

appreciable increase in the total flora was noted if the temperature of this phase was held at 18° C. (64.4° F.) or less. Even with 21° C. (69.8° F.) as the thawing temperature an appreciable increase in count did not occur until the third freezing and thawing cycle. In the series in which the thawing temperature was 32° C. (89.6° F.) a rapid and gradual increase in flora was found through each succeeding cycle of freezing and thawing.

An increase of the thawing cycle to 12 hours resulted in a more rapid increase in count, Figure 5, during the freezing and thawing cycles than when 21° C. (69.8° F.) and 32° C. (89.6° F.) were employed as the thawing temperatures. In those cases in which the thawing temperature was 18° C. (64.4° F.) or lower, an appreciable increase in count was noted when the chicken pies were passed through 3 freezing and thawing cycles.

It may be noted, Figure 3, that approximately 8 hours is the maximum time of holding at 32° C. (89.6° F.) before growth is initiated while a little longer is required at a temperature of 21° C. (69.8° F.). These results, similar to those reported earlier (1957) for frozen

vegetables, indicate that alternate freezing and thawing of frozen chicken pies will not affect an increase in count unless the thawing phase is at a temperature and time which will initiate growth.

#### CONCLUSIONS

1. Alternate freezing and thawing of commercial frozen chicken pies did not increase the total flora unless the conditions of the thawed phase initiated growth. An increase in count occurred when thawing chicken pies were held at 2° C. (35.6° F.), 7° C. (44.6° F.) and 18° C. (64.8° F.) for 48 hours.

2. Approximately 50% of the commercial frozen chicken pies showed the presence of coliform types.

3. When held at 21° C. (69.8° F.) and 32° C. (89.6° F.) chicken pies showed the first increase in total flora after approximately 10 hours.

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## Pectin Hydrolysis in Certain Fruits During Alcoholic Fermentation

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MANY FRUITS ARE FERMENTED INTO alcoholic drinks: e.g., grapes into wines and apples into cider. Similarly, many cannery wastes from both fruits and vegetables are utilized for the production of ethanol by fermentation. Because most of these plant materials contain varied amounts of pectin, it often happens that during their alcoholic fermentation, or probably just before it, some of the pectin substances are hydrolyzed, the methoxyl groups split off, and the resulting drink then contains greater or lesser amounts of methanol alongside with ethanol.

Although grapes, for instance, are known to contain pectin, wines normally are free of methanol except perhaps for traces. However, it has come to our knowledge that some wines recently imported into the United States from other countries were refused admission due to excessive methanol content. Apples and citrus fruits present another interesting case: whereas fermented apple cider contains no methanol, citrus juices after fermentation exhibit the presence of considerable amounts of it. Moreover, citrus wastes when serving as raw material for ethanol production, both lined and non-lined, result in spirits practically unfit for human consumption because of their high methanol content, unless properly rectified. An additional example is the case of sugar beets, which were recently used in Israel for the production of ethyl alcohol before the sugar refinery was completed. Although sugar beets contain

final product while others got a considerable amount of it.

On the strength of these and similar examples the writers thought it proper to make a preliminary survey of the entire field in order to elucidate the following points:

- (a) Can the pectin be demethoxylated by yeast fermentation alone?
- (b) Why do two plant materials containing equal large quantities of pectin differ so utterly in their methanol content after fermentation?
- (c) What is the reason for the wide differences in methanol content after fermenting one and the same plant material when treated under different conditions of pH and temperature?

The answers to all three questions are to be found in the vast literature on pectic substances and pectolytic enzymes; however, they are rather scattered, and a clearer view on the situation would be of great practical value to the processor and the distiller.

#### EXPERIMENTAL

The experiments that follow were primarily undertaken with a view to seek confirmation of various facts already known about pectin and pectolytic enzymes. They were designed in accordance with the 3 questions posed above.

**Methods employed.** All fermentations were carried out in ordinary baker's yeast (*Saccharomyces cerevisiae*) at 30° C. in Ehrlenmayer flasks of 500 ml. each which had been previously

after distilling 100 ml. of the fermented media. Estimates of methanol were made by the Klett-Summerson Photoelectric Colorimeter using the green filter (5000 Å) which exhibits the most convenient range, according to the method of Denigès, described by A.O.A.C. (6), the solutions having been prepared according to Woodman (7). For comparison, standard solutions were prepared containing pure methanol dissolved in 5% ethanol-water in various proportions. Readings on the arbitrary scale of the Klett photocolormeter were plotted against these standards and being proportional to the optical density it was easy to establish the relation between the scale reading, the percentage of light transmission, and the percentage of methanol in solution.

Measurements of pectin esterase (PE) activity were made by means of a Beckman Potentiometer with a glass electrode according to the method originally developed by Kertesz (3) and subsequently modified by others (5) in which the increase of free carboxyl groups was determined by the amount of 0.1 N NaOH consumed during 30 min., in order to keep a given pectin solution at pH 7.

#### A. Experiments in model solutions

The question whether yeasts alone can cause the demethoxylation of pectin was tested on a series of model solutions all containing 10% sucrose, some ammonium phosphate (1% of the sucrose) and various amounts of pectin. All samples were brought to pH 3.5 by means of citric acid, sterilized as above, and baker's yeasts were added after cooling. In all, two series of 4 experiments each were run, every experiment comprising three equally set up test flasks. The results are presented in Table 1—experiments 1 to 8.

When all the above experiments showed negative results, further series of the same tests were run with the addition of fungi such as *Aspergillus niger* (experiments 9 to 13 in the above table) and *Penicillium glaucum*. The fungi were left to act on the pectin solutions for 3 days at the end of which the whole was sterilized, inoculated with baker's yeast and methanol contents estimated after the fermentation. (See Table 1, experiments 14 to 18).

#### B. Experiments with yeast juice

A series of experiments was then run with triturated yeast cells in a solution containing 10% sucrose, 0.5% pectin at pH 4.5. The yeast cells were triturated by known methods in a Fisher disintegrator using (a) carborundum (SiC), (b) silicic acid (SiO<sub>2</sub>) and (c) corundum (Al<sub>2</sub>O<sub>3</sub>). A microscopic examination after trituration showed that 78–80% of the yeast cells were completely broken. To these three runs a blank was added using untrituated yeast for control.

TABLE 1

Test No. <sup>1</sup>	Pectin content % of T.S.S.	Action of fungi, hrs.	Fermentation time, hrs.	pH	% Methanol	% Ethanol
1	none		72		none	4%
2	0.25		72		none	4%
3	0.50		72		none	4%
4	0.70		72		none	4%
5	none		96		none	4.5
6	2.5		96		none	4.5
7	5.0		96		none	4.5
8	7.5		96		none	4.5
9*	none	72	120		none	none
10	0.50	72	120		> 0.1	none
11	1.25	72	120		0.1 to 0.2	none
12	2.50	72	120		0.3 to 0.4	none
13	5.0	72	120		well over 0.5	none
14	5.0	72	96	0.6	none	no fermentation
15	5.0	72	96	2.2	> 0.1	4%
16	5.0	72	96	6.0	over 0.5	4%
17	5.0	72	96	12.0	over 0.5	no fermentation
18	5.0	72	96	13.0	over 0.5	no fermentation

<sup>1</sup> Each test comprised 3 parallel runs.

\* Each of the following tests comprised in addition a blank containing no fungi for control.

rate. After fermentation the mixture was distilled and the distillate showed a negative reaction for methanol.

#### C. Experiments with different pectin containing plant materials

Following the experiments in model solutions, a series of tests were set up with the following pectin-containing fruits and vegetables: apples, beets, sugar beets, oranges, lemons, grapefruit, guavas and tomatoes. In each case, the material was disintegrated in a Waring blender and brought up to a slurry in which the solution showed approximately 10<sup>6</sup> Brix. Baker's yeast was then added and the whole was set at different pH, namely 3, 5, 6 and 7. Fermentation was in all cases terminated after 72 hours and the ethanol contents varied between 4% and 5% by volume. Only citrus fruits (oranges, lemons and grapefruit) and tomatoes exhibited the presence of methanol (<0.1%).

When tested for the presence of PE by the method of Kertesz (3), again only citrus fruits and tomatoes gave a positive reaction for this enzyme.

#### D. Influence of pH

On one and the same plant material the influence of pH was tested on citrus peels with a high content of PE. These were disintegrated as before and fermented with baker's yeast at various pH levels. The amounts of methanol received in the distillates are shown in Figure 1.

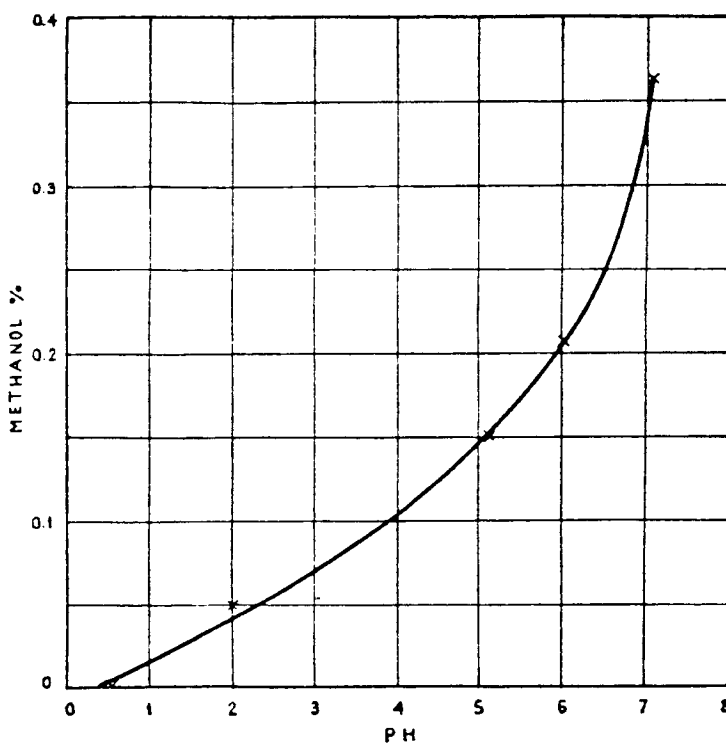


Figure 1. PE activity in citrus peel juice (measured as percent of methanol) at different pH.

### RESULTS AND DISCUSSION

Results in Table 1 clearly show that model solutions of sucrose containing various quantities of pectin had no methanol after fermentation. Indeed, if the yeasts themselves would not split the methoxyl groups there would hardly be any other cause to account for, because any amount of PE possibly present in the model solution, would have been inactivated by sterilization.

On the other hand, the introduction of fungi, such as *Aspergilli* or *Penicillia*, quickly changed the picture: after a comparatively short time the pectin present was demethoxylated by the fungi. This probably also explains why wines may often contain some methanol

after fermentation: if the grapes carried with them some fungi, the latter's PE contents would be sufficient to hydrolyze the pectin.

Because of the failure of the yeasts to exhibit the action of PE it was thought that the many hydrolytic enzymes present in yeast are all intracellular components (2), whereas the fungi work apparently extracellularly. This point was worth further elucidation. It may well be that if the hydrolytic enzymes of yeast are present intracellularly, they could not attack the pectin molecule which is quite large and cannot penetrate the semipermeable yeast membrane. The above reasoning called for a fermentation experiment in which yeast juice could be used instead of intact yeast cells, thus enabling the intracellular hydrolytic enzymes, if present, to act on the pectin molecule.

However, as mentioned above, the experiments with yeast juice gave also negative results. Thus, the baker's yeast used by us contained virtually no PE, not even intracellularly. This confirms previous observations by Kertesz and others (5) who were unable to show the presence of PE in *Saccharomyces cerevisiae*.

Turning from model solutions to fresh fruits and vegetables, it was found, as expected, that only citrus fruits and tomatoes, which actually exhibited the presence of PE gave place to pectin hydrolysis. It is obvious, then, that at acid or neutral pH and at normal temperatures, only pectin-containing materials which have also at the same time the PE can give rise to methanol on fermentation. No methanol will appear in the absence of the enzyme.

When investigating specifically the influence of pH on the same plant material, it was observed that in the range of pH up to 2, there were no appreciable amounts of methanol (0 to less than 0.1%). From pH 2.0 up to pH 7.0 the content of methanol rises quickly with the rise of pH. These findings are in agreement with the general statement by Kertesz (4) summarising different opinions on this matter. At higher pH ranges the PE activity is said to be even stronger. However, it is obvious that in such cases the demethoxylation can be related rather to the action of the alkali than to PE alone.

As far as thermal influence is concerned, we could only say that in the experiments carried out by us, no demethoxylation occurred while heating pectin solutions at 100° C., 110° and 115° C. for time periods of up to 2 hours, but on further elevation of the temperature to 120° C. traces of methanol were detected. These experi-

ments were performed in three media: (a) models of sugar and pectin, (b), apple pulp and (c) sugar beets.

However, the alcohol produced from sugar beets by one plant in Israel contains very considerable amount of methanol. This plant disintegrates the beets by the Henze process, which consists of cooking them in an autoclave at a pressure of 4 atm. followed by a quick release of the pressure. The demethoxylation of the pectin in this case can only be explained by the treatment at this high temperature. Further experiments in this direction are now in progress.

### SUMMARY

A preliminary survey was made in order to elucidate several points regarding pectin hydrolysis in certain fruits during alcoholic fermentation.

Apparently pectin is not hydrolyzed by ordinary baker's yeast during fermentation, nor does it become hydrolyzed with yeast juice.

Unless the fruit in question contains pectin esterase or the medium is contaminated by fungi, which in most cases contain this enzyme, the pectin is not hydrolyzed during fermentation and no methanol is formed.

Differences in preparation procedures before fermentation, such as liming and thermal treatment are largely responsible for the wide differences in methanol content of alcohol produced from one and the same plant material.

The influence of pH on the PE activity of citrus pectin juice was explored.

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