Reclaiming Used Cherry Brines

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Reclaiming Used Cherry Brines

DARRELL V. BEAVERS, CARL H. PAYNE, M. R. SODERQUIST,
KJELL I. HILDRUM, and R. F. CAIN

SUMMARY

Brines used for bleaching, curing, and preserving sweet cherries can be made re-usable by treatment with activated carbon. The used brine contains dissolved pigment which must be removed before re-use. In a pilot-plant test the amount of dissolved pigments was reduced to 1/900 of the initial level by the treatment—from 41.700 mg/1 to 0.047 mg/1. During this treatment the concentration of sulfur dioxide fell only 26 ppm, the pH rose only 0.09, and the soluble solids decreased only 0.17 percent.

Cherries packed in the reclaimed brine were of higher quality than those used in the control, showing a significant decrease in solution pockets, brine cracks, torn pitter holes, and soft fruit. It appears that the brine could be reclaimed and re-used several times. Savings would result not only from lower requirements of chemicals for making brine, but also from reduced sewerage charges when discharging into a municipal sewerage system.

Before any brines are reconditioned for re-use, they should be tested for the presence of polygalacturonase enzymes. These enzymes could result in softening of the cherries. If present, polygalacturonase enzymes should be inactivated by heat.

INTRODUCTION

Sweet cherries for manufacture into maraschino, cocktail, or glace fruit are preserved, bleached to a bright light yellow, and cured in a brine solution of sulfur dioxide and various calcium salts. In the Pacific Northwest, the brine is a 1.25 to 1.5 percent sulfur dioxide solution which is partially neutralized with calcium hydroxide or calcium carbonate to pH 2.7 to 3.0 and to which calcium chloride may be added in amounts up to 3 percent. After the cherries have cured and equilibrated in this brine for a period of several months, the brine will contain approximately 0.5 percent SO₂ at pH 3.2 to 3.6 and will have a soluble solids content of 6 to 12 percent. The soluble solids consist principally of dextrose sugar, acids, anthocyanins, polyphenols, and other soluble constituents which have diffused from the cherries.

Dissolved anthocyanins and polyphenols in the brine usually will prevent the production of a bright yellow color in the cherries when
old brine is re-used for brining fresh cherries the second year. Apparently the SO_2 brine has a capacity to bleach only a limited amount of phenolic-type pigments successfully; when this amount of pigments is exceeded in the brine, a dull, dark, or grey colored fruit results when the fruit is leached for finishing.

Because of the color problems involved in re-using old brine, it has become standard practice to waste old brine, discharging it directly into rivers and estuaries or into the sewerage systems of cities and towns. The high organic content (soluble solids) of the brine creates very serious pollution problems when the brine is discharged directly into streams. The very high biochemical oxygen demand (BOD) of this organic material depletes the oxygen in the streams and can lead to fish kills. Microbiological metabolism or direct oxidation of sulfite results in the formation of sulfuric acid (2H_2SO_3 + O_2 \rightarrow 2H_2SO_4), which markedly lowers the pH of the water and changes the aquatic environment. Chemical poisoning of marine life by the high SO_2 concentrations adds to the seriousness of the pollution problem.

Discharging used brines into city sewerage systems will cause problems, depending upon the size and type of the system. Discharging large volumes of high concentration brines into the sewerage systems of small towns has caused complete disruption and failure of the systems by either inhibiting or killing the active digestive microorganisms or by modifying the environment so that undesirable organisms preferentially grow. Discharging into a sewerage system in a large city produces fewer problems, as the dilution with other sewage and waste water is usually sufficient to minimize the adverse effects of the brine. However, whenever sulfite wastes are discharged into a sewerage system, several adverse effects are likely to occur.

The high organic levels in the waste stimulate microbiological growth in the sewerage system and can quickly deplete the dissolved oxygen in the liquid, leading to anaerobic conditions. This anaerobiosis is conducive to the growth of organisms (such as Desulfovibrio sp.) which evolve hydrogen sulfide and other malodorous reduced sulfur compounds, resulting in serious odor problems. Concomitant with H_2S production is pH depression due to acid formation through anaerobic metabolism. Thus, the process is self-sustaining as long as the sulfur supply lasts and the liquid remains anaerobic.

As shown in Figure 1, the H_2S that evolves can lead to problems in a poorly ventilated pipeline. The poor ventilation (which is common) can lead to the accumulation of condensate along the crown (top) of the pipe. Hydrogen sulfide will tend to dissolve in this water and the ubiquitous H_2S-oxidizing bacteria (such as Thiobacillus sp.) likely will flourish in this medium, generating sulfuric acid. The acid, in turn, can lead to crown corrosion, as sewer lines are usually con-
Structured of portland cement-type concrete. The concrete is attacked by the acidic sulfite wastes as well as by the acids which are generated under anaerobic conditions.

Solutions to these problems are to avoid anaerobic conditions by providing short detention times and adequate ventilation in the sewer lines or by adding nitrate, which serves a metabolic function similar to oxygen in the microorganism. Anaerobic treatment processes should be avoided in the design of municipal facilities expected to accommodate sulfur-bearing wastes. Another solution would be to eliminate, or at least minimize, the discharge of sulfur-bearing wastes into the sewerage system.

In view of the problems brought about by discharging waste cherry brines into streams or into municipal sewerage systems, it was decided to investigate the possibility of treating or reconditioning used cherry brine to make it suitable for re-use.

* Numbers in parentheses refer to Literature Cited, page 19.
PRELIMINARY EXPERIMENTS

In 1967, it was determined that addition of decolorizing carbon (Norite A) successfully removed anthocyanins and polyphenols (2), (3), (4) with minimal reductions in soluble solids and SO₂ or change in pH. Sufficient reconditioned cherry brine had not been obtained for a series of brining experiments for the 1968 season, so in order to simulate reconditioned old brine, fresh cherries were harvested, held for six hours at ambient temperature, and packed in a standard calcium hydroxide or calcium carbonate brine at pH 2.75 and 1.25 percent SO₂, to which 6, 9, and 12 percent dextrose were added. After five months, the samples were evaluated for texture, percent solution pockets, percent brine shrink, percent pitting loss, and percent total loss. Texture measurements were made with a modification of the Hunter Texture Instrument (Figure 2). Measurement of the texture of the cherry consisted of measuring the force in grams required to compress the fruit 3/16 inch with a 7/16 inch disc. Solution pockets were calculated

Figure 2. Hunter Texture Instrument.
as percent by count. Percent brine shrink and percent pitting loss were calculated on the fresh fruit basis. The total loss was the sum of these two factors.

**Results of preliminary experiments**

There was an increase in the cherry texture, and the solution pockets decreased by one-half in the calcium hydroxide (Table 1) and by one-third in the calcium carbonate brines (Table 2).

The increased texture, decreased solution pockets, decreased brine shrink, and total loss in the calcium hydroxide brines very strongly suggest that using reconditioned brines may have a beneficial processing effect on the fruit.

In order to develop a satisfactory method of brine reconditioning, laboratory experiments were conducted in 1969 with insoluble polyvinylpyrrolidone (5), with a commercially formulated decolorizing agent (G.A.F. Polyclar AT), and with water-treatment-grade activated carbon (6), (7) (Calgon Filtrasorb, Type 300, mesh size 8 x 30). The use of the carbon gave the most encouraging results. This activated carbon, although not the ideal type for a liquid with the characteristics of spent cherry brine, nonetheless proved to be an effective

<table>
<thead>
<tr>
<th>Table 1. Addition of Dextrose to Calcium Hydroxide Brines</th>
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</thead>
<tbody>
<tr>
<td><strong>Texture</strong> (gms compression force)</td>
</tr>
<tr>
<td>Control (0%) ..................................</td>
</tr>
<tr>
<td>Dextrose 6% ...................................</td>
</tr>
<tr>
<td>Dextrose 9% ...................................</td>
</tr>
<tr>
<td>Dextrose 12% ..................................</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Addition of Dextrose to Calcium Carbonate Brines</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Texture</strong> (gms compression force)</td>
</tr>
<tr>
<td>Control (0%) ..................................</td>
</tr>
<tr>
<td>Dextrose 6% ...................................</td>
</tr>
<tr>
<td>Dextrose 9% ...................................</td>
</tr>
<tr>
<td>Dextrose 12% ..................................</td>
</tr>
</tbody>
</table>
decolorizing and reconditioning agent. It is absolutely essential to use iron- and copper-free activated carbon, as serious color problems in the brined cherries result if iron or copper is added as a contaminant during the reconditioning process.

The unregenerated brine was filtered and scanned in the visible range, using a Beckman Model DB Spectrophotometer. The minimum transmittance (2% transmittance) in the brine was noted at a wavelength of 335 mµ. After treatment, the wavelength of minimum transmittance had not shifted appreciably, but the relative transmittance value had risen to 74 percent. To the eye, the treated brine appeared clear.

The success of the bench-scale trials prompted the investigators to advance to the pilot-scale phase, to determine the effectiveness of the regenerated brine in bleaching commercial lots of cherries. Ten barrels of used dark-colored Black Republican cherry brine were obtained from a local processor and reconditioned as described below.

PILOT-SCALE REGENERATION

The activated carbon regenerant was housed in a 10-foot high, 8-inch diameter Plexiglas column fitted with 1-inch-diameter inlet and outlet hoses. Placed on the bottom was a coarse screen under a plug of glass wool. The column was filled with water-treatment-grade activated carbon (Calgon Filtrasorb, Type 300, mesh size 8 x 30) and the spent brine was passed downward through the column under an average head (at the top of the column) of 6 feet. The flow rate was initially about 2 gpm/ft², but as the top of the column became laden with debris (leaves and other trash), the flow rate fell to about 1 gpm/ft². In a full-scale application of this process, some type of prefiltration should be practiced.

Each of the 10 drums of brine was monitored for free SO₂, pH, and soluble solids content before and after reconditioning. The results of the analyses were encouraging (Table 3). The average free sulfur dioxide level fell only 26 ppm, the average pH rose only 0.09 units, and the soluble solids content fell 0.17 percent. The anthocyanin content, determined according to the method of Swain and others (8), fell from 41.700 mg/l to 0.047 mg/l, a nine-hundred fold decrease.

COMMERCIAL TESTS

The 10 drums of reconditioned 1968 brine were transported to a commercial briner and held until two days prior to brining the fresh fruit in 1969. At this time the separate brines were combined in one lot, the brine strength was adjusted to 1.40 percent SO₂, and the pH was adjusted to 2.8 to 2.9 with calcium hydroxide. Two hundred and
<table>
<thead>
<tr>
<th>Drum no.</th>
<th>Sulfur dioxide (ppm)</th>
<th>pH</th>
<th>Soluble solids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Reduction</td>
</tr>
<tr>
<td>1</td>
<td>4,930</td>
<td>4,860</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>4,285</td>
<td>4,420</td>
<td>..</td>
</tr>
<tr>
<td>3</td>
<td>5,445</td>
<td>5,250</td>
<td>195</td>
</tr>
<tr>
<td>4</td>
<td>5,000</td>
<td>4,930</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>5,190</td>
<td>5,190</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>5,120</td>
<td>5,120</td>
<td>0</td>
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<tr>
<td>7</td>
<td>5,120</td>
<td>5,190</td>
<td>..</td>
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<td>8</td>
<td>5,445</td>
<td>5,375</td>
<td>70</td>
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<td>9</td>
<td>5,190</td>
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<tr>
<td>10</td>
<td>5,630</td>
<td>5,570</td>
<td>60</td>
</tr>
<tr>
<td>Average</td>
<td>5,136</td>
<td>5,110</td>
<td>26</td>
</tr>
</tbody>
</table>
forty pounds of Royal Anne cherries and 230 pounds of brine were packed in 55-gallon drums on June 18, June 19, July 2, and July 3, 1969. Sixteen drums with reclaimed brine and nine drums of controls (new brine) were packed and held four months before evaluation. The initial soluble solids content of the cherries was determined and after four months, the drained weight, percent brine shrink, brine pH, percent solution pockets, percent brine cracks, texture, percent pitting loss, percent pits, percent of pits left in fruit (unpitted), percent large torn pitter holes, percent soft fruit, percent of grades No. 1, 2, 3, and rejects (No. 4's), percent brine soluble solids, and anthocyanin content were determined.

Drained weight was determined by draining drums five minutes on a stainless steel screen. In scoring solution pockets, an attempt was made to score only those pockets which showed a subepidermal flesh break which was serious enough to have commercial significance. Solution pockets having an over-all length of less than one-fourth inch, or which did not penetrate deeply into the flesh of the cherry, were not considered serious and were not scored. The cherries were pitted with a Dunkley Pitter style SP and reweighed to determine pitting loss. Pits were weighed separately. All data were calculated on a fresh fruit basis. The experimental results on the controls (new brine) and the reclaimed brine were averaged and the statistical significance was determined at the 5 percent level (Table 4).

Seven months after brining, the anthocyanin content (8) was determined on individual samples of Royal Anne cherries and the brine from highly pigmented (mature) fruit which had been packed July 3, 1969. The control sample contained 2.86 mg/l in the brine and 1.24 mg/kg in the cherries. The sample packed in reconditioned brine contained 4.07 mg/l in the brine and 3.61 mg/kg in the cherries. Although the anthocyanin content of the re-used brine was almost twice the level of the control, it was less than 10% of the original amount prior to reconditioning.

Cherries packed in the reconditioned brine showed a significant decrease in solution pockets, brine cracks, torn pitter holes, and soft fruit. There was a slight decrease in brine shrink, but the decrease was not significant and there was no significant difference in pitting loss or total loss. (The slight decreases in brine shrink and total loss in the 1968 laboratory-scale experiments with calcium hydroxide brines may not have been significant due to the small sample size.) Although there was a slight increase in No. 1 grade, and in No. 1 and No. 2 grades, the increase was not significant. There was a very slight increase in off-colored fruit, but the color difference was not significant. No detrimental results could be determined as a result of brining cherries in reclaimed brine, whereas the increase in quality factors not only dem-
Table 4. EVALUATION OF CHERRIES BRINED IN NEW BRINE (CONTROL) AND RECLAIMED BRINE

<table>
<thead>
<tr>
<th>Soluble solids of fruit</th>
<th>Brine pH</th>
<th>Solution pockets</th>
<th>Brine cracks</th>
<th>Texture (gms comp. force)</th>
<th>Brine shrink</th>
<th>Pitting loss</th>
<th>Total loss</th>
<th>Pits Unpitted</th>
<th>Torn pitter holes</th>
<th>Soft</th>
<th>Soluble solids of brine</th>
</tr>
</thead>
<tbody>
<tr>
<td>New brine (control)</td>
<td>14.0-18.4 (Avg. 16.45)</td>
<td>3.37</td>
<td>24.4</td>
<td>24.7</td>
<td>456</td>
<td>10.5</td>
<td>11.0</td>
<td>21.5</td>
<td>7.35</td>
<td>1.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Reclaimed brine</td>
<td>14.0-18.4 (Avg. 16.45)</td>
<td>3.35</td>
<td>15.0</td>
<td>4.9</td>
<td>519</td>
<td>9.4</td>
<td>11.9</td>
<td>21.3</td>
<td>7.34</td>
<td>0.6</td>
<td>1.55</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>0.04</td>
<td>7.2</td>
<td>11.2</td>
<td>53</td>
<td>2.7</td>
<td>1.2</td>
<td>2.0</td>
<td>.83</td>
<td>1.3</td>
<td>1.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade No. 1</th>
<th>Grade No. 2</th>
<th>Grade No. 3</th>
<th>Grade No. 4 (Rejects)</th>
<th>Off color</th>
<th>Grade No.1 and No. 2</th>
<th>Grade No.1, No. 2, and No. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>New brine</td>
<td>74.3</td>
<td>5.8</td>
<td>13.9</td>
<td>6.0</td>
<td>4.2</td>
<td>80.1</td>
</tr>
<tr>
<td>Reclaimed brine</td>
<td>79.9</td>
<td>5.4</td>
<td>9.8</td>
<td>4.9</td>
<td>4.9</td>
<td>85.3</td>
</tr>
<tr>
<td>LSD</td>
<td>14.4</td>
<td>3.2</td>
<td>11.8</td>
<td>5.1</td>
<td>3.2</td>
<td>11.5</td>
</tr>
</tbody>
</table>

LSD = Least significant difference at the 5% level.
NS = No significant difference.
* = Significant difference at the 5% level.
onstrated its feasibility, but indicated that use of reclaimed brine would have considerable economic value.

At the present time, it is postulated that used brine could be re-
claimed and re-used several times over a period of several years, and its use would produce a product of better quality. Furthermore, sav-
ings would be realized through reduced sewerage charges and lower chemical requirements.

**ENZYMATIC SOFTENING**

One facet of the use and re-use of reconditioned brine is poten-
tially hazardous to the briner. If any portion of the brine were con-
taminated with a polygalacturonase (PG) enzyme, the entire bulk stock of brine could become contaminated by mixing of the brines dur-
ing the reconditioning process. Re-use of this brine could lead to en-
zymatic softening.

The incidence of enzymatic softening has not followed a definite pattern in the cherry industry. The outbreaks have been sporadic. The source or sources of the pectinolytic enzymes are not entirely known, and the literature regarding the activity of these enzymes in SO₂ brines reveals varied results among investigators. Brekke and co-
workers (9) reported that the PG activity of brine from softened commercially brined cherries persisted for over one year, and cherries brined the second year in this brine produced soft fruit. Yang and Steele (10) found that the PG enzymes remained active in the brine for a period of only 13 to 25 days.

Softening of commercial lots of cherries occasionally has oc-
curred several months after brining. In 1969, a commercial sample of brined cherries was obtained which showed a PG activity three months after brining, but the enzymes became inactive after six months in the brine.

Brekke and others (9) induced softening of brined cherries under controlled conditions by adding commercial pectinolytic enzymes (Pectinol A) to the brine. However, commercial pectinolytic enzyme preparations consist of different types of polygalacturonases (exo, endo, and transeliminases) and some pectin methylesterase (PME). The PME will interfere with or change the activity of the PG enzymes by acting on and changing the pectin substrate (11).

According to McClendon and Hess (12) and Endo (13), the degree of activity and the period of activity of the pectinase enzymes are determined by the source and type of enzymes. Studies in 1969 by one of the authors of this bulletin showed that a purified fungal PG enzyme remained active for 15 weeks in cherry brine. The activity of this purified enzyme and its effect on pectins and the texture of the cherries are presented in Appendix I.
Brekke and others (9) determined that calcium chloride in the brine, in concentrations of 2 percent and greater, prevented enzymatic softening even though PG enzyme in the brine evidenced potent activity. This is believed to be due to complete calcium saturation of all available carboxyl sites on the polygalacturonic acid chain, thereby blocking off all possible positions subject to PG enzymatic attack. If this hypothesis is correct, then the calcium in the reaction is in equilibrium with hydrogen ions and the concentration of calcium chloride necessary for enzyme blockage is pH dependent, more CaCl$_2$ being required at a lower pH.

At the present stage of knowledge, the degree of activity and the period of activity of pectinolytic enzymes is not predictable. Hence, tests for the presence of polygalacturonase enzymes always should be conducted on each lot of brine before it is released for re-use. The details of the cup plate method assay for polygalacturonase enzymes are given in Appendix II of this bulletin.

A safe method of insuring against losses from enzymatic softening would be the inactivation of enzymes by heat. The used brine could be flash pasteurized to 190° F (14) and then flash cooled in a closed system. Since the pH of the used brine is usually quite high (3.2 to 3.6), insoluble calcium sulfite may be precipitated at the pasteurization temperature (15). To prevent the precipitation of calcium sulfite, it may be necessary to slightly acidify the brine with SO$_2$ to a pH of 2.8 or below before subjecting it to pasteurization temperatures. The pasteurized brine then could be treated with activated carbon in the usual manner to remove anthocyanins and other polyphenolic compounds.

**APPENDIX I**

**Action of Purified Polygalacturonase Enzyme on Pectin and Texture of Brined Cherries**

To study the effect of PG on brined cherries, a sample of purified polygalacturonase enzyme from fungi was obtained. Enzyme assay showed no PME activity, so no further purification was made.

Firm cherries which had been brined for eight months were selected. The brine was drained off the cherries and was autoclaved to inactivate enzymes if present. Purified PG enzyme was added to this brine in a concentration of 1 mg/ml and cherries were packed in the brine on a one to one w/w basis. The samples were stored at 70° F and were analyzed after 1, 3, 6, and 10 weeks in storage. At each sampling period, total pectin, water-soluble pectin, and EDTA-soluble pectin fractions were extracted according to the method of Postlmayer and others (17) and the anhydrogalacturonic acid of the extracts was measured colorimetrically by the carbazole procedure of McCready.
and McComb (18). Enzyme activity was determined according to the cup plate method of Dingle and others (16) and texture was determined with the Hunter Texture Instrument.

Figure 3 gives the total pectin and shows that PG had no effect upon total pectin content (calculated as anhydrogalacturonic acid).

Figure 4 shows the changes in the water-soluble fraction. Although the water-soluble pectin doubled after 10 weeks, the absolute amount of pectin remained insignificantly low.

Figure 5 shows the changes in EDTA-soluble pectin. The EDTA-soluble pectin is not clearly defined in the literature, but it has been associated with short, low-esterified pectin chains, bound in the tissue mainly as calcium pectate. The large amounts of calcium often found in the EDTA-soluble fractions might indicate this. Deuel and others (19) stressed, however, that the effect of calcium is not to be entirely attributed to the presence of calcium bridges between the free carboxyl groups in pectin. They found that the complete precipitation of a 50 percent esterified pectinic acid required four to five times the quantity of calcium calculated from the free carboxyl groups. As noted in Figure 5, the PG led to the pectin being almost totally extracted in the EDTA fraction after only three weeks in the brine.

The appearance of the major portion of the pectin in the EDTA fraction suggests that the EDTA extraction includes more than the

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**Figure 3.** Influence of pure polygalacturonase enzyme on total pectin.
Figure 4. Influence of pure polygalacturonase enzyme on water-soluble pectin.

Figure 5. Influence of pure polygalacturonase enzyme on EDTA-soluble pectin.
calcium pectate portion since very little de-esterification could have taken place in the absence of pectin methylesterase enzymes.

Figure 6 shows the loss in activity of the PG enzyme in storage. After 10 weeks, the activity was 22 percent and after 15 weeks in the brine the PG enzyme was inactive.

Table 5 shows the comparison of water-soluble and EDTA-soluble fractions from a commercial sample of enzymatically softened cherries and the sample to which the pure PG enzyme was added. This indicates that the enzymes in both instances converted most of the pectin into the EDTA-soluble fraction and that the water-soluble fraction increased only slightly.

Table 5. PECTIN FRACTIONS OF COMMERCIAL SOFT CHERRIES AND CHERRIES WITH ADDED PG ENZYME

<table>
<thead>
<tr>
<th>Pectin fractions (Percent of total pectin)</th>
<th>Commercial soft cherry sample</th>
<th>Pure PG enzyme added</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Med. soft fruit</td>
</tr>
<tr>
<td>Water-soluble pectin</td>
<td>2.6</td>
<td>5.8</td>
</tr>
<tr>
<td>EDTA-soluble pectin</td>
<td>46</td>
<td>78</td>
</tr>
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</table>
These data indicate that the purified fungal polygalacturonase enzyme (free from pectin methylesterase) can cause softening of the brined cherry and that the enzyme eventually becomes inactivated in the brine (Figure 6).

Figure 7 shows that a decrease in texture accompanies the PG activity. However, in this particular instance the enzyme apparently did not completely hydrolyze the pectin, as the cherries were still sufficiently firm to process after 10 weeks in the brine.

The apparent failure of the pure PG enzyme to completely break down the texture of the brined cherries is not necessarily incongruous. Schubert (20) showed that polygalacturonase activity is promoted by the presence of pectinesterases and the degree of pectin breakdown is enhanced by their presence. Doesburg (11) stated that the action of pectin enzymes are influenced by the presence and action of other pectic enzymes as well as by their substrate.

Since the pectinolytic enzymes rarely occur in the pure form but usually are associated with other pectinolytic enzymes, the variations in activity and action found by various researchers easily could be accounted for by the inter-reaction of the total pectinolytic enzyme systems working together to cause softening. Variations in systems could cause the differences found by different researchers.

![Figure 7. Texture of brined cherries treated with pure polygalacturonase enzyme.](image-url)
APPENDIX II

Cup Plate Method of Analysis for Polygalacturonase Enzymes

The following is the cup plate method for polygalacturonase enzyme assay according to the method of Dingle and others (16) as given by Watters and others (14).

One thousand milliliters of medium is prepared as follows:
1. To 500 ml distilled water in a one-quart blender jar, add 2.50 g of polygalacturonic acid.
2. While blending add 75 ml of 0.1N sodium hydroxide. (Check pH, which should be 4.0 ± 0.1).
3. Add 2.5 g ammonium oxalate and 10.0 g potassium acid phthalate. Continue to blend until completely dissolved.
4. Transfer to a 1-liter Erlenmeyer.
5. Blend 20.0 g Bacto Agar into 425 ml of distilled water and add 0.25 g thymol.
6. Transfer to a second 1-liter Erlenmeyer.
7. Steam both flasks about 30 minutes or until dissolved. (Agar will become clear above 97° C).
8. Cool contents of both flasks to 50° C; mix in a 2-liter flask.

Small stainless steel collars (8 mm dia.) may be imbedded in the media to provide cups into which a few drops of brine can be placed. The collars (¼ inch long) can be cut from stainless steel tubing.

With an eye dropper or similar device, dispense four or five drops of test sample into a cup. Incubate overnight at 35° C. At the end of the incubation period, pour 6N hydrochloric acid over the media in the plates. Allow to stand one hour and observe. If polygalacturonase is present, a clear zone will appear around the cup; the diameter will vary depending on the concentration of the enzyme.

Polygalacturonase activity is evaluated by measuring the diameter of the clear zone, including the diameter of the cup. Control samples of solutions of pectin enzymes in water or brine should be included in any series of tests. Solutions containing 1, 0.1, and 0.01 percent of pectin enzyme will give clear zones of different diameters around the cup. By comparing the sample of cherry brine under test with the enzyme solutions, one can estimate the degree of polygalacturonase activity in terms of concentration of the pectic enzyme used. A clear zone 10 mm in diameter indicates a strong positive test for polygalacturonase; samples with sufficient pectinolytic enzyme added to soften the cherries experimentally will show a clear zone of 10 mm diameter or larger. However, a brine which shows any enzyme activity whatsoever either should be discarded or be treated to inactivate the enzyme before reclaiming for re-use.
LITERATURE CITED


