbiol. 3 (6): 346-353.

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EXPERIMENTAL PROCEDURE

Preparation of Culture

Micrococcus MS 102 was used as the test organism. Twenty-four hours prior to use, 10 tubes of trypticase agar were seeded with cells of MS 102 cultured at 98.6° F. (37° C.) for 36 hours. After incubation at the same temperature for 24 hours, each slant was washed with 5 ml. of M/15 phosphate buffer. The washings were pooled in a single bottle, thoroughly shaken, and filtered through a sterile fiber glass filter. This suspension was used for testing immediately after preparation.

Preparation of the Substrate

Homogenized milk was used as the substrate. Three hundred ml. of milk were placed in a sterile, stainless steel container equipped with a stirring device. The sterile container and the milk were placed in a constant-temperature water bath and given a laboratory pasteurization equivalent to 30 minutes at 160° F. (71.1° C.) as an initial phase of a test.

Following pasteurization, the container was removed from the water bath and placed in a –25° F. (–30.1° C.) freezer for 1 hour to bring the temperature of the homogenized milk from 160° to 70° F. (71.1 to 21.1° C.). The uniform 300 ml. of milk was inoculated by adding 10 ml. of the MS 102 suspension to the 300 ml. of laboratory pasteurized milk while in the stainless steel container. The container and product were then agitated for 3 minutes after which the sample was divided into two representative samples of 150 ml. each. These aliquots were identified as aliquot A and aliquot B.

Irradiation

Ten ml. samples were removed from aliquot A by means of a sterile hypodermic needle and deposited in sterile 8.5 cm. square bottomed petri dishes. These prepared samples were taken to the irradiation chamber where they were arranged on the irradiation conveyor. The glass covers were removed and replaced with polyethylene covers.

It was necessary to limit the quantity of milk in the individual dishes since the irradiation was being supplied by a 1-million volt, resonant transformer-type electron beam machine with penetration not exceeding .2 inches.

Following irradiation, the samples were re-combined to make one composite sample by transferring the irradiated milk from the petri dishes to a single sterile container. All tests were carried out at least in duplicate; four irradiation levels were used: 10,000; 20,000; 50,000; and 80,000 rep.

Heat Treatment

Five ml. of milk were placed in sterile screw-top vials. Six tubes were prepared from irradiated milk and six from non-irradiated milk. Five of the six tubes for each treatment were immersed in a constant-temperature water bath at 160° F. (71.1° C.). Tubes were removed

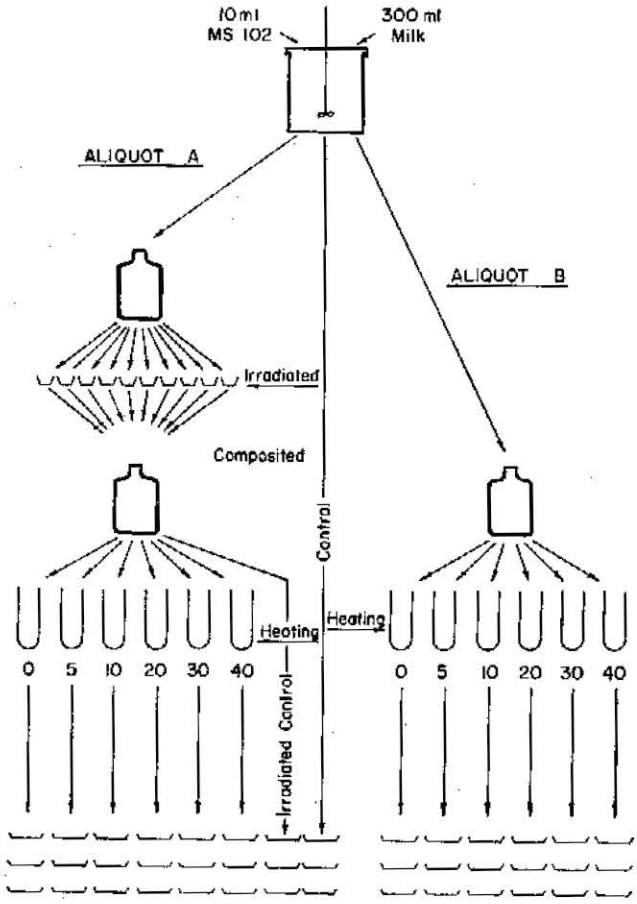

Fig. 1. Schematic diagram of procedure followed in carrying out experiment.

after 5, 10, 20, 30, and 40 minutes, and immediately placed in an ice bath until ready for plating. The sixth tube of milk served as a secondary control for errors that might accompany the heating and cooling operations. The primary control was a sample of milk from the original dilution placed in the ice bath during the heating operation.

Obtaining Bacteriological Results

Plate counts in three dilutions were made according to standard methods. The number of organisms present was determined in the initial sample, the composite irradiated sample, the five tubes of irradiated milk that were heated, the irradiated control, the five tubes of non-irradiated milk that were heated, and the control.

The plate counts were made by plating an aliquot on trypticase agar. After pouring, plates were placed in a 98.6° F. (37° C.) incubator for 72 hours after which they were counted and recorded. Fig. 1 (page 165) illustrates graphically the procedure followed in this project.

Results

The results are summarized in Table 1. (Results of replicate tests have been averaged arithmetically and these averages are presented in Table 1.)

TABLE 1—Summary of Results

Treatment	Heating time (minutes)	Number of organisms (10,000 rep.)	(20,000 rep.)	(50,000 rep.)	(80,000 rep.)
Control		15,000,000	30,600,000	21,600,000	24,200,000
Irradiated		11,400,000	14,200,000	1,800,000	1,220,000
Heated, but not irradiated	0	16,250,000	32,000,000	17,000,000	18,300,000
	5	8,700,000	19,100,000	7,900,000	12,300,000
	10	5,220,000	13,200,000	7,370,000	7,300,000
	20	1,787,000	7,610,000	562,000	44,200
	30	594,000	2,730,000	33,400	8,950
	40	21,700	186,000	708	930
Irradiated then heated	0	10,510,000	21,400,000	981,000	850,000
	5	1,593,000	6,560,000	316,000	70,200
	10	444,000	1,600,000	55,200	3,090
	20	151,000	947,000	542	400
	30	14,400	107,000	40	84
	40	2,530	10,500	10	12

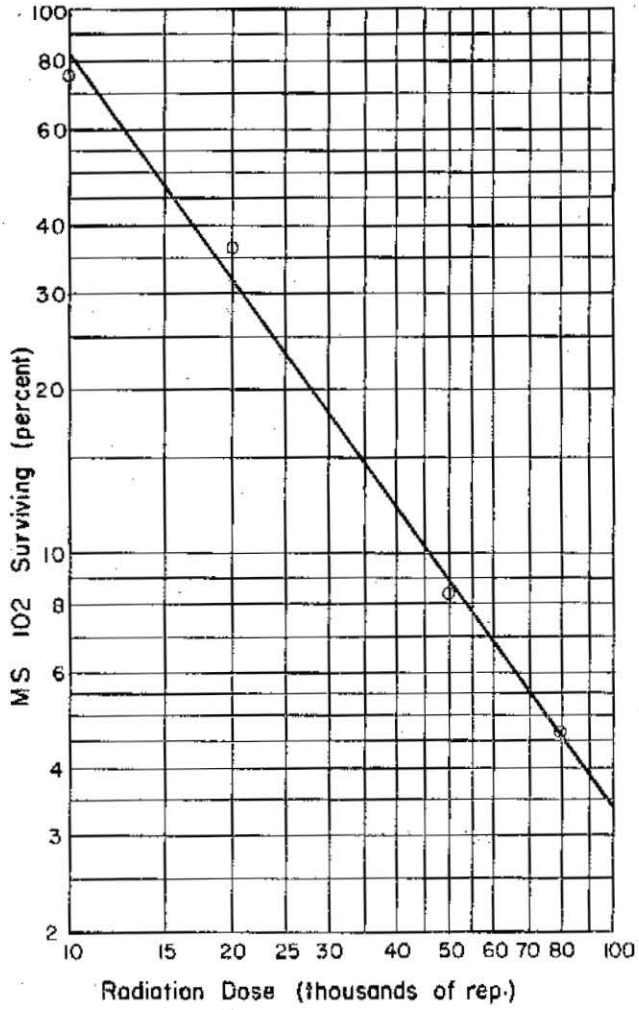

Fig. 2. Radiation survivor curve of MS 102.

It was found in these tests with MS 102 that there was a definite reduction in population due to irradiation. Fig. 2 illustrates the relationship of the irradiation dose and the reduction in population due to this irradiating dose plotted on log-log paper. A straight line fits the limited data quite well.

Table 2 lists the results obtained by comparing the heat destruction rates of populations of MS 102 that had been irradiated, with populations that had not been irradiated. The evaluation was made using average D values (D value is defined as the direction of a

survivor curve; one D value is the time in minutes required to reduce the bacterial population by 90 percent). The procedure used to obtain D values was to plot the number of survivors as a function of time for each test and then draw the best straight line through the points representing the number of survivors. The D value or direction of the rate of destruction curve was determined directly from this line. The D values in Table 2 are the average values for a given irradiation dosage.

TABLE 2—Heat destruction rates for irradiated and non-irradiated suspension of MS 102

Irradiation dose (rep.)	Average D value (min.)		Reduction in D of irradiated sample
	Irradiated	Control	
			(percent)
10,000	12.8	21.9	58.3
20,000	11.6	26.2	51.4
50,000	7.1	13.9	52.2
80,000	7.1	19.0	42.3

DISCUSSION

It was previously pointed out that a new culture was prepared for each test. Since the resistance of micro-organisms to heat varies between cultures, the D value of a culture grown one day cannot be compared directly to the D value of another culture. However, it was found that the reduction in D value, due to irradiation, was approximately 50 percent in all cases. While there was a difference between the 10,000 rep. dose and the 80,000 rep. dose, these are not significant at the 5 percent level even though there appears to be a trend. These results indicate that irradiation not only reduces the population, but increases their death rate after irradiation or reduces their D value. In the samples tested there was an average reduction in D value of about 50 percent.

SUMMARY

Micrococcus sp. MS 102 was studied regarding the effect of irradiation and irradiation combined with heat.

Four irradiation levels of 10,000, 20,000, 50,000, and 80,000 rep. were studied and all tests were carried out at least in duplicate. The

heat resistance of irradiated and non-irradiated samples of MS 102 was determined. It was found that irradiation itself reduces the bacterial population and appears to sensitize the bacteria in such a way that the rate of destruction of the organism is approximately doubled after irradiation.

Acknowledgment

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