The Growth of Yeasts in Grape Juice Stored at Low Temperatures[1]

I. Control of Yeast Growth in Commercial Operation

C. S. Pederson, M. N. Albury, Dorothy C. Wilson, and N. L. Lawrence

New York State Agricultural Experiment Station, Cornell University, Geneva, New York

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The grape juice industry has shifted almost entirely from the old method of storing juice in 5-gallon carboys to cool storage in tanks at 22 to 28 F (−5.5 to −2.2 C). Excess tartrates are precipitated during storage. When juice was stored in carboys, the carboys were filled at processing temperature, and either spray-cooled or allowed to cool slowly at the cellar storage temperature. For the modern cool storage, the juice is flash heated at 175 to 185 F (70 to 85 C) and flash cooled to about 32 F (0 C) before pumping it into storage tanks. After the tartrates and other argols are precipitated, the juice is repasteurized and bottled for retail sale. By this method of storage, heating effects are minimized. A general improvement in quality has been effected by this change in method of storage. However, problems of yeast and mold contamination and growth have been serious at times. Since quality is retained better when stored at the cool temperature, processors would like to hold juice in tanks until bottled juice is needed. They would also like to distribute bottling throughout the year and, at times, hold juice into the following season to equalize differences in supply resulting from variations in production of grapes. They would prefer to use a cool storage temperature high enough to prevent the juice from freezing solidly.

The mere presence of yeast is unimportant in itself. However, if yeasts grow and multiply sufficiently to alter the product, they will cause an economic loss to the producer. Although it was originally assumed that yeasts would not grow at low storage temperatures, it is now well known by all processors that they will grow. In fact, it has been demonstrated that some of the varieties of yeast will grow better at a low temperature of 1 C than at room temperature, 21 C (Lawrence *et al.*, 1959).

The temperature used for pasteurization is sufficient to kill all yeasts. It is possible to pack juice without having viable yeast present (Pederson *et al.*, 1959).

This study was instituted in an effort to determine the degree and the sources of contamination in the handling and storage of grape juice in order that methods may be employed to eliminate yeast contamination and growth from future operations.

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Results and Discussion

Yeast counts from juice in tanks. During the past 4 years, 833 grape juice samples have been obtained from tanks in 19 storage rooms maintained by 11 processing companies cooperating in this study. Each set of samples from the several tanks of a storage room was assigned a series number. When tanks in the storage rooms were resampled later, samples were obtained from the same tanks when possible. These samples then received a new series number. Because tanks of juice yielding the highest counts were usually processed first, many of the tanks were unavailable for sampling a second or third time. Comparison of series from a single storage room as the season progressed gave an approximate indication of change in numbers and types of yeast. The results can be compared only on a general basis from year to year because samples cannot be obtained at identical periods each year and were not necessarily taken from the same tanks.

Samples were withdrawn with sterile pipettes. Usually about 10 to 15 ml were removed but sometimes 4-ounce samples and in a few cases, 1-gallon samples were obtained. They were packed in ice, brought to the laboratory, and plated in triplicate as soon as possible (Pederson *et al.*, 1959). Plates were ordinarily incubated at 32, 18, and 1 C for 3 days, 3 to 5 days, and 3 to 4 weeks, respectively. After counting colonies, a general attempt was made to classify the yeast types according to colonial morphology. Initially, replicate plates were incubated at 37, 32, 25, 18, and 1 C. However, the 25 and 37 C incubations were later omitted since they yielded no additional information.

The 24 samples from the 1953 pack, series 1, 2, 3, and 4, were obtained in November, April, and May from three storages (table 1). These results demonstrated the seriousness of the problem and the losses that may result. Obviously, if a high alcohol content resulted, such juices could not be used for bottled grape juice or concentrate. Of course, some types of yeast do not produce alcohol but will affect quality.

From the 1954 and 1955 packs, 286 and 182 samples,

TABLE 1

Range of yeast plate counts from some of the series of tank samples of various storage rooms

Series No.	Storage Room	Approximate Storage Time in Months	No. Of Tanks Sampled	Range of Yeast Colony Counts Among Samples when Plates Incubated at:						No. of Tanks Showing Counts in Excess of 500,000
				32 C		18 C		1 C		
				Low	High	Low	High	Low	High	
		1953 Pack								
1	I	<1	4	100,000–	TMC*	100,000	TMC		TMC	2
3	J	6	10	0	340,000	1,000	TMC	1,500	TMC	4
4	A	7	5	70	1,000	400	140,000	2,700	140,000	0
		1954 Pack								
11	E	5	18	0	19,000	8,000	TMC	8,300	TMC	8
6	H	<2	8	20	570	500	47,000	880	32,000	0
18	H	9	5	0	0	1,700	110,000	3,200	24,000	0
		1955 Pack								
20	B	<1	10	0	0	0	5,700	0	4,800	0
32	B	2	17	0	30	10	260,000†	0	700,000	1
21	G	<1	16	0	480	0	3,900†	0	12,000†	2
27	G	2	17	0	450	510	260,000	220	190,000	0
22	A	<1	6	10	240	0	180	0	0	0
31	A	2	12	0	170	3,900	TMC	4,800	TMC	3
28	C	2	27	0	2,800	640	TMC	270	TMC	18
		1956 Pack								
35	A	<1	12	0	250	0	700	0	600	0
48	A	2	11	0	31†	230	TMC	1,400	TMC	3
57	A	4	11	0	190	1,800	390,000	5,000	TMC	4
33	B	<1	13	0	38	0	24	0	38	0
42	B	2	14	0	1,000	0	1,400	0	75,000	0
53	B	4	7	0	18	6	130†	1,000	5,000†	0
41	I	<1	11	1	58	0	1,800	0	1,600	0
50	I	3	7	2	100	2,000	TMC	1,300	TMC	2
38	J	<1	14	0	57	0	46	0	19	0
51	J	3	15	3	1,600	240	130,000†	100	50,000†	1
36	C	<1	7	0	2	2	210	0	0†	0
44	C	2	14	0	2,500	0	20,000	0	TMC	6
55	C	4	6	0	38	0	220	200	40,000†	0
34	G	<1	11	0	480	0	330†	0	120	0
43	G	2	14	12	540†	10	2,400†	0	7,200	0
54	G	4	7	10	3,200	4	4,000	10,000	35,000	0
		1957 Pack								
61	A	<1	9	2	50	0	66	0	185	0
70	A	>1	9	2	80	15	7,600	25	16,000	0
74	A	5	8	0	6	220	32,000	2,400	70,000	0
64	I	<1	22	0	4	0	870†	0	5,000	0
73	I	3	10	0	42	4,400	TMC	1,200	TMC	4
59	B	<1	7	0	4†	0	4†	0	1†	0
65	B	>1	6	0	12	1	2,700	1	1,200	0
76	B	5	8	0	32	200	TMC			
72	K	2	18	1	7,300†	2,800	TMC	2,100	TMC	9
60	C	<1	14	0	2	0	1	0	0	0
71	C	>1	20	0	8,500	0	26,000	0	500,000	2
75	C	5	13	0	TMC	7,700	TMC	150,000	TMC	8
63	G	<1	9	3	28	2	120	0	540	0
67	G	>1	3	2	6	1	7	2	25	0
78	G	5	4	0	11	12,000	230,000	9,000	TMC	1

* TMC indicates a count in excess of 500,000 per ml.

† One or two samples yielded counts considerably greater or, in a few cases, less than the range indicated.

respectively, in 28 series representing most of the storages, were plated at different times during these years. The results re-emphasized the gravity of the situation. In 1954, the juices from 48 tanks and, in 1955, from 39 tanks yielded counts of 500,000 or more. In many cases an economic loss was suffered. Many of the juices yielded these high counts shortly after the tanks were filled. The juice of many of the tanks had to be repasteurized, cooled, and replaced in the vats for storage. Counts from 2 tanks of the more serious conditions, series 11 in 1954 and series 28 in 1955, are shown in table 1. Many other tanks in other storages presented problems. The rapid increase in counts in the 1955 pack is illustrated in table 1, in the sets of series 20 and 32; 21 and 27; and 22 and 31, taken from the same storage a little over 1 month apart. In other series of samples, however, juices from some tanks never yielded positive colony counts, showing that juice can be processed and stored with complete freedom from yeast growth.

The 1954 juices yielded somewhat higher counts than the 1955 juices, but they had been sampled later during the storage period. Plate counts in the light of those reported elsewhere (Pederson *et al.*, 1959) strongly indicated certain sources of contamination. During progress of the study, the several plant operators observed certain known or obvious sources of contamination. Attempts were made to correct some of these conditions within individual plants. Colony counts from samples of the 1956 and 1957 packs from individual factories compared with those of the three previous seasons indicated that an improvement had been effected in some factories where attention was given to elimination of possible sources of contamination.

The interpretation of results is essential to an understanding of the problem. Reports by Lawrence *et al.* (1959) and Pederson *et al.* (1959) have shown that four genera of yeasts are commonly found as contaminants in grape juice. Differences in types of yeast were noted early in the study and it was felt that a study of the yeasts' characteristics was essential. The processor judges the condition of a tank of juice in storage by its content of alcohol. The fermentative yeasts, that is, the yeasts of the genera *Saccharomyces*, *Hanseniaspora*, and *Torulopsis*, produce alcohol. The yeasts of the first genus are the most active and are most likely to be the ones responsible for the fermentation in so called "wild tanks." Since the yeasts of the genus *Candida* are not alcohol producers, their presence would not be indicated by alcohol content. However, like the other yeasts, they affect flavor and also remain in juice when it is siphoned from a tank. In pasteurization the yeasts are killed and settle to form a fine precipitate in the bottom of a bottle of juice. Ordinary filtration removes a high percentage but not necessarily all yeast.

It became apparent that a distinct change in predominant flora occurred in many instances during storage of juice. This change confused the interpretation of results. During the storage in 1954 and 1955, it was observed that counts of yeast plates incubated at 32 C ordinarily did not increase as rapidly as counts of yeast plates incubated at 18 and 1 C. In certain instances, very rapid increases were noted at 18 and even more so at 1 C, whereas at 32 C the count often remained constant or decreased. In some instances, however, marked increases were noted at 32 C incubation. The identification of many of the isolates indicated the yeasts of the genus *Candida*, the nonfermenters, often multiplied more readily during prolonged storage than did yeasts of the other genera. Therefore with increased storage time, frequently a proportionately higher percentage of the colonies were the dry, rough, wrinkled types associated with the genus *Candida*. This apparently occurred more frequently with the 1956 and 1957 packs than with the 1954 and 1955 packs. A further analysis of results for 1956 and 1957 presented in table 1 may partially clarify this statement. It should be pointed out that plate counts at 32 C seldom exceeded 10,000, which indicated failure of some of the fermentative types of yeast to grow.

The set of series 35, 48, and 57 (table 1) represents counts obtained from 11 of the same tanks in storage room A. Series 35 samples were obtained soon after filling, series 48 after about 1½ months, and series 57 after 4 months. The platings of the 12 tanks of series 35 yielded nearly all fermentative types of yeast colonies, although the maximum count was only 700 per ml. During storage, a shift in flora occurred in that the nonfermentative type developed and, as shown in series 57 of table 2, were by far the predominant type, particularly among the high count juices. The detailed colony counts from the 11 tanks in series 57, table 2,

TABLE 2

Individual plate counts of 11 samples after approximately 4 months' storage

(Series 57, storage room A, 1956 pack)

Tank	Incubation Temp			Approx *Candida* Colonies
	32 C	18 C	1 C	
				%
A	190	200,000	160,000	95
B	0	7,200	47,000	10
C	19	380,000	1,300,000	95
D	0	3,000	12,000	80
E	2	320,000	2,000,000	95
F	0	150,000	150,000	95
G	0	3,400	40,000	20
H	0	1,800	5,000	60
I	0	150,000	3,000,000	70
J	0	100,000	500,000	70
K	0	7,800	13,000	70

show striking differences. Such counts are representative of the wide variation in counts obtained from different juices in many other series. During the storage period of 1½ to 4 months, a marked increase in numbers of yeast occurred in 3 tanks, C, E, and F, but a marked decrease occurred in tank K. Other tanks yielded approximately the same counts at 1 and 18 C. With the exception of 1 tank, the average counts at 32 C dropped from 8 to 3.

Similar detailed results could be presented for consecutive sets of series sampled from tanks of single storage rooms in other companies to illustrate the shift in flora from the fermentative to the nonfermentative types of yeast during storage. Series 33, 42, and 53 yielded similar results even though many of the tanks of juice were repasteurized between the second and fourth month storage period. Results were similar with series 41 and 50, and 38 and 51, but the percentage of *Candida* colonies were somewhat lower in the final platings. Among 1957 juices, in the set of series 61, 70, and 74, series 74 yielded almost 100 per cent *Candida* colonies at 18 C but a high percentage of mucoid colonies at 1 C. In the set of series 59, 65, and 76, and 64 and 73, a high percentage of *Candida* were obtained from the platings in the 73 and 76 series. In general, in each series, the juices that yielded the lower counts yielded the lower percentage of *Candida* colonies.

In contrast, many tanks of juice, even after prolonged storage, yielded very high percentages of fermentative types of yeast. The fermentative types seemed to have persisted longer among 1954 and 1955 juices than in 1956 and 1957 juices. For example, the 27 tanks in series 28 of the 1955 pack yielded very high counts of the mucoid type of yeast associated with fermentation. In series 72, 2 of the 18 tanks yielded practically 100 per cent fermentative types of colonies, the other tanks yielded *Candida* types. The 13 tanks in series 75 in 1957 yielded the tiny mucoid colonies. Presumably, strains of the genus *Hanseniaspora* were predominant even though the juices from the same storage room, series 55, yielded a high count of *Candida* colonies. The juices from all 4 tanks in series 78 yielded fermentative types, whereas juices the previous year had yielded nonfermentative types.

Uniform samples are difficult to obtain from a tank. Because it is desirable to disturb a tank as little as possible, usually a single sample was taken. However, 11 samples were obtained from 1 tank. Counts at 1 C ranged from 860 to 26,000; at 18 C, from 940 to 23,000; and at 32 C, 0 to 90. The highest counts were obtained from the samples at the greatest depth. In contrast, some counts obtained by the laboratory of one of the companies showed the highest count from samples near the top. In a series of 5 tanks, sampled from surface level and at 2 to 3 foot depths, 2 tanks yielded counts at the 2 to 3 foot levels of 46,000 and 24,000, but at the surface level only 5000 and 1500, when plates were incubated at 1 C.

Possible Sources of Contamination

There is ample evidence that juice is adequately pasteurized and can be delivered from enclosed coolers in a sterile condition (Pederson *et al.*, 1959).

The sources of contamination may be many. Contaminated equipment or air will introduce yeasts to the juice. Any place where juice is trapped to remain for an indefinite period and any place where foam forms should be considered possible sources of contamination.

It is difficult for a plant operator to visualize the extremely small size of yeast cells and the ways in which they are harbored in the air or equipment. It should be realized that 100 to 150 yeasts in a row may pass through a hole the size of a small pin; that 10,000 to 15,000 yeast cells may be required to fill this hole of 1 cell thickness; or that possibly 1,000,000 cells could exist in the hole if it were 1 pin size in depth. Liquids present in small openings tend to remain there due to capillary action. Yeast will grow in such juice. The potential contaminating influence and growth possibilities of these cells in contact with fresh grape juice are tremendous. Furthermore, yeasts may sporulate in restricted areas and be carried over from season to season.

Among important possible sources of contamination are the pores of wood, cement, or other materials used in construction of tanks. Others, including the air in the room, foam on the surface of the juice in troughs of surface coolers, bypass valves, control valves, thermometer wells, gauges, gaskets, sampling apparatus, pipeline dead ends, T and Y connections, and intermediate holding tanks, have been apparently implicated in contamination. It would be difficult to assign relative degrees of significance to the various sources of contamination or prove in all instances that they are sources of contamination. The possibility that they are potential sources can be indicated, however, by the demonstration of the presence of yeast in these areas. From results presented (Pederson *et al.*, 1959), it is obvious that the tanks themselves are one of the major sources of contamination. Data summarized and shown in table 1 present further evidence of this source of contamination. Many of the sanitizing agents would remove or destroy these yeasts if such agents could be brought into contact with the yeasts.

Among the 13 one-gallon samples of juice obtained from surface coolers, 6 were taken from the trough below the coolers. Ordinarily the juice in the trough is covered with foam formed when the juice splashes into this trough. Such foam can warm up to room temperature readily and, since it remains there, yeast grows rapidly in the foam.

Since the data presented by Pederson *et al.* (1959)

seemed to implicate the foam on the surface of troughs of surface coolers, more attention was given to this as a source of contamination. Such foam is subject to air contamination. Since in commercial practice this foam is not removed except at cleanup periods, it may remain on the surface of juice for several hours. It warms up rapidly to the temperature of the room and contaminating yeast develops rapidly. Two samples of foam from the trough yielded appreciable counts (table 3). Six samples of juice from troughs with foam on the surface yielded appreciable counts (table 3). Four samples from a trough where foam was constantly removed yielded low or zero counts. Two samples of foam removed from such troughs yielded counts of 2 and 300. Among 11 samples taken from the surface coolers before the juice had come in contact with the trough, only 5 yielded colony counts, the highest count being 30 (table 3). It must be realized that the surface cooler is subject to air contamination.

Similar conditions undoubtedly exist wherever foam is allowed to accumulate in this manner, such as the surface of intermediate storage or surge tanks. One such instance was previously cited. Other instances undoubtedly occur.

TABLE 3

Yeast counts of grape juice and foam samples from surface coolers and troughs below coolers

Samples from:	Yeast Counts when Plates Incubated at		
	32 C	18 C	1 C
Foam from trough	600	—	183
	21,000	—	9,800
Juice from trough	2,500,000	—	90,000
	100,000	99,000	240
	280,000	200,000	500
	160	170	130
	640	640	540
	410	280	0
Juice from trough when foam was removed continually	0	0	0
	0	0	0
	3	0	0
	4	0	0
Foam from trough when foam was removed continually	—	300	—
	—	2	—
Juice before entering trough	0	0	0
	0	0	0
	0	10	0
	0	0	0
	0	0	0
	0	0	0
	30	0	0
	2	0	0
	0	0	0
	0	2	0
	0	1	0

Air should never be presumed to be sterile unless it is filtered or otherwise sterilized. Even the air in entirely closed tanks, unless it has been sterilized or filtered, presents a source of contamination. The air in tank rooms is an important source of yeast and mold contamination for open tanks. Plates exposed 20 to 30 min in various tank rooms have invariably yielded yeast and mold colonies (table 4). Five agar plates exposed 20 min in one storage room yielded a total of 42 yeast colonies; 8 plates in another plant yielded 35 yeast colonies. Inasmuch as a plate has a diameter of 3.5 in. and a total surface area of 9.6 square in., whereas a 16 foot tank has a surface area of 200 square feet, chances of contaminating a tank are about 3000 times as great. It can readily be seen that air may be a major source of contamination. Although it is true that in many operations ice is eventually formed on the surface, contamination would have occurred before the surface had frozen.

As previously stated, it is difficult to attach degrees of significance to the various sources of contamination, or even prove in some instances that they are sources. Only by personal observation can it be assumed that contamination may have arisen from any one point. In these studies, evidence indicated that a wooden, intermediate holding tank was a source of contamination. An enclosed cooler in which poorly fitted gaskets were used was obviously a source of contamination in another instance. Juice obtained from this equipment yielded an appreciable yeast count. Appreciable yeast counts were obtained from a T joint and valve arrangement in an otherwise enclosed line from an enclosed cooler. A special sampling device was obviously implicated in another instance. In one installation, the operators observed contamination from the pressure valve. Un-

TABLE 4

Yeast counts obtained from plates exposed in cool storage rooms in 1955 and 1957

No. of Plates Exposed*	Range of Plate Counts at 18 C	
	Yeast	Mold
4	1–4	1–3
10	0–7	1–18
12	0–9	1–20
10	14–28	0–7
10	0–7	0–14
5	5–moldy	5–moldy
5	6–16	4–12
4	0–19	4–12
4	0–4	1–1
8	1–26	0–8
7	0–3	0–4
4	0–1	2–7
5	0–7	0–6
5	1–5	0–6
5	2–15	20–43

* Plates exposed, in 1955, 30 min; in 1957, 20 min.

doubtedly there are other sources of contamination such as pipelines with nonsanitary fittings. Again, it should be emphasized that any point where juice remains for a period of time and any foam that forms should be considered possible sources of contamination.

There may be some question as to the quality of juice in which high numbers of yeasts existed. Three of the predominant types of yeast were typical alcohol producers. These typical alcohol and carbon dioxide producing species have been the main contaminants in some series, such as no. 28 (table 1). They often yielded counts at 32 C as high as those obtained at 18 or 1 C. They produced typical mucoid colonies on plates and were readily recognized. In those cases where alcohol determinations have been made, the amounts of alcohol were very low unless the counts approached or exceeded 500,000. The *Candida* species are not considered to be either carbon dioxide or alcohol producers. When growing in juice, they first impart a pleasing ester-like odor, which eventually may become offensive. This type of yeast has been the main contaminant in some juices such as those in series 72 (table 1). They were readily recognized because they produced a dry, rough colony. Many times they grew only at 1 C as in series 42, 43, and 54.

SUMMARY

Storage of grape juice at −5.5 to −2.2 C has presented a serious sanitation problem. Yeasts and mold may grow during storage and cause an economic loss to the industry. However, grape juice has been processed and stored commercially for long periods of time with no yeast contamination or growth. With proper cognizance of the sources of contamination, followed by precautions to reduce their effects, it is felt that losses can be reduced.

Rates of growth and fermentation are slow at the low storage temperature. At times growth apparently ceases and some of the yeast cells die. The presence of yeast, however, is always a source of potential fermentation or alteration in flavor.

The rates of growth of the yeasts commonly encountered vary among strains of four genera. Although in the early stages of storage fermentative types of yeast are more commonly encountered, later the nonfermentative types commonly predominate. In order to determine the entire flora present, it was necessary to make replicate plates so that they could be incubated at 1, 18, and 32 C.

It is important that every precaution be taken by plant operators to avoid contamination. Contamination of grape juice arises from residual yeasts harbored in the pores of wood or coatings of tanks, the air in the room, foam on the surface of containers, intermediate holding tanks, pipelines with crevices where juice can accumulate, improperly made gaskets, valves, and any other places where juice with a few yeast cells can accumulate and growth of these yeast occur.

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