

THE CONTROL OF BACTERIAL CONTAMINATION IN MAPLE SAP STORED IN FIELD STORAGE TANKS BY ULTRAVIOLET IRRADIATION

J. C. KISSINGER AND C. O. WILLITS

*Eastern Utilization Research and Development Division, ARS
United States Department of Agriculture
Philadelphia, Pennsylvania*

(Received for publication April 23, 1966)

SUMMARY

During the maple sap seasons of 1964 and 1965, sap collected from roadside stands of trees was stored in field holding tanks for as long as eleven days without deterioration, by continually irradiating the stored sap with germicidal ultraviolet lights emitting in the range of 260-300m μ . The bacterial populations of the stored, irradiated sap did not exceed 4.0×10^8 organisms per ml, and sirup made from the sap was light amber in color (fancy grade) with an excellent flavor.

The storage of maple sap is one of the more important problems of the maple sirup industry. Unlike other crops, there is no warning or indication as to the amounts of sap that will be produced per day in a sugar grove. Often, the amounts produced far exceed the capacity of the evaporators, causing the sap to be held in storage for periods up to five days during which time the sap, because of its perishable nature, deteriorates with considerable financial loss to the producer. Due to this, the sap producer must have a method whereby the sap can be held for several days without deterioration.

In recent years, sap yields have been increased through (a) improved sugar bush sanitation procedures (8), (b) the use of germicidal taphole pellets (2), and (c) the use of plastic tubing for sap collection (9).

Raw maple sap is a highly perishable commodity. Although it is sterile as it flows from the tubules of the tree, subsequent contamination of the sap with bacteria, yeasts and molds invariably occurs at the woods site or at the evaporator plant during its collection and transportation. The degradation of sap due to microbial growth results in the production of darker, lower grade sirup (4), the production of off-flavors (8) and poor texture (ropy) sirup (8). All of these effects result in a lower sirup price and decrease farm income.

Maple sap producers try to hold spoilage to a minimum by processing the sap within 24 hours after collection, but often this is not possible. When sap is held beyond 24 hours, microbial growth, even at low temperatures, is sufficient to produce fermentation products that result in low quality sirup.

The use of germicides are unsatisfactory for controlling microbial growth in sap since (a) they result in the addition of chemicals to a food product, and (b) the chemical additive is concentrated 30-40 fold in the concentration of sap to sirup.

Ultraviolet irradiation, on the other hand, is ideally suited for controlling the growth of microorganisms in maple sap. Since it is a physical method there are no problems of chemically induced off-flavors or harmful chemical residues. Ultraviolet irradiation has no effect on the flavor precursors in sap nor on the delicate maple flavor in the resulting sirup.

Sap, a water solution containing only 1-4% dissolved solids, is comparable to water in clarity and in the transmission of ultraviolet light. Thus, it is an excellent medium or irradiation with the actinic rays of ultraviolet light derived either from sunlight or ultraviolet lamps that produce wavelengths of 260-300 m μ which are known to be lethal to microorganisms. Frank and Willits (3) and Schneider, Frank and Willits (7) showed the effectiveness of these actinic rays for the control of microbial growth in sap under laboratory conditions. In 1965, Kissinger and Willits (5) reported on the industrial application of irradiation of a flowing stream for the control of microorganisms in flowing maple sap, adapting commercially available ultraviolet irradiation units used to control microbial growth in cane sugar solutions and domestic water supplies (6).

This paper presents the results obtained on the control of microorganisms in sap stored under field production conditions by continuous irradiation of the static sap with actinic rays of ultraviolet lamps.

METHODS

Apparatus.

Tanks. Two round, galvanized iron, free-standing storage tanks 4 ft high by 4 ft in diameter with tight-fitting covers.

Tank covers. The metal covers were equipped with 18 in. diameter man-heads for inspection and sampling. The man-heads were covered with tight-fitting, metal lids.

Ultraviolet light source. A 30 watt, 36-inch tubular ultraviolet lamp, General Electric model G30T8¹ (1, 8), with re-

TABLE 1. THE EFFECTS OF ULTRAVIOLET IRRADIATION ON BACTERIAL POPULATIONS IN MAPLE SAP HELD IN FIELD STORAGE TANKS—1964

Sap flow period	Date	Days in storage	Tank 1				Tank 2			
			Sap temp. (° F)	Sap vol. (gal)	Sap depth (inches)	Bacterial count/ml	Sap temp. (° F)	Sap vol. (gal)	Sap depth (inches)	Bacterial count/ml
1	2/4	1	33	4	½	0	33	4	½	50
		3	41	9	1½	45	41	64	8	45
		4	47	9	1½	2	47	64	8	2
2	2/14	1	36	3	¾	10	36	24	3	10
		4	33	20	2½	10	33	100	12½	13
		6	33	40	5	0	33	132	16½	12
3	3/2	1	44	48	6	1	44	144	18	150
		2	44	48	6	1	44	144	18	TNTC ^a
4	3/9	1	50	4	¾	3	50	30	3¾	20
		5	50	28	3½	0	48	105	13¾	TNTC ^a
		9	65	40	5	3				
		10	60	46	5¾	33				
5	3/20	1					40	12	1½	30
		2					45	38	4¾	3.8 x 10 ³
		3					65	74	9¾	3.5 x 10 ⁴
		4					60	76	9¾	TNTC ^a
6	4/1	1	40	16	2	1	40	31	3¾	1.2 x 10 ³
		3	42	43	5¾	2	42	89	11¾	1.9 x 10 ³
		5	65	56	7	4.0 x 10 ⁵	65	128	16	4.0 x 10 ⁴

^aTNTC = Colonies Too Numerous To Count at a dilution of 10⁵.

flectors was mounted on the underside of each of the tank covers 4 ft above the bottom of the tank so that the entire inner surface of the tank's walls and bottom were irradiated.

Sap. This was supplied to the tanks from 50 taps in roadside grown sugar maples. Tank 1 received sap from young trees, 10 inches D. B. H. (diameter at breast height) which had not been previously tapped. Tank 2 received sap from mature trees, 18-25 inches D. B. H., which had been tapped each year for the preceding ten years. Each taphole contained a paraformaldehyde pellet (2).

Plastic tubing. The tubing, 7/16 inch I.D. (9), was used to conduct the sap from the tapholes to the holding tanks.

Sampling

Samples for bacterial counts were taken aseptically from the holding tanks each day during the storage periods with the exception of days when ice in the tanks prevented sampling. At the time of sampling, the volume, depth and temperature of the sap were recorded.

Bacterial counts

Tryptone glucose extract agar (Difco¹) was used as the culture medium for all bacterial counts.

All incubations for bacterial counts were carried out for 48 hrs at 30 C.

A Quebec¹ colony counter was used for making bacterial counts.

RESULTS AND DISCUSSION

In 1964, the first year these experiments were conducted, weather conditions conducive to the flow of maple sap occurred intermittently from February 4 to April 6. The effects of continuous ultraviolet irradiation on bacterial populations in sap held in the field holding tanks during this period are shown in Table 1. During these storage periods, the temperature sometimes was below freezing. This caused interruption of sap flows and also caused the stored sap to freeze which prevented sampling on those days. Hence, the data in Tables 1 and 2 refer only to those days on which the sap was liquid or partially frozen. The first and second sap flows of 1964 were stored for four and six days, respectively. Sap temperatures ranging from 33 F to 47 F for the first run and 33 F to 36 F for the second run reflected average seasonal norms. Bacterial counts were very low (0-50 organisms per ml) in the sap stored in both tanks, and the sirup made from the sap was delicately flavored, light amber in color and graded table sirup Fancy grade.

On March 1, 1964 a large volume of sap was produced (sap flow number 3).. Within 48 hrs, the sap held in tank 2 developed a heavy turbidity with microbial counts very high and a musty odor. Sirup made from this sap had a dark color and a strong,

¹Mention of company or trade names does not indicate endorsement of the Department over other commercial items not named.

musty off-flavor. The sap was discarded, and the tank was sanitized with hypochlorite solution (0.5%). The sap held in tank 1 during this same period, however, remained very low in bacterial population; and a light amber, delicately-flavored sirup was made from it.

During the fourth sap flow period, the sap in tank 1 was held for 10 days without deterioration. During this time, sap temperatures ranged from 36 F to 65 F with ambient temperatures reaching as high as 72 F, and the bacterial counts throughout this period did not exceed 33 organisms per ml. The sirup made from sap which had been stored for the 10 days had an excellent maple flavor and was of light amber color (Fancy grade).

The sap in tank 2 collected during this same period had bacterial counts that remained below 30 per ml. for the first three days. Then, they rapidly increased to high populations by the fifth day. The tank was taken out of service for the remainder of period 4 to permit washing and sanitizing.

During the fifth flow period, tank 1 was taken out of service for cleaning and sanitizing since it had become highly contaminated due to a break in the sap supply line. In this same flow period (March 20-24), the bacterial count in sap stored in tank 2 remained low (below 400 organisms per ml) for the first two days and then increased rapidly through the fifth day. These high bacterial counts in the sap in tank 2 were unexplainable until it was discovered that the reflector of the ultraviolet fixture, which was not readily observed from the "man-head", had shifted so that most of the surface of the sap was shielded

from the germicidal rays of the lamp. The reflector was readjusted, and both tank and plastic tubing lines were sanitized. During the sixth flow period, bacterial counts in the sap held in both tanks were again low (below 4×10^3 organisms per ml) but they showed a slight though steady increase during the five days of storage. This increased count reflected the effect of the high sap temperature which rose from 40 F on the first day to 65 F on the fifth day. Thus, even with sap temperatures favorable to bacterial growth, the final bacterial counts in both lots of stored sap were kept sufficiently low so that no deterioration of the sap occurred as determined by the grade of the sirup made from it. These counts were comparable to the counts reported in fresh sap delivered to a central evaporator plant (4). The depth of the sap in the tanks varied from 1/2 inch to 13 1/8 inches. These different depths appear to have no appreciable correlation with the effectiveness of the germicidal lamps on the control of bacteria in the stored, irradiated sap. All of the sirup made from the sap stored during the different periods (except for that of run 4 and 5 in tank 2) was light amber and had a good maple flavor.

During the 1965 sap season which occurred from February 2, 1965 to March 28, 1965, sap storage studies were carried out in exact duplication of those conducted for the 1964 season using the same two tanks (equipped with the germicidal lamps) with sap supplied from the same trees. This season's data are presented in Table 2. During the first sap flow period, sap was held in both tanks for nine days. Daily temperatures were normal for the sea-

TABLE 2. THE EFFECTS OF ULTRAVIOLET IRRADIATION ON BACTERIAL POPULATIONS IN MAPLE SAP HELD IN FIELD STORAGE TANKS—1965

Sap flow period	Date	Days in storage	Tank 1				Tank 2			
			Sap temp. ($^{\circ}$ F)	Sap vol. (gal)	Sap depth (inches)	Bacterial count/ml	Sap temp. ($^{\circ}$ F)	Sap vol. (gal)	Sap depth (inches)	Bacterial count/ml
1	2/12	1	47	1	3/8	6	47	32	4	1
		4	33	44	5 1/2	0	33	120	15	0
		6	44	60	7 1/2	0	44	154 ^a	19 1/2	0
		9	43	112	14	1.3×10^3	45	226	28 1/2	0
2	3/12	1	44	3	3/8	1	44	16	2	2.9×10^3
		2	48	36	4 1/2	0	48	90	11 1/2	8×10^2
		3	44	40	5	0	44	100	12 1/2	1.6×10^3
3	3/18	1	34	12	1 1/2	0				
		4	43	32	4	25				
		8	33	70	8 1/2	5				
		9	37	84	10 1/2	3.5×10^3				
		11	44	92	11 1/2	3.6×10^3				

^a24 gallons of sap removed from tank for experimental use.

son with sap temperatures ranging from 33 to 47 F throughout the period, and as predicted from the 1964 data, the bacterial counts remained at a very low level ranging from 0-130 organisms per ml. Sirup made from the sap which has been stored for 9 days, had an excellent maple flavor and was light amber in color (Fancy grade). The storage of sap collected in tank 2 during the second sap flow period was terminated after three days, because of vandal damage to the tank. The sap stored in both tanks for these three days was again low in bacterial count ranging from 0-1000 organisms per ml with sap temperatures remaining about 45 F. The sirup made from this sap was fancy grade.

At the end of the second flow period, tank 1 was sanitized. Sap collected during the third flow period was held for eleven days. The temperature of the sap ranged from 33 to 45 F with ambient temperatures as high as 71 F. The bacterial counts remained below 25 per ml through the seventh day and then increased to 3.6×10^3 per ml by the eleventh day when storage was terminated. The sirup made from this sap was again of Fancy grade.

These results indicate that sap can be stored for periods of at least eleven days without deterioration, and the resulting sirup will be of Fancy grade, when the sap is irradiated continuously with germicidal lamps emitting actinic rays in the range of 260-300 m μ . The bacterial counts in sap can be kept below 4×10^3 organisms per ml even when the ambient temperature and the temperature of the sap are relatively high. Bacterial populations below 4×10^4 cells per ml in sap have no deleterious effect on the sirup made from the sap. However, sap having high bacterial counts (tank 2, sap flow 3, 1964 where the counts reached a high level after two day's storage) produced dark-colored, low grade sirup.

During the second year, sap held in storage in tank 2 reached a depth of 28 1/4 inches (flow period 1), but the bacterial count in the stored sap did not exceed 20 organisms per ml during the 9-day storage period. These results indicate that the effectiveness of the ultraviolet lamps on the control of microbial growth was independent of the sap depth. Likewise, the distance between the lamps and the surface of the sap varied with the changes in depth of sap, but there was no apparent effect with the change in these distances.

CONCLUSIONS

Maple sap can be held in storage for as long as eleven days in field storage tanks without deterioration, if the sap is constantly irradiated with ultraviolet light of 260-300 m μ . The entire surface of the sap must be continually illuminated by the ultraviolet light.

The storage tank and its feeder lines should be initially sanitized with hypochlorite solution or other equally effective and safe sanitizing solution.

Increases in stored sap temperatures cause small increases in bacterial populations, which are not great enough to affect the quality of the sirup produced.

High quality sirup can be made from sap stored as long as eleven days under ultraviolet irradiation. This was independent of the time when the sap flowed during the sap season.

The depth of the sap (up to 28 inches) and the distance that the germicidal lamps are mounted above the sap surface (up to 48 inches) are independent of the control of bacterial growth in sap by the actinic rays of the lamps.

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