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Sugar and Acid Tolerance of Spoilage Yeasts from Sweet-Cucumber Pickles[a]

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What is believed to be the first preservation-prediction chart for yeast spoilage in sweet-cucumber pickles has been developed through studies of 35 yeast cultures, responsible for gaseous-type spoilage, which were isolated from 15 samples of sweet pickles made by 3 pickle manufacturers in different locations. The yeasts were identified as being closely related to the species Zygosaccharomyces globiformis. The prediction chart should aid the pickle manufacturers in standardizing sweetening formulas, in reducing spoilage, and in saving sugar.

It is well known that sugars and organic acids play an important role in the preservation of certain food products — especially in the manufacture of sweet-cucumber pickles. Where pasteurization and such chemical agents as sodium benzoate are omitted, the pickle product must contain sufficient quantities of sugar and acid, which are usually cane sugar and vinegar, to preserve it properly.

The methods for sweetening cucumber pickles have been more of an art—handed down from one pickle manufacturer to another — than a science based on knowledge of spoilage organisms of high sugar and high acid tolerance. Fabian and Demain *(4)* reported a wide variation in the amounts of sugar and vinegar that existed among different commercial sweet-pickle products. Manufacturers have raised or lowered the quantities of sugar and vinegar needed usually on a trial and error basis.

Because of this lack of exact formulas to insure preservation, the possibility of microbial spoilage of their products has been a problem to pickle manufacturers. Fabian and Switzer *(5)*, in a paper on the classification of pickles, warned manufacturers of spoilage in sweet pickles which finish below 20 grains of vinegar (2.0% acetic acid). Etchells and Jones *(2)* cited an occurrence of yeast spoilage in the finishing of sweet pickles which they attributed to low acidity, but they were unsuccessful in attempts to isolate the yeast because it was dead at the time of observation.

A number of workers *(1, 6, 10, 12, 16)* have reported on the preserving and germicidal action of sugars and organic acids, but they were in most instances studying yeasts and other organisms which did not have a history of causing spoilage in acid foods such as pickles. One cannot assume, as did Fellers and his co-workers *(10, 12, 16)*, that the "bread-yeast" *Saccharomyces cerevisiae* will represent the characteristics of all species of yeasts. Yeast growth occurring in foods other than sweet pickles, such as honey, maple sirup, molasses, and dried fruits, has been thoroughly reviewed by Henrici *(7)* and Mrak and Phaff *(13)*, who showed that species of the genus *Zygosaccharomyces* were most frequently found in high-sugar content substrates.

The present study is believed to be the first in which the yeasts causing spoilage in commercial sweet pickles have been isolated and studied with respect to their acid, sugar, and benzoate tolerances. In this work a sweetening chart for the prediction of preservation or spoilage was developed which is based on the biochemical properties of spoilage yeasts isolated from commercially prepared sweet cucumber pickles. This chart is presented here for the aid it may offer pickle manufacturers in standardizing their sweetening formulas, in reducing spoilage, and in saving sugar.

EXPERIMENTAL PROCEDURE

Isolation and identification of spoilage organisms. In a period of a year 15 samples of spoiled sweet pickles were received by our laboratory from 3 manufacturers in different sections of the country. Spoilage was characterized by slight gaseous pressure under the caps, off-flavor, and lens-type bloaters (hollow cucumbers). Microscopic examination of the liquor showed a high non-viable yeast population compared to a low viable count. The liquors from the jars were either plated or streaked on acidified dextrose-agar media using the technique of Etchells and Jones *(3)*, and from these plates 35 isolates were obtained. Their source as to type of pickle product, sugar and vinegar levels is given in Table 1.

TABLE 1

Origin of 35 yeast cultures from spoiled sweet pickles from 3 commercial pickle plants

Plant	No. of jars	Product	Range in acidity as acetic	Range in sugar	Spoiled pickle yeast (SPY), culture numbers
			%	%	
A	4	Sweet chips	1.5-1.6	23	SPY-1 to 13
B[b]	7	Sweet mix	0.7-0.8	12-16	SPY-14 to 31
C	4	Sweet whole	1.8-1.9	21	SPY-32 to 35

[b] Product contained 0.1% sodium benzoate, according to manufacturer.

Taxonomic studies were made on the 35 isolates which will be referred to as "SPY" for "Spoiled Pickle Yeasts." These yeasts were identified as belonging to the genus *Zygosaccharomyces*, and they were found to be closely related to the species *Z. globiformis* Kroemer et Krumbholz which was described and studied in detail in 1931 by Krumbholz *(9)*. Five cultures (SPY 9, 15, 21, 29, 32) were selected as being representative of the group, and these were used in the tests for fermentation and growth.

MATERIALS AND METHODS

The preserving agents tested for inhibition of growth using the test cultures were sucrose (cane sugar), glucose, acetic acid (vinegar[c]), and sodium benzoate. These are chemical compounds used by the commercial cucumber-pickle companies. Sucrose in the presence of acetic acid and heat was considered as hydrolyzed to invert sugar *(8, 14, 15)*. Similarly, it could be assumed that the sucrose as used by the commercial companies

[a] A cooperative project with the Department of Horticulture of the North Carolina Agricultural Experiment Station.

[c] Distilled vinegar, 110 grains (11.0% acetic acid) was used. Referred to in this paper in terms of percent acetic acid.

for making sweet pickles would also be inverted due to the presence of acetic acid and usually some mild heat treatment. The basal broth used to supply the necessary nutrients for yeast growth consisted of 0.5% Bacto peptone, 0.25% Bacto yeast extract and 0.5% sodium chloride. To the basal broth, sucrose or glucose on a percent by weight basis, and acetic acid as vinegar on a percent by volume basis were used to make the test broths. The concentrations for sucrose were from 2 to 65%, and for glucose from 2 to 40%. Acetic acid concentrations varied from 0 to 4%. Sodium benzoate, in conjunction with sucrose and acetic acid, was also tested in concentrations from 0.05% to 0.4%.

Fermentation tests for the various sugar, acid, and benzoate broths were conducted in Durham tubes, which consisted of a small inverted tube (9 x 75 mm.) inserted into a larger tube (16 x 150 mm). The tubes with broth were sterilized at 15 pounds of pressure for 15 minutes, followed by prompt cooling in water. The inoculated broths were observed frequently for gas and growth; the latter was determined by turbidity and sediment. After 2 to 4 weeks, microscopic counts for viable and non-viable yeast cells were made with an improved Neubauer hemacytometer counting chamber used as described by Mills *(11)*. He reported a sharp distinction between viable and non-viable yeast cells in solutions of methylene blue, methyl green, and erythrosin, using 1: 10,000 concentrations of the dyes in phosphate buffer at pH 4.6. Mills also preferred methylene blue for best results. However, our experience with yeasts in high salt content brines and high sugar content liquors has proved erythrosin to be the most satisfactory for giving a sharp distinction between living and dead cells and ease in counting.

RESULTS

Fermentation and growth tests. In preliminary studies, the test yeasts grew and produced gas in broths containing the basal nutrients and the following ingredients:

Sucrose, 60% (by weight).
Glucose, 40% (by weight).
Sucrose, 2%; acetic acid, 3.6% (at pH 3.0).
Sucrose, 15%; acetic acid, 0.8%; and sodium benzoate, 0.3%.
Sucrose, 10%; acetic acid 1.5%; and sodium benzoate, 0.1%.

In these broths, the yeasts demonstrated unusually high tolerance to the above individual preserving agents. Additional tests were conducted to obtain the exact limitations for growth in various concentrations and combinations of these ingredients.

In one experiment, sucrose was held constant at 10% with the levels of acetic acid increased to approximately 3.5% in increments of 0.5%. The acetic acid levels with pH values in parentheses for the uninoculated broths were as follows: 0% acetic acid (6.52); 0.5% (3.68); 1.0% (3.42); 1.5% (3.28); 2.0% (3.12); 2.5% (3.10); 3.0% (2.96); and 3.6% (2.92). The effect on yeast cell production and gas evolution was very noticeable. The viable and non-viable yeast counts, after 14 days' growth, are illustrated in Figure 1. The non-acid broth gave a population of over 40 million viable yeast cells per ml. with a very low non-viable count. The acidified broths gave a decrease in viable cells as compared to an increase in non-viable cells until the point of inhibition was reached. Yeast populations were rapidly suppressed beyond the 2.5% acetic acid level, and no viable cells were observed at 3.6% acetic acid. Gas from yeast growth filled the insert tubes in all the broths except that at 3.6% acetic acid level, which was

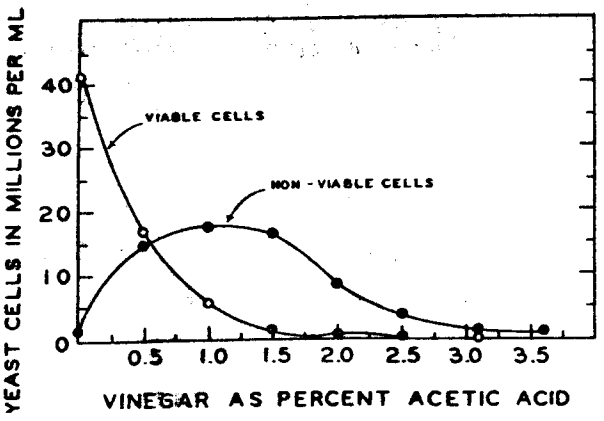

Figure 1. Yeast populations at fourteen days in ten percent sucrose and various concentrations of acetic acid. (Test yeast strain used Spy 29.)

negative. Two days were required to fill the insert tubes for 0 through 1.5% acetic acid; 5 days for 2.0%; 6 days for 2.5%; and 7 days for 3.1%.

The results of other tests are shown in Table 2. Here the concentrations of sucrose and glucose as well as that of acetic acid were varied and the broths inoculated with the representative cultures. With sucrose, growth and gas were observed at all levels of the sugars through 1.5% acid. In 2.0% acid, growth and gas were observed in concentrations through 35% sucrose, but not at 40%. Further inhibition for growth and gas were shown in 2.5% acid; the limitation of growth was between 20% and 30% sugar concentrations. When glucose was substituted for sucrose, a greater inhibition was noticed using the same levels of sugar. For example, 2.0% acid and 30% of each sugar gave growth and gas for sucrose but none for glucose. This finding confirms that of Erickson and Fabian *(1)*, and Tarkow et al. *(16)* that glucose, at concentrations equal to sucrose, is more inhibiting for certain organisms.

TABLE 2
Yeast growth and gas in broths containing sugar and acetic acid

Test broths containing		Sucrose		Glucose	
Acetic acid	Sugar	Growth [a]	Gas [b]	Growth [a]	Gas [b]
%	% by weight				
1.0	10	3+	+ (3)	3+	+ (5)
	20	3+	+ (3)	3+	+ (7)
	30	3+	+ (5)	3+	+ (9)
	40	2+	+ (7)	2+	+ (16)
	10	3+	+ (3)	3+	+ (9)
	20	3+	+ (4)	3+	+ (11)
1.5	30	2+	+ (5)	2+	+ (16)
	40	2+	+ (11)	—	—
	45	1+	+ (28)	—	—
	10	3+	+ (4)	3+	+ (21)
	20	3+	+ (5)	2+	+ (28)
2.0	30	2+	+ (15)	—	—
	35	2+	+ (16)	—	—
	40	—	—	—	—
	10	2+	+ (6)	2+	—
2.5	20	2+	+ (15)	—[c]	—
	30	—	—	—	—
	40	—	—	—	—

[a] Growth: Sediment and turbidity of broth, 3+ = good; 2+ = moderate; 1+ = scant; — = none. Five representative cultures used in this experiment.
[b] Days required for maximum evolution of gas.
[c] One of the five test cultures gave very scant growth. Repeated tests gave same result.

The yeast populations (viable and non-viable) for 4 levels of acetic acid in combination with 4 levels of sucrose are shown in Figure 2. It is important to point out that the SPY isolates produced a greater number of cells at the 1.0% acetic acid level in sugar concentrations of 20% and 30% than at either 10% or 40%.

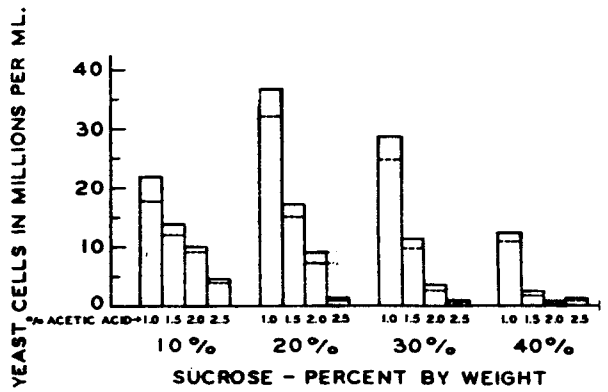

Figure 2. The effect on total yeast populations caused by increasing concentrations of sucrose and acetic acid. The dotted line in each bar represents the non-viable cell count, and above the dotted line the viable count. (Counts made at 28 days using strain Spy 29.)

This is probably due to the osmophilic nature of these yeasts. Further, at 2% sucrose concentration without acid, this group of yeasts assimilates the carbon source for energy but may or may not produce gas. However, acidification of sucrose results in its inversion to simple sugars which are readily fermented by the isolates.

Three concentrations of sodium benzoate were tested in 5 concentrations of acetic acid with 10% sucrose (Table 3). Growth and gas were produced at all 5 levels—0.5% to 2.5% acetic acid at the .05% benzoate level. The 0.10% benzoate inhibited the growth at 2.5% acid, and 0.20% benzoate permitted growth only in the 0.5% acid.

Preservation-prediction lines and formulas. By making additional fermentation and growth tests with various concentrations of the 4 compounds (glucose, sucrose, acetic acid and sodium benzoate), as well as using the tests previously described, certain predictions for preservation seemed possible. In plotting the individual sugar concentrations against the individual acetic acid concentrations, lines were drawn which divide the areas of fermentation and growth from those of no fermentation and no growth. Three lines are presented for the compounds tested[1] which were (A) sucrose and acetic acid, (B) glucose and acetic acid, and (C) sucrose, acetic acid, and 0.1% sodium benzoate (Figure 3). The areas above the lines represent preservation: the areas below, spoilage.

TABLE 3

Yeast growth and gas in 10% sucrose broths containing acetic acid in combination with sodium benzoate

Acetic acid	Concentration of sodium benzoate					
	0.05%		0.10%		0.20%	
	Growth [a]	Gas [b]	Growth [a]	Gas [b]	Growth [a]	Gas [b]
%						
0.5	3+	+ (3)	3+	+ (3)	2+	+ (10)
1.0	3+	+ (3)	2+	+ (5)	—	—
1.5	3+	+ (5)	1+	+ (10)	—	—
2.0	2+	+ (7)	1+	—	—	—
2.5	1+	+ (10)	—	—	—	—

[a] Growth: Sediment and turbidity of broth, 3+ = good; 2+ = moderate; 1+ = scant; — = none.
[b] Days required for maximum evolution of gas.

Commercial test of prediction line. The validity of the prediction line (Figure 3) was substantiated by cooperative tests over a 2-year period by a large pickling company on 37 small-scale commercial lots of sweet-cucumber pickles. The pickles were equalized with sucrose (cane sugar) and acetic acid (distilled vinegar) over a range covering both the preservation and the spoilage sides of the prediction line. Quart samples of each lot were sent to our laboratory and analyses for sugar, acid, and yeast populations were made. When the sugar and acid concentrations of the 37 lots were plotted, 28 were on the spoilage side of the prediction chart and the remaining 9 fell on the preservation side (Figure 4).

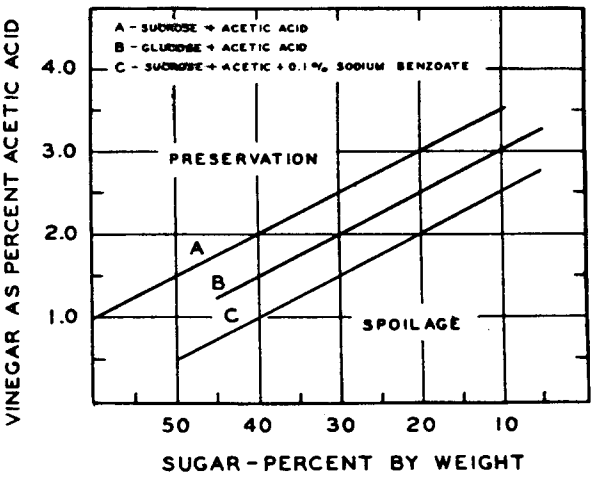

Figure 3. Preservation prediction chart; the area above each line represents preservation for that particular combination of acid, sugar, and/or sodium benzoate; the area below each line represents the spoilage zone.

[1] The following mathematical formulas for the 3 lines can be applied in calculating the quantities of sugar or acid required to prevent fermentation:

(a) Sucrose + Acetic Acid

$$S_s = 80 - (20 \times A)$$

or

$$A = \frac{80 - S_s}{20}$$

(b) Glucose + Acetic Acid

$$S_g = 70 - (20 \times A)$$

or

$$A = \frac{70 - S_g}{20}$$

(c) Sucrose + Acetic Acid + Benzoate

$$S_s + B = 60 - (20 \times A)$$

or

$$A + B = \frac{60 - S_s}{20}$$

Where:

A = % acetic acid
S_s = % sucrose by weight
S_g = % glucose by weight
B = 0.1% Sodium benzoate

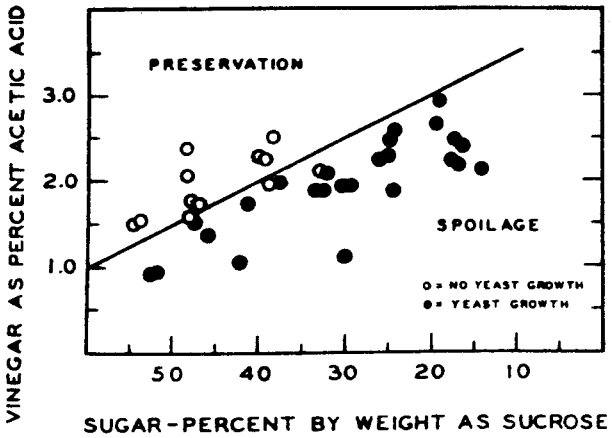

Figure 4. Yeast growth in commercially prepared lots of sweet cucumber pickles at different concentrations of sucrose and acetic acid.

Next, all lots were inoculated with the test yeast to give a final count of about 5,000 viable cells per ml. If, after 3 to 6 weeks from inoculation, there were no viable yeast cells, and the non-viable count had not increased, then the lot was considered preserved. On the other hand, if the total count of either viable or non-viable cells increased, then the pickles had supported growth and were considered spoiled. Most of the lots which were predicted to spoil were either fermenting or had fermented prior to reaching the laboratory.

All 9 lots which were predicted to be preserved were found to be negative for yeast growth and obeyed the sugar-acid prediction formula. Twenty-five of the 28 below the line supported yeast growth and followed through to spoilage as predicted. The authors have no explanation for the 3 lots that did not support yeast growth other than that they were border-line cases.

Ten of the 20 lots made in 1949 were prepared from partly cured salt-stock. It had been the opinion of the manager of the cooperating pickle plant that these lots would be more difficult to preserve and probably take more sugar and acid. However, this was not found to be the case.

DISCUSSION

The isolation of the 35 yeasts from spoiled sweet-cucumber pickles not only helped explain the gaseous-type of spoilage for this product but also gave the opportunity to study the underlying principles of preservation by sugar and acid that have been long used in the art of making sweet pickles. The data obtained, in offering sweetening formulas by which ratios of glucose or sucrose with acetic acid may be determined for the preservation of pickles, should prove to be a useful working tool in processing plants. It should be emphasized, however, that the preservation-prediction lines now being proposed were made from one group of yeasts isolated from products manufactured in 3 sections of the country, and are valid only until such time as other organisms definitely associated with pickle spoilage are shown to be more sugar-and acid-tolerant. The chances of finding additional strains of *Z. globiformis* or other organisms of higher sugar tolerances than the strains studied by the authors should not be ruled out.

It is believed that research workers in other food laboratories may use the representative yeast isolates mentioned in the report in tests of other food products.[1] It is likely that there are many other food preservatives, including additional sugars and organic acids as well as spices and essential oils, which should be tested against highly resistant test organisms such as the yeast cultures used in this study.

SUMMARY

Thirty-five yeast cultures were isolated from commercially prepared sweet-cucumber pickles and were identified as being closely related to the species, *Zygosaccharomyces globiformis* Kroemer et Krumbholz. This yeast was responsible for gaseous type of spoilage in the pickle products examined.

Representative yeast isolates were tested for growth and gas formation in various concentrations of sucrose or glucose with acetic acid. The effect of sodium benzoate was also tested in combination with sucrose and acetic acid. By plotting the sugar and acid concentrations required to inhibit yeast growth, preservation-prediction lines were made. In laboratory tests with the yeast, it took less glucose than sucrose on a percent by weight basis to inhibit growth. The addition of sodium benzoate reduced the amount of sucrose required for preservation.

The validity of the prediction line was substantiated with commercially prepared lots. Thirty-seven small-scale lots of sweet pickles were made by a commercial pickle company so that the sucrose and acid contents after equilibrium ranged on both sides of the preservation prediction line. All lots which were predicted to be preserved did not support yeast growth. Twenty-five lots out of the 28 predicted to spoil were found to have yeast growth.

The preservation-prediction lines should help the pickle manufacturers in standardizing their sweetening formulas, in reducing spoilage, and in saving sugar.

Acknowledgments

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