Cathode Radiation as a Means of Sterilizing Distiller's Barley Malt

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Cathode radiation was investigated as a means of sterilizing barley malt for use in converting distillery grain mashes. Irradiation dosages were varied from 0.05 to 2.0 $\times$ 10^6 rep. The resulting malts were examined for viable bacteria and residual α-amylase and were evaluated in laboratory scale grain alcohol fermentations. Irradiation dosages of 0.5 $\times$ 10^6 rep and above reduced the bacterial counts to less than 2% of those present in the untreated malts. Dosages of 1.0 $\times$ 10^6 and above reduced alcohol yields. An elevated temperature and prior grinding of the samples accelerated the death rate of bacterial contaminants. A dosage of 0.2 $\times$ 10^6 rep did not appreciably affect α-amylase content nor yield of alcohol, but greatly lowered lactic-type flora developing in the mash during fermentation. While "cleaner" fermentations were obtained with cathode-irradiated malts, this method is not now considered commercially practical.

GRAIN ALCOHOL FERMENTATIONS in which barley malt is used for saccharification are subject, as a consequence of bacterial contamination, to the potential development of abnormal acidity, off-flavored distillates, and reduced alcohol yields. Previous reports from this laboratory have been concerned with media and methods for counting these contaminants (mainly *Lactobacilli*) (*8*), their general description and the production of acrolein by certain types (*14*), and the evaluation of antibiotics as contamination-control agents (*7*).

Barley malt with a bacterial count normally ranging between 1,000,000 and 10,000,000 per gram is the primary source of the *Lactobacilli* characteristically associated with grain alcohol fermentations. The malt conversion of grains (148° F. for 30 minutes in the Peoria distillery) after cooking greatly reduces the number of these bacteria but does not sterilize the mash. A practical method of sterilizing malt without inactivating malt amylase would be desirable in distillery operations. Increased sulfur dioxide treatment of malt during kilning and the use of formaldehyde in malt slurries as described by Atwood (*2*) have been found of very limited effect. The relative stability of enzymes and the susceptibility of microorganisms to cathode rays as shown by O'Meara (*10*) suggested an investigation of malt sterilization by this means.

Effects of ionizing radiations from high voltage cathode rays on bacteria and enzyme systems have been of interest since the early work of Coolidge (*4–6*). A brief study of the effects of x-rays upon certain bacteria was reported by one of the authors in 1929 (*3*). A broad program of the feasibility of using high voltage x-rays and cathode rays for commercially sterilizing foodstuffs, pharmaceuticals, and other biological substances was initiated at the Massachusetts Institute of Technology in 1942 (*13*). Comparisons have been made there (*11*, *12*) of commercial-type radiation units, including the resonant transformer type of the General Electric Co.

Cathode rays are electrons which are artificially accelerated and directed. Particle accelerators, such as Van de Graaff generators and resonant transformers, produce cathode rays at efficiencies great enough to make sterilization by ionizing radiation feasible where depths of penetration within the range of 0.2 to 1 inch are adequate. In the fall of 1952, the X-Ray Department of the General Electric Co. installed a 1-mev. cathode ray unit at its Milwaukee plant to explore industrial applications. The study reported here was carried out with malts irradiated with this unit.

Materials and Methods

Malt samples, packaged in 24-gram aliquots in irradiated polyethylene bags, measured 2 $\times$ 8 $\times$ 0.2 inch in thickness. They were exposed on a metal conveyor, as described by Knowlton, Mahn, and Ranftl (*9*). Current and exposure time were varied to provide dosages from 50,000 to 2,000,000 rep for each malt. Malt sample A was exposed at 45° F. in meal form, the meal having been prepared by passage through a Labconco attrition mill. Sample B was divided into two parts; sample B-1 was irradiated at 78° F. in whole berry form and sample B-2 was treated at the same temperature in meal form. Sample C was irradiated in whole berry form at 145° F. The sieve analysis of the ground malt samples was as follows:

On 12-mesh screen, 0.3%
On 40-mesh screen, 45.3%
Through 40-mesh screen, 54.4%

After irradiation, whole malts were ground aseptically in a Wiley mill to pass a 20-mesh screen. They were analyzed for α-amylase content and residual bacteria and were evaluated as saccharifying agents in laboratory scale grain alcohol fermentations. Lactic acid organisms were determined in shake tubes of tomato juice agar as described by Garey and others (*8*) and aerobic organisms by plating in nutrient agar. Colony counts were made after 48 hours' incubation at 37° C. Numbers of viable bacteria reported are the average of triplicate malt samples exposed at each irradiation level.

α-Amylase was determined by a modification of the method of the American Society of Brewing Chemists (*1*), in which 0.47% sodium carbonate was used for extracting the enzyme. Values presented are the average of duplicate determinations.

Laboratory scale grain mash fermentations were carried out by adding 77.7 grams of corn meal and 0.7 gram of barley malt meal to 450 grams of water at 140° F. contained in a 1-liter Florence flask. These grain slurries were cooked in an autoclave at 260° F. for 45 minutes, cooled to 147° F., and saccharified with 5.5 grams of the experimental malt meal. This level of conversion malt—6.6% of the grain—is near-minimum for complete saccharification, but was desirable in these studies to detect slight differences in the saccharifying power of irradiated malts. After saccharification for 45

minutes, mashes were cooled to 75° F., adjusted to pH 4.8 with lactic acid, and inoculated with 3% by volume of distiller's yeast grown in a malt sirup medium. The final volume in each flask was made up with water to 535 ml. Fermentations were conducted for 72 hours at 90° F., after which an aliquot was distilled and alcohol determined by pycnometer. Alcohol yields, which are reported as proof gallons of alcohol per 56 pounds of dry grain, are the average of triplicate fermentations.

Results

Data presented in Figure 1 and Table I show the effects of different temperatures during irradiation and prior grinding of malt samples upon the destruction of malt bacteria by varying dosages of cathode rays. *Lactobacilli* were of major importance in this study because of their growth in grain mashes and sporadic effect on the yield and/or quality of alcohol distillates. The effect of irradiation upon this group of organisms is shown in Figure 1 and in part of Table I.

Although complete sterilization is difficult to assess and may not have been achieved with even the highest dosage level used—2.0 × 10^6 rep—there was in all treated samples a substantial reduction of the malt lactic-type flora. For example, in sample A (column 2, Table I) which had an initial count of 3,760,000 lactics per gram, exposure to 0.05 × 10^6 rep reduced the viable count to less than 10% and exposure to 2.0 × 10^6 rep reduced the count to some 700 per gram of malt—i.e., less than 0.02% of the original. Viable organisms in samples B-1 and B-2 (columns 4 and 5, Table I), irradiated whole and in meal form at 78° F., were reduced to 200 and 100 organisms per gram, respectively, by exposure to 1.0 × 10^6 rep. Sample C, irradiated whole at 145° F. with 0.2 × 10^6 rep, contained less than 100 organisms per gram of malt (column 8, Table I).

Comparison of the three temperatures at which samples were irradiated (Figure 1) reveals that, at the lower levels of irradiation, the bacteria-killing effectiveness was enhanced roughly from 10- to 100-fold by raising the temperature from 45° to 145° F. With the higher radiation levels, survival was low at all exposure temperatures.

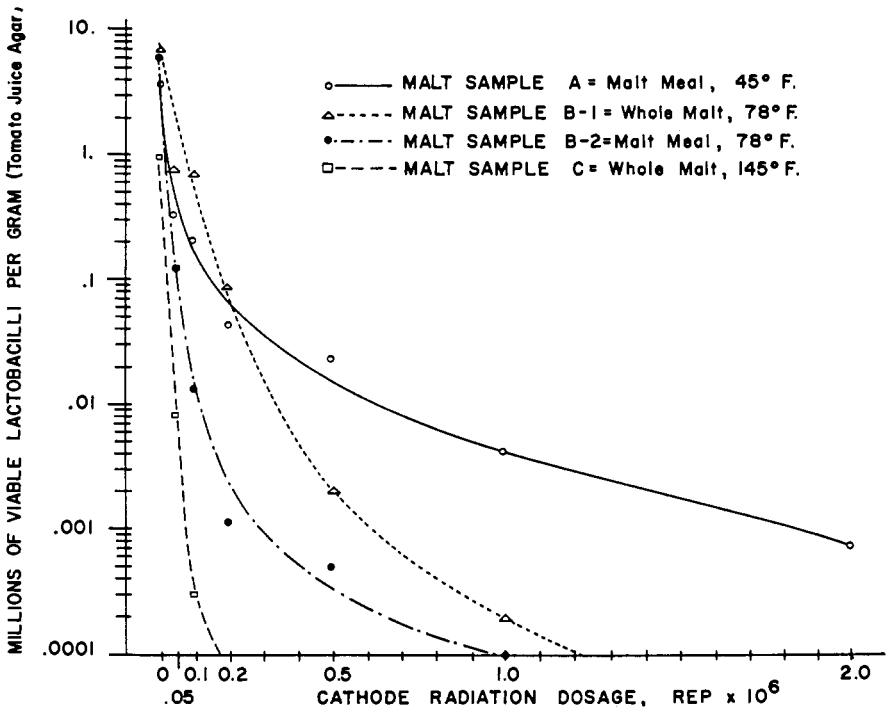

Figure 1. Effect of cathode irradiation dosage and temperature upon destruction of bacteria in whole and ground malt

Ground malt was definitely more susceptible (compare samples B-1 and B-2) to cathode radiation than whole malt at low to intermediate dosage levels, whereas at the higher intensities, a high percentage kill was obtained with both types of samples. These results suggest that the whole malt berry affords some protection to bacterial contaminants.

Total aerobic counts as determined in nutrient agar plates are presented in columns 3, 5, 7, and 9 of Table I. These bacteriological data are similar to those obtained with the *Lactobacilli* as to the effects of both temperature and grinding. The aerobic flora appeared to be about equally susceptible to radiation, with the possible exception of sample A, in which the higher dosages—i.e., 0.5 to 2.0 × 10^6 rep—destroyed a lower percentage of aerobic organisms than *Lactobacilli*.

α-Amylase and yields of alcohol from grain were determined on treated malts as an indication of enzyme destruction. Only a part of the malt saccharogenic system, α-amylase, has been shown (*15*) to be essential for the production of high yields of alcohol from grain mashes. Amylase inactivation was found (columns 2, 4, 6, and 8, Table II) to be relatively low compared with the effect of this form of radiation upon bacteria. Particularly was this true of dosages up to 0.5 × 10^6 rep in which the samples retained over 90% of their original α-

Table I. Effect of Radiation Dosage and Temperature upon Destruction of Bacteria in Whole and Ground Malt

Radiation Level Rep × 10^6	Sample A, Malt Meal, 45° F.		Sample B-1, Whole Malt, 78° F.		Sample B-2, Malt Meal, 78° F.		Sample C, Whole Malt, 145° F.	
	Lactobacilli[a]	Aerobic bacteria[b]	Lactobacilli	Aerobic bacteria	Lactobacilli	Aerobic bacteria	Lactobacilli	Aerobic bacteria
	Bacteria, % of Original Malt Count							
1	2	3	4	5	6	7	8	9
None	100	100	100	100	100	100	100	100
0.05	8.9	28.7	11.2	16.6	2.1	6.9	0.85	0.016
0.1	5.2	7.4	10.1	13.6	0.22	1.6	0.03	0.068
0.2	1.1	2.6	1.2	3.9	0.019	0.10	0.01	0.002
0.5	0.63	1.77	0.03	0.01	0.008	0.007	0.01	0.002
1.0	0.10	0.72	0.003	0.002	0.002	0.005	0.01	0.002
2.0	0.019	0.24	0.001	0.002	0.001	0.001	0.01	0.002

[a] Determined in tomato juice agar shake tubes by method of Garey and others (*8*). Untreated malts contained following *Lactobacilli* (millions per gram): sample A, 3.76; sample B-1, 6.81; sample B-2, 5.92; sample C, 0.95.

[b] Determined by plating in nutrient agar. Untreated malts contained following aerobic bacteria (millions per gram): sample A, 4.18; sample B-1, 4.30; sample B-2, 5.45; sample C, 4.35.

Table II. Residual Amylase Activity and Alcohol Yields from Irradiated Malts

Radiation Level, Rep × 10^6	Malt Meal, 45° F.		Whole Malt, 78° F.		Malt Meal, 78° F.		Whole Malt, 145° F.	
	Amylase, % of untreated malt[a]	Proof gal. alcohol/-56 lb. dry grain[b]	Amylase, % of untreated malt[a]	Proof gal. alcohol/-56 lb. dry grain	Amylase, % of untreated malt[a]	Proof gal. alcohol/-56 lb. dry grain	Amylase, % of untreated malt[a]	Proof gal. alcohol/-56 lb. dry grain
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
None	100	5.98	100	6.00	100	6.05	100	6.05
0.05	97.4	6.01	99.7	5.99	98.7	6.05	98.7	6.07
0.1	97.7	5.99	97.8	5.94	96.3	6.04	97.5	6.04
0.2	93.2	5.98	94.8	5.97	93.3	6.04	92.0	6.07
0.5	89.5	5.97	89.1	5.93	85.9	5.94	85.2	6.03
1.0	82.1	5.89	85.5	5.84	79.8	5.88	75.4	5.92
2.0	74.4	5.65	75.9	5.63	65.2	5.63	59.6	5.52

[a] α-Amylase content of untreated malts in dextrinizing units/g. was: sample A, 35.2; sample B-1, 36.6; sample B-2, 37.6; sample C, 48.0. [b] Mash bill composed of 92.6% corn, 0.8% premalt, 6.6% conversion malt; set with 3% by volume of distiller's yeast in malt sirup; fermented 72 hours at 90° F.

amylase content. As with bacteria, however, destruction of enzyme increased when the exposure temperature and radiation dosages were raised.

Alcohol fermentations carried out with irradiated malts and untreated control malts are reported in columns 3, 5, 7, and 9 of Table II. Saccharifying action was not materially affected until dosages of 1.0 and 2.0 × 10^6 rep were used. Reduction of alcohol yields was pronounced in malts receiving 2.0 × 10^6 rep, indicating the inactivation of one or more malt enzymes.

Normally, the small percentage of *Lactobacilli* which survives the grain mashing process develops rather rapidly and attains appreciable numbers by the time the alcoholic fermentation is complete. It was of considerable interest, therefore, to determine the effect of irradiating malt upon the subsequent development of the typical lactic flora. Bacteriological data for such a series, representative of a number of fermentations, are shown in Table III. Mashes saccharified with malts receiving dosages from 0.05 to 0.1 × 10^6 rep were markedly delayed in their development of lactic types. Furthermore, mashes converted with malts receiving from 0.2 to 2.0 × 10^6 rep showed less than 100 bacteria per ml. of mash after 144 hours of alcoholic fermentation. Such fermentations would be considered exceptionally "clean" and would probably give excellent distillate character, although this was not determinable with the small volumes of mashes fermented. It would appear that irradiation decreased the ability of surviving *Lactobacilli* to initiate growth in grain mashes.

This study has shown that cathode radiation is an effective method of reducing the bacterial flora of barley malt. Malts so treated would be expected to give more uniform alcohol yields and distillate quality. The capital investment and operating cost of a cathode unit of the size needed to irradiate malt for a moderate sized to large distillery would make the process uneconomic at the present time. However, continued interest in this sterilization method by the distilling industry is indicated; it may become practical as improvements are made and costs reduced.

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Literature Cited

(1) *Am. Soc. Brewing Chem. Proc.* **1952,** 152.
(2) Atwood, H., U. S. Patent **2,222,306** (November 1940).
(3) Clark, G. L., Boruff, C. S., *Science* **70,** 1803 (1929).
(4) Coolidge, W. D., *Ibid.*, **62,** 441 (1925).
(5) Coolidge, W. D., Moore, C. N., *Gen. Elec. Rev.* **35,** 413 (1932).
(6) Coolidge, W. D., Moore, C. N., *J. Franklin Inst.* **202,** 722 (1926).
(7) Day, W. H., Serjak, W. C., Stratton, J. R., Stone, L., J. AGR. FOOD CHEM. **2,** 252 (1954).
(8) Garey, J. C., Rittschof, L. A., Stone, L., Boruff, C. S., *J. Bacteriol.* **49,** 307 (1945).
(9) Knowlton, J. A., Mahn, G. R., Ranftl, J. W., *Nucleonics* **11** (11), 64 (1953).
(10) O'Meara, J. P., *Ibid.*, **10** (2), 19 (1952).
(11) Proctor, B. E., Goldblith, S. A., *Advances in Food Research* **3,** 119 (1951).
(12) Proctor, B. E., Goldblith, S. A., Dept. Food Technol., Mass. Inst. Technology, 11th Annual Meeting, Institute of Technologists, New York, June 20, 1951.
(13) Proctor, B. E., Van de Graaff, R. J., Fram, H., U. S. Army Quartermaster Contract Projects, Research Report, p. 217, July 1942–June 1943.
(14) Serjak, W. C., Day, W. H., Van Lanen, J. M., Boruff, C. S., *J. Appl. Microbiol.* **2,** 14 (1954).
(15) Whitehouse, Katherine, Adams, S. L., J. AGR. FOOD CHEM. **2,** 1040 (1954).

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Table III. Effect of Irradiation of Malt upon Numbers of Bacteria Developing in Laboratory Grain Mash Fermentations

(Tomato juice agar tubes)

Radiation Level Rep × 10^6	Viable Bacteria in Fermenting Mashes[a], Millions per Ml.			
	Set ferm.	72 hr.	96 hr.	144 hr.
None	<0.0001	0.013	0.515	1.590
0.05	<0.0001	0.011	0.019	0.136
0.1	<0.0001	<0.0001	<0.0001	<0.0001
0.2	<0.0001	<0.0001	<0.0001	<0.0001
0.5	<0.0001	<0.0001	<0.0001	<0.0001
1.0	<0.0001	<0.0001	<0.0001	<0.0001
2.0	<0.0001	<0.0001	<0.0001	<0.0001

[a] All mashes converted with irradiated salt sample C.