in fruits, vegetables, and ornamentals from countries other than the United States. The hot-air treatment appears to be useful to disinfest many different commodities. A disadvantage of the treatment is that air, compared with water, is a poor conductor of heat. Thus, several hours are required to heat commodities with air before disinfestation occurs. Also, fruit must be arranged so that resistance to air flow is not a factor that contributes to an excessively long exposure time.

A commercial scale heat treatment facility was built for packinghouses in Hawaii. Williamson and Winkelman (1989) reported that the best heating of fruits was obtained using five trays each with a single layer of fruit and treating the fruit with a reversible vertical air flow. Properly heating fruits for a time period that provides quarantine security and does not damage fruit quality are major constraints that must be addressed. Until a commercial hot-air treatment facility is economical to build and operate, industry will be reluctant to use the technology.

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METHODS TO LIMIT ELLAGIC ACID PRECIPITATION IN MUSCADINE JUICE AND WINE

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Additional index words. Vitis rotundifolia, phenolic compounds.

Abstract. **The production of muscadine grape juice and wine continues to expand in Florida. However, the formation of an ellagic acid sediment in these products limits their overall quality. A series of studies were conducted to evaluate the effects of processing and storage conditions on ellagic acid sediment formation. Heat pasteurized juice had much more sediment formation than sterile-filtered juice, and sediment formation was greater at higher storage temperatures. Ultrafiltration of the juice through a 30,000 or 10,000 MWCO membrane also reduced sediment formation. The addition of PVPP or gelatin reduced sediment formation, and sediment formation was greater in juice treated with a commercial pectinase. Red muscadine wines had much more sediment formation than juice, and sediment levels increased significantly with longer skin fermentation times (4 or 6 days).**

Muscadine grapes (*Vitis rotundifolia*) are the main species of grapes grown in Florida. These grapes are used commercially as fresh fruit and for wine and juice production (King et al., 1988; Morris, 1981). The muscadine grape industry has expanded in the Southeast, but is still limited by several problems (Olien, 1990). One of the major quality defects hindering muscadine juice and wine is the formation of a precipitate or sediment after bottling (Boyle and Hsu, 1990). Researchers have examined this sediment and concluded that it is composed entirely of ellagic acid (Boyle and Hsu, 1990; Lin and Vine, 1990).

Ellagic acid is a phenolic compound found in several fruits such as strawberries and is reportedly an antimutagen and anticarcinogen (Maas et al., 1991). The only other record of ellagic acid sedimentation found in wines or juices was in a loganberry wine (Singleton et al., 1966). Ellagic acid has not been reported in grape species other than *Vitis rotundifolia*, but wines aged in oak may contain ellagic acid derived from the wood (Quinn and Singleton, 1985). Ellagic acid is likely present in muscadine wines and

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juices as hydrolyzable tannins or polymeric phenolic compounds, which can undergo hydrolysis to yield ellagic acid (*Quinn and Singleton,* 1985). Free ellagic acid is very insoluble and would precipitate, resulting in sediment formation.

Processing and storage conditions can significantly affect the phenolic levels in grape juice and wine (Macheix et al., 1991). Fining agents such as gelatin, polyvinylpolypyrrolidinone (PVPP), egg albumen and casein have been shown to reduce the phenolic levels in juices and/or wines (Rossi and Singleton, 1966; Singleton, 1967; Ough, 1960; Zoecklein et al., 1990). Gelatin and PVPP have been reported to reduce ellagic acid levels in muscadine juice, but the effects of these treatments on sediment formation were not reported (Lin and Vine, 1990). Ultrafiltration (UF) membranes have also been shown to alter the color and phenolic levels of wines and juices (Peri et al., 1988; Sims et al., 1988). The extent of pressing or juice extraction and skin fermentation (for red wines) could also have major impacts on the amount of ellagic acid sediment formation since the seeds and skins of muscadines are major sources of ellagic acid (Boyle and Hsu, 1990).

The objectives of this research were to determine the effects of temperature, fining agents, ultrafiltration and storage on ellagic acid precipitation in white muscadine juice, and the effects of skin fermentation time on ellagic acid precipitation in red muscadine wines.

Material and Methods

Processing and Storage Temperature Study. Dixie muscadine grapes were crushed, treated with 50 mg SO_2 (as potassium metabisulfite)/kg, pressed in a hydraulic basket press, and the juice frozen at –20°C until needed. Thawed juice was filtered through a 0.45 μm membrane cartridge and divided into two equal portions. One portion was bottled into 200 ml glass bottles, pasteurized for 10 min at 100°C and air cooled. The other portion was treated with 200 mg sorbic acid/liter juice, filtered through a sterile 0.45 μm membrane cartridge and bottled. Heat-pasteurized samples were stored at 1.5°, 25° and 40°C, while sterile-filtered samples were stored at 1.5°C only. Samples were analyzed in duplicate every 2 mo for soluble ellagic acid, total phenolics and ellagic acid sediment in the juice.

To assess the effects of heat-acid hydrolysis on the extent of ellagic acid precipitation, juice was filtered through a 0.45 μm membrane, acidified to pH 2.0 with sulfuric acid, and heated in an autoclave at 121°C for 10 min. The pH of the juice was adjusted back to the original with 1 N NaOH, and the juice was filtered through a 0.45 μm membrane, bottled in 200 ml glass bottles and pasteurized at 100°C for 10 min. Samples were stored at 25°C and the amount of ellagic acid sediment was measured after 2 mo.

Ultrafiltration Study. Thawed white muscadine juice was filtered through a 0.45 μm membrane cartridge and then ultrafiltered through one of three molecular weight cut-off (MWCO) ultrafiltration membranes (Amicon spiral-wound cartridge type SLY10, SLY30 and SLY100 with MWCO of 10,000, 30,000 and 100,000 daltons, respectively). The juice was bottled in 200 ml glass bottles, pasteurized at 100°C for 10 min and air cooled.

Juice was stored at 25°C and analyzed every 2 mo as described above. All treatments were duplicated and all analyses were performed in duplicate. Data were analyzed by analysis of variance using SAS, and least significant difference (LSD, 5% level) was used to separate means.

Press Fraction Study. White muscadine grapes (a breeding line, AD3-42) were crushed, treated with 50 mg SO_2/kg and divided into two equal portions. One portion was enzyme treated with 200 mg/kg pectinase (pectinol 80-SB, Genencor, South San Francisco, CA) and then pressed in a hydraulic basket press, while the other portion was not enzyme treated before pressing. Crushed grapes were poured in the press and the free run juice was collected. The grapes were then pressed at 300 psi and the extracted juice collected separately (hard press). Juice was filtered though a 0.45 μm membrane cartridge into 200 ml glass bottles. After heat pasteurization at 100°C for 10 min, juice was stored at 25°C and analyzed every 2 mo as described above. All treatments were duplicated and the data were subjected to analysis of variance as previously described.

Fining Agents Study. Thawed white muscadine juice was treated with two levels of each of three fining agents, stored overnight at 1.5°C and then filtered through a 0.45 μm membrane into 200 ml glass bottles. Fining agents used were egg albumen (0.5 and 1.0 g/liter juice; from fresh eggs), polyvinylpyrrolidone (Polyclar AT; 0.1 and 0.2 g/liter juice), and gelatin (75 bloom; 0.05 and 0.4 g/liter juice). Gelatin was hydrated in warm water to obtain a 1% solution before adding to juice, while the PVPP was added directly to the juice. Egg whites were separated from whole eggs and added to the juice at the levels mentioned above. The juices were pasteurized at 100°C for 10 min, stored at 25°C and analyzed every 2 mo as described above. All treatments were duplicated and analyses were performed in duplicate. Data were subjected to analysis of variance as described above.

Red Wine Study. Red muscadine grapes (Noble) were crushed, treated with 50 mg/kg SO_2, and inoculated with Prise de Mousse yeast. A portion of the grapes was pressed immediately (no skin fermentation) while the remaining crushed grapes were allowed to ferment with the skins at 27°C for 2, 4, or 6 days before pressing. After pressing, the juices were adjusted to 20% soluble solids (based on the original soluble solids) with cane sugar and allowed to ferment to dryness in glass carboys at 13°C. The wines were then cold-stabilized, filtered and bottled. Ellagic acid sediment and total phenol levels were determined after 1 year of bottle aging.

Analyses. Preliminary studies (data not shown) and previous research (Boyle and Hsu, 1990) indicated that the sediment formed in muscadine juice under normal storage conditions was composed only of ellagic acid. As a consequence, the amount of sediment was estimated by measuring the amount of ellagic acid in the sediment by HPLC. The sediment in a 200 ml juice sample (or a 750 ml wine sample) was isolated by filtering juice through a 0.45 μm membrane (47 mm, nylon). The membrane was washed with 35 ml water to remove any water soluble compounds. The membrane was then carefully removed and immersed in 50 ml methanol, stirred for 1 hr, allowed to stand 12 hr, and stirred again for another 1 hr. The methanol extract was brought to a final volume of 50 ml, filtered through a 0.45 μm membrane and used for HPLC analysis for ellagic acid.

Ellagic acid in the methanol extract and juice was quantified using a HPLC procedure (Boyle and Hsu, 1990).

The HPLC system was a Hewlett Packard liquid chromatograph model 1090 controlled by a HP 85B personal computer, a C_{18} reverse phase μ-bondapack column (10 micron packing, 2.5 × 300 mm) (Phenomenex, Rancho Palos Verdes, CA), and a Phenomenex μ-bondaclone precolumn (3.9 × 20 mm). The effluent was monitored with a diode array detector (set for a wavelength of 255nm) and a DPU multichannel integrator. The binary solvent system used was a linear gradient from 10% (B) to 98% (B), at a flow rate of 1 ml/min in a 15 min period. Solvents were extra-purified water (A) adjusted to pH 2.5 with 0.6 M perchloric acid and 100% methanol (B). All analyses were carried out at room temperature (ca 25°C). Injection volume was 10 μl of the methanol extract or juice. Standard curves for ellagic acid were used for quantification.

Total phenolics in the juice were determined by the Folin-Ciocalteu assay (Zoecklein et al., 1990). Ellagic acid was used as the standard, and total phenolic concentration was expressed as mg/liter ellagic acid.

Results and Discussion

Processing and Storage Temperature Study. Higher storage temperatures accelerated sediment formation (Fig. 1), with juice stored at 1.5°C having a very slow rate of sediment formation. The apparent decrease in ellagic acid sediment at 40°C after 6 and 8 mo could have been due to hinderance in ellagic acid extraction from the sediment by heat-unstable proteins that likely precipitated at this high storage temperature. The non-heated juice did not develop detectable levels of ellagic acid sediment during storage at 1.5°C for 8 mo, while the levels of ellagic acid in the heated juice started increasing after the second month and reached a level of ca 5 mg of sediment per liter of juice after 8 mo (data not shown).

Juice subjected to a heat-acid hydrolysis showed a very rapid (less than 2 weeks) and substantial precipitation of ellagic acid when compared to the non-hydrolyzed control, which did not develop a sediment during 2 mo. This suggests that the most probable source of ellagic acid in the juice is the hydrolysis of a tannin containing ellagic acid. Free ellagic acid released from the hydrolysis of the tannin would probably precipitate very quickly due to its insolubility. From a practical standpoint, heat could be used to hasten sediment formation before bottling, provided flavor and color degradation were not a problem. Cold storage could likewise be used to delay sediment formation.

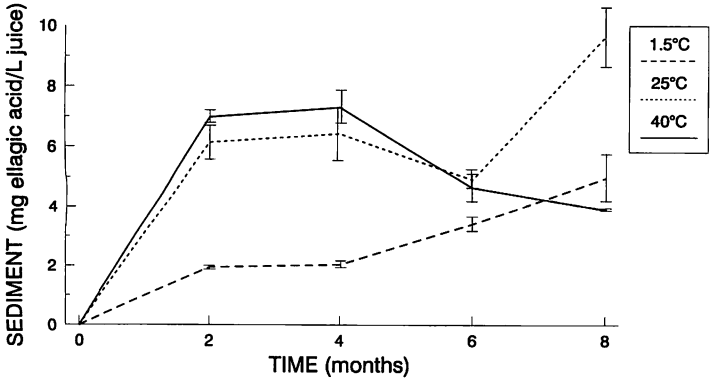

Fig. 1. Ellagic acid sedimentation in heat-pasteurized white muscadine grape juice at different storage temperatures. Bars at each sampling represent standard deviations.

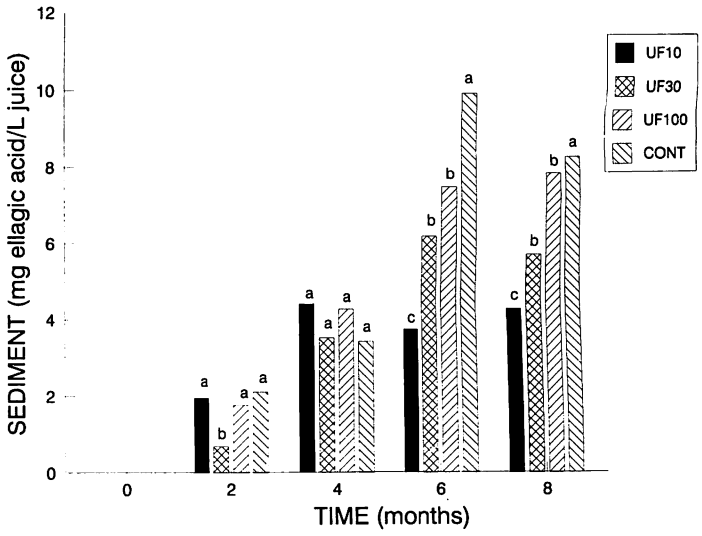

Fig. 2. Ellagic acid sedimentation in white muscadine grape juice as influenced by ultrafiltration (CONT = control, UF100 = 100 000, UF30 = 30 000 and UF10 = 10 000 MWCO membranes). Storage temperature was 25°C. Means within each storage time followed by the same letter are not significantly different (LSD, 5% level).

Ultrafiltration Study. Juice filtered through the 10,000 MWCO membrane had the lowest level of sediment after 6 and 8 mo, followed by juice filtered through the 30,000 and 100,000 MWCO membranes (Fig. 2). The amount of sediment formed did not relate to the total phenolic levels in the juice since no significant or consistent differences were found in total phenolic levels between the UF and non-treated samples (data not shown). The UF membranes could have retained ellagic acid containing tannins, but the concentrations of these compounds could have been too small to be detected by this non-specific total phenolic assay.

Soluble ellagic acid was not detected in the juices until 4 mo, but there was no consistent effect of UF on ellagic

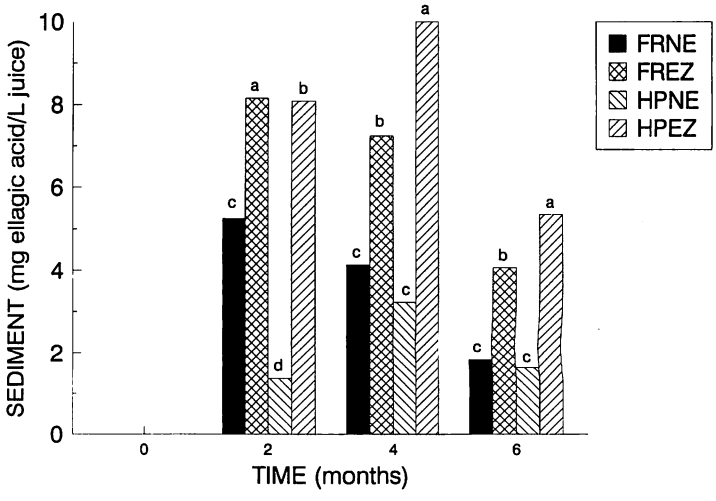

Fig. 3. Ellagic acid sedimentation in white muscadine grape juice as influenced by press fraction (FR = free run, HP = hard press) and treatment with a commercial pectinase (NE = no enzyme, EZ = enzyme treatment). Storage temperature was 25°C. Means within each storage time followed by the same letter are not significantly different (LSD, 5% level).

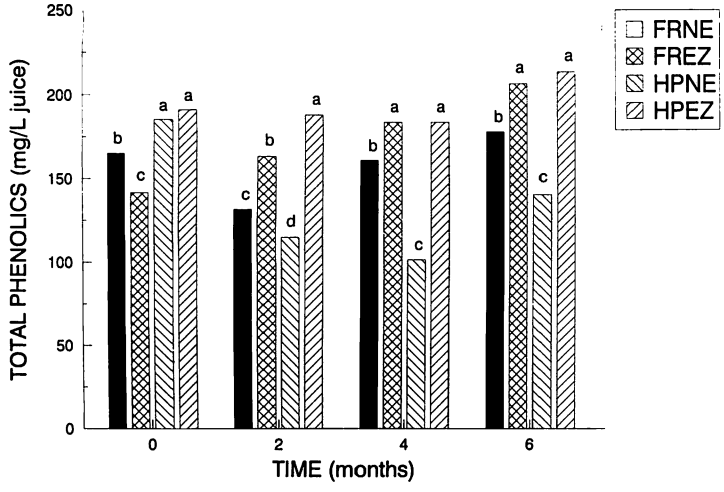

Fig. 4. Total phenolic levels in white muscadine grape juice as influenced by press fraction (FR = free run, HP = hard press) and treatment with a commercial pectinase (NE = no enzyme, EZ = enzyme treatment). Storage temperature was 25°C. Means within each storage time followed by the same letter are not significantly different (LSD, 5% level).

acid levels, nor was there any correlation with sediment formation (data not shown). Apparently, very little free ellagic acid remains soluble in the juice after being released from the tannins.

Press Fraction Study. The use of pectinase significantly increased sediment formation after all storage times (Fig. 3). The free run fraction had similar sediment formation as the hard press fraction when pectinase was not used, but the hard press fraction from enzyme-treated grapes had significantly more sediment formation than the free run.

The amount of sediment in the juice related fairly well to total phenolics in the juice after 2, 4 and 6 mo (Fig. 4). Juice from enzyme-treated grapes had significantly higher levels of total phenolics than juice from the non-treated

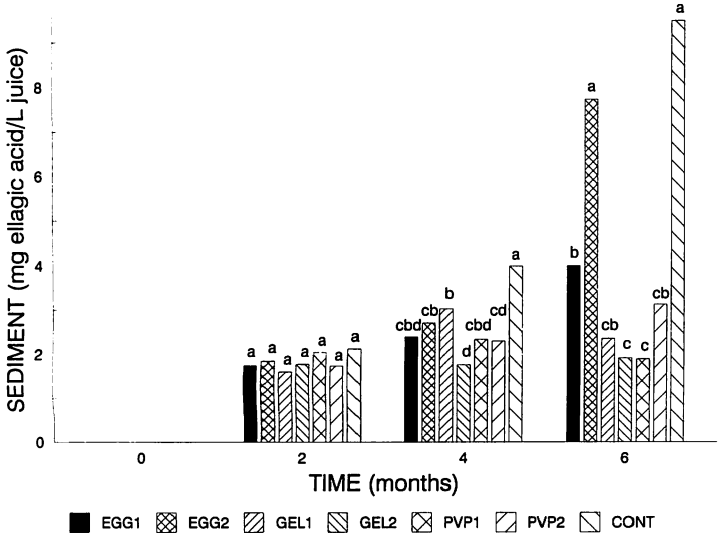

Fig. 5. Ellagic acid sedimentation in white muscadine grape juice as influenced by fining agents (EGG1 and EGG2 = fresh egg albumen at 0.5 and 1.0 g/L juice; GEL1 and GEL2 = gelatin at 0.05 and 0.4 g/L juice; PVPP1 and PVPP2 = polyvinylpyrrolidone at 0.1 and 0.2 g/L juice; CONT = control). Storage temperature was 25°C. Means within each storage time followed by the same letter are not significantly different (LSD, 5% level).

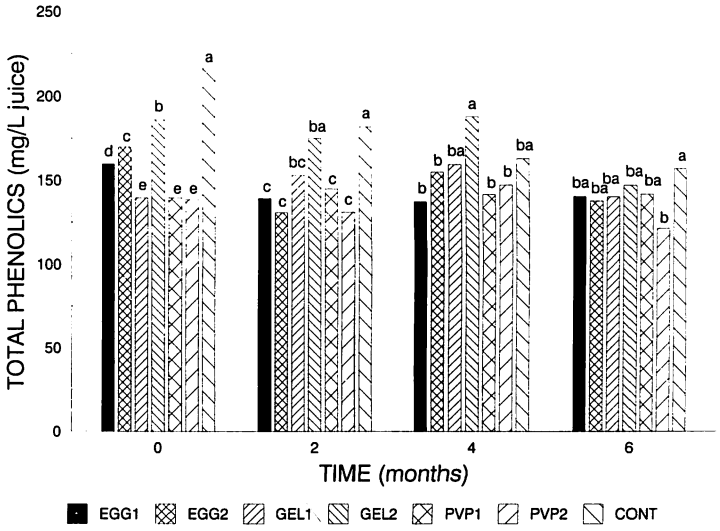

Fig. 6. Total phenolic levels in white muscadine grape juice as influenced by fining agents (EGG1 and EGG2 = fresh egg albumen at 0.5 and 1.0 g/L juice; GEL1 and GEL2 = gelatin at 0.05 and 0.4 g/L juice; PVPP1 and PVPP2 = polyvinylpyrrolidone at 0.1 and 0.2 g/L juice; CONT = control) Storage temperature was 25°C. Means within each storage time followed by the same letter are not significantly different (LSD, 5% level).

grapes after 2, 4 and 6 mo. Hard press juice from non-treated grapes had lower levels of total phenolics than the free run fraction, while the opposite was observed in juice from enzyme-treated grapes. No obvious trend was observed in the amount of soluble ellagic acid found in the juice (data not shown), which agrees with the results from the previous study.

Most of the total phenols in grapes are contained in the skin and seeds (Singleton and Esau, 1969). However, since muscadine grapes have a very thick, tough skin, extraction (press) pressure used in this study was probably insufficient to extract significant levels of phenolic compounds from the skins unless pectinase was used. Harder pressing regimes, or greater skin maceration, would probably result in more ellagic acid sediment formation.

Fining Agents. Sediment formation in the juice was reduced by all fining treatments after 4 and 6 mo (Fig. 5). Gelatin and PVPP were generally more effective in reducing sediment formation than egg albumin. There were no significant differences in sediment formation between the 2 levels of gelatin or PVPP, which seems to indicate that the concentrations used may have been excessive. All fining agents reduced the total phenolic levels initially and after 2 mo (Fig. 6), but there was little difference in the total phenolic levels among the treatments after 4 and 6 mo. The ellagic acid-containing tannins were probably reduced by the initial decrease in total phenolics by the fining

Table 1. Effects of skin fermentation time on the total phenols and ellagic acid sediment in Noble wine after 1 year of aging.

Skin fermentation time (days)	Total phenols (mg/L)	Ellagic acid sediment (mg/L)
0	171 d[z]	0.8 c
2	752 c	7.1 b
4	1114 b	27.4 a
6	1301 a	26.8 a

[z]Means followed by the same letter are not signfificantly different (LSD, 0.05).

agents, which resulted in lower amounts of ellagic acid precipitate. The levels of soluble ellagic acid in the juice did not relate very well to the sediment formation in the juice (data not shown), which agrees with results from the previous studies.

Red Wine Study. Skin fermentation time had a large impact on the ellagic acid sediment formation in a red muscadine wine. Wines fermented on the skins had much higher levels of ellagic acid sediment than the immediate press wine (no skin fermentation) (Table 1). Levels of ellagic acid sediment increased significantly through 4 days of skin fermentation, then remained relatively constant between 4 and 6 days. Total phenol levels in the wine also increased with skin fermentation time, with the largest increase occurring between 0 and 4 days. Since grape skins are known to contain relatively high levels of phenolic compounds, longer skin fermentation time probably extracted more of the ellagic acid containing tannins. The levels of sediment formed in the red muscadine wine were considerably higher than levels formed in a white juice, which tends to illustrate the contribution of skin tannins to the sediment problem.

Conclusions

Heat pasteurization and high storage temperatures accelerate ellagic acid sediment formation in white muscadine juice. Treatment of the juice with the fining agents gelatin, egg albumen and PVPP reduced sediment formation. A commercial pectinase added to crushed grapes increased total phenolics and sediment formation in a white muscadine juice. Ultrafiltration membranes (10,000, 30,000 and 100,000 MWCO) were also effective in reducing sediment formation in the white juice. A red muscadine wine had much more sediment than the white juice, and longer skin fermentation times greatly increased sediment formation and total phenols in a red muscadine wine. More research is needed to determine the specific phenolic and tannin composition of muscadine grapes.

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CHANGING TECHNOLOGY IN CITRUS PROCESSING

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Abstract. **Changes in citrus juice market demands over the past 60 years have driven developments in technology which have resulted in higher quality juices. These changes in market demands are discussed and their impact on technological development is also explored. Specific technologies in extraction, finishing, concentration, pasteurization, automation, juice quality enhancement and by-product recovery are discussed. Finally, future trends in processing technology and fresh fruit packing technology, such as automated harvesting and non-thermal pasteurization of juice, are reviewed and several potential emerging technologies are discussed.**

Changes in Citrus Juice Markets

The development of the commercial citrus industry started with Spain's cession of Florida to the United States in 1821. The propagation of citrus in both Florida and California followed. Later, the industry concentrated in four states (Florida, California, Arizona, and Texas). Originally, the citrus industry was developed for the fresh fruit market. Fruit production increased at an accelerated pace by the 1930's, creating a fresh fruit oversupply. This surplus of fruit was the major factor in the development of different citrus products to increase consumption.

The unsalable fruit began to be expressed and sold as single-strength fresh orange juice which was processed and packed in glass and cans. Although the introduction of these two different products allowed the expansion of the shelf life and distribution of orange juice, the quality of these products was poor and their market never expanded