An Examination of the Effectiveness of Various Sanitizing Agents against Yeasts Isolated from Liquid Sugar and Sucrose-Corn Syrup Blends

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In recent years, the market for liquid sugar and sucrose-corn syrup blends has greatly increased. Along with this market increase has come a relative increase in bacteriological and mycological problems associated with the preparation and distribution of these products. Yeasts have been isolated which grow readily at sugar-blend concentrations of 72 to 75 Brix. Once these yeasts contaminate mixing tanks, storage tanks and transmission lines, it is difficult to sanitize all areas which contact the sugar products to eliminate contamination which will again seed the next batch of product produced.

The purpose of the investigation to be reported by this paper has been twofold: First, to test the activity of various sanitizing agents against three different colonial types of yeast isolated from liquid sugar products, and second, to compare the sanitizing efficiency of these agents as tested under laboratory conditions.

Materials and Methods

Yeasts: Three distinctively different colonial types of yeast have been isolated from liquid sugars and liquid sugar blends during routine microbiological examination of submitted samples. The yeast most commonly found in blends grows as a small white colony on mycophil agar. Usually the number of microorganisms per 10 grams of dissolved sugar equivalent is high because this organism will multiply in the high concentrations of carbohydrate involved (Figure 1). A second yeast, seen less often, grows anaerobically in a "star burst" type of colony (Figure 2). At the surface, under aerobic conditions, the colonial form is fluid and thinly mucoid in its early stages of growth. The colony enlarges rapidly, and if the agar plate is tipped at an angle of more than 30° from the horizontal, the colony will flow across the agar surface. Upon standing, the colony loses it fluid characteristic and develops a black pigment. This pigment first appears as a ring at or near the center of the colony, and then as the culture ages the pigment extends throughout the entire colonial mass.

[1] Department of Bacteriology, College of Medicine, University of Utah, Salt Lake City, Utah.

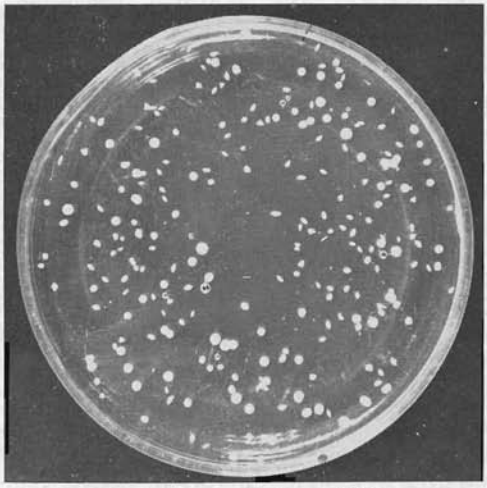

Figure 1.—Colonial morphology of first type yeast.

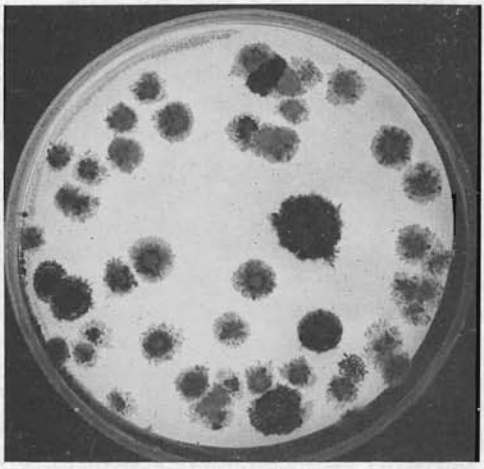

Figure 2.—Colonial morphology of second type yeast.

The frequency of the appearance of the black yeast is greatest in the summer months, and has been seen very infrequently during the winter months.

The third yeast has been observed with some regularity in routine samples, but seldom in large numbers. It appears to be confined to sucrose syrups. It is possible that this colonial type is the same as the first organism, but shows the different characteristics in the different environment.

The first described yeast was used almost exclusively for the germicidal efficiency tests. All three were tested for sensitivity to germicide.

A stock culture of each yeast type was maintained on acidified mycophil agar slants. All stock cultures were stored at 4° C. Test cultures were grown in mycophil broth for 24 hours at room temperature (22° C ± 5°) and at a pH of 4.8 to 5.2.

All tests were carried out with 24-hour yeast culture suspensions.

Germicidal Efficiency Test: The germicidal efficiency of the several sanitizing agents as well as the sensitivity of the yeast cultures to the agents was tested by the method of Chambers (1)[2].

Each agent was prepared at a predetermined concentration and 100 ml placed in a 250 ml Erlenmeyer flask. One ml of the 24-hour broth yeast suspension was added to the flask. Fifteen seconds later and at intervals of 30 seconds, 1, 2, 5, 10, 20 and 30 minutes, a one ml portion of the germicide-culture mixture was removed and placed into 9.0 ml of neutralizer which consisted of lecithin suspended in a solution of Tween 80. Thus the 1 ml aliquot samples were removed and transferred to neutralizer after 15 and 30 seconds, and 1, 2, 5, 10, 20, and 30 minutes exposure to the sanitizing agent.

As soon as possible after removal of the aliquots to the neutralizer, 1 ml of the contaminated neutralizer was transferred to each of two tubes of mycophil broth. The latter were incubated for a period of 72 hours at room temperature (22° C ± 5°) and then examined for yeast growth. The results of tests are reported as killing effect after an exposure of a given time.

The effect of the neutralizer on the test organism was observed by adding 1 ml of culture to 100 ml of sterile distilled water. After thorough mixing, 1 ml of diluted culture was transferred to a tube of neutralizer. One ml of neutralizer culture suspension was then transferred to each of two tubes of mycophil broth. Two more tubes of mycophil broth were inoculated from the same neutralizer culture suspension after completion of the test run. Growth was always positive in all tubes.

The effectiveness of the neutralizer was observed by adding 1 ml of sanitizing agent to 9.0 ml of neutralizer. This was then inoculated immediately with yeast culture and 1.0 ml aliquots transferred to mycophil broth. Growth was always positive in all control tubes and it appears that the lecithin in Tween 80 was equally effective as a neutralizer against the iodine compounds as well as the quaternary ammonium compounds.

It was shown that added sucrose decreased the killing effectiveness of both Hyamine and Weladyne (see results). The following titrimetric methods were carried out to show that this

[2] Numbers in parentheses refer to literature cited.

effect was accompanied by a loss in titratable iodine. The effect of sucrose on titratable Hyamine 3500 was also tested but the results were equivocal. The carbohydrate interfered with the argentimetric titration; therefore, no results are reported.

Zero, 10, 20, 30, 40 and 50 grams of sucrose were added respectively to 100 ml of a solution of 100 ppm (9.8×10^{-4} N) Weladyne in distilled water. Each of these solution mixtures was added from a burette into 5 ml of 0.001 N Sodium thiosulfate in which starch had been added as an indicator.

It was recognized that volume error was introduced into the above titrations. Therefore in a second experiment, a different volume of sugar in each case was added to a given volume of iodine solution. Another titration was carried out as above and the volume effect of added sugar taken into account. A second solution containing 100 ppm of available iodine was prepared and percentile solutions of sugar at 0, 10, 25, 35, and 50% were prepared with the iodine solution. Each of these was titrated into 5 cc of 0.001 N Sodium thiosulfate.

Results

Ten different germicidal agents were first tested at concentrations recommended by the manufacturers. In all experiments in the absence of added carbohydrate, all agents were effective and test organisms were killed in less than a 15-second exposure.

Many of these agents were then tested at concentrations of 5 and 10 ppm. The iodine compounds at this low concentration were much superior to the quaternary ammonium compounds.

After scanning several agents, two which demonstrated the greatest effectiveness in both classes of compounds were settled upon for more intensive study, namely Hyamine 3500[3], a quaternary ammonium compound, and Weladyne[4], an iodine-detergent complex. The comparative effectiveness of these two compounds is demonstrated in Table 1. The effectiveness of the iodine complex at 10 ppm. titratable iodine, is comparable to that of the quaternary ammonium compound at 200 ppm.

As it was proposed that these agents were to be used to sanitize tanks and transmission lines where rather large residual amounts of sucrose and sucrose syrups would be extant, it was imperative that the agents be germicidal in the presence of rather high concentrations of carbohydate. Hyamine 3500 was tested, first at 100 ppm in 5% and 10% sucrose solution and then at 200 ppm in 5%, 10% and 15% sucrose solutions with

[3] Hyamine 3500 supplied from stocks of the Amalgamated Sugar Company. The compound is manufactured by Rohm and Haas, Inc., Philadelphia, Penn.

[4] Weladyne supplied by the Chilean Iodine Information Bureau. The compound is manufactured by the West Chemical Co., Long Island City, N.Y.

Table 1.—A comparison of two sanitizing agents.

Germicide	Germicide concentration ppm	Exposure time to kill
Hyamine 3500	20	30 min
	40	20 min
	60	10 min
	80	10 min
	100	2 min
	200	15 sec
Weladyne	5	2 min
	10	15 sec
	50	15 sec
	80	15 sec

Table 2.—The effect of sucrose on the germicidal efficiency of Hyamine 3500 and Weladyne.

Germicide	Germicide concentration ppm	Sucrose concentration %	Exposure time to kill
Hyamine 3500	100	5	2 min
	100	10	5 min
	200	5	1 min
	200	10	2 min
	200	15	5 min
Weladyne	50	5	15 sec
	50	10	15 sec
	50	20	15 sec
	50	30	30 sec

the results shown in Table 2. The activity is markedly reduced as compared with solutions containing no carbohydrate. Also, as the carbohydrate is increased, the activity of the agent decreases.

Weladyne was also examined at 50 ppm in the presence of 5, 10, 20, and 30% sucrose solutions with results as presented in Table 2.

It is obvious that the iodine-detergent compound was much less affected at comparable concentrations of sucrose than was the quaternary ammonium compound.

These two compounds were further examined by maintaining the sugar concentration at 50% and the concentration of the agents varied from 40 through 100 ppm in increments of 10 ppm. The result is shown in Table 3. The iodine complex again was shown to be less affected by the very high concentration of carbohydrate.

Table 3.—A comparison of the germicidal properties of Hyamine 3500 and Weladyne in the presence of 50% sucrose.

Germicide	Germicide concentration ppm	Sucrose concentration %	Exposure time to kill
Hyamine 3500	50	50	30 min
	60	50	30 min
	70	50	30 min
	80	50	20 min
	100	50	20 min
Weladyne	50	50	30 min
	60	50	30 min
	70	50	20 min
	80	50	5 min
	100	50	2 min

Germicidal tests against the "black yeast" showed comparable results except the "black yeast" was more sensitive to the sanitizing agents than the yeast found regularly in blend. Tests for sensitivity of the third yeast to germicides indicate it to have a sensitivity comparable to that of the first yeast described.

The results of the two iodine titrations are graphically presented in Figures 3 and 4. By adding 50 grams of sugar to 100 ml of solution, the normality of the iodine dropped from 9.8×10^{-4} N to 5.0×10^{-4} N, a loss of half the available iodine. Where more sugar was added per unit volume of solution, as demonstrated by preparing percentile dilutions of sugar, the fall off in titratable iodine was much more precipitous (from 10.8×10^{-4} N to 1.8×10^{-4} N).

It was also shown by further laboratory experimentation that both Hyamine 3500 and Weladyne would deteriorate if stored in open vessels at dilutions recommended for use as sanitizing agents. The chemically titratable activity is lost and the germi-

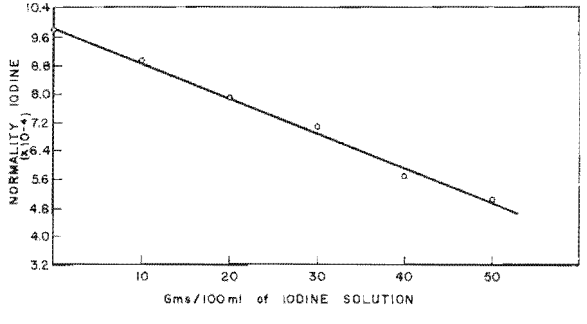

Figure 3.—The effect of sucrose on titratable iodine.

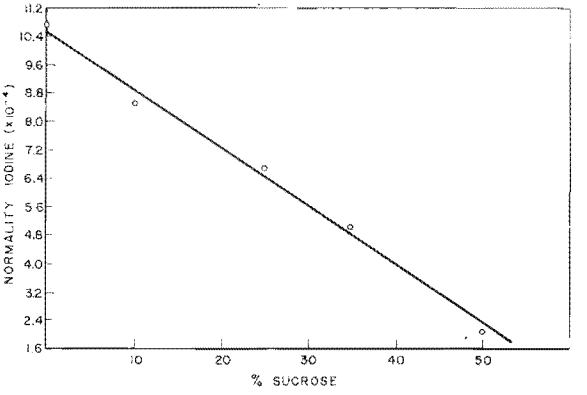

Figure 4.—The effect of sucrose on titratable iodine.

cidal efficiency of both is impaired. Further, the rapidity of deterioration is increased if the storage temperature is higher than 22° C.

Discussion and Conclusions

It has been demonstrated that yeasts commonly found as contaminants in liquid sugar syrups and in liquid sugar-corn syrup blends are readily killed in recommended concentrations of several different sanitizing agents at present on the market. It was also demonstrated that two of these, Hyamine 3500 and Weladyne were especially active, even in the presence of sucrose, but the efficiency of both was impaired. The carbohydrate affected the iodine compound (Weladyne) less than it did the quaternary ammonium compound (Hyamine 3500).

It was also shown that both the iodine and quaternary ammonium compound in water solutions deteriorated upon standing and that the rate of deterioration was affected by temperature. As would be expected, warm temperatures increased the rate of deterioration. Iodine compounds were less stable than the quaternary ammonium compounds, but have the advantage of a built-in indicator. As long as color is present the iodine is relatively active. One can therefore "see" whether or not the sanitizing agent is still at active concentration.

Literature Cited

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(2) PIERCE, HAENISCH & SAWYER, Quantitative Analysis, 4th Ed., Wiley & Sons, New York.