Studies in the Industrial Control of Microorganisms in Granulated Sugar

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Thermophillic Bacteria

Sugar was first suspected as a contaminant of non-acid canned foods during the canning season of 1926. Since that time it has been proved that sugar may contain organisms which cause two of the recognized types of spoilage, which in commercial canneries act mainly through the establishment of foci in the apparatus with resulting contamination of foods passing through that equipment.

These organisms are thermophilic in nature, characterized by their ability to withstand temperatures higher than those at which most foods are processed.

Recognizing the dangers of using contaminated sugar, the National Canners Association has established standards which would tend to minimize these dangers. They are as follows:

Table 1.—National Canners' Standards.

Total thermophiles
In 10 grams of sugar an average of not over 125 for 5 samples and a maximum of not more than 150 for any 1 of the 5.

Flat sours
In 10 grams of sugar an average of not more than SO for 5 samples and a maximum of not more than 75 for any 1 of the 5.

Anaerobes not producing H_2S
In 4 grains of sugar not over 67 percent of the tubes positive for any 1 of 5 samples and not over 3 of 5 samples 60 percent positive.

We in the sugar industry believe these specifications to be reasonable and accept them in every respect.

The thermophiles with which we are concerned are divided roughly into two groups.

1. Aerobic bacteria.
2. Anaerobic bacteria.

The first class or aerobic type requires oxygen in order to live and reproduce. Most of the thermophiles which contaminate sugar are in this class. Here is found the acid-producing organism which causes the industrial spoilage called "flat sour" in non-acid foods. The microbe secretes an acid product that causes a definite bitter "off flavor."

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The number of thermophiles of this class in any given product is determined by the plating method. One hundred cc. of distilled water are measured into a 250-cc. Erlenmeyer flask, a cotton plug inserted and the flask and contents autoclaved for 20 minutes at 15 pounds pressure. The flask is removed, cooled, and into it is weighed 20 grams of sugar or its equivalent. The solution is then gently boiled at atmospheric pressure for 5 minutes to destroy all microorganisms, except the thermophillic types. The volume at this point should be 100 cc. The solution is cooled slightly, and with a sterile 10-ce. serological pipette, 2 cc. (equivalent to 0.4 gram of sugar) are seeded in each of 5 petri plates and nutrient brom-agar of the following composition is added:

15 grams agar,
10 grams tryptone,
5 grams dextrose,
1 liter of distilled water.

The p1l is adjusted to exactly 6.8 with 10-percent NaOH or HC1, and 1 cc. of 4-pereent alcoholic solution of brom-cresol purple added.

The agar in the media causes it to set to a jelly-like consistency holding the individual bacteria in one place. The plates are then incubated at 55° C. for 48 hours and each cell develops into a colony of sufficient size to be visible to the unaided eye. These colonies, each of which represents a single organism, are counted on all 5 plates, the total multiplied by 5 giving results in terms of total thermophiles per 10 grams of sugar. Colonies of the flat-sour type may be distinguished on these plates by their characteristic deep-red center surrounded by a yellow halo which is produced by the action of the secreted acid on the purple indicator.

The second class, the anaerobes, comprises a group which is unable to sustain life in the presence of oxygen. The particularly detrimental microbe which is encountered here produces what is known in the canning industry as "hard-swell" spoilage. These organisms produce within the can a gas composed of carbon dioxide and hydrogen in varying proportions. A pressure is built up resulting in bulging of the can and eventual bursting. Since these organisms cannot grow in contact with oxygen, their presence is detected by inoculating beef-liver, peptone, broth culture tubes prepared in the following manner:

0.75 pound of beef-liver is put through a fine meat grinder, 750 cc. distilled water added, and the mixture boiled for 1 hour. It is then filtered, the filtrate made up to 1 liter, brought to a boil, and 10 grams of peptone added. The p1l is adjusted to 6.7 and 1 gram of K_2HP0_4 added, bringing the pH to 6.8. The extracted liver is dried and ground to pass a 100-mesh screen. One-eighth inch of this dried ground material is placed in the tubes and covered with about 1 inch of the broth. Twenty cc. (equivalent to 4 grams sugar) of the same

solution with which the petri plates were seeded, are divided equally between 6 tubes. Effective sealing is accomplished by carefully pouring in about 1 inch of 2-percent sealing agar of pH *6.8* containing 0.4 percent tryptone. If, after 72 hours incubation at 55° C, the agar seal has been forced up and away from the beef broth by the pressure of the gases mentioned, the tube is positive and the unknown is said to contain thermophillic anaerobes. An unchanged tube of course denotes their absence, (figure 2, picture 4).

Most of these thermophiles have their origin in the soil and are introduced with the sugar beet itself. This is evidenced by the fact that counts of 400 to several thousand were made in 10 grams of raw juice from the diffusion battery. It was found that considerable numbers of these organisms entering with the diffusion juice are eliminated in the earbonation process with its subsequent filtrations but many go through to become centers of contamination in later stages of the process. In all o£ our examinations, unfiltered sirups and accumulations of sugars and juices throughout the factory showed extremely high counts, (table 2).

Product*	Total thermophiles	Flat sours	Anaerobes not producing H_2S
			percentage
Diffusion juice	420	20	67
Diffusion juice	800	00	67
Diffusion juice	25000	0	33
2nd press juice	10	0	0
Thin juice	50	10	0
Thick juice	40	0	0
High melter	200	30	17
High wash	525	15	33
Intermediate sirup	1135	10	66
Dust box	1100	40	0

*A11 counts arc on the basis of 10 grrams of the product.

During the campaign of 1939, experiments were devised at Toppenish to determine whether a single filtration of standard liquor using diatomaceous earth could produce thermophile-free masseeuite and subsequent sugar. Two colors of filter-aid were used. "Dicalite 20," which is brown in color, was used for precoating. Table 3 shows an average of a series of counts on the filtrate while precoating.

Thus it was concluded that feeding the filter-aid into the unfiltered liquor at the rate of 35 pounds per hour resulted in a suitable preeoat about 1/16-inch thick in 45 minutes. Opening the press gave further evidence that the precoat was very uniformly distributed over the entire cloth surface.

For the duration of the, press cycle, "Speed Plus," which is white, was fed continuously at the same rate. Series of examina-

Table 3.

Time minutes	Pressure (lb. per sq. inch)	Total thermopliiles	Flat sours	Anaerobes (percentage of tubes positive)
2	2	160	10	84
10	2	120	10	17
20	3	80	0	0
25	4	55	0	0
30	5	20	0	0
40	11*	50	0	0
45	12*	5	0	0

•Pump applied.

tions were inade through many complete press cycles and it was found that an occasional press would run thermophile-free filtrate for 20 hours and suddenly counts would jump to several hundred.

Upon investigation it was found that the press cake had pulled away from the cloths and white patches of "Speed Plus" showed up next to the cloth and in back of the brown precoat. In some instances patches of the cake had fallen to the bottom of the frame. The reason for this was easily determined. When the pump was allowed to take away the juice from the tank supplying the presses at a faster rate than it entered, the pressure on the filter presses would fluctuate from 0 to 50 pounds within a few seconds. This sudden drop in pressure would cause the cloths to sag away from the frames and the two inner surfaces of the cake to stick together. The next upward surge in pressure would force the cloths back against the frames, pulling the cake away from one or both sides, and leaving some uncoated cloth through which poorly filtered sirup could pass.

This condition was remedied by installing float signals on the supply tank to enable the operator to maintain an even pressure on the presses.

Table 4 shows typical results obtained from faulty press operation compared with counts observed after corrective measures had been applied.

In spite of very close supervision at the press station, some thermophillic spores pass through to the later stages of the process where they rapidly develop into serious sources of contamination. Samples taken from the foam which accumulates on the sides of the standard liquor tanks and accumulations on the braces in the white mixer ran as high as 1,100 in 10 grams. Samples of the high-raw sugar which is introduced into the standard liquor just before filtration, ran into the hundred thousands per 10 grams. To alleviate this situation the following program was adopted:

1. Both white and high-raw mixers were fitted with tight monel-clad covers and steam was introduced to keep the fillmass from collecting on the sides, braces, etc.

Table 4.

Time hours	Total thermophiles	Flat sours	Anaerobes
	Poor Filtration		
2	50	20	**0**
3	330	10	**0**
4	**100**	10	0
5	820	10	0
6	80	0	**0**
Resultant sugar	125	0	**0**
	Proper Filtration		
1	10	**0**	**0**
2	5	0	**0**
4	5	**0**	0
8	**0**	**0**	**0**
24	0	**0**	0
White massecuite	5	0	0
Resultant sugar	0	0	0

2. Standard liquor tanks on the pan floor were washed and sprayed with a solution of calcium hypochlorite equivalent to 1-percent available chlorine.

3. Scrupulous cleanliness was observed around the press station. Before dressing a press, the frames, plates, troughs, and floor were carefully washed and sprayed with 1-percent chlorine. In every batch of press cloths washed, 2 quarts of this solution were used in the final rinsing.

4. Similar sanitary measures were taken at the centrifugals. Buckets containing the paddles were steamed out after each strike and the water for washing the paddles treated with 1 quart of the chlorine solution.

An examination of the average counts of thermophillic bacteria in sugar produced the last 4 seasons at Toppenish, strikingly illustrates the value of bacteriological research and control. From figure 1 it is observed that in the sugar produced in the campaign of 1937, the average count on total thermophiles was 125 per 10 grams. This figure increased to 139 in the following year. In 1939, the season in which bacteriological control was established, the average dropped to 10 and was further reduced last campaign to an average of 3 thermophiles per 10 grams.

Mesophillic Bacteria

Solution of the problem of producing sugar which is uniformly very low in thermophiles directed our attention to the mesophillic bacteria found in sugar. Of prime importance to the manufacturer of beverages are these mesophiles, for in high concentration they may destroy both the flavor and appearance of his product. Both carbonated and uncarbonated drinks may be affected by one or more species of these organisms found in sugar.

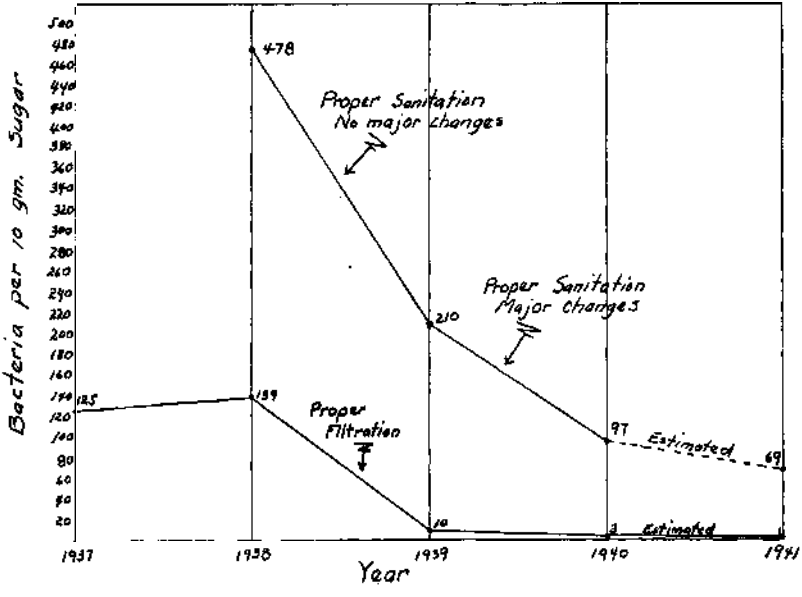

Figure 1.—Average bacteria count by years.

To diminish the possibility of contamination from this source, tentative beverage standards have been set up, (table 5).

Table 5.—Tentative beverage standard.

Bacteria at 37° C. In 5 samples an average of not more than 200 per 10 grams of sugar.
Molds In 5 samples an average of not more than 13 per 10 grams of sugar.
Yeasts In 5 samples an average of not more than 13 per 10 grams of sugar.

From this table may be deduced the classifications into which we divide the mesophiles.

The first class, bacteria at 37° C., may produce sediment and "rope" in beverages, especially in those which are not carbonated. The total number of bacteria of this type in a given product is determined as in total thermophiles by the plating method. The media in this case is a nutrient agar composed of: 15 grams agar, 3 grams beef extract, 5 grams tryptone, 3 grams dextrose, 1 liter distilled water. Adjust pH to exactly 6.8.

To make the determination, a 250-cc. Erlenmeyer flask containing 45 cc. of distilled water is stoppered with cotton and sterilized in the autoclave. After cooling, 24 grams of sugar are added bringing the volume to 60 cc. Each cc. of this solution contains 0.4 gram of sugar. With a sterile pipette, 2 petri plates are seeded with 2.5 cc. each and nutrient agar added to a depth of about ⅛ inch. After mixing and setting, the plates are incubated at 37° C. for 48 hours. At the end of this time the developed colonies on both plates are counted and the total multiplied by 5. This result is the total mesophillic bacteria in 10 grams of sugar. The 48-hour incubation period is not long enough for yeast and mold colonies which would ordinarily develop on these plates to become large enough to be seen. Development of thermophillic bacteria is likewise inhibited by the nutrient agar which is not rich enough to promote their growth and by the comparatively low incubation temperature.

Figure 2, pictures 1 and 2, are photographs of plates showing mesophillic bacteria in sugar from different sources. Each white spot is a bacterial colony. Number 1, a sample of Bottlers' Tested Sugar, has a total count of 70. The estimated count on sample number 2 is 16,000 per 10 grams.

Yeasts and molds are of special importance to the soft-drink bottler because they may tolerate acidities as high as pH 2. These organisms produce stringy sediments in beverages in which the acidity would inhibit most bacteria. In plating these organisms, this tolerance toward low pH values is utilized to prevent other bacterial growth.

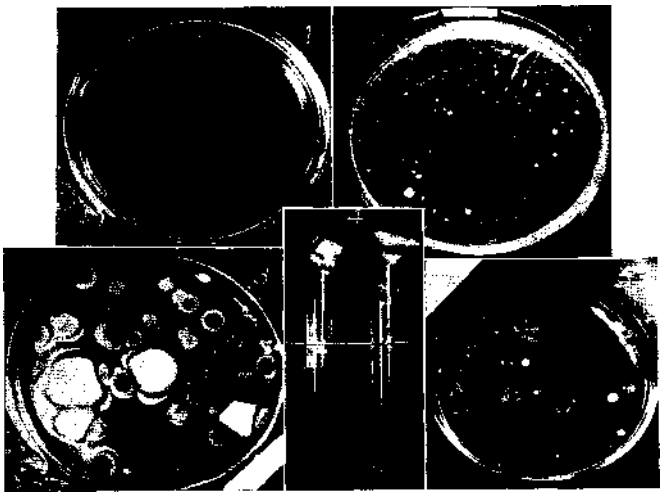

Figure 2.—Pictures 1 and 2 show mesopbillis bacteria in sugar; picture 3, molds, picture 4, shows both a positive and a negative test; and picture 5 shows the different types of colonies which may develop from a single sample of sugar.

The determination is made in the same manner and on the same solution used for plating the mesophiles. Each of 2 petvi plates is seeded with 2.5 ec. of this sugar solution. Agar made up as follows is added: 1 pint beer, 15 grams agar, 8 grams NaCl, 10 grams dextrose, 5 grams peptone. The beer is autoclaved for 20 minutes to drive off the alcohol, the above components added and the volume brought to 1 liter.

Just prior to pouring the plates, this media is brought to pH 4.0 with sterile 10-percent lactic acid. The media thus acidified is added to the seeded plates, allowed to set, and incubated for 5 days at 32° 0. Both the yeast and mold colonies are multiplied by 5 to obtain their respective counts.

Figure 2, picture 53, illustrates molds in sugar and as this plate contains 1 gram of sugar, the count would be 400 per 10 grams for that particular sample. Such a sugar would of course be very undesirable for soft-drink bottling.

Figure 2, picture 5, was included to show the different types of colonies which may develop from a single sample of sugar. At the side may be seen a spreader type, a species which grows very rapidly

and may sometimes cover the entire surface of the media obscuring other colonies. In the center are observed very irregular and perfectly round colonies; large mucoid colonies, and at the bottom of the plate some approaching a pin point in size.

Throughout this paper we have laid special emphasis on exact control of pH values. The reason for this lies in a series of experiments completed during the beginning of the 1941 campaign. In all of our bacteriological work before that time, pH values were determined by the colorimetric method using brom-thymol blue indicator and standard color comparators. In September of 1941 an electric pH meter equipped with a shielded glass electrode and temperature compensator was obtained. This instrument has the advantage that the pH of the agar media may be checked after it has cooled and set. Culture media which tested pH 6.8 on the color comparator was found on the pH meter to be 7.05. A series of comparisons were made on 2 culture media of pH 6.8 and 7.05, respectively. The results are shown in table 6.

Table 6.—Comparisons on bacterial counts at pH 7.05 and 6.8.

Sample number	Bacteria at 37° C. pH 7.05	pH 6.8	Difference	Percentage of difference
1	100	145	+45	+31.0
2	155	160	+ 5	+ 3.1
3	80	75	— 5	— 6.7
4	115	115	0	0
5	80	90	+10	+12.5
6	140	150	+10	+ 7.1
7	95	110	+15	+13.6
S	100	115	+15	+13.0
Total	865	960	+95	+ 0-9

These results indicate that average counts at the optimum pH of 6.8 were about 10 percent higher than at pH 7.05. For this reason we believe that small variations in the pH of the media should be avoided if maximum accuracy is to be maintained.

While pH 4.0 is not necessarily the optimum pH for plating yeasts and molds, from the results we have obtained it was concluded that this pH is very near the ideal working point. Dower than this point the media hydrolizes and becomes liquid. At higher pH values some of the mesophiles develop in the 5-day incubation period.

We have found that elimination in the plant of mesophillic bacteria, yeasts and molds is strictly a problem of sanitation. Since only a few of the mesophiles and none of the yeasts and molds can survive in the sirups at their processing temperatures, it is not difficult to produce white fillmass practically free of these organisms. However, starting at the centrifugal station, contamination may take place at any point thereafter until the sugar is in the bag. It was found that

contamination could occur wherever the sugar came in contact with a free circulation of unfiltered air and that countless numbers would develop wherever moist conditions prevail. Table 7 shows typical counts before any control was established.

Table 7.

Product and source of sample	Bacteria at 37° C.	Yeasts	Molds
Swab from paddle at centrifugals	75	0	0
Water in bucket holding paddle	200	0	0
Wet sugar at centrifugals	65	0	0
Centrifugal wash water	0	0	0
Wet sugar to scroll	85	0	0
Wet sugar at eud of scroll	800	0	10
Sugar standing in scroll	2300	0	0
Sugar lying in boot of wet elevator	10,000	0	30
Swab from side of wet hopper	80	0	105
Poorly filtered air to granulator			
Swab from fan blades	GCO	40	60
Swab from walls	»60	40	200

It is self-evident that sanitary measures taken in the control of thermophillic bacteria play an important part in the control of mesophiles. In addition, however, a program was adopted in 1939 as follows:

1. Covers were placed over and 2 feet above the standard liquor tanks 011 the pan floor. This restricted opening allowed the steam to escape without condensing and prevented the entrance of dust-laden air.

2. Great care was exercised at the centrifugals to prevent water and sugar accumulation on the curb of the machine from dripping into the basket during purging of the strikes.

3. Cleaning the wet-sugar scroll every shift was made a matter of routine. Similarly the wet elevator was washed periodically.

4. All windows and doors that allowed outside air currents to come in contact with exposed sugar or filtered sirup were equipped with air filters or kept closed as much as possible. Special care was exercised in this respect during windstorms.

5. The practice of cleaning up the sugar scales and sewing machine with a blast of compressed air was found to be extremely bad practice and was discontinued.

6. The granulator air filters were overhauled and all places which might admit unfiltered air were sealed over.

A glance at figure 1 shows that under this program we succeeded in decreasing the average mesophillic bacteria count from 478 in 1938 to 210 in 1939. However this was not enough and it was found necessary to make some changes in existing equipment:

1. The wet-sugar scroll was covered in such a way as to exclude all drips from the floor above and at the same time carry away any condensate formed on the inside of this cover.

2. Both sugar elevators, which were so constructed that their high speed and tight housings set up very definite air currents, were enlarged and run at slower speeds.

3. The wet-sugar hopper was discarded and the sugar discharged directly from the wet elevator into the granulator.

4. The booster fan in back of the air filters was so regulated that a slight pressure was maintained at all times on the granulator. This, we believe to be of considerable importance as it prevents tinfiltered air from entering through openings in the granulator housings.

Referring again to figure 1, installation of these mechanical improvements and maintenance of strict bacteriological control over the centrifugals, wet-sugar-conveying machinery, and bagging room resulted in an average bacteria content of 97 for the 1940 campaign as compared to 210 the previous season.

We have not found yeast contamination to be a problem at any time during our work. In fact we have never observed more than 2 yeast colonies in any sample of sugar examined and those colonies only in very isolated cases, on the other hand, mold contamination is a constant menace. Generally speaking, all of the precautions observed in the production of sugar low in bacteria at 37° C. are applicable to the making of sugar low in mold spores. Dust and burlap fuzz which, to some extent is unavoidable in the sacking and sewing room, are large contributors to high mold counts. For example, a piece of burlap 3 inches square from a new bag analyzed as follows:

Thermophillic aerobes 9 Square Inches		Thermophillic anaerobes			
Total count	Flat sours	Percentage of tubes positive	Bacteria at 37° C.	Yeasts	Molds
2,880	100	100	2,500,000	0	1,000

The answer to this situation is obviously to bag more sugar in cotton or paper bags in a room isolated from the rest of the bagging equipment.

Table 8 contains a brief summary of the foregoing discussion.

Disinfecting Granulated Sugar

Because of the difficulty experienced with existing equipment in producing sugar free of molds, attempts were made in our laboratory to destroy with ozone these spores in the finished sugar. For this experiment a special 20-tube, blower-type ray ozone generator was obtained. The sugar was treated at 70 and 110 volts, in dry, moist, and wet conditions. Table 9 shows averages of the results which were obtained.

Table 8.--Summary.

Micro-organism	Oxygen requirements	Incubation temperature	Incubation time	Plating PH	Method of determination	Industrial spoilage	Origin within the plant	Control
Total thermophiles	Aerobic	55°C.	48 hr.	6.8	Plating on dextrose tryptone agar	Plat sour in non-acid packs	Soil-borne with the sugar boot	Filtration
Thermophillie Anaerobes Dot producing H_2S	Anaerobic	55°C.	72 hr.	6.8	Sealed beef-liver tubes	Hard swell in no n-acid packs	Soil-horne with the sugar beet	Filtration
Mesophillic bacteria	Aerobic	37°C.	48 hr.	6.8	Plating on nutrient agar	Sediment and "rope" in beverages	Air-borne on dust particles-conden-sation	Sanitation
Yeasts	Aerobic	320 C.	5 days	4.0	Plating on beer agar	Sediment in beverages	Air-borne on dust particles—conden-sation	Sanitation
Molds	Aerobic	320 C,	5 days	4.0	Plating on beer agar	Stringy sediment in beverages	Air-borne—burlap fuzz and dust	Sanitation; use of paper or cotton bags

Table 9.—Sugar treated with ozone.

Sample number	Time of treatment	Voltage	Condition	Molds per 10 gm. sugar	
				Treated	Untreated
1	30 sec.	70	dry	8O	65
2	1 min	70	dry	15	15
3	1 min	110	dry	0	5
4	2 min	110	dry	15	25
5	2 niin	110	moist	0	5
6	1 min	110	wet	75	75
7	2 min	110	wet	25	40
8	30 sec.	110	vibrating	45	40

It may be noted that in most cases there was a very slight decrease in mold count. However, this decrease was no greater than the probable experimental error. Also it may be observed that in no case, where the original contamination was above the maximum set *by* the standards, did the ozone treatment bring the sugar within the accepted specifications.

Ultra-violet radiation has been tried in several factories at various stages throughout the process with some success. This prompted an experiment in our laboratory in which we attempted to lower the mesophile and mold count on sugar which had been stored in the warehouse for about 6 months. For this purpose we obtained a standard sterilamp with an effective tube length of 20 inches, operating on 110 volts A. C. current which produced 80 percent of the radiant energy in the region of 2550 angstrom units. In sterilization of sugar by a lamp of this type, all surfaces of the crystal should be exposed to the direct ray. To accomplish this, several set-ups were devised, one of which will suffice for illustrative purposes.

A trough was constructed 3 inches wide, 3 inches deep, 3 feet long, open at the top and closed at both ends. This trough was mounted horizontally upon a vibrating machine and a weighed amount of sugar introduced. The tube of the sterilamp was fastened directly above the trough and 5 inches from the sugar. The vibrator was started and the trough was made to incline slightly causing the sugar to travel slowly back and forth. This was so arranged that the sugar traveled a distance of 6 feet each minute. Thus it formed a very thin, evenly distributed layer as the vibrating crystals traveled from one end of the trough to the other. In both tests shown in table 10 the exposure time was 20 minutes and the sugar made 20 trips back and forth under direct exposure of the full length of the tube. This is equivalent to a total effective exposure of 65 feet with the crystals in constant motion.

All of our experiments with ultra-violet light gave approximately the same results as were obtained in table 10. In no case did the radiant energy have any effect on the mold spores nor did the treated

Table 10.—Sugar treated with ultra-violet light.

Sample	Weight of sugar	Bacteria at 37° C	Molds
No. 1 untreated		150, spreaders	5
No. 1 treated	100 gm.	140, spreaders	15
No. 2 untreated		480	55
No. 2 treated	50 gm.	510	55

sugar become low enough in bacteria at 37° C. to be acceptable for beverage manufacture.

It is an established fact that many of the mesophillic bacteria contained in dry sugar die off during long storage periods. However, the extent of this decrease does not seem to be an arithmetical function of the total count before storage. Generally speaking, those bacteria which are able to survive the long dry storage periods are undoubtedly spore formers and the fewer these spore-forming types originally present the greater will be the decrease in the stored sugar. We should not expect a decrease in thermophillic contamination since in this case only the spores are counted. Moreover, we have not observed any noticeable decrease in mold count after storage.

The fact that all spores are much more resistant to destruction than bacteria in the vegetative state, perhaps explains the reason that considerable numbers of bacteria are killed in newly produced sugar by means of radiant energy, while under controlled experimental conditions we were able to show very little destruction of those spores remaining after storage.

Conclusion

In the modern sugar refinery, thermophillic bacteria enter the process with the sugar beet. Mesophillie bacteria, yeasts and molds originate at the white mixer, centrifugals, sugar-conveying equipment, and bagging room. From a consideration of the origin of these two groups of micro-organisms, it is evident that there is no correlation between their respective counts in the final granulated sugar.

It is our opinion that treatment of granulated sugar for the purpose of lowering the bacterial count by ozone or radiant energy is not commercially practicable.

We believe the answer to this problem is to produce bacteria-free sugar by:

1. Elimination of thermophiles by efficient filtration.

2. Elimination of the mesophiles, yeasts, and molds by strict sanitation and bacteriological control at the sources of contamination between the white pan and the bagged sugar.