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MALT PASTEURIZATION

Sterilization of Barley Malt with Gamma Radiation

LLOYD L. KEMPE and JOHN T. GRAIKOSKI

Department of Chemical Engineering, The University of Michigan, Ann Arbor, Mich.

J. R. STRATTON[1] and W. H. DAY

Hiram Walker and Sons, Inc., Peoria, Ill.

This study has shown that barley malt can be effectively pasteurized with gamma radiations. When malts irradiated with 0.112 megarad of gamma rays were used in grain alcohol fermentations, saccharifying enzyme activity was not impaired, and no lactobacilli developed within 144 hours. Such malts should provide yields about equivalent to nonirradiated malts with some improvement in distillate quality and uniformity. Estimates based upon extrapolation of published costs for disinfestation of grain suggest a price from $1.79 to $2.77 per ton for pasteurization of grain with 0.1 megarad of gamma rays from cobalt-60.

OFF-FLAVORED DISTILLATES are occasionally produced during the processing of grain mashes to beverage alcohol. In many cases, these off-flavors can be traced to metabolic activities of bacteria that survive the saccharification step in mashing in which the mash is maintained at 145° to 148° F. for a short time. Barley malt is the principal source of these bacteria, which are mainly lactobacilli (*9*). It would be desirable to kill such bacteria before using the malt, but heating, which is the most common sterilizing technique, also inactivates enzymes of the malt. Other possible methods for controlling the growth of these contaminants include the use of antibiotics in the fermenting mashes (*4*) and presterilization of the malt with ionizing radiations. Such radiations selectively kill vegetative cells of bacteria at dosages that do not materially affect enzymes present in the grain. Cathode radiations have been investigated for this purpose (*10*); but gamma rays could be more effective since their penetrating ability is much greater, thus permitting more nearly uniform irradiation of the grain (*6*).

Materials and Methods

Malt samples were irradiated in the large, cobalt-60, gamma-ray source at the University of Michigan. This source has been adequately described elsewhere (*3*). For irradiation, the malt was sealed in tin cans and placed either in the center-well of the source or in locations around the periphery. The cobalt-60 was raised into the radiation room for the required time interval and then returned to the well beneath.

Radiation dosages were determined with Fricke dosimeters (*11*) placed in the center of test cans. When grain was irradiated outside the source, No. 2 tin cans were used. In these positions, gamma radiations came from one side only, so the cans were placed on turntables to produce essentially uniform dosages throughout the grain. For irradiations in the center-well, the grain was sealed into No. 1 picnic cans; here no turntables were needed.

The dose rate in the center-well was essentially uniform and amounted to approximately 0.23 megarad per hour during these experiments. (The terms rad and rep are used in this paper. Rad = dissipation of 100 ergs per gram of tissue; rep = 93 ergs per gram of tissue.) Outside the source, the dose rate varied as a function of distance from the cobalt-60 rods. By placing the cans on turntables at selected distances from the source, dose rates were obtained that permitted the desired amount of radiation to be delivered in the grain over an interval of 2 to 4 hours. The exact interval used varied between these time limits in the various irradiations.

The temperature of the barley was not precisely controlled during irradiation, but averaged about 5° C. Immediately after irradiation, the cans of barley malt were shipped to Peoria for analysis. Changes in numbers of lactobacilli and aerobic bacteria in the malt, as well as effects upon amylase activity and ethyl alcohol yields in test fermentations, were determined and evaluated as a function of radiation dosage.

Malt samples were aseptically ground 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 *et al.* (*5*) and aerobic organisms by plating in nutrient agar. Colony counts were made after 48 hours of incubation at 37° C. Numbers of viable bacteria reported are the average of triplicate malt samples 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 expressed in dextrinizing units per gram.

[1] Present address: St. Petersburg, Fla.

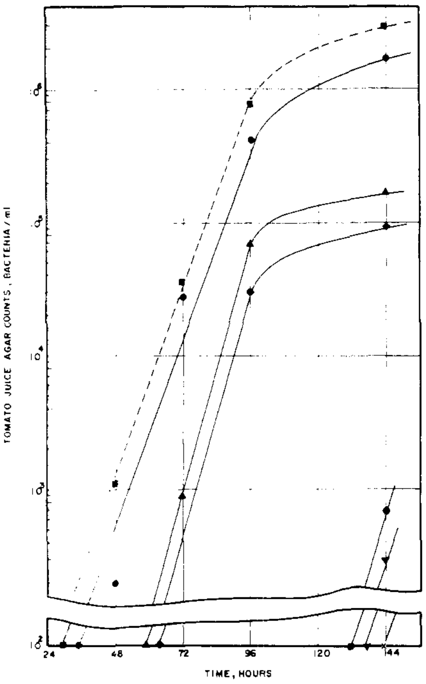

Figure 1. Bacterial populations developing in laboratory corn mash fermentations saccharified with gamma-irradiated barley malt. (Saccharification at 145° F. for 45 min., counts in tomato juice agar)

■, no irradiation; ●, 4.65 × 10^3 rad; ▲, 9.3 × 10^3 rad; ◆, 1.86 × 10^4 rad; ⬮, 3.72 × 10^4 rad; ▼, 7.45 × 10^4 rad; ×, 1.12 × 10^5 rad, and more

Table I. Effect of Gamma Radiations upon the Viable Bacteria, α-Amylase Contents of Barley Malt, and Alcohol Production from Grain Mashes

Gamma Ray Dosage Level, Rad	Viable Bacteria, Millions per Gram of Malt: Tomato juice agar tubes	Viable Bacteria, Millions per Gram of Malt: Nutrient agar plates	α-Amylase, Dextrinizing Units per Gram	Proof, Gal. Alcohol per 56 Lb. Dry Grain
0.93 × 10^6	<0.0001	<0.0001	42.7	6.03
6.98 × 10^5	<0.0001	<0.0001	41.2	6.02
4.65 × 10^5	0.0009	<0.0001	43.2	6.04
2.32 × 10^5	0.001	0.0040	44.5	6.02
1.12 × 10^5	0.003	0.0013	48.6	6.04
7.45 × 10^4	0.005	0.0140	47.6	6.02
3.72 × 10^4	0.04	0.0920	50.6	6.03
1.86 × 10^4	0.19	1.3400	50.5	6.08
0.93 × 10^4	0.9	1.3000	49.8	6.06
4.65 × 10^3	0.8	1.6000	50.4	6.12
None	1.0	1.7000	49.4	6.13

Laboratory-scale grain fermentations were carried out by adding 79.3 grams of corn meal and 0.4 gram of barley malt meal to 450 ml. of water at 140° F. contained in a 1-liter Florence flask. The mashes were precooked at 180° F. for 20 minutes, then cooked at 260° F. for 45 minutes, cooled to 145° F., and saccharified with 4.2 grams of the test malt for 45 minutes. The 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 syrup medium. The final volume in each flask was made up to 535 ml. with water. Fermentations were conducted for 72 to 144 hours at 90° F. after which an aliquot was distilled and alcohol determined by a pycnometer. Alcohol yields, which are reported as proof gallons per 56 pounds of dry grain, are also averages of triplicate fermentations in each instance. Bacterial populations in the fermenting mashes were determined at various times using the tomato juice agar and procedures described for bacteriological analyses of the malts.

Results and Discussion

Data in Table I show that 0.112 megarad of gamma radiations reduced the bacterial counts of malts in both tomato juice and nutrient agar to very low levels whereas no significant reduction in α-amylase or alcohol yields occurred. Higher dosages of radiation resulted in substantially complete sterilization of the malts but also in some destruction of their amylase activities.

In normal grain distillery operations, a small fraction of the bacterial flora of malt survives the time and temperature of the saccharification step and develops to attain numbers in the range of millions per milliliter (tomato juice agar counts) by the time the fermentation is complete.

This pattern is altered by pre-irradiation of the malt as shown in Figure 1 which indicates progressively lower maximum bacterial populations and lengthened lag phases as more radiation was used; however, the rate of multiplication in the exponential growth phases was not influenced by the amount of radiation. These observations duplicate in principle the results given in another paper describing the growth of psychrophylic bacteria during refrigerated storage of irradiation pasteurized ground beef (*8*).

Since heating subsequent to irradiation has been shown to have a synergistic lethal effect on bacteria (*7*, *10*), it was of interest to determine whether a similar effect would be noted in this study. The data in Table II show no significant difference between irradiated and nonirradiated malts or of saccharification temperature upon alcohol yields. In some trials, there appeared to be a tendency toward higher alcohol yields from irradiated malts used at a saccharification temperature of 125° F., suggesting that some destruction of malt amylase occurs by saccharifying at 145° F. With regard to the development of bacteria during fermentation, it is

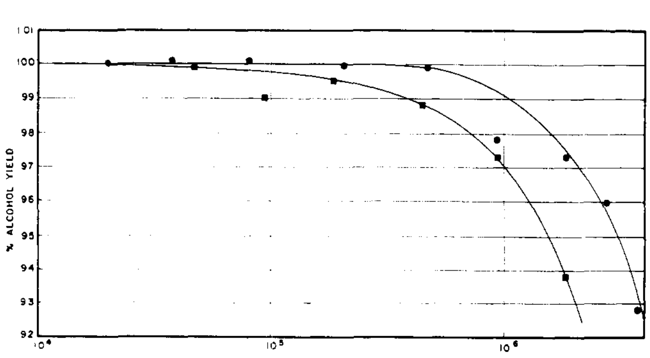

Figure 2. Effect of gamma irradiation of barley malt on yields from grain alcohol fermentation

●, gamma radiation; ■, cathode radiation

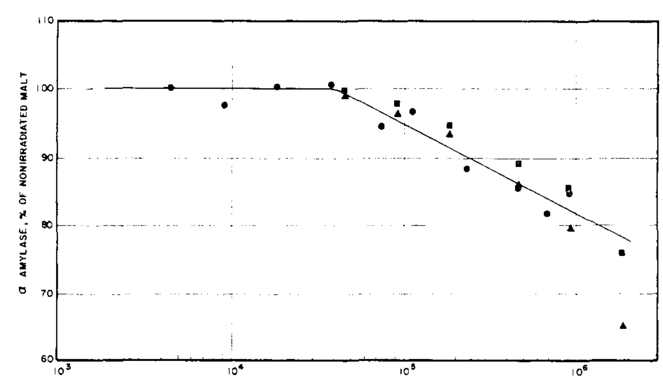

Figure 3. Effect of gamma irradiation on residual α-amylase content of barley malt

●, gamma radiation; ■, ▲, cathode radiation

Table II. Effect of Gamma Irradiation of Barley Malt and Saccharification Temperature upon the Yield of Alcohol and the Development of Bacteria during Grain Mash Fermentations

	Fermentation 1	2	3	4	5	6
Gamma-ray dosage, megarad	None	None	None	0.465	0.465	0.465
Saccharification temp., °F.	145	135	125	145	135	125
Alcohol yield, proof gal. per bu. (dry basis)	5.99	6.03	6.04	5.94	5.99	5.96
Viable bacteria per ml. during fermentation (tomato juice agar tubes)						
0 hr.	<100	<100	<100	<100	<100	<100
24 hr.	<100	<100	<100	<100	<100	<100
144 hr.	1.17×10^6	1.11×10^6	1.36×10^6	<100	<100	<100

apparent from Table II that the anaerobic flora of the irradiated malt, if indeed small numbers did survive, were unable to grow in the fermenting grain mashes. Even after 144 hours, the counts were below 100 compared with more than 1,000,000 per ml. in the control mashes. In this study, malts irradiated at 0.112 megarad and above consistently gave very low tomato juice agar counts during the course of alcoholic fermentation.

The data in Table I show typical results for yields from grain alcohol fermentations when the mashes are saccharified with malt that has previously been irradiated with less than 1 megarad of gamma rays. No significant reduction is caused by this amount of gamma radiation. Similar data are shown in Figure 2 where results using malts treated with higher levels of both gamma and cathode rays can be compared. The cathode ray data are for whole malt irradiated at 78° F. and are taken from the data of Stratton *et al.* (*10*). It was necessary to convert rep to rad for this comparison; one rep is equivalent to 0.93 rad. The curves in Figure 2 show that a 1% reduction of alcohol yield is caused by 0.4 megarad of cathode rays and by 1.1 megarad of gamma rays. Beyond the amount of radiation causing 1% reduction, the yields drop rapidly with either kind of radiation.

The curves in Figure 3 show that the α-amylase content of malt is not significantly reduced by irradiation with less than 0.04 megarad of either gamma or cathode rays. Beyond 0.04 megarad, however, the α-amylase content decreases rapidly with both kinds of radiations, no significant difference being apparent between the action of gamma and cathode rays on this enzyme. The data for cathode rays were also from Stratton *et al.* (*10*); the gamma-ray curve was based upon data in Table I, assuming 50.4 dextrinizing units as 100% enzyme activity.

A renewed interest is developing in the irradiation of grain for disinfestation. In fact, an entire meeting of the International Atomic Energy Agency was devoted to this subject in May of 1962. Dosages of 10,000 to 20,000 rads are advocated for disinfestation. This dosage is an order of magnitude lower than the approximately 100,000 rads needed to prevent the growth of lactobacilli within 144 hours in grain alcohol fermentations. Predictions of the cost for treating barley malt with this amount of gamma radiation must still be considered estimates. Fortunately however, rather detailed cost estimates have recently been made for the disinfestation of grain.

Jefferson and Rogers (*6*) compared the use of gamma radiations from cobalt-60 and 1 m.e.v. electrons for this purpose. They calculated a cost of 44.4¢ per ton for irradiating 20 tons per hour of grain to an average dose of 16,000 rads with gamma rays from cobalt-60. Their installation would require 100,000 curies of cobalt-60 valued at $70,000 and a plant costing $30,000. Continuous operation for 8000 hours per year was assumed; if the plant operated 2400 hours per year, the irradiation cost would rise to 76.3¢ per ton. Using linear extrapolation of their continuous operation data to permit pasteurization of grain at 0.1 megarad rather than disinfestation at 16,000 rads, the authors estimate a cost of $2.77 per ton for pasteurization. A linear extrapolation appears justified since cobalt-60 represents most of the cost of the installation. With a somewhat differently designed irradiator, Brownell (*2*) estimated a cost of 0.5¢ per bu. for disinfestation of grain using 10,000 rads. A similar extrapolation of his estimate suggests a cost of $1.79 per ton for a 0.1 megarad irradiation of grain. Brownell assumed continuous operation and cobalt-60 at $1.00 per curie. Even these costs may still be too high for radiation pasteurization of malt in beverage alcohol fermentations. Actually a cost of $1.00 per ton could be met by using approximately 0.06 megarad of irradiation. According to data in Figure 1, this level of irradiation should provide relatively clean fermentations but the effectiveness of this irradiation level would need further testing. Also, a cost of $1.00 per ton would result if the price of cobalt should drop to approximately 50¢ per curie.

In a comparison of gamma radiations from cobalt-60 and 1 m.e.v. electrons for irradiation of grain, Jefferson and Rogers (*6*) calculated only minor differences in the cost and size of the necessary installations. They stated that discontinuous operation favors the use of electrons, but that cobalt-60 irradiation provides a more uniform radiation dosage. The data in Table I indicate that the α-amylase activity in malt fall rapidly as the radiation dosage exceeds 0.11 megarad. Close control of dosage therefore would be required when radiation pasteurizing barley malt for use in beverage alcohol fermentations. Such control appears more feasible with the penetrating gamma rays from cobalt-60 than with 1 m.e.v. electrons.

Acknowledgment

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