Thermal Resistance of Microorganisms and Polyphenol Oxidase as Related to Solar Pasteurization of Concord Grape Juice


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(Received for publication February 9, 1981)

ABSTRACT

Pasteurization conditions for grape juice were examined and the concept of pasteurizing grape juice utilizing solar energy was explored in this study. The z-values of four selected microorganisms in grape juice were approximately 5-8 C, whereas polyphenol oxidase in fresh grapes had a z-value of 8.0 C at pH 3.4. Using a 0.5 m² solar collector test module constructed for this study, 2.5 h were required to heat the grape juice from 10 to 85 C. The solar pasteurized juice and commercially pasteurized juice were different in flavor and color but not in pH, titratable acidity or soluble solids.

Recently solar energy has received considerable attention as an alternative energy source (23). However, the feasibility of using solar energy as a supplemental energy source in direct pasteurization of fruit juices has not been tested. In general, cost effectiveness of a solar heating system increases as temperature decreases (16). Therefore, if solar energy is to be utilized, low pasteurization temperatures that do not affect the quality and storage stability of fruit juices should be used.

Commercially, grape juice is most often pasteurized after extraction, then cooled and stored in bulk tanks. After precipitation of the tartrates the juice is repasteurized and bottled. The pasteurization temperature is usually between 80 and 90 C. However, relatively little research has been published on pasteurization temperature of grape juice. Much of the earlier work was done by Pederson and coworkers (11,12,13,14,22). Their studies showed that pasteurizing grape juice at high temperature (88-90 C) was unnecessary, was detrimental to the product and resulted in a cooked flavor. They concluded that heating grape juice to 74 C was adequate for pasteurization.

Microorganisms found naturally in fruit juices include yeasts, molds and lactic and acetic acid bacteria, and these are generally heat sensitive (7,9). However, a problem which has received considerable attention over the last 10 years concerns Byssochlamys which produces heat-resistant ascospores (5). Since Byssochlamys ascospores withstand normal pasteurization temperatures, fruit juices should be screened for heat resistant molds (10). With the exception of a few heat-resistant molds (8,9), little information is available regarding thermal resistance of microorganisms in fruit juices.

The objectives of this study were to examine the thermal resistance of selected microorganisms and polyphenol oxidase in grape juice, and to explore the concept of direct pasteurization of grape juice with solar collectors.

MATERIALS AND METHODS

Grape juice samples

Concord grape juice samples were collected in October, 1980 from the Welch Foods, Inc. plant in Grandview, WA while fresh grapes were being processed. The grape juice was collected immediately before and after the first pasteurization. Another sampling of Concord grape juice was collected from the Welch Foods, Inc. bottling plant in Kennewick, WA the same week, but samples were taken immediately before the second pasteurization and immediately after bottling. The juice samples were transferred to sterile containers and kept on ice during transport to Washington State University for analyses.

For comparison purposes, commercial grape juice samples (Welch Foods, Inc.) were also purchased from a local store (Pullman, WA).

Microbial analysis

Standard Plate Counts of the grape juice were determined using Plate Count agar (PCA). Yeast and mold counts were determined using Potato Dextrose agar (PDA) acidified to pH 3.5 with 10% tartaric acid. The incubation temperature was 22 C (20). The fresh grape juice and the grape juice collected after first pasteurization and storage in a bulk tank were screened for moderately heat-resistant microorganisms. To
screen microorganisms for heat resistance, the juice samples were incubated at 32°C for 48 h and transferred to 2-ml ampules. The ampules were sealed and heated in water bath for about 1 min at 60°C. The samples were cooled immediately in ice and plated on PCA and PDA. Microorganisms surviving this heat treatment were isolated and used for thermal resistance studies. The thermal resistance of *Leuconostoc mesenteroides* (Department of Bacteriology and Public Health, Washington State University), *Saccharomyces cerevisiae* var. *Montrichard* (Department of Food Science and Technology, Washington State University) in grape juice, was also determined.

The thermal death time (TDT) tube method was used for the thermal resistance study (27). Commercial grape juice was sterilized at 121°C for 15 min in an autoclave. Sterile grape juice was inoculated with a known quantity of microorganisms and incubated at 32°C for 48 h. The juice was transferred aseptically to 2-ml ampules (TDT tube), sealed and heated in a water bath. The water bath temperature (50-70°C) was controlled to within ±0.8°C by a Haake Model D-1 circulator. The temperature of a juice sample was monitored using a copper-constantan thermocouple together with an Esterline Angus Model D-2020 Digital Data Acquisition System. When the desired temperature was reached in the juice, duplicate samples were removed, immediately cooled in ice and used to correct for the death of microorganisms during the preheating period. The time required for grape juice to reach the desired temperature was about one minute. Duplicate samples were taken at selected time intervals and cooled in ice immediately. The surviving microorganisms were enumerated using Nutrient agar, PDA or Eugon agar (Difco). *Leuconostoc mesenteroides* was plated between two layers of Eugon agar to reduce the oxygen tension.

**Polyphenol oxidase**

Fresh Concord grapes were obtained from a local store. Enzyme extraction of grape tissue was a modification of the method of Flurkey and Jen (4). Grapes (100 g) were blended for 1 min with 300 ml of cold acetone and 5 g of polyethylene glycol 6000 as a phenolic scavenger in a prechilled Waring Blender. The mixture was centrifuged at 14,000 × g for 10 min. The dried precipitate (acetone powder) was mixed with 200 ml of 0.1 M potassium phosphate buffer, pH 7.0, or 200 ml of 0.1 M potassium phthalate buffer, pH 3.4, for 20 h at 4°C using a magnetic stirrer. The mixture was centrifuged at 20,000 × g for 20 min. The filtrate was used as the crude enzyme extract. Polyphenol oxidase (PPO) activity was determined in duplicate using 1ml of 0.1 M catechol and 1 ml of the crude enzyme extract. One unit of enzyme activity was defined as the amount of enzyme extract causing a change in absorbance (420 nm) of 0.001 OD/min at 22°C (2).

To determine the thermal degradation rates of PPO, 2 ml of the crude enzyme extracts were placed in 2-ml ampules. Ampules were sealed and heated in a water bath at various controlled temperatures, removed at selected time intervals and chilled immediately in ice. Correction for thermal lag time was determined as described in the microbial analysis section. The PPO activity of the heated enzyme extract was assayed as described above. The D- and z-values were calculated from linear regression curves; standard deviation was calculated to give a direct estimate of variability about the z-value.

**Solar pasteurization**

The solar collector test module was constructed from four Owens-Illinois SUNPAK™ solar collector tubes connected in series and four aluminum reflectors. The individual collector tubes were spaced 102 mm on center and placed in front of cylindrical-shaped aluminum reflectors that directed solar radiation onto the tubes (Fig. 1). Both clear and coated tubes were used to heat the grape juice. For the coated tubes, a special selective coating on the absorber surface of each collector tube absorbed the incident solar energy and converted it to heat. The clear tubes were identical to the coated tubes except for the lack of the selective coating.

Each collector tube had three concentric circular layers of glass (Fig. 2). A vacuum region between the outer two layers reduced heat loss and protected the selective coating. Grape juice entered a tube between the intermediate and inner glass layers and traveled from the lowest end to the highest end of the tube as it absorbed heat from the intermediate glass layer. The heated juice flowed down the inner glass tube and to the next collector tube where it was heated further. The total effective surface area of the test module for intercepting solar radiation was approximately 0.5 m². During any given test the solar collector module remained in a stationary position inclined 25° from the horizontal and facing south. Maximum radiation intensities on the collector surface were approximately 1100 Watt/m² within two days of collection, about 4 L of fresh Concord grape juice or once pasteurized grape juice were heated in the solar pasteurization module. The grape juice was recirculated in the solar collector until it reached the desired temperature. Juice samples were withdrawn aseptically from the collector at selected temperatures for SPG and yeast and mold counts. Separate pasteurization tests were conducted using clear collector tubes and tubes with selective coatings. After pasteurization, the grape juice was withdrawn from the collector and stored at 4°C for quality evaluation.

**Quality evaluation of grape juice**

The solar pasteurized juices, the bottled juice obtained from Welch and the commercial juices purchased from a local store were analyzed for pH, titratable acidity and soluble solids. Titratable acidity (TA) was determined by titrating with 0.1N NaOH to an end point of pH 8.1 using a pH meter (1). The soluble solids (SS) were determined using an Abbe refractometer (Bausch and Lomb). The visible spectrum of the diluted juice samples was obtained with a Beckman Model 35 scanning spectrophotometer. Duplicate samples were used in the analysis.

To determine whether the solar pasteurized grape juice and commercial juice were different in flavor and aroma, the juice heated to 80°C

![Diagram of Collector Tube](https://example.com/collector_tube_diagram.png)

**Figure 1. Cylindrical shaped aluminum reflectors under collector tubes.**

**Figure 2. Fluid flow direction inside collector tube.**
in the coated tube collector and grape juice purchased in a local store were subjected to two separate triangle tests. The panelists consisted of 17 members of the Department of Food Science & Technology at Washington State University. In both trials, each panelist was given two sets of juice samples. Each set consisted of two identical samples and one different sample. Color was not masked because we were unable to detect color differences among preliminary samples. The panelists were asked to identify the odd sample in each set based on flavor and aroma. They were also asked to comment on the differences among the juices and the reasons for their selection. The juice was served at temperatures between 5 and 10 °C.

RESULTS AND DISCUSSION

Microbial analysis

Five moderately heat-resistant microorganisms which survived at temperatures higher than 60 °C were isolated from Concord grape juice samples collected before and after initial pasteurization using PCA and Eugon agars. These five microorganisms were incubated in sterile grape juice at 32 °C for 48 h before receiving the heat treatment.

Two organisms were isolated from raw grape juice before pasteurization. The first organism was a gram-negative rod, possibly belonging to genus Acetobacter, which did not grow after incubation in grape juice. It was inactivated after 3 min of heating at 60 °C. The second organism was a gram-positive, sporeforming Bacillus which survived 10 min of heating at 80 °C. It did not grow in sterile grape juice. Three other microorganisms were isolated from grape juice which had undergone the first pasteurization in the processing plant. One organism was a gram-positive, non-sporeforming rod which was inactivated after 2 min of heating at 76 °C. Another organism was a brown pigmented yeast which was inactivated after 1 min at 74 °C. The fifth organism, a nonpigmented yeast, was inactivated after 2.5 min at 60 °C. The population of the last three microorganisms was 10^2-10^5/ml after incubation. The brown and nonpigmented yeasts were used for thermal death time studies later.

Survivor curves for L. mesenteroides at four different temperatures are shown in Fig. 3. The curves for the other microorganisms were similar and are not presented. L. mesenteroides showed little heat resistance at temperatures above 65 °C. Plots of D-values vs. temperature for L. mesenteroides, S. cerevisiae, the brown pigmented yeast (Y1) and the nonpigmented yeast (Y2) isolated from grape juice are shown in Fig. 4. The z-value for L. mesenteroides was 7.68 ± 0.14 °C. The z-values for the brown and nonpigmented yeasts were 5.62 ± 0.51 °C and 5.44 ± 0.01 °C, respectively. These z-values are comparable to those obtained for Byssochlamys fulva asc (z = 5.7 °C) in buffer at pH 3, and Penicillium sclerotia (z = 4.8 °C) in diluted blueberry juice (9). Saccharomyces cerevisiae is less heat resistant than the other two microorganisms. It has a D-value of 55 s at 48 °C and a z-value of 5.6 ± 0.09 °C between 45 and 55 °C. These relatively small z-values indicate that the thermal destruction of these organisms is highly temperature dependent.

None of the four organisms studied had a D-value above 5 s at 70 °C (Fig. 4). The problem with heat-resistant mold spores, such as B. fulva, as indicated by Meyrath (9) and Murdock and Hatcher (10), was not observed in our samples. The extensive clarification and filtration treatment used in the plant probably helped to eliminate this problem.

Polyphenol oxidase

Figure 5 shows the thermal inactivation curves of PPO in fresh grapes at pH 3.4 and 7.0. The variation of PPO activity of duplicate samples was within 10%. PPO activity was higher at pH 3.4 (300 units per g of fresh grape) than at pH 7.0 (140 units/g). However, PPO was more heat sensitive at pH 3.4 than at pH 7.0. The z-values of PPO in Concord grape juice were 7.9 °C at pH 3.4 and 13.8 °C at pH 7.0. At pH 7.0, there was little or no decrease in PPO activity during the come-up time. This was not the case at pH 3.4. For example, the PPO activity for the unheated sample and the zero-time sample at 115 °F were 300 and 200 units/g, respectively.

The thermal stability of PPO in pears was investigated by Halim and Montgomery (6) who found the D-value of the crude enzyme extract (pH 7.0) to be 8.4 min at 80 °C. PPO in pears is considerably more heat stable than was PPO in the Concord grades used in this study. Relatively little information is available on the thermal stability of PPO in grapes. No PPO activity was detected in grape juice collected after the first pasteurization and storage. Apparently PPO is not a problem in grape juice pasteurization.
Solar pasteurization

Two or more pasteurization tests were conducted for each sample. Typical heating times and maximum temperatures reached during pasteurization of the grape juice are shown in Table 1. The coated tubes were more efficient than the clear tubes in absorbing solar radiation and heating the grape juice. The selective coating was designed to improve absorption of solar energy. The efficiency of the solar collector was dependent on incident radiation and heating requirements of the system. About 2.5 hours were required to heat 4 L of grape juice from 10 to 85°C in four coated tubes. When using four clear tubes, 3 hours or more were needed to heat four tubes of juice to 70°C under similar radiation conditions. It should be noted that the solar pasteurization tests were conducted in October, which was a time when the solar radiation intensity was relatively low. The heating time could have been reduced considerably if pasteurization was done during June, July or August, if the collector surface area per unit of juice was increased, or if the required temperature between entering and exiting juice was decreased.

Since grapes are harvested in fall, solar pasteurization does not appear to be practical for pasteurization of raw grape juice. Solar radiation could be used as a supplemental energy source for repasteurization of grape juice after bulk storage. Further research is being done using a 24-tube solar energy collector equipped with a double-tube heat exchanger.

**TABLE 1.** Titratable acidity, pH and soluble solids of grape juices before and after solar pasteurization.

<table>
<thead>
<tr>
<th>Grape juice</th>
<th>Heating time (min)</th>
<th>Maximum temp (°C)</th>
<th>TA (meq/100ml)</th>
<th>pH</th>
<th>SS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>-</td>
<td>-</td>
<td>11.8</td>
<td>3.30</td>
<td>18.3</td>
</tr>
<tr>
<td>Before</td>
<td>170</td>
<td>87</td>
<td>11.5</td>
<td>3.30</td>
<td>17.8</td>
</tr>
<tr>
<td>After</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-pasteurized</td>
<td>-</td>
<td>-</td>
<td>11.6</td>
<td>3.35</td>
<td>15.0</td>
</tr>
<tr>
<td>Before</td>
<td>160</td>
<td>85</td>
<td>11.3</td>
<td>3.30</td>
<td>15.0</td>
</tr>
<tr>
<td>After</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commercial (store)</td>
<td>-</td>
<td>-</td>
<td>10.3</td>
<td>3.40</td>
<td>16.0</td>
</tr>
<tr>
<td>Before</td>
<td>180</td>
<td>70</td>
<td>10.3</td>
<td>3.40</td>
<td>15.0</td>
</tr>
<tr>
<td>After</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. Thermal Destruction curves of selected microorganisms in grape juice.

Figure 5. Thermal inactivation curve of polyphenol oxidase in Concord grapes.
TABLE 2. Change in microbial counts of raw and pre-pasteurized grape juice during solar pasteurization.

<table>
<thead>
<tr>
<th>Heating time (min)</th>
<th>Maximum temp (°C)</th>
<th>SPC (CFU/ml)</th>
<th>Yeast/Mold count (CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Raw juice</td>
<td>Pre-pasteurized juice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>88</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>102</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>115</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>130</td>
<td>75</td>
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<td></td>
<td></td>
<td>140</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>165</td>
<td>85</td>
</tr>
</tbody>
</table>

Quality evaluation of grape juice

Table 1 shows the mean TA, pH and SS values of grape juice samples before and after pasteurization. These values did not vary for replicate samples pasteurized on different days. As shown in Table 1, little or no change in pH, TA and SS of the grape juice samples was observed after solar pasteurization. The apparent loss of solids in grape juice during bulk storage may be attributed to crystallization of tartrates and sedimentation. The raw and pre-pasteurized juices were heated in the coated tubes, whereas the commercial juice purchased from the store was heated in the clear tubes. Typical microbial counts of the grape juice samples for the raw and pre-pasteurized grape juice withdrawn from solar collectors at 60-85 °C are given in Table 2. Microorganisms in the raw grape juice were generally more variable and heat resistant than the yeasts observed in the pre-pasteurized juice. Heating the grape juice to 75 °C in about 2 hours was adequate for pasteurization.

Figure 6 shows the visible spectra of the pre-pasteurized juice sample taken immediately before the second pasteurization (pre-pasteurized), the sample after the second pasteurization and bottling (bottled), and the pre-pasteurized sample after being heated in the solar collector to 80 °C (solar pasteurized). The anthocyanin pigments that predominate in grape juice color have a maximum absorbance at about 520 nm in aqueous solution (3,14). Heating resulted in decreased anthocyanin pigments which give the characteristic red color of the grape juice. Based on the absorbance at 520 nm, more color loss was expected and observed during solar heating than during commercial pasteurization. We also recognize that temperature or degree of heat treatment may have a significant effect on retention of anthocyanins (15,18,19).

Grape juice pasteurized in the solar collector and in the commercial plate heat exchanger were subjected to two separate triangle tests. Preliminary trials indicated that color differences between the solar-pasteurized and the commercial samples were not noticeable. Panelists were asked to select the odd sample based on flavor and aroma. In the first trial, 18 out of 34 judgements were correct in choosing the odd sample. In the second trial, 19 out of 34 judgements were correct. Both tests indicated that there was a significant difference (P<0.01)
in flavor between the two juices (17). Comments from panel members who made the correct judgements indicated that solar pasteurized grape juice had less flavor intensity than commercially pasteurized grape juice.

CONCLUSIONS

Based on thermal resistance data obtained in this study, most spoilage organisms in grape juice showed little heat resistance at 70 °C or higher. A pasteurization temperature of 85 °C could be attained with the laboratory solar collector module constructed. Coated tubes were more efficient than clear tubes in heating grape juice. Even with the coated tubes, however, about 2.5 hours were required to heat 4 L of grape juice from 10 °C to 85 °C when the effective solar collector area was 0.5 m². Heating grape juice to 70 °C in about 2 hours with the solar collector appeared to be adequate for pasteurization. Triangle tests indicated that the taste of solar-pasteurized grape juice and commercially pasteurized grape juice was significantly different in flavor and aroma. Substantial improvement in performance of the solar collector is needed before it can be utilized for adequate pasteurization of grape juice. Since the first pasteurization is done only during the harvest season for Concord grapes, the concept of solar pasteurization is practical only for pasteurization of grape juice out of bulk storage which is done throughout the year.

ACKNOWLEDGMENTS

The authors thank Dr. C. W. Nagel and Dr. H. M. Nakata for providing cultures. This study was supported in part by a grant from the U.S. Department of Agriculture (Grant No. 58-7B30-9-54).

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