AN ABSTRACT OF THE THESIS OF

Jose H. Flores Gaytan for the degree of Master of Science in Food Science and Technology presented on December 16, 1983

Title: The Influence of Different Processing Procedures on Strawberry Juice and Wine Quality

Abstract approved: Dr. David A. Heatherbell

This study was conducted to optimize the extraction of juice and color from strawberry fruit without using thermal processing and to investigate the color quality and color stability of the strawberry wines fermented from the juices extracted.

The effect of different milling (Hammer mill) and pre-press pulp treatments, and of three different commercial pectinases, a cellulase and a protease, on the extraction of juice and color from frozen strawberry fruit was investigated. The optimum pre-press milling and enzyme pulp treatment conditions for release of free run juice (FRJ) determined on a laboratory-scale were incorporated into larger scale pilot-plant press trials using a Willmes bag press.

The nature of the fruit (fresh or frozen), milling speed and mill screen size all affected release of FRJ from crushed fruit. Highest yields were obtained when excessive crushing was avoided by milling partially thawed fruit at low speeds.

Pectinases containing high pectin esterase activity were most effective in releasing FRJ. Pre-press enzyme treatment of the pulp

increased yields of FRJ from 28% (untreated pulp) up to 56%. However, when press juice was combined, total juice yields of approximately 86% were obtained for all treatments.

Pre-press enzyme treatment of the pulp increased anthocyanin pigment extraction by up to 50%. The anthocyanin pigment extracted varied with the different enzymes, the pectinases with known "side-activities" and in combination with proteases, extracting the highest concentration of anthocyanins. All juices had low polymeric color, and percent polymeric color, indicating that most of the pigmentation of these juices is due to monomeric anthocyanins.

The influence of different processing procedures on the quality of strawberry wine, in particular on wine color quality and stability, was investigated. Wines were prepared by fermenting juices extracted by standard press (Willmes Bag Press) procedures including different pre-enzyme treatments, by pulp fermentation and by processing in the presence and absence of sulfur dioxide (SO_2). The following color parameters were determined in the fermented juice ("young" wine) and in the bottled wine during a four month storage period at 25°C: anthocyanin pigment content, polymeric color, color density, browning, haze and Hunter L, a, b values. In addition SO_2 and ascorbic acid were also determined.

Large decreases in anthocyanin pigment content and color density occurred as a result of fermentation.

Storage time had a significant effect ($p<0.05$) on all the color parameters (except color density), sulfur dioxide, and ascorbic acid, for all the different processing procedures and enzyme treatments.

The different processing procedures and enzyme treatment had a significant effect ($p<0.05$) on color density, polymeric color, browning, haze and Hunter L, a, b values. Anthocyanin pigment content was significantly affected ($p<0.05$) by the different enzyme treatments. However, none of the different processing procedures and enzyme treatment helped to stabilize anthocyanin degradation throughout the storage period. Enzyme wines had lower Hunter L, a, b values than the other processing procedures. Sulfur dioxide had a protective effect against anthocyanin degradation during mid-storage period. Likewise, SO_2 had an inhibition effect on browning and polymeric color development.

The following correlations were found in all wines: a) SO_2, anthocyanin pigment content, and ascorbic acid were positively correlated, b) ascorbic acid was negatively correlated with browning index and polymeric color, c) polymeric color, percent polymeric color and browning index were positively correlated, d) anthocyanin pigment content was positively correlated with Hunter a values, and e) SO_2 was negatively correlated with polymeric color.

Sensory evaluation of the wines determined significant differences in clarity among the different processing procedures, and in color intensity, hue and clarity among the different enzyme treatments. In addition, correlation analysis of the sensory evaluation data vs. the parameters analyzed on the wines gave the following correlations: a) Browning was negatively correlated with color density, polymeric color, browning index, and Hunter values a and b, b) hue was positively correlated with color density, polymeric color and browning index, and

c) clarity was negatively correlated with SO_2 and Hunter b value. Significant differences in aroma and taste were detected among the different processing procedures whereas none was determined among the different enzyme treatments.

In conclusion, this study indicates that although pre-press enzyme treatments could increase anthocyanin extraction by up to 50%, none of the different processing procedures and enzyme treatments investigated prevented eventual degradation of anthocyanins during storage. Further research is required into methods for stabilizing anthocyanin pigments in strawberry wines.

The Influence of Different Processing Procedures on Strawberry Juice and Wine Quality.

by

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INTRODUCTION

Major problems confronted in the production of strawberry juice and wine products include the extraction of juice and color from the fruit, and the loss of fresh fruit aroma with thermal processing. When crushed, strawberries yield a highly pectinous pulp containing up to 1.4% total pectin, which releases little free run juice (28,29,35,39). In addition, strawberry wines are regarded as the most difficult fruit wine to process (39), being noted for their poor color quality and poor color stability on storage (33).

The purpose of part I of this study was to optimize the extraction of juice and color from strawberry fruit without using thermal processing. The application of different processing procedures, in particular different pre-press enzyme treatments, was investigated. Part II of this study investigates the color quality and color stability of the strawberry wines fermented from the juices extracted in part I.

REVIEW OF LITERATURE

AN INTRODUCTION TO THE USE OF ENZYMES IN FRUIT JUICE AND WINE PROCESSING

Different fruit varieties often show clearly different chemical and physical properties and consequently they require different juice extraction technologies. Juice has been extracted from crushed grapes (pulp or mash), and wine from their fermented pulps, by simple means since ancient times. The method has been essentially the enclosure of the crushed fruit in a cloth, basket or other perforated container, and the application of mechanical pressure. Juice has similarly been pressed from "hard" fruits after they have been disintegrated by milling or grinding.

Although crushed grapes and the pulps of apples and pears readily yield juice when subjected to pressure, many berry fruits such as currants, strawberries and raspberries give highly pectinous pulps, from which there is poor juice drainage. Whereas apple juice usually contain less than 0.1% of soluble pectin, berry juices may contain up to five times this amount (35).

The total pectic content varies widely in this type of fruit; for example strawberries, blackberries, and raspberries contain 0.5 - 1.4%, 0.7 - 1.2%, and 0.6 - 1.0%, respectively. A high percentage (50-95%) of the pectic substance is in the soluble form even when the fruit is

not completely ripe, and essentially all of the pectic constituents are soluble in fully ripe fruit (29,35).

Pectins, especially in berry fruit can hinder the extraction of juice from the mash: because of the extreme viscosity, the juice drains very slowly. Irrespective of the pressure applied much pectin-bound juice remains in the mash and in so doing retains juice components including with it color, sugar, and acids. The mash is resilient and difficult to handle. Pectin acts as a coloidal stabilizer of the suspended matter in the juice so that the latter cannot be clarified by centrifuging or filtering. It is also impossible to produce highly concentrated juices which contains pectin. They gel and burn during concentration (38).

Enzymes are widely used commercially for the treatment of fruit pulps to expidite handling, in particular to facilitate extraction and pressing, to increase juice yield and to clarify the juice or wine obtained from the fruit (28,29). The commercial enzyme preparations commonly used in the processing of fruit and vegetables are mostly mixtures of pectinases, cellulases and hemicellulases (52). The general assumption that enzymes acting on pectic substances i.e., pectinases, played the dominant role in clarification, was confirmed by Japanese workers who demonstrated that apple juice and grape juice could be clarified by highly purified pectic enzymes (17,18). Nevertheless, the effectiveness of commercial pectinase preparations in some applications is influenced by the "side activities" of other enzymes present such as Cellulases and Hemicellulases.

EXTRACTION OF JUICE BY ENZYMES:

The application of enzymes to increase release of free run juice has been reported in studies made in various parts of the world, but the amount of increase has been quite variable, and in a few cases no increases has been obtained (12,19,30,53). The inability to demonstrate an increase in free run juice in some experiments has been due to not optimizing enzyme concentration and time of reaction. Other factors, such as temperature, degree of crushing, variety and maturity, and source of enzyme, may be equally important. Reports on the effect of pectic enzyme treatment on total juice or wine yield (free run plus press juice) usually indicate less dramatic and more variable increases than free run. In addition to the enzyme and fruit factors responsible for variation in free run juice, control of conditions associated with pressing influence the total yield (28). A main advantage in the increase of the quantity of free run juice is to increase the press capacity of the plant and shorten the press cycle.

Increase in free run and total juice, viscosity reduction, and turbidity decrease generally follow an increase in pectic enzyme concentration, but some deviations are apparent. Excessive enzyme treatment can even reduce release of free run juice. Wine produced from enzyme extracted juice has been reported to have an increase in dry extract, while having a reduced viscosity (28).

USE OF ENZYMES IN CLARIFICATION:

The first report on the use of pectolytic enzymes for the clarification of fruit juices was performed by Kertesz in 1930; Since then a relatively large number of commercial pectic enzyme preparations have been used successfully for the clarification of apple juice as well as other juices. Proponents of clarified juice note that a clarified juice remains clear during long periods of shelf storage and is free of the darker color and cooked flavor which may arise from degradation of the pectin and haze components during pasteurization of hazy juice. In addition, clarified juices are easier to filter and concentrate.

Initially pectic enzymes were added to wines to obtain more rapid clarification or as a desperate attempt to clarify wines that did not respond to other methods of clarification utilizing bentonite or gelatin. Pectic enzymes are now widely used, but they have not been universally adopted for clarification.

Observers generally agree that wines prepared from pectic enzyme-treated grapes clarify more rapidly, and, in addition, most workers report increases in free run juice or wine as well as in total juice or wine.

COLOR EXTRACTION BY ENZYMES:

In thermovinification of red grape mash the color extraction process is largely a consequence of the heat process. However, pectinases promote the maceration of the grape skins and can extract a higher yields of color (51). Cruess et. al., 1955 reported that red wines prepared commercially from pectic enzyme-treated (52-55°C) grapes showed a good color and stability. However there are conflicting reports; for instance, samples of Bulgarian wine prepared from pectic enzyme-treated Zartchine grapes showed almost 18% more color than a control wine when 0.3% enzyme was used for treatment, but 60% less color than the control when 0.7% of the same enzyme was used for treatment (28).

One possible answer to the rather inconsistent pattern observed with red wine color is suggested by the finding that fungal enzymes may contain an enzyme capable of hydrolyzing anthocyanins. It has been demonstrated that fungal preparations derived from Aspergillus niger and A. oryzae may contain an enzyme that hydrolyzes colored anthocyanins to anthocyanidins and glucose followed by transformation of the aglycone to colorless derivatives (2,14,16). The rate of decolorization found in red wine could depend not only on the amount of Anthocyanase (β-Glucosidase) present in the pectic preparation and the amount retained in the wine but also on the pigment composition of the grape variety (28). There is a lack of information on the application

of enzymes in the color extraction of berry fruits, the information being mainly restricted to technical bulletins from enzyme companies.

USE OF ENZYMES IN WINEMAKING:

Despite the apparent advantage to be gained from the use of pectic enzymes in winemaking, they were not readily adopted for winemaking and this was on account of the low level of purification of the commercially available pectic enzymes. Baumann (1974) pointed out that enzyme manufacturers devoted considerable time and effort in the 1960's to developing relatively pure and concentrated preparations of pectic enzymes, and therefore it is unfair and largely irrelevant to consider earlier reports on the performance of pectic enzymes in winemaking. It is interesting to note that there were no significant publications in the scientific literature between 1959 and 1972 on pectic enzymes and winemaking. However, in 1972 Dittrich reported that the use of pectic enzymes resulted in a better filtration of musts; increased yields from mash; higher concentration of tannins, total extract, sugar-free extract and ash in the juice; and improved filtration and clarification of young wines (37).

In 1974 Ough and Berg (1974) using two commercial enzymes demonstrated an increase in yield of white grape juices as well as improved clarity and filterability. There was no effect on color. They also stated that pectic enzymes increased the quantity of settleable solids as well as the juice yield (30).

Ough et al., (1975) also reported that pectic enzyme treatment of red grape musts can reduce the rate of fermentation and speed pigment extraction and, to a lesser degree phenolics extraction. The only significant effect on wine quality was the increased intensity of wine color. No significant trends were observed in the rates of filtration of wines.

The use of commercial pectic enzymes preparations does increase the methanol content of wines as a result of pectin de-esterification. Although the use of these preparations increased the methanol content proportionately more for white varieties, the final methanol content is less than that for similarly treated red varieties (8).

Similarly in 1979, Ough and Crowell reported that treating crushed white grapes with pectic enzymes, increased juice yield and methanol production. Other chemical changes which were attributable to the pectic-enzyme treatment were very minor. It was also reported that temperature and time of skin-contact are extremely important factors in analytical and quality changes independent of enzyme additions.

More recently Heatherbell et al., (1980), reported that pectolytic enzyme treatment of kiwifruit pulp before pressing increased juice yields as well as inducing desirable Riesling aroma formation during fermentation of juice to wine.

FRUIT WINE:

Fruit wines are beverages obtained by alcoholic fermentation of fruits (with the exception of vine grapes) or of juices thereof, with an alcohol content of usually between 8-18% (v/v), and some times even more. Fruit wines are made mainly out of pome fruits, berry fruits and stone fruits, and less frequently out of citrus and other fruits. The above definition does not include alcoholic fruit-beverages like Cider or Perry, since these have a lower alcohol content (ca 5-7% v/v) than the fruit wines (19).

STRAWBERRY COLOR:

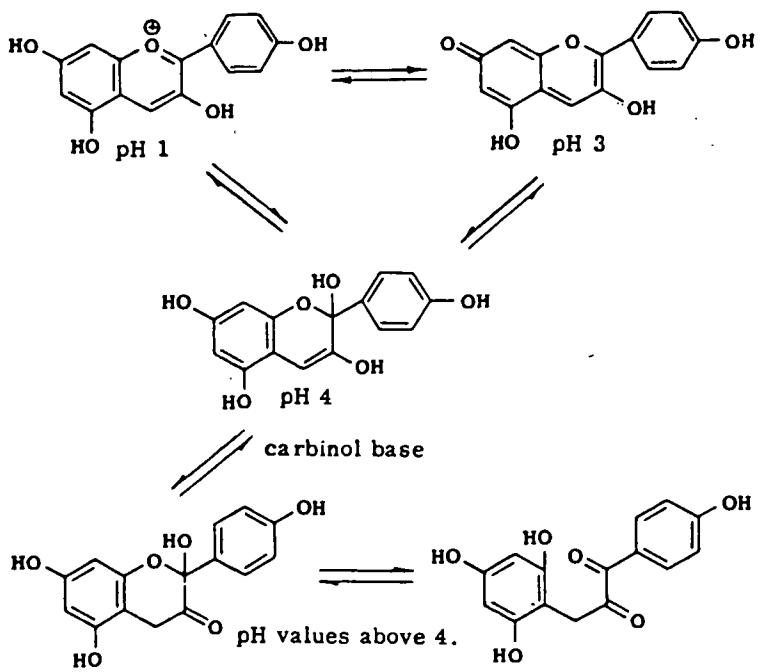
The strawberry and strawberry products owe their red color to the anthocyanin pigments and the color quality of these products is influenced by many factors but mainly due to anthocyanin pigment instability.

Strawberries are known to contain Pelargonidin-3-glucoside as a major pigment (46) and Cyanidin-3-glucoside as a minor pigment (25).

FACTORS AFFECTING THE DEGRADATION OF ANTHOCYANINS:

It is well known that the color of anthocyanin solutions depends on several factors including pigment concentration, solvent, temperature, pH, structure of the pigment, and the presence of substances known to react reversibly or irreversibly with anthocyanins.

The highly reactive nature of the anthocyanins is due to their electron deficient flavylium nucleus. The rate of anthocyanin destruction is pH dependent, being greater at higher pH values. Reactions usually produce decolorization of the pigments:

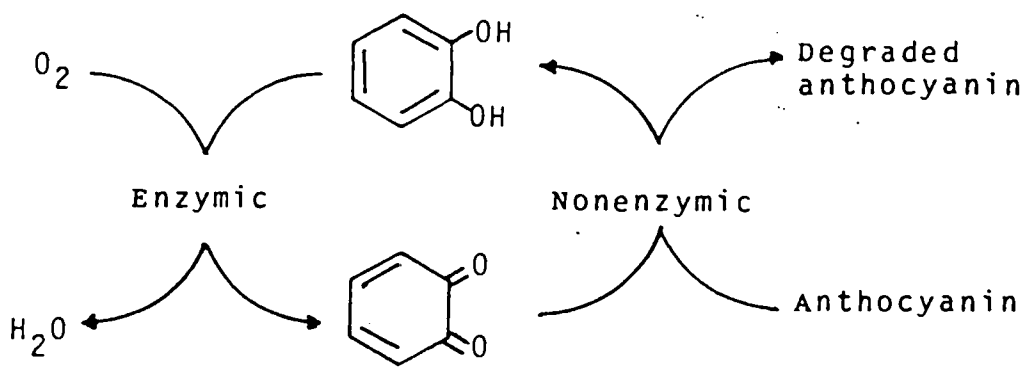


TEMPERATURE: The effect of temperature on the stability of anthocyanins in model systems and in food products has been studied by many investigators and the general consensus is that anthocyanin

pigments are readily destroyed by heat during the processing and storage of foods. Meschter showed that processing a strawberry preserve at 100°C for 1 hr resulted in 50% destruction of the fruit anthocyanin (27). During the storage of the preserve at 38°C, the half life of the pigment was 10 days; at 20°C, it was 54 days. Markakis and co-workers, found that the temperature effect on a solution of pure Pelargonidin-3-glucoside, was not very different from that described by Meschter, and recommended a short time/ high temperature process for best pigment retention. The opening of the heterocycle (pyrilium ring) and the formation of a chalcone as the first step in the degradation of anthocyanins was postulated by Markakis, et al., 1957. However, Adams in 1973 contended that anthocyanins heated in the pH range 2 to 4 first undergo hydrolysis of the glycosidic bond (position 3), followed by conversion of the aglycon to a chalcone, which subsequently yields an α-diketone. It is assumed that further degradation leads to brown products, especially in the presence of molecular oxygen. When a buffered solution (pH 3.4) of (^{14}C) Pelargonidin-3-glucoside was stored at room temperature until virtual decolorization, a brown precipitate was formed containing most of the radioactivity of the anthocyanins. Several investigators have established that the red pigment of strawberry products is unstable on heating and storage (Kertesz and Sondhelmer, 1948; Mackinney and Chichester, 1952). However, the blanching of strawberries has been reported to provide a protective effect on the anthocyanin pigment, color and other chemical constituents (Wrolstad et al., 1980; Hassanein, 1982).

ENZYMES: Color deterioration is also attributed to anthocyanin decolorizing enzyme systems which have been found in molds, roots, leaves and in fruits. Hang in 1955, attributed the enzymatic decoloration to glycosidases, referred earlier as "Anthocyanases", which hydrolyze the sugar moeity of the anthocyanin with the subsequent transformation of the aglycone in to a colorless derivative.

Probably the most investigated enzyme in the browning and decolorization of fruits, vegetables and their products is Polyphenol oxydase (PPO,Phenolase). Grommerck and Markakis, 1964, reported a peroxidase-catalyzed anthocyanin degradation. Although phenolases are capable of reacting directly with anthocyanins Peng and Markakis (1963) proposed a scheme of sequential reactions to explain the effect of mediating phenolics: the o-phenolic substrates are first oxidized by PPO to o-quinones; then the anthocyanin is oxidized non-enzymically to a colorless product



Wesche-Ebeling (1981) reported that the enzyme was most active on d-catechin which is present in strawberries.

ASCORBIC ACID: Ascorbic acid concentration in strawberry fruit range from 28.5-94.3 mg/100g fruit (55), and in the hood variety has been reported to contain a reasonable high concentration of 48.2 mg/100g fruit (1).

It is well known that ascorbic acid has a destructive effect upon the anthocyanin pigments, but the nature of the converse effect, the effect of anthocyanins on ascorbic acid stability, is not well defined. Beattie et al., (1943), and Pederson et al., (1947), were among the first to show the parallel disappearance of the anthocyanin and ascorbic acid during storage of fruit juices. Similar observations were reported by Sondheimer and Kertesz, (1952); Meschter, (1953); Markakis et al., (1957) and Starr and Francis, (1968).

Because hydrogen peroxide (H_2O_2) is formed when ascorbic acid is oxidized in the presence of oxygen and copper, and H_2O_2 is known to decolorize anthocyanins, it is thought that ascorbic acid-induced anthocyanin degradation is mediated by H_2O_2. It is also well known that traces of copper catalyze the oxidation of ascorbic acid in acid solutions, and that the effect is enhanced by iron (50). Sondheimer and Kertesz (1953) presented evidence in which conditions that decrease the rate of the oxidation of ascorbic acid, such as lack of oxygen and the addition of thiourea, also decelerate the rate of anthocyanin destruction. Therefore, they suggested that the accelerating effect on the degradation of the pigment is associated

with the oxidation of ascorbic acid. Sondheimer and Kertesz (1953) further postulated that the H_2O_2 produced in the oxidative breakdown of ascorbic acid was probably responsible for the pigment degradation. Reports by Starr and Francis (1968) and Shrikhande and Francis (1974) also indicate that the detrimental effect of ascorbic acid on anthocyanins was primarily due to the interaction of its oxidation products, H_2O_2, with anthocyanins. Jurd (1966), demonstrated that H_2O_2 is capable of oxidizing anthocyanin and related flavylium salts in model systems. While such a mechanism is possible when ascorbic acid is oxidized in the presence of oxygen and copper, there is another mechanism proposed by Jurd (1972) where a condensation of ascorbic acid and anthocyanin is involved. Such condensation products are unstable and degradate to colorless compounds. Poei-Langston and Wrolstad (1981) working with model systems of Pelargonidin-3-glucoside reported that ascorbic acid had a significant effect on the pigment, Hunter color parameters, browning and polymeric color. They also present evidence that anthocyanin pigment and ascorbic acid degrade though a direct mechanism of condensation.

It is generally recognized that the main cause of ascorbic acid degradation is due to oxidation under aerobic conditions, but this does not preclude anaerobic decomposition which may also result in many undesirable side effects. The anaerobic destruction of ascorbic acid may proceed by a variety of mechanisms that have been postulated but not verified.

Hooper and Ayres (1950) assumed that the stability of ascorbic acid is probably due to the capability of naturally occurring anthocyanins and flavonols to protect ascorbic acid against oxidation. Davidek, (1960); Clegg and Morton, (1968); Horper et al., (1969); Shrikhande and Francis, (1974), further confirmed the protective effect of flavonols on ascorbic acid.

SULFUR DIOXIDE, (SO_2): Sulfur dioxide in solution in fermenting grapes has a number of effects. It causes the grape cells to release anthocyanins and other phenols, but in high concentrations it decolorizes wine pigments. It helps prevent oxidation of wine phenols, but it becomes bound during fermentation so that the effective concentration changes. Ascorbate, SO_2 and phenols can interact in different ways, but there are conflicting reports about their reactions in juices and wines (4,23,24). The concurrent loss of SO_2 and ascorbic acid can be explained on the basis of the auto and coupled oxidation of ascorbate. Kielhofer has demonstrated that in wines and model wine solutions ascorbic acid oxidizes to dehydroascorbic acid with the formation of hydrogen peroxide, (H_2O_2) (23,24). This reaction can be coupled with the oxidation of free SO_2 to sulfate ($SO_4^{=}$) (47). Kielhöfer has also shown that low concentrations of SO_2 (= 8 mg/L), protected ascorbic acid but that higher concentrations of SO_2 were able to bind dehydroascorbic acid displacing the equilibrium in favor of dehydroascorbic acid, resulting in increased oxidation of ascorbic acid (24). This was also

demonstrated more recently by Heatherbell et al. (1980), in kiwifruit wine.

POLYMERIZATION: Anthocyanins readily condense with themselves and with other organic compounds during their degradation. Somers and Evans (1979) proposed the presence of pigment complexes between anthocyanins and related phenolics in red grape juice, that owe their stability to intermolecular hydrogen bonds, which break when ethanol is produced resulting in a significant red color loss by the end of vinification. Somers (1968, 1971) contends that in ageing red wines a gradual formation of condensed or polymeric pigments occurs. This occurrence is attributed to the irreversible conversion of the monomeric anthocyanin pigments to more stable polymeric forms. They are reddish, much less sensitive to pH change than the anthocyanins, and quite resistant to decolorization by sulfur dioxide.

In the course of the first months following vinification, new wine undergoes numerous transformations, many of which are oxidative in nature. The transformation are accompanied by loss of anthocyanins. This decrease is dependent upon both the storage conditions of the wine and its initial composition in phenolic substances (36).

The decrease in anthocyanin concentration during aging, may be due to incorporation of the monomeric anthocyanins into polymeric complexes of leucoanthocyanins and other flavonoid units (42), or due to the reaction of anthocyanins with other phenolic components like procyanidins (11). Studies on strawberry juice and concentrate has

shown that anthocyanin pigment exhibit similar degradative patterns to that described for grape wine pigments by Somers in 1971 (56).

TITLE: STRAWBERRY JUICE AND WINE PROCESSING: I EFFECT OF DIFFERENT PROCESSING PROCEDURES AND PRE-PRESS ENZYME TREATMENTS ON THE EXTRACTION OF JUICE AND COLOR FROM STRAWBERRY FRUIT.

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RUNNING HEAD: STRAWBERRY JUICE AND WINE PROCESSING

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STRAWBERRY JUICE AND WINE PROCESSING: I EFFECT OF DIFFERENT PROCESSING PROCEDURES AND PRE-PRESS ENZYME TREATMENTS ON THE EXTRACTION OF JUICE AND COLOR FROM STRAWBERRY FRUIT.

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ABSTRACT

The effect of different milling (Hammer mill) and pre-press pulp treatments on the extraction of juice and color from frozen strawberry fruit was investigated. The effectiveness of three different commercial pectinases, a cellulase and a protease were compared. The optimum pre-press milling and enzyme pulp treatment conditions for release of free run juice (FRJ) determined on a laboratory-scale were incorporated into larger scale pilot-plant press trials using a Willmes bag press.

The nature of the fruit (fresh or frozen), milling speed and mill screen size all affected release of FRJ from crushed fruit. Highest yields were obtained when excessive crushing was avoided by milling partially thawed fruit at low speeds.

Pectinases containing high pectin esterase activity were most effective in releasing FRJ. Pre-press enzyme treatment of the pulp increased yields of FRJ from 28% (untreated pulp) up to 56%. However, when press juice was combined, total juice yields of approximately 86% were obtained for all treatments.

Pre-press enzyme treatment of the pulp increased anthocyanin pigment extraction by up to 50%. The anthocyanin pigment extracted varied with the different enzymes, the pectinases with known "side-activities" and in combination with proteases, extracting the highest concentration of anthocyanins. All juices had low polymeric color, and percent polymeric color, indicating that most of the pigmentation of these juices is due to monomeric anthocyanins.

INTRODUCTION

Major problems confronted in the production of strawberry juice and wine products include the extraction of juice and color from the fruit, and the loss of fresh fruit aroma with thermal processing. When crushed, strawberries yield a highly pectinous pulp containing up to 1.4% total pectin, which releases little free run juice (10,11,14,19). In addition, strawberry wines are regarded as the most difficult fruit wine to process (19), being noted for their poor color quality and poor color stability on storage (13).

The purpose of part I of this study was to optimize the extraction of juice and color from strawberry fruit without using thermal processing. The application of different processing procedures, in particular different pre-press enzyme treatments, was investigated. Part II of this study (6) investigates the color quality and color stability of the strawberry wines fermented from the juices extracted in part I.

MATERIALS AND METHODS

Strawberry fruit (Hood variety) which had been mechanically harvested, were spray washed thoroughly, individually quick frozen at -40°C, placed in polyethylene bags and stored at -10°C in plastic containers until processed.

PRE-PRESS TREATMENT: Frozen or partially thawed fruit was crushed in a Hammer mill (Hammer mill model D comminuting machine, The W. J. Fitzpatrick Co., Chicago) using screen sizes from 1/2 to 1 in and milling speeds from 100 to 800 rpm. The crushed fruit (pulp) was subjected to the following pre-press treatments:

1) Reacted for 15-60 min at 25°C with 250-2,000 mg enzyme/kg pulp. The following enzymes provided by Rohm Tech Inc. New York (subsidiary of Rohm GmbH Darmstadt, Fed. Rep. Germany) were used: Pectinase Rohapect D5s, Rohapect B-20, Rohapect VR, Cellulase 2240, and an experimental fungal protease EL57-59.

2) Pulp fermentation. The pulp was inoculated with a 3% v/v exponentially-growing pure yeast culture (Champagne strain, VI-A-DRY, Scott labs, San Rafael Cal.) and allowed to ferment for 3 days at 18°C with daily punching down. At this stage of near complete fermentation (3°Bx) the fermented pulp was pressed with a Willmes bag press.

FREE-RUN JUICE: The free run juice test described by Rohm (17) was used to determine release of juice from pulps produced by the different thawing and milling conditions and the different enzyme treatments.

PRESSING PROCEDURE: Pressing conditions were standardized as follows unless otherwise specified. A 2% cellulose press aid (by weight) was added to 4.5 kg lots of pulp, allowed to swell and placed in a Willmes type 60 Bag Press. Free run juice was allowed to drain for 30 min, before a first stage press was obtained by gradually increasing the pressure up to 3 bars over a 20 min period. Remaining juice was extracted by increasing the pressure up to 5 bars over a 15 min period. Juice fractions were collected and measured.

ANALYSIS: Enzymatic pectin degradation was controlled by determining the residual pectin by the alcohol test (16). Total acidity, pH, soluble solids (Abbe refractometer) were determined by the procedures described by Amerine and Ough (2). Filterability of juice was determined by measuring the volume of juice which passed by gravity flow in 30 min through a 9.0 cm-diameter no.1 Whatman filter paper in a conical filter funnel. Anthocyanin pigment content was determined using the pH differential method as described by Wrolstad (1976). Anthocyanin concentration was expressed as mg of pelargonidin-3-glucoside per L juice using a molar absorptivity of 22,400. Color density, polymeric color, percent polymeric color, and

browning index were determined following the metabisulfite method developed by Somers (1972). Color density, the sum of absorbances at 420 and 510 nm, gave a measure of the total sample color. Polymeric color was the sum of the absorbances at 420 and 510 nm of the bisulfite treated samples. Percent polymeric color was defined as the percent ratio of polymeric color to that of color density. The absorbance measurements at 420 nm of the bisulfite-treated sample were taken as an index of the browning. β-Glucosidase activity was determined using the method described by Duerksen (1958), using p-Nitrophenol-β-d-glucoside as substrate at a pH of 6.8, and reporting activity as an optical density change of 0.001 units per minute per 3 ml of reaction mixture.

RESULTS AND DISCUSSION

Several advantages may result from increasing release of free run juice (FRJ) including increased yield, easier pressing with reduced press-time, increasing "pressing capacity", and increased juice quality.

Therefore, before press-trials were conducted, preliminary experiments were undertaken to determine optimum conditions for release of FRJ. Initial experiments using the laboratory-scale FRJ test determined that thawing for approximately 3 hrs at 20-23°C followed by milling at 180 rpm with a 3/4" screen size gave optimum release of FRJ. Fruit that was completely frozen, or thawed to a lesser degree, or

milled at higher rpm, or with smaller screen sizes resulted in excessive crushing of the fruit with a subsequent reduction in release of FRJ. Large screen sizes did not retain the fruit resulting in insufficient crushing. Likewise, it was also found that there was no advantage in enzyme pulp treatment times in excess of 1 hr for concentrations used, as demonstrated by pectinase D5s (Fig. II.1) and reaction conditions were standardized to 60 min at 25°C. These conditions also produced a FRJ which gave a negative test for residual pectin. The removal of pectins represents a real advantage if the juice is going to be filtered, centrifuged or concentrated. For example, the filterability of D5s treated juice increased approximately 8 folds (800%) over untreated control juice.

The relative effectiveness of different enzyme preparations is compared in Fig. II.2. Increasing the concentration of D5s, VR, and B-20 enzymes had a very similar effect on the release of FRJ. The D5s and VR had an optimum effect at concentrations of 1,250 mg/kg pulp, and the B-20 and Cellulase at 1,000 mg/kg pulp, the cellulase being very much less effective than the other enzymes. In all instances, there was no advantage in the use of excessive enzyme treatment and in addition the use of higher concentrations may not be cost effective. Therefore for press trials (refer below) the enzyme pulp treatments were standardized to 250 mg enzyme/kg pulp for 1 hr at 25°C.

PRESS-TRIALS: The optimum pre-press milling and enzyme pulp treatments determined by the laboratory-scale FRJ test were

incorporated into larger scale pilot-plant press trials using a Willmes bag press. A comparison was made between pressing untreated pulp, pulp treated with a standard commercial pectinase Rohapect D5s, and fermentation of the pulp (Table II.1). In addition, different enzyme preparations were also compared (Table II.2).

Pre-press enzyme treatment of the pulp was found to increase yields of FRJ from 28% (untreated pulp) up to 56% (pectinase D5s). These are large increase compared with those reported for some other fruits juices (7,12). Although differences were obtained in the release of FRJ, the D5s releasing the most (56%), when press juice was combined, total juice yields from all treatments were essentially identical. It should be emphasized that this study was conducted using frozen fruit, which is commonly used in the industry. However, more pronounced differences would have been obtained between different treatments if fresh fruit was available for the study, as cell rupture resulting from freezing and thawing increases juice release. For instance in preliminary trials using fresh fruit there was a 20% reduction in the FRJ when compared with that obtained from frozen fruit. In addition, pectinase D5s treatment of fresh fruit gave approximately 15% increases in both free run and total juice yields over that obtained from untreated fruit. Juice settled solids were observed to be <3% v/v and did not contribute significantly to juice volumes. This is attributed to the use of the 2% press aid.

The different pectin esterase (PE) activities present in the different enzyme preparations may account for their different behavior

in releasing FRJ. The native pectins in strawberry fruit are highly esterified (4,22). Therefore it was not surprising that D5s which has the highest concentration of PE (18) released the highest yields of FRJ. This higher PE in D5s would increase the de-esterification of the pectins in the strawberry allowing further pectin degradation by hydrolytic pectinases (polygalacturonases, and polymethylgalacturonases) resulting in a higher FRJ yield. Furthermore, the results in Table II.2 demonstrate that B-20 and VR which are known to contain lower concentration of PE (18) released lesser amounts of FRJ.

COLOR EXTRACTION: Preliminary experiments used for determining optimum conditions for release of free run juices indicated pre-press enzyme treatment of pulps increased color extraction. In particular the D5s:Protease and VR pre-press enzyme treated juices had a greater "apparent color intensity" than the other juices. When press juices were analyzed for anthocyanin content it was found that anthocyánin concentration was greater in the enzyme treated samples, with the exception of the B-20 enzyme, than either the control (untreated pulp) or pulp fermented samples (Fig. II.3). The total anthocyanin extracted varied with the different enzymes used. The mixture pectinase D5s:Protease extracted the highest (174.44 mg/L; an increase of 50% with respect to the control) followed by VR (163.61 mg/L), D5s (155.44 mg/L), D5s:Cellulase 2240 (133.05 mg/L), and B-20 (68.46 mg/L). The higher anthocyanin concentration in D5s:Protease and VR juices

coincided with the "apparent greater color intensity" of these juices. Increased color in pre-press enzyme treated juices is mainly attributed to extraction of color by pectinases, which promote the maceration of fruit skins causing a permeabilisation of the cell wall and the diffusion of coloring matter ensuring a higher yield of color. In contrast, in pulp fermentation, it is the alcohol which causes this diffusion. Urlaub has reported that when grape mash is treated with pectinases the diffusion of coloring substances starts at once and is independent of alcohol concentration (21). Our study indicates that nonpectic enzyme activities such as β-glucosidases and the "side activities" in VR enzyme may contribute to color extraction (the VR enzyme is known to consist of side activities of proprietary nature in addition to the standard commercial pectinase Rohapect D5s). The VR enzyme has also found application for improving clarification and color extraction in red wine processing (3,15). The cellulase did not increase anthocyanin extraction. Although nonpectic enzyme activities may contribute to color extraction they may also contribute to color instability. For instance, β-glucosidase activity is known to destabilize anthocyanins (1,8,9). However, only low β-glucolsidase activities were found in the preparations D5s, B-20, VR, and Cellulase 2240 (Table II.3). Such activities can be considered insignificant particularly since the enzyme concentrations assayed were the highest recommended by the manufacturer for processing use.

In an attempt to obtain objective measurements of apparent color differences between juice samples, the color analysis procedure

developed by Somers for red wine (20) was applied to the extracted juices (Table II.4). Although the interpretation of color parameters is complex (20) the following conclusions can be obtained from this data. The pulp fermented sample had the lowest color density (1.8 units) whereas B-20 pre-press enzyme treated juice had the highest (6.6 units). All juices had low polymeric color, ranging from 0.200 units for the D5s:Protease pre-press enzyme treated juice to 0.620 units for the VR treatment. Therefore, polymeric color could be considered unimportant at this stage of processing. Likewise, all the juices also had low percent polymeric color, pulp fermented sample having the highest value (16.3%) and D5s:Protease the lowest (4.1%), indicating that at this stage most of the pigmentation of the juices is due to the monomeric anthocyanin. With the exception of the B-20 and VR treatments, there was no increase in the browning index of the extracted juices. The high browning index of the VR juice is possibly due to the presence of "side activities".

As all the juices had a low pH and were within the range pH 3.17±0.12, pH was not considered a factor in color differences in this study

A more detailed discussion of color and color stability in the juices extracted by the above procedures, during fermentation and storage as wine is discussed in part II of this study.

Fig. II.1 Effect of enzyme reaction time on yield of free run juice from strawberry pulp. ●-● Rohapect D5s treated pulp: 250 mg Rohapect D5s/kg pulp, 25°C. o-o Untreated (no enzyme) pulp. All results are the average of at least two separate determinations.

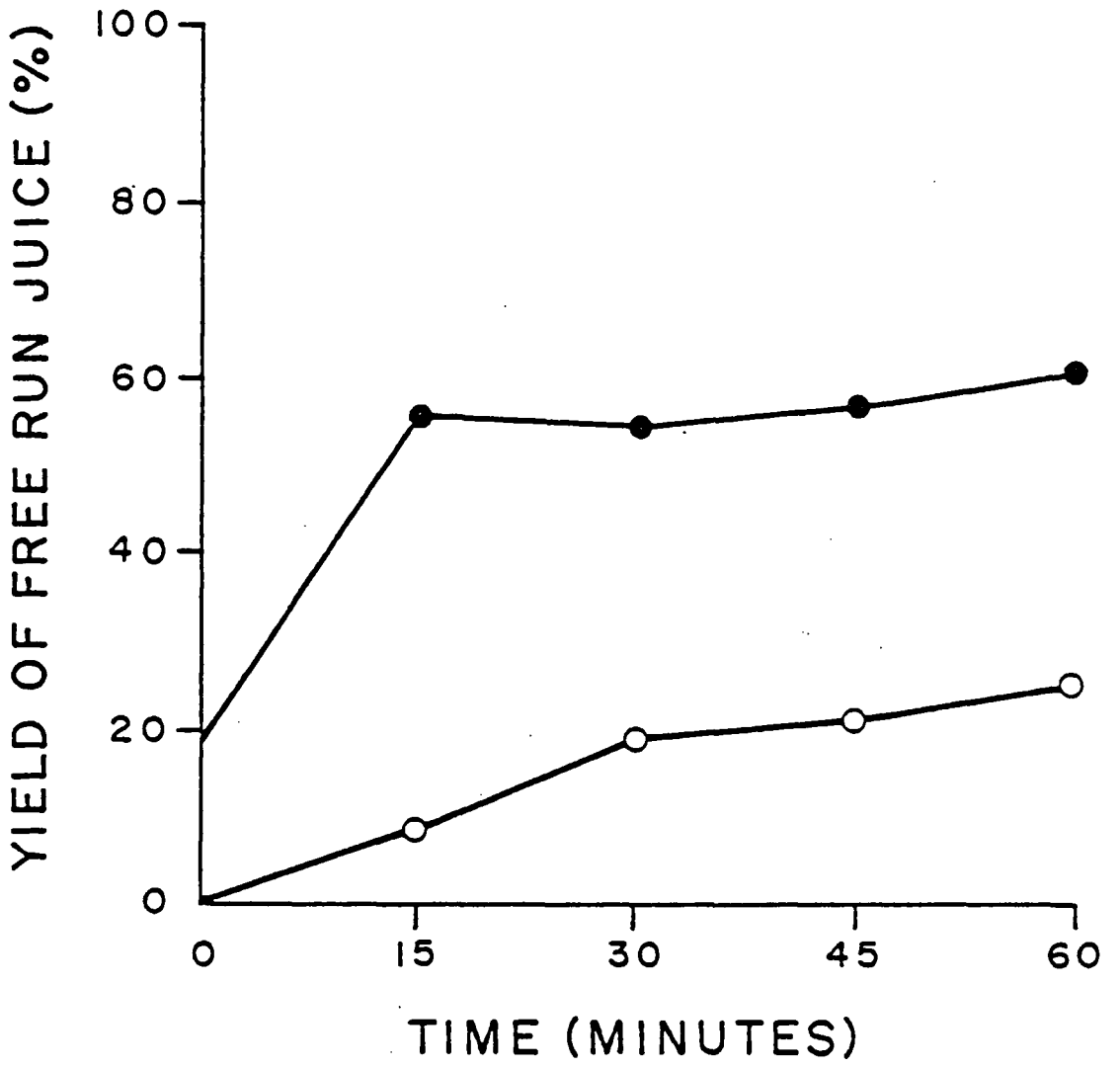
YIELD OF FREE RUN JUICE (%)
100
80
60
40
20
0
0
15
30
45
60
TIME (MINUTES)

Fig. II.1

Fig. II.2 Effect of different enzymes and enzyme concentration on yield of free run juice from strawberry pulp, reacted for 1 hr at 25°C. All results are the average of at least two separate determinations.

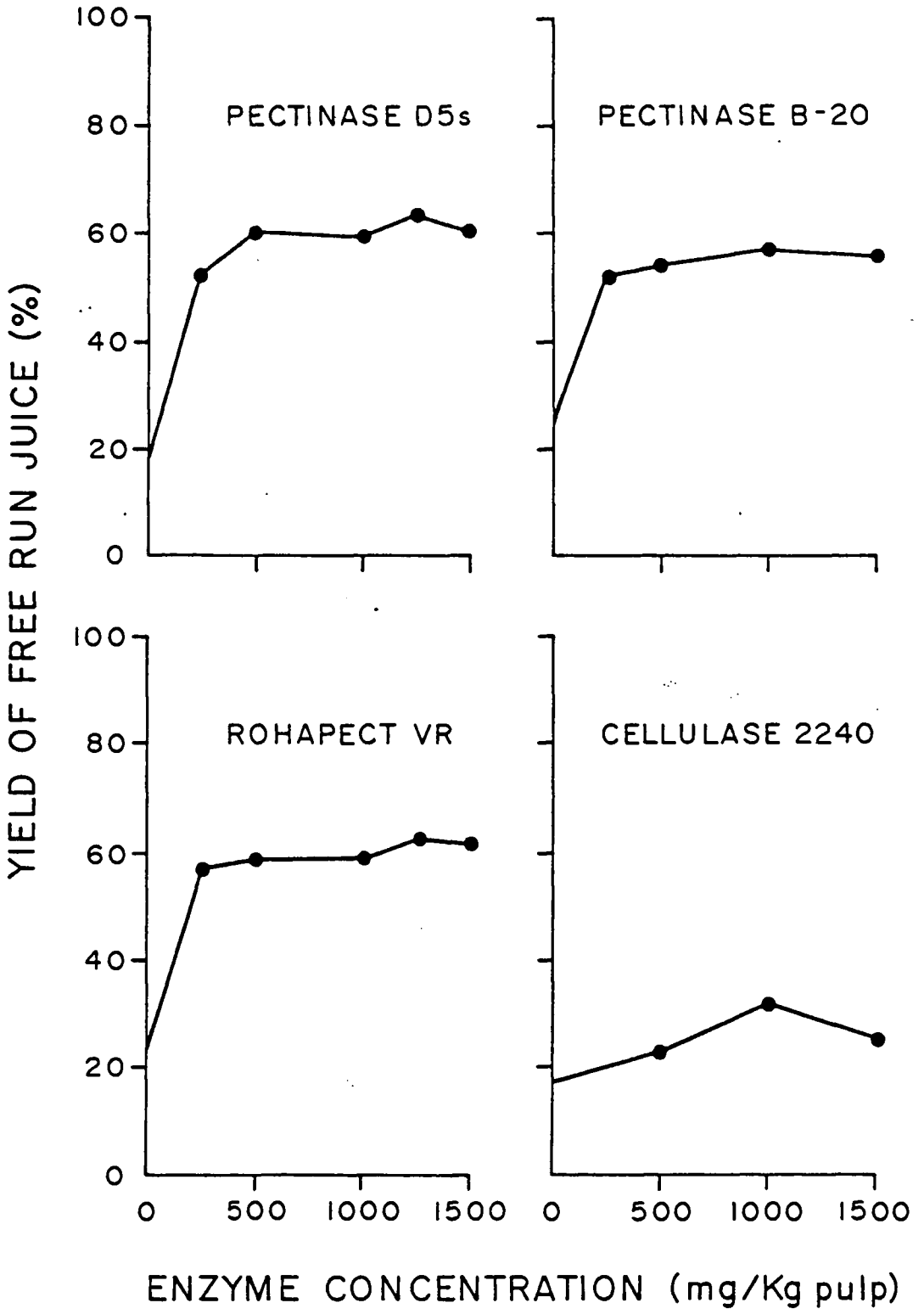
PECTINASE D5s
PECTINASE B-20
ROHAPECT VR
CELLULASE 2240
YIELD OF FREE RUN JUICE (%)
ENZYME CONCENTRATION (mg/Kg pulp)
0
20
40
60
80
100
0
500
1000
1500

Fig. II.2

Fig. II.3 Effect of pre-press pulp treatment on concentration of anthocyanin in press juice. Juices processed as described in Tables II.1 and II.2. All results are the average of at least two separate determinations.

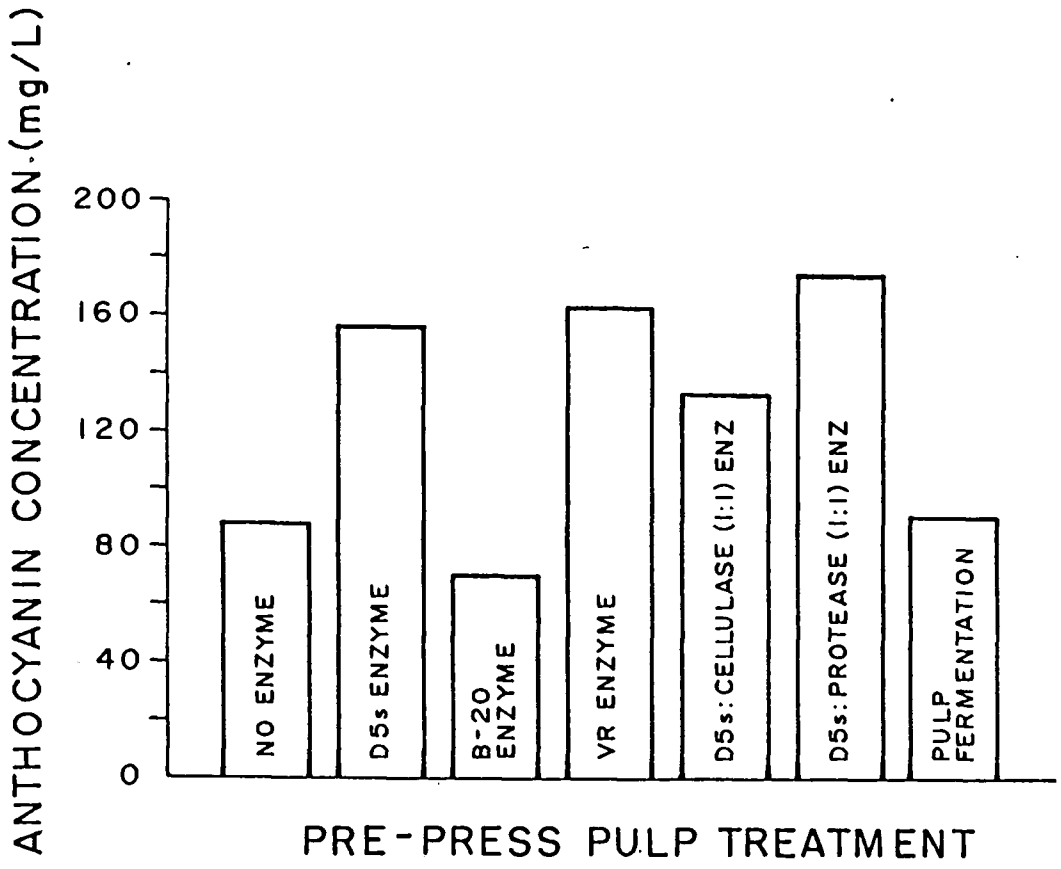
ANTHOCYANIN CONCENTRATION (mg/L)
200
160
120
80
40
0
NO ENZYME
D5s ENZYME
B-20 ENZYME
VR ENZYME
D5s:CELLULASE (1:1) ENZ
D5s:PROTEASE (1:1) ENZ
PULP FERMENTATION
PRE-PRESS PULP TREATMENT

Fig. II.3

TABLE II.1 EFFECT OF PRE-PRESS PULP TREATMENT ON THE YIELD OF STRAWBERRY JUICE[a].

PULP TREATMENT[b]	FREE RUN JUICE[c] (% BY VOLUME)	PRESS JUICE[d] (% BY VOLUME)	TOTAL JUICE (% BY VOLUME)
UNTREATED PULP	28	56	84
PECTINASE D5s[e]	56	30	86
PULP FERMENTATION[f]	45	40	85

[a] Average of two separate determinations.
[b] All treatments included 2% cellulose press aid.
[c] 30 min drain time.
[d] Willmes bag press, 35 min press, 5 bars.
[e] 250 mg/kg pulp, 25°C, 1 hr.
[f] Pulp fermented for 3 days at 18°C.

TABLE II.2 EFFECT OF DIFFERENT PRE-PRESS ENZYME TREATMENT ON THE YIELD OF STRAWBERRY JUICE[a].

ENZYME TREATMENT[b]	FREE RUN JUICE[c] (% BY VOLUME)	PRESS JUICE[d] (% BY VOLUME)	TOTAL JUICE (% BY VOLUME)
PECTINASE D5s	56	30	86
PECTINASE B-20	46	39	85
ROHAPECT VR	43	43	87
D5s:CELLULASE (1:1)	51	34	86
D5s:PROTEASE (1:1)	50	34	84

[a] Average of at least two separate determinations.
[b] 250 mg/kg pulp, 25°C, 1 hr.
[c] 30 min drain time.
[d] Willmes bag press, 35 min press, 5 bars.

TABLE II.3 β-GLUCOSIDASE ACTIVITY IN COMMERCIAL ENZYME PREPARATIONS.

ENZYME PREPARATION	β-GLUCOSIDASE ACTIVITY[a]
ROHAPECT D5s	1.0
ROHAPECT B-20	4.0
ROHAPECT VR	5.8
CELLULASE 2240	1.5

[a] Average of at least two separate determinations. Activity measured as optical density change of 0.001 units per minute per 3 ml of reaction mixture.

TABLE II.4 COLOR ANALYSES OF STRAWBERRY JUICE[a].

PRE-PRESS TREATMENT[b]	COLOR DENSITY[c]	POLYMERIC COLOR[d]	% POLYMERIC COLOR[e]	BROWNING INDEX[f]
UNTREATED PULP	2.8	0.300	10.6	0.240
PECTINASE D5s	3.8	0.240	6.4	0.180
PECTINASE B-20	6.6	0.460	6.9	0.380
ROHAPECT VR	5.2	0.620	11.8	0.480
D5s:CELLULASE(1:1)[g]	3.5	0.240	6.9	0.240
D5s:PROTEASE (1:1)[g]	4.8	0.200	4.1	0.200
PULP FERMENTATION	1.8	0.300	16.3	0.220

[a] Average of at least two separate determinations.

[b] As described in Tables II.1 and II.2.

[c] $A_{420} + A_{510nm}$.

[d] $A_{420} + A_{510nm}$ of the bisulfite treated sample.

[e] Percent ratio of polymeric color to that of color density.

[f] A_{420nm} of the bisulfite treated sample.

[g] 1:1 (250 mg of enzyme/kg pulp:250 mg of enzyme/kg pulp).

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TITLE: STRAWBERRY JUICE AND WINE PROCESSING: II EFFECT OF DIFFERENT PROCESSING PROCEDURES AND ENZYME TREATMENTS ON WINE COLOR AND COLOR STABILITY.

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Corvallis, Oregon 97331

RUNNING HEAD: Strawberry Juice and Wine Processing.

Technical Paper No._____ from the Oregon Agricultural Experiment Station.

STRAWBERRY JUICE AND WINE PROCESSING: II EFFECT OF DIFFERENT PROCESSING PROCEDURES AND ENZYME TREATMENTS ON WINE COLOR AND COLOR STABILITY.

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and

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ABSTRACT

The influence of different processing procedures on the quality of strawberry wine, in particular on wine color quality and stability, was investigated.

Wines were prepared by fermenting juices extracted by standard press (Willmes Bag Press) procedures including different pre-press enzyme treatments, by pulp fermentation and by processing in the presence and absence of sulfur dioxide (SO_2). The following color parameters were determined in the fermented juice ("young" wine) and in the bottled wine during a four month storage period at 25°C: anthocyanin pigment content, polymeric color, color density, browning, haze and Hunter L, a, b values. In addition SO_2 and ascorbic acid were also determined.

Large decreases in anthocyanin pigment content and color density occurred as a result of fermentation.

Storage time had a significant effect ($p<0.05$) on all the color parameters (except color density), sulfur dioxide, and ascorbic acid, for all processing procedures and enzyme treatments.

The different processing procedures and enzyme treatment had a significant effect ($p<0.05$) on color density, polymeric color, browning, haze and Hunter L, a, b values. Anthocyanin pigment content was significantly affected ($p<0.05$) by the different enzyme treatments. However, none of the different processing procedures and enzyme treatments helped to stabilize anthocyanin degradation throughout the

storage. Enzyme wines had lower Hunter L, a, b values than the other processing procedures. Sulfur dioxide had a protective effect against anthocyanin degradation during the mid-storage period. Likewise, SO_2 had an inhibitory effect on browning and polymeric color development.

In all wines the following correlations were found: a) SO_2, anthocyanin pigment content, and ascorbic acid were positively correlated, b) ascorbic acid was negatively correlated to browning index and polymeric color, c) polymeric color, percent polymeric color and browning index were positively correlated with each other, d) anthocyanin pigment content was positively correlated to Hunter a values, and e) SO_2 was negatively correlated to polymeric color.

Sensory evaluation of the wines determined significant differences in clarity among the different processing procedures, and in color intensity, hue and clarity among the different enzyme treatments. In addition, correlation analysis of the sensory evaluation data vs. the wine parameters analyzed gave the following correlations: a) browning was negatively correlated to color density, polymeric color, browning index, and Hunter a and b values, b) hue was positively correlated to color density, polymeric color and browning index, and c) clarity was negatively correlated to SO_2 and Hunter b values. Significant differences in aroma and taste were detected among the different processing procedures whereas none was determined among the different enzyme treatments.

Although pre-press enzyme treatments could increase anthocyanin

extraction by up to 50%, none of the different processing procedures and enzyme treatments investigated prevented eventual degradation of anthocyanins during storage.

INTRODUCTION

Strawberry wines are noted for being difficult to process and in particular, for their poor color quality and lack of color stability on storage (16,20). In addition to anthocyanin degradation, browning can be a major contributor to loss of color quality and acceptance in strawberry products (1).

Part I of this study (5) compared the extraction of juice and color from strawberry fruit by different processing procedures. The purpose of this investigation was to 1) compare the color quality of the wines fermented from the juices extracted in part I, 2) determine the effect of storage on wine color parameters, 3) determine which factors contribute to the development of browning during wine storage, and 4) conduct a sensory evaluation on the stored wines.

MATERIALS AND METHODS

JUICE EXTRACTION AND VINIFICATION: The following strawberry (Hood variety) juices extracted by different processing procedures and pre-press enzyme treatments as described in part I (refer tables II.1 and II.2) of this study (5), were fermented (refer below):

1).- Juices extracted without pre-press pulp treatment in the presence and absence of SO_2 (+/-SO_2).

2).- Juices extracted by the following pre-press enzyme treatments (+SO_2), D5s, B-20, VR, D5s:Cellulase, D5s:Protease.

3).- The "juice" extracted by pulp fermentation (wine fermented to 3°Bx before pressing) This press "juice" (wine) was then fermented to dryness as described below (+SO_2).

A 1% solution of sodium metabisulfite ($K_2S_2O_5$) was used to add 25 mg SO_2/L to all the juice treatments labelled as (+SO_2). Juices were allowed to stand for 2 hrs, before ameliorating to 22° Brix., by adding sucrose (LSI Liquid Sugars Northwest Type "O"). One percent of $(NH_4)_2PO_4$ (J.T. Baker, Co.) was added as nutrient. Four and one half liter lots were transferred into 3 gallon glass carboys and inoculated with a 3% v/v exponentially-growing pure yeast culture (Champagne strain, VI-A-DRY, Scott labs, San Rafael, Cal.). Containers were sealed with fermentation locks and the juices fermented to dryness at 16°C. (less than 0.5% residual sugar as determined by "dextrocheck" tablets).

Wines were then racked, stored at 3°C in full, sealed containers until bottling.

Twenty-five mg/L of SO_2 and 250 mg/L of sodium sorbate were added to finished wines which were bottled without filtration (750 ml bottles). Wines were stored in the dark at 25°C and sampled for analysis at intervals during a 16 week period.

ANALYSIS: Anthocyanin pigment content, polymeric color, percent polymeric color, color density, browning, pH, titratable acidity, and soluble solids were determined as previously described (5). Sulfur dioxide was determined by the method of Rankine and Pocock (18), using oxygen-free nitrogen for the aspiration of SO_2. Ascorbic acid was determined by the dinitrophenylhydrazine method (19). Hunter L, a, b color parameters values were determined in the transmission mode using a Hunter Model D25 P-2 Color Difference Meter which was standardized against a white tile (No. DC 122, L=+94.02, a=-0.9, b=+1.2). All measurements were made with the light source in the normal, aligned position (arrangement I) for the diffuse transmittance only, excluding the specular component, and using a 1 cm, light path. Haze measurement was calculated as the ratio of Y in arrangement I over Y in arrangement III, (YI/YIII). A One-Way Analysis of Variance (ANOVA) and simple correlation were performed on all analytical data obtained.

Sensory analysis of the color quality of the different wines was conducted after 16 weeks of storage at 25°C. The 11 member panel consisted of representatives from Oregon fruit wineries as well as OSU

wine research personnel. The wines were evaluated as to color intensity, hue, clarity, browning and over-all color and appearance on a desirability scale (Appendix 1). A reference-difference scale was used to evaluate color, aroma, and taste, scored from one (same as reference) to nine (extremely different from reference) and a hedonic scale of from nine (like extremely) to one (dislike extremely) was used for over-all desirability, (Appendix 2). Wines were evaluated in two categories: Those produced by (1) The different processing procedures, and (2) The different enzyme treatments. The data were analyzed by multiple comparisons (using a two-way analysis of variance) based on treatment least significant differences (LSD) at 1 and 5% levels.

RESULTS AND DISCUSSION

COLOR CHANGES DURING FERMENTATION: Large decreases in anthocyanin pigment content (Table III.1) and color density (Fig. III.2; Part I, Table II.4) occurred as a result of fermentation. There was an average decrease of 92% in anthocyanin pigment content in pre-press enzyme treated wines compared with 85% for the untreated pulp wines. The least loss occurred in the pulp fermented wine (82%), and this is believed to be due to the higher phenolic content in these wines (27).

There was less color density loss in wines produced by pulp fermentation (38%) than in wines produced by fermenting pre-press enzyme treated juices (80% average) and untreated pulp (+/-SO_2) juices (70% average).

The decrease in color density may be attributed to adsorption and precipitation of anthocyanins by solids and yeasts, by combination of anthocyanins with proteins and reduction of unstable pigments forms. In addition, Somers has reported that the phenomenon of major color loss during the making of red wine is also due to the extreme instability, in the presence of ethanol, of deeply colored structures initially present in the extracted juice (26).

The greater retention of color density in the wine produced by fermenting the pulp may be explained on the basis of higher phenolic extraction by fermenting the pulp than by the pre-press enzyme treatment of the pulp. The possibility of a linkage between the flavylium ion of the natural anthocyanins with phenols and thus retaining the visible red color has been suggested by Jurd (12). Moreover, Timberlake and Bridle (1976) reported a color augmentation due to the formation of highly colored compounds believed to consist of anthocyanins and phenolics linked by CH_3CH bridges.

EFFECT OF WINEMAKING PROCEDURES AND STORAGE TIME ON COLOR PARAMETERS:

Anthocyanins : There were no significant differences in anthocyanin stability in wines produced by the different processing procedures (D5s enzyme treated juice, pulp fermentation, and untreated juices (+/-SO_2). However, there was significant differences ($p<0.05$) between the B-20 wine and the D5s wine (Table III.3a).

Storage time had a significant effect ($p<0.05$) on anthocyanin degradation in all treatments (Tables III.2a and III.3a).

In grape wines, the initial red wine color is very largely due to monomeric anthocyanins (24). Sulfur dioxide is known to decolorize anthocyanins (11,27) and this is demonstrated in Fig. III.1. Although the final anthocyanin pigment concentration is essentially the same for the different processing procedures, differences can be observed during storage (Fig. III.1). For instance, pulp fermented wine completed fermentation with a higher anthocyanin concentration than the others. In contrast, there was less anthocyanin degradation during the mid-storage period in the untreated pulp wine containing SO_2. This protective effect of SO_2 against anthocyanin degradation can be explained by the inhibition of PPO by SO_2 (4,6,15,29).

Except for D5s-wine, all the enzyme wines completed vinification (Table III.1) and storage (Fig. III.1) with a lower anthocyanin concentration than the different processing procedures although, some of these treatments started vinification with a higher anthocyanin concentration. The possibility exists that side activities contained in the enzymes such as β-glucosidase may contribute to the anthocyanin degradation by the removal of sugar moieties from their anthocyanidins, followed by the transformation of the aglycon to colorless derivatives (2,9,10). However, only very low β-glucosidase activities were detected in the different enzyme preparations (5).

Color Density : The different processing procedures and enzyme treatments had significant effects ($p<0.05$) on color density (Fig. III.2, Tables III.2b and III.3b). However, storage time had no

significant effect on color density. Pulp fermented wine had the highest color density of all the treatments, the D5s:Protease wine having the highest of the different enzyme treatments.

Color density in untreated pulp wine was reduced by processing in the presence of SO_2. This is attributed to the formation of colorless anthocyanin sulfonic acid derivatives (11).

The apparent increase in color density in Fig. III.2, which was expected from previous reports (16,23,24,25), is however not significant.

In this study there was no correlation between color density and anthocyanin content, which is consistent with the findings of Somers and Evans (1974).

Polymeric Color : Different processing procedures as well as the different enzyme treatments had a significant effect ($p<0.05$) on polymeric color. Likewise, storage time had a significant effect ($p<0.05$) on all treatments (Fig. III.3, Tables III.2c and III.3c). An increase in polymeric color during storage for all the wines is consistent with the report of Somers for red grape wines, where monomeric anthocyanin, initially responsible for wine color, is displaced progressively and irreversibly by more stable polymeric pigments during wine ageing (7,22,24,28). The differences among the different processing procedures and among the different enzyme treatments could be attributed to the degree of extraction of phenolic compounds by the different treatments. A high phenolic content in pulp fermented wine would account for the higher degree of polymerization in

this wine. As expected, untreated pulp processed with SO_2 produced wines with less polymeric color, due to inhibition of PPO (4).

Percent Polymeric Color : No significant effect was observed by the different processing procedures and the different enzyme treatments on percent polymeric color (PPC) of the wines. However, storage time showed a significant effect ($P<0.05$) on the change of this parameter in all the wines (Fig. III.4, Tables III.2d and III.3d). The increase in PPC, was as expected and consistent with the other analysis, where a decrease in monomeric anthocyanin and total color of the wine was accompanied by an increase in polymeric pigment. Similar results were reported by Somers for red grape wines (24). After 16 weeks 70-85% of the color in the wine was due to polymeric color whereas in the original juice only 4-16% of the color was PPC (5).

Hunter L, a, b values : Hunter measurements were taken in an attempt to measure the lightness, L; redness, a; and yellowness, b; of the wines.

The different processing procedures and enzyme treatments significantly affected all values (Tables III.2e,f,g and III.3e,f,g). Although, Hunter L values presented a varied behavior, storage time had a significant effect ($p<0.05$) on this parameter for the different processing procedures and enzyme treatments (Fig. III.5, Tables III.2e and III.3e). Only the untreated (no enzyme) pulp ($+SO_2$) and the D5s:Protease wines continued to increase in lightness at the end of the storage. The loss of color, anthocyanins, and the steady browning formation and precipitation may explain the increase of Hunter L

values. Pre-press enzyme wines presented lower Hunter L values than the other processing procedures. This reflects less bleaching and lightening of the wines made by these procedures. Untreated pulp wine ($+SO_2$) had the highest L value by the end of storage.

Storage time had a significant effect ($p<0.05$) on Hunter b value for both different processing procedures and enzyme treatments (Fig. III.6, Tables III.2f and III.3f). This corresponds to the increase in browning and polymeric color reported in figures III.3 and III.8. Enzyme wines presented lower b values than the other processing procedures, indicating lower yellownes development of those wines during storage.

Storage time significantly affected ($p<0.05$) Hunter a value in the different enzyme treatments (Fig. III.7, Table III.3g). The decrease of this parameter during storage may be due to the precipitation of the larger polymers formed. When compared to the enzyme wines, the different processing procedures had higher Hunter a values at the beginning of storage, and maintained higher values throughout the storage period. This could be attributed to higher levels of anthocyanins at the completion of fermentation and thus the formation of more stable compounds (12,28). The observed bleaching effect of SO_2 is apparent when comparing untreated pulp ($+/-SO_2$) treatments (Fig. III.7).

Browning Index : There were significant differences ($p<0.05$) in browning of wines produced by the different processing procedures and enzyme treatments (Fig II.8, Tables III.2h and III.3h). As expected,

there was a significant increase ($p=0.05$) in browning with storage for all treatments. Pulp fermented wines had the highest browning index.

Due to the fact, that the browning of some of treatments containing SO_2 had higher browning index than the wine processed with no SO_2 (Fig. III.8), it is evident that enzymes (PPO) are not the only factor responsible for the browning of strawberry wine. Browning can also be caused by non-enzymic reactions (Maillard reaction), the caramelization reaction and by ascorbic acid browning reactions.

Analysis of variance of the change of browning throughout the storage adjusted for ascorbic acid for all the different processing procedures and enzyme treatments, shows that ascorbic acid has a significant effect ($P<0.05$) on browning. Moreover, regression analysis for each one of the wines, indicates that polymeric color is the independent variable most related to browning, followed by ascorbic acid. The selection of polymeric color was as expected, as this is a measurement of browning. A negative correlation was found between ascorbic acid and browning for all the different wines tested with the exception of the untreated pulp wine ($+SO_2$).

These data supports the conclusion that ascorbic acid is an important contributor to browning (17).

The different processing procedures and enzyme treatments had no significant effect on ascorbic acid and SO_2. However, storage time had a significant effect ($P<0.05$) on these two parameters for all treatments (Fig II.8, Tables III.2i,j and III.3i,j).

When additional SO_2 was added to both new and stored wines, the

free SO_2 rapidly disappeared. For example, when 50 mg SO_2/L was added, within 24 hrs this was entirely accounted for as bound SO_2 and this coincided with an approximate 6% reduction in anthocyanin pigment content. Sulfur dioxide may also be bound by other compounds in the wine (3).

A rapid loss of ascorbic acid during storage occurred in all treatments. Likewise, there was a similar loss of SO_2 during storage (Fig. III.8).

The concurrent loss of SO_2 and ascorbic acid can be explained on the basis of the auto and coupled oxidation of ascorbate. Kielhöfer has demonstrated that in wines and model wine solutions ascorbic acid oxidizes to dehydroascorbic acid with the formation of hydrogen peroxide (13,14). This reaction can be coupled with the oxidation of free SO_2 to sulfate ($SO_4^{=}$) (21). Similar conclusions were reported by Heatherbell et al. for kiwifruit wine (8).

CORRELATION ANALYSIS: Simple correlation, testing Ho:ρ=0; Ha:$\rho\neq$0, $r_{0.05,8}$=0.632, was used to determine interrelationships among the wine parameters analyzed (Table III.4). Likewise, testing Ho:ρ=0; Ha:$\rho\neq$0, $r_{0.05,6}$=0.707, showed the interrelationships between parameters measured and the sensory scores of the wines (Table III.5).

Sulfur dioxide (SO_2), anthocyanin content, and ascorbic acid were positively correlated with each other for all treatments with the exception of untreated pulp wine ($-SO_2$). In addition, ascorbic acid was negatively correlated to browning index and polymeric color in

all treatments again with the exclusion of untreated pulp wine ($-SO_2$). This confirmed the significant effect of ascorbic acid on browning during storage. Moreover, polymeric color, percent polymeric color and browning index were positively correlated in all wines studied.

Anthocyanin content and Hunter a values correlated positively in all enzyme treatments. Likewise, a positive correlation was found between ascorbic acid and Hunter a values in all the different enzyme treatments except B-20 enzyme treated wine.

Sulfur dioxide was negatively correlated to polymeric color and browning index in all the different wines excluding the B-20 enzyme treated wine and the untreated pulp wine ($+SO_2$), the SO_2 being correlated only to polymeric color on the former, and to polymeric color and color density on the latter.

The hue measured by sensory analysis was positively correlated to color density, polymeric color and browning index. Likewise, browning was negatively correlated to color density, polymeric color, browning index, and Hunter values a and b. Over-all color and appearance was negatively correlated to ascorbic acid content and clarity was found to be negatively correlated to SO_2 and Hunter b value.

SENSORY ANALYSIS: No significant sensory differences (in color intensity, hue, browning and over all color and appearance) were detected among the different processing procedures, (Table III.6). However, significant differences in desirability of clarity were found

between the wine processed with SO_2 and both the pulp fermented wine and the enzyme treated wine (pectinase D5s) at the 1% level of significance. Pulp fermented wine and the enzyme treated wine (pectinase D5s) were the most desirable. No significant differences in desirability of browning, over-all color and appearance were detected among the different enzyme treatments (Table III.7). However, D5s enzyme treated wine and B-20 enzyme treated wine presented significant differences in desirability of color intensity and hue at the 5% level of significance. D5s enzyme treated wine was more desirable. Likewise, significant differences at the 5% level of significance were detected, between VR enzyme treated wine and D5s enzyme treated wine, between the B-20 enzyme treated wine and the D5s:Cellulase enzyme treated wine, and between the B-20 enzyme treated wine and the D5s:Protease enzyme treated wine, in the desirability of their color intensity, hue, and clarity, respectively.

Significant sensory differences in aroma and taste were detected among the different processing procedures at the 1% level of significance, (Table III.8). However, there was no significant difference in likability (nine points hedonic scale) within the same processing procedures. The D5s enzyme treated wine differed significantly (1% LS) in aroma from wines processed with and without SO_2. Likewise, significant difference in taste was found between the D5s enzyme treated wine and both the pulp fermented wine (5%LS) and the untreated pulp wine with no SO_2 (1%LS).

Significant differences in the color of the wines produced by the

different processing procedures and the different enzyme treatments were detected by the panel (Tables III.8, III.9). The pectinase treated wine differed from all the other processing procedures and the pulp fermented wine differed from the untreated pulp wine ($+SO_2$). Likewise, B-20 treated wine differed from all the other enzyme treated wines.

In contrast there were no significant differences in their aroma and taste among the different enzyme treated wines, (Table III.9). Nevertheless, significant differences in likability (nine points hedonic scale) were detected between the D5s:cellulase enzyme treated wine and all the other enzyme treated wines.

Because of the limited number of judges (breadth of sampling), the results of the likability test (nine points hedonic scale) should not be interpreted as a consumer preference test.

In conclusion, this study indicates that although pre-press enzyme treatments could increase anthocyanin extraction by up to 50%, none of the different processing procedures and enzyme treatments investigated prevented eventual degradation of anthocyanins during storage. Further research is required into methods for stabilizing anthocyanin pigments in strawberry wines.

Fig. III.1 Effect of different, A) Processing procedures, and B) Enzyme treatments, on changes of total anthocyanin content in strawberry wine during storage at 25°C. Wines prepared by fermenting juices extracted by the different pre-press pulp treatments (refer part I, tables II.1 and II.2). All results are average of at least two separate determinations.

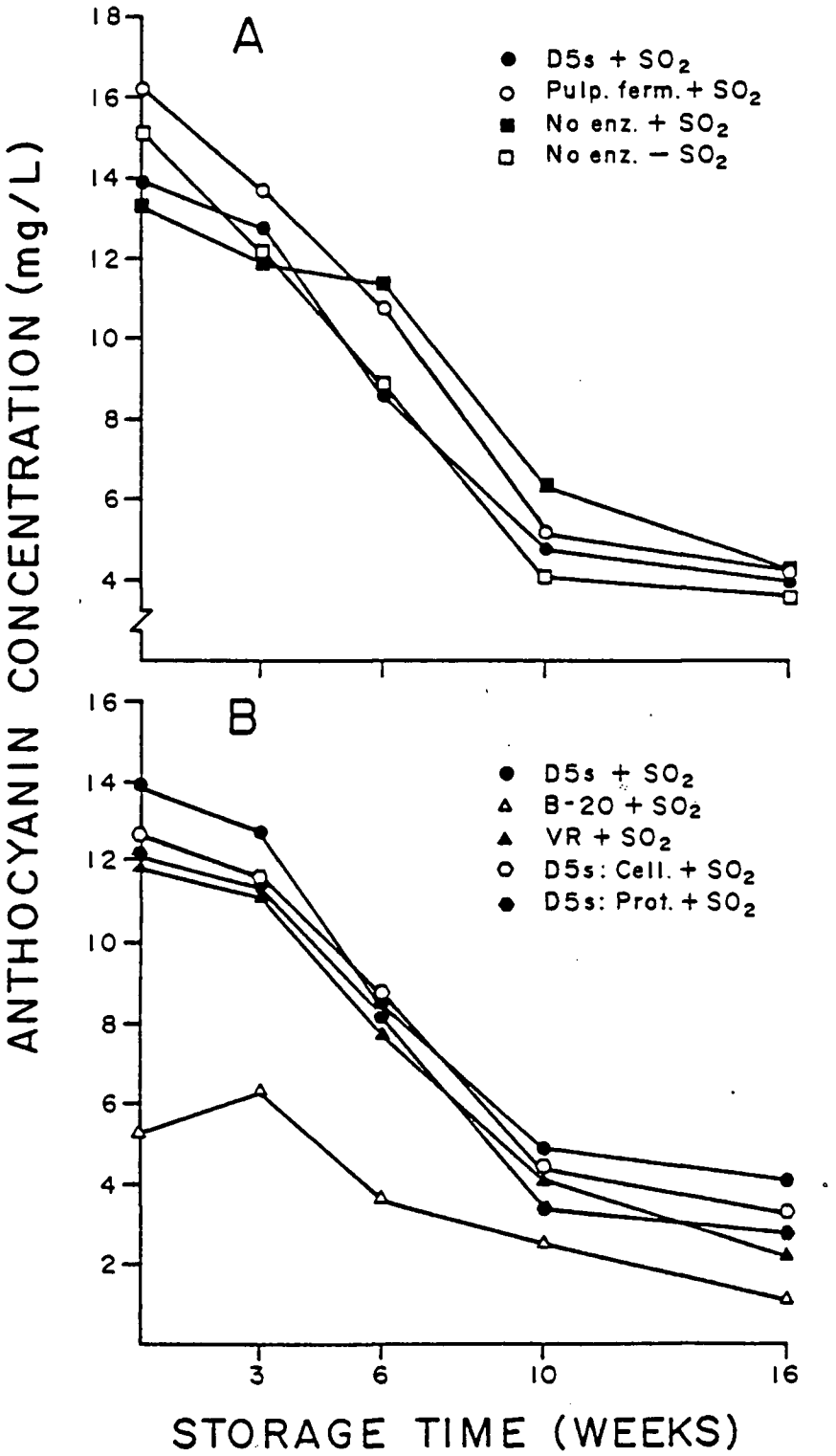
A
D5s + SO2
Pulp. ferm. + SO2
No enz. + SO2
No enz. − SO2
B
D5s + SO2
B-20 + SO2
VR + SO2
D5s: Cell. + SO2
D5s: Prot. + SO2
ANTHOCYANIN CONCENTRATION (mg/L)
STORAGE TIME (WEEKS)
18
16
14
12
10
8
6
4
2
3
6
10
16

Fig. III.1

Fig. III.2 Effect of different, A) Processing procedures, and B) Enzyme treatments, on changes in color density in strawberry wine during storage at 25°C. Wines prepared by fermenting juices extracted by the different pre-press pulp treatments (refer part I, tables II.1 and II.2). All results are average of at least two separate determinations.

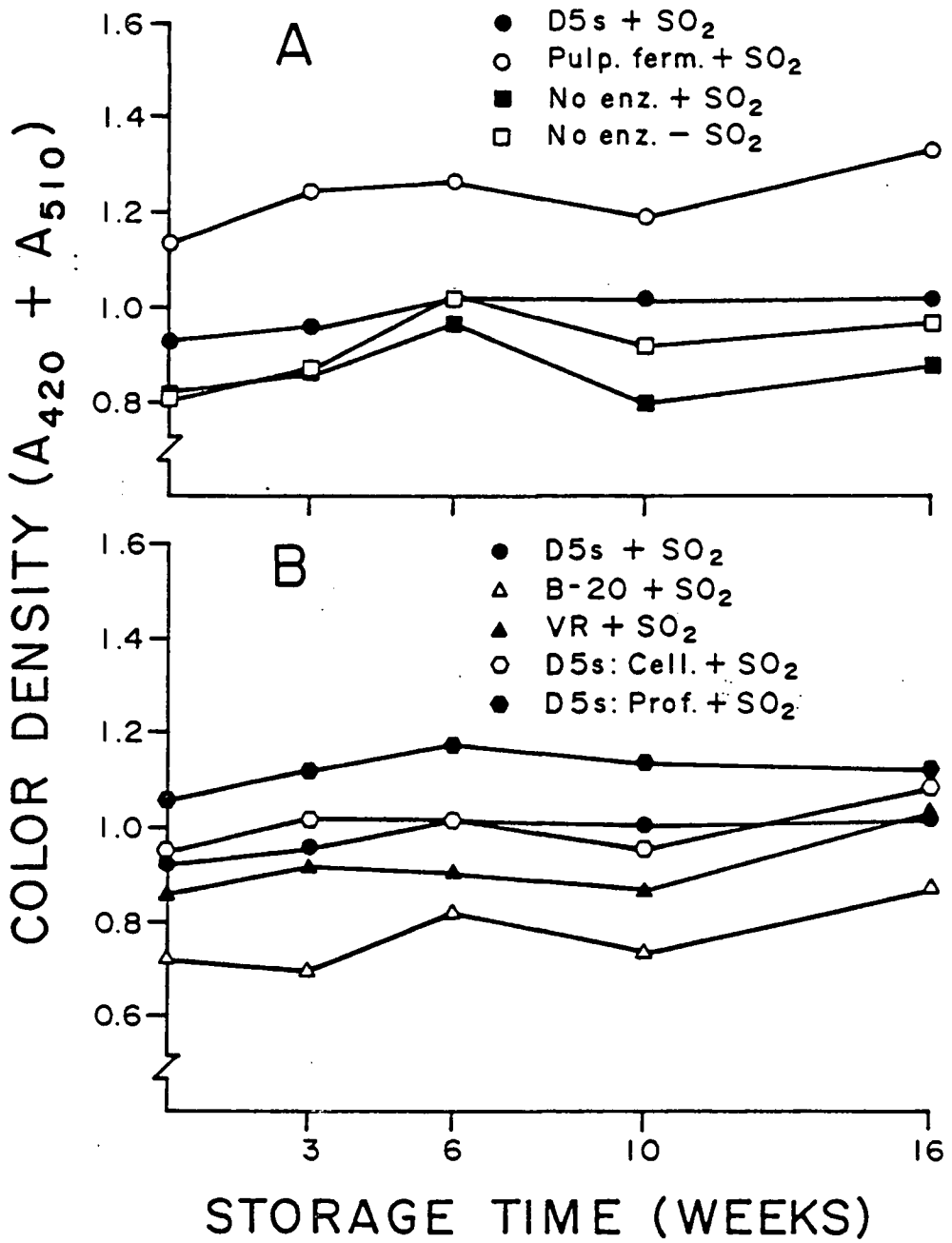
A
B
D5s + SO2
Pulp. ferm. + SO2
No enz. + SO2
No enz. − SO2
D5s + SO2
B-20 + SO2
VR + SO2
D5s: Cell. + SO2
D5s: Prof. + SO2
COLOR DENSITY (A420 + A510)
1.6
1.4
1.2
1.0
0.8
0.6
3
6
10
16
STORAGE TIME (WEEKS)

Fig. III.2

Fig. III.3 Effect of different, A) Processing procedures, and B) Enzyme treatments, on changes in polymeric color in strawberry wine during storage at 25°C. Wines prepared by fermenting juices extracted by the different pre-press pulp treatments (refer part I, tables II.1 and II.2). All results are average of at least two separate determinations.

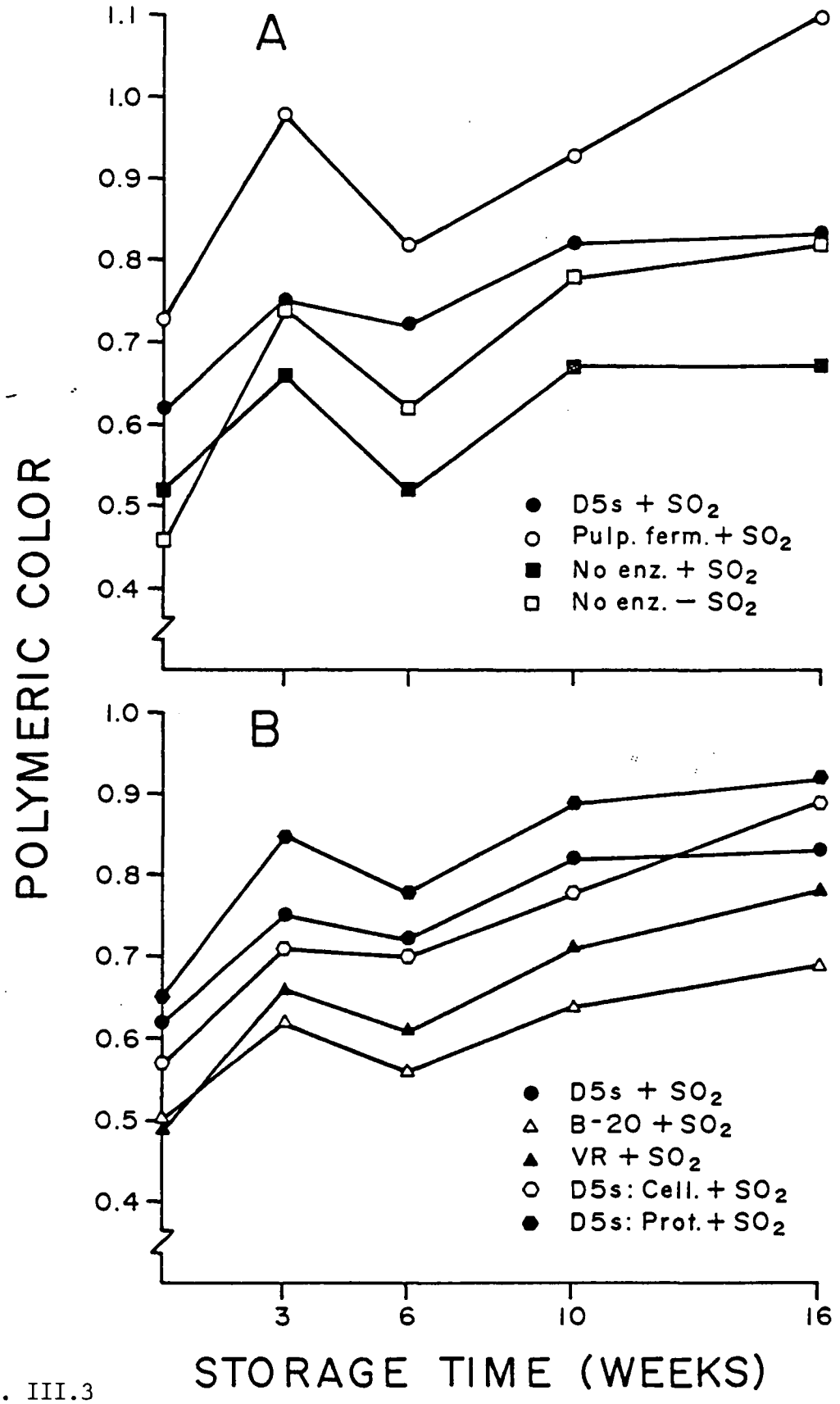
A
B
POLYMERIC COLOR
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
D5s + SO2
Pulp. ferm. + SO2
No enz. + SO2
No enz. − SO2
D5s + SO2
B-20 + SO2
VR + SO2
D5s: Cell. + SO2
D5s: Prot. + SO2
3
6
10
16
STORAGE TIME (WEEKS)

Fig. III.3

Fig. III.4 Effect of different, A) Processing procedures, and B) Enzyme treatments, on changes in percent polymeric color in strawberry wine during storage at 25°C. Wines prepared by fermenting juices extracted by the different pre-press pulp treatments (refer part I, tables II.1 and II.2). All results are average of at least two separate determinations.

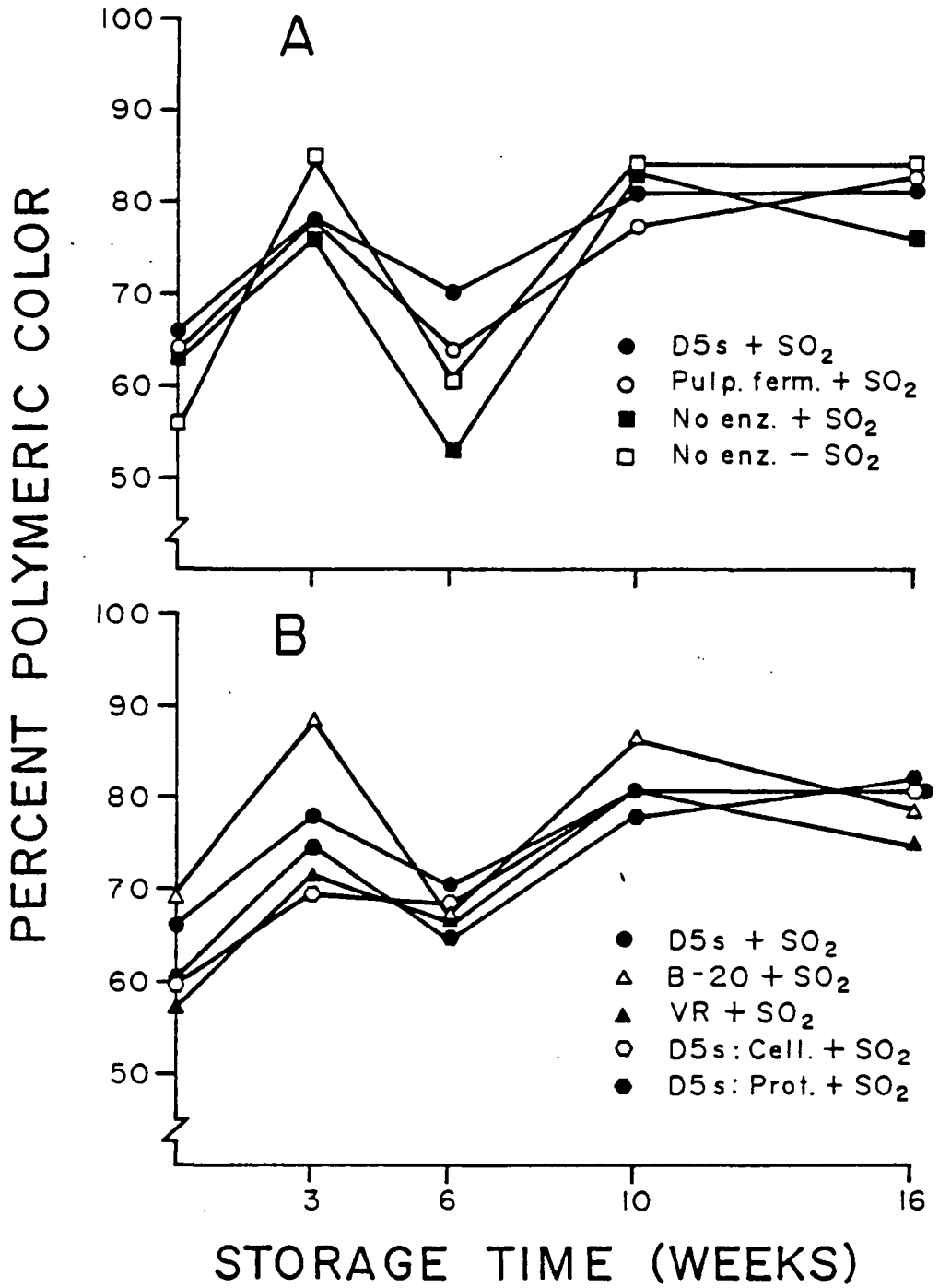
A
B
100
90
80
70
60
50
PERCENT POLYMERIC COLOR
D5s + SO2
Pulp. ferm. + SO2
No enz. + SO2
No enz. − SO2
B-20 + SO2
VR + SO2
D5s: Cell. + SO2
D5s: Prot. + SO2
3
6
10
16
STORAGE TIME (WEEKS)

Fig. III.4

Fig. III.5 Effect of different, A) Processing procedures, and B) Enzyme treatments, on changes in Hunter L values in strawberry wine during storage at 25°C. Wines prepared by fermenting juices extracted by the different pre-press pulp treatments (refer part I, tables II.1 and II.2). All results are average of at least two separate determinations.

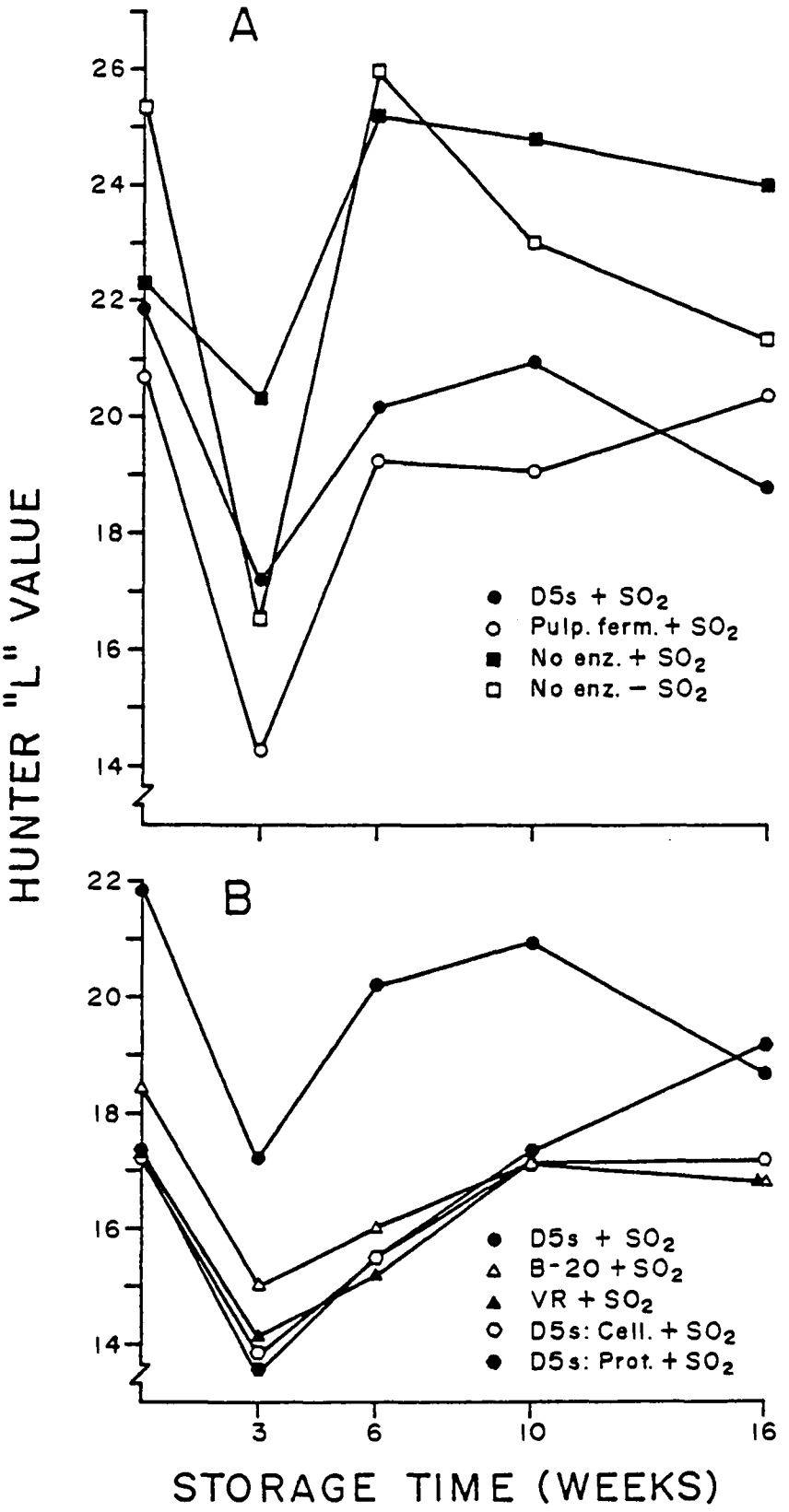
A
B
HUNTER "L" VALUE
STORAGE TIME (WEEKS)
D5s + SO2
Pulp. ferm. + SO2
No enz. + SO2
No enz. − SO2
B-20 + SO2
VR + SO2
D5s: Cell. + SO2
D5s: Prot. + SO2
26
24
22
20
18
16
14
3
6
10
16

Fig. III.5

Fig. III.6 Effect of different, A) Processing procedures, and B) Enzyme treatments, on changes in Hunter b values in strawberry wine during storage at 25°C. Wines prepared by fermenting juices extracted by the different pre-press pulp treatments (refer part I, tables II.1 and II.2). All results are average of at least two separate determinations.

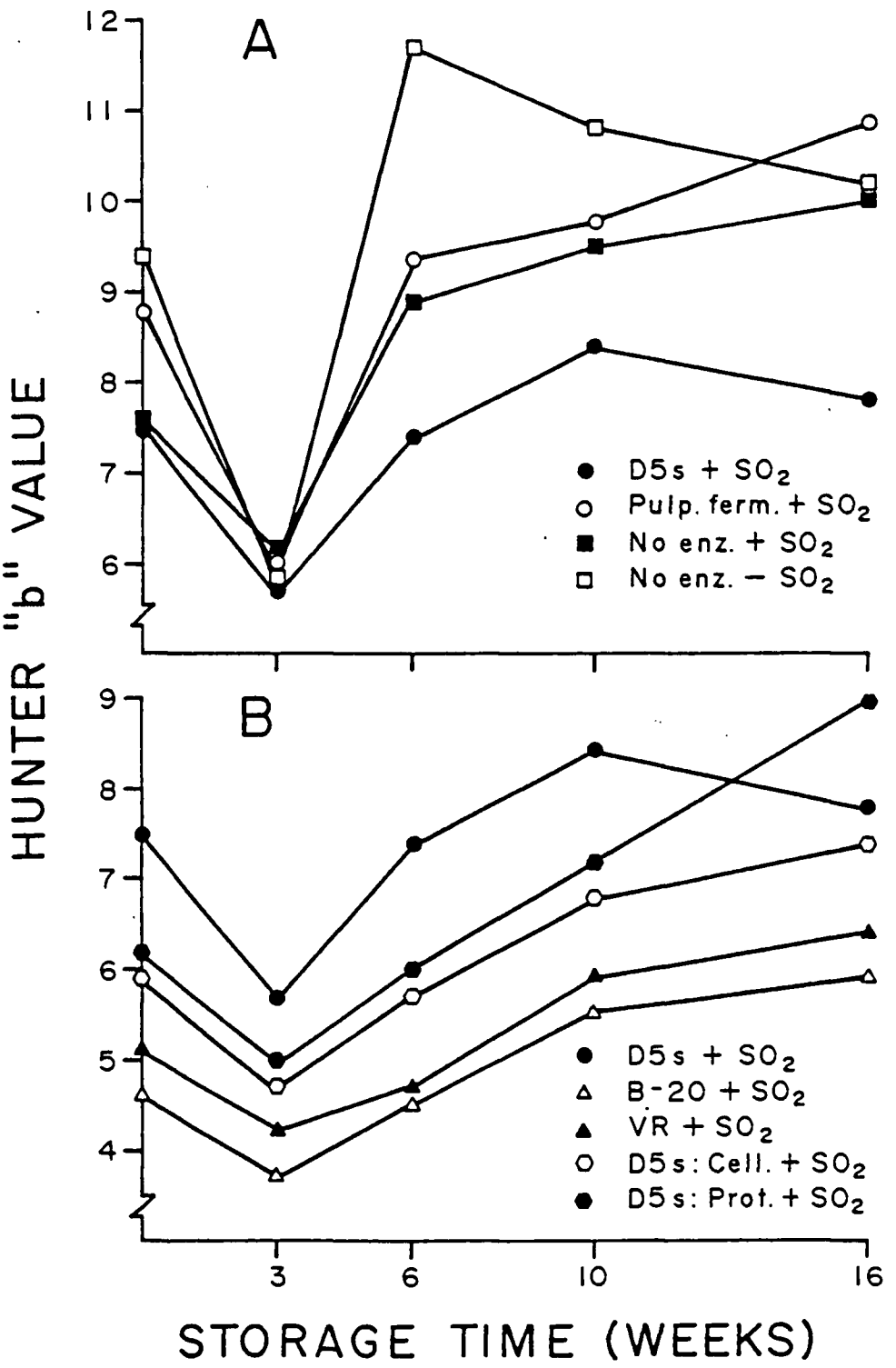
A
B
HUNTER "b" VALUE
STORAGE TIME (WEEKS)
D5s + SO2
Pulp. ferm. + SO2
No enz. + SO2
No enz. − SO2
D5s + SO2
B-20 + SO2
VR + SO2
D5s: Cell. + SO2
D5s: Prot. + SO2

Fig. III.6

Fig. III.7 Effect of different, A) Processing procedures, and B) Enzyme treatments, on changes in Hunter a values in strawberry wine during storage at 25°C. Wines prepared by fermenting juices extracted by the different pre-press pulp treatments (refer part I, tables II.1 and II.2). All results are average of at least two separate determinations.

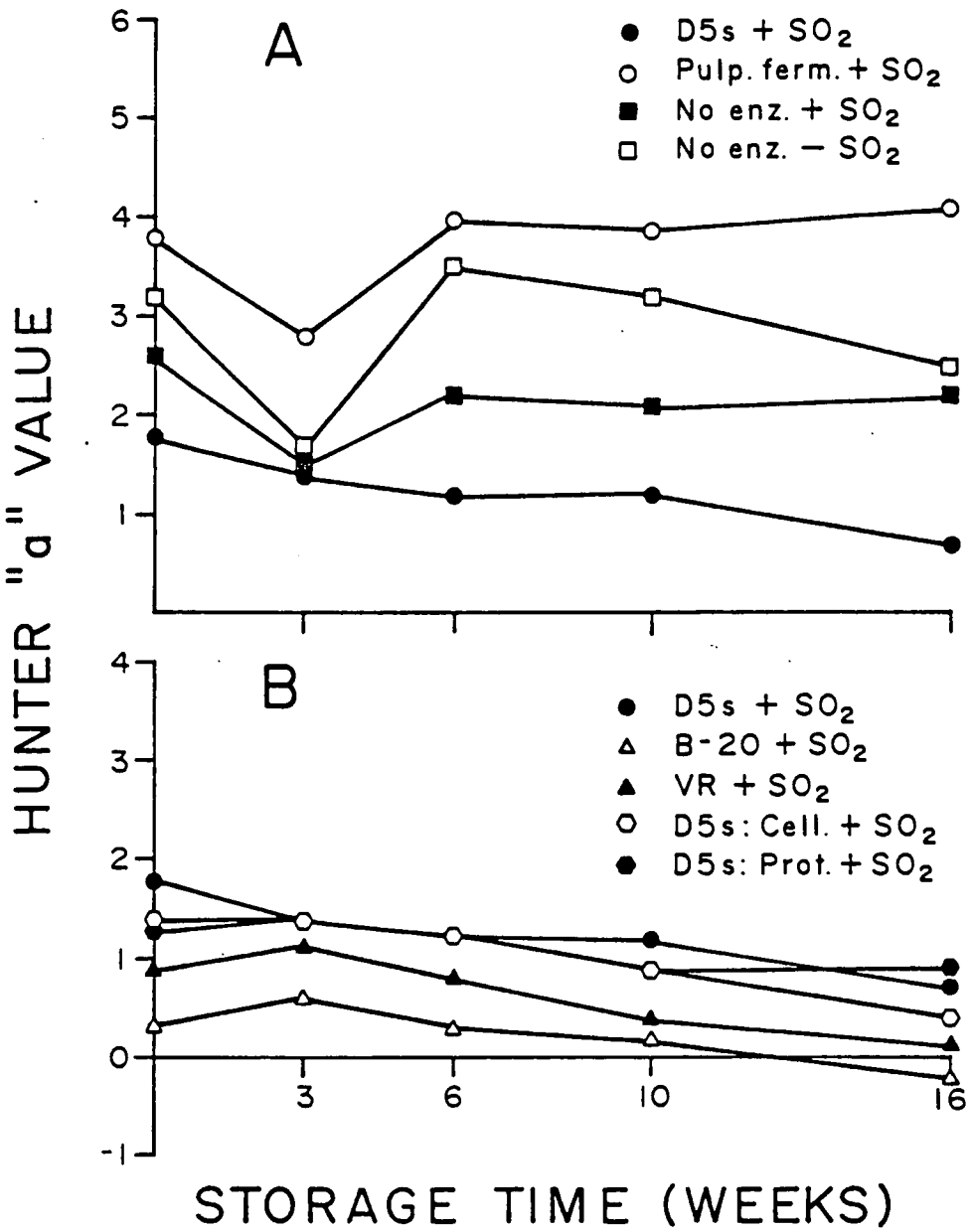
A
D5s + SO2
Pulp. ferm. + SO2
No enz. + SO2
No enz. − SO2
B
D5s + SO2
B-20 + SO2
VR + SO2
D5s: Cell. + SO2
D5s: Prot. + SO2
HUNTER "a" VALUE
STORAGE TIME (WEEKS)
6
5
4
3
2
1
0
-1
3
6
10
16

Fig. III.7

Fig. III.8 Effect of the different processing procedures, on the change of ascorbic acid, browning index, and sulfur dioxide in strawberry wine during storage at 25°C. Wines prepared by fermenting juices extracted by the different pre-press pulp treatments (refer part I, tables II.1 and II.2). All results are average of at least two separate determinations.

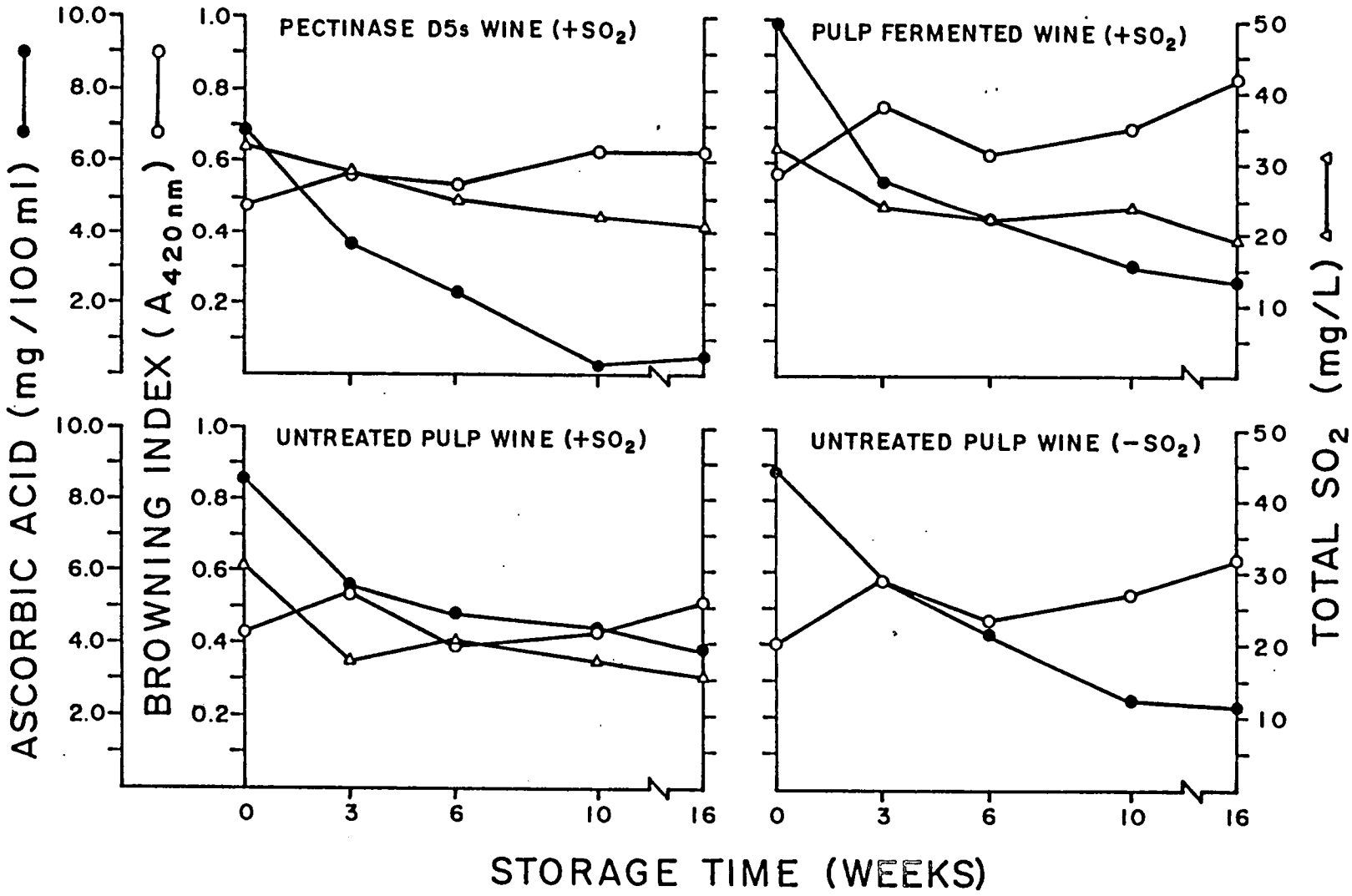
PECTINASE D5s WINE (+SO2)
PULP FERMENTED WINE (+SO2)
UNTREATED PULP WINE (+SO2)
UNTREATED PULP WINE (−SO2)
ASCORBIC ACID (mg/100 ml)
BROWNING INDEX (A420nm)
TOTAL SO2 (mg/L)
STORAGE TIME (WEEKS)
10.0
8.0
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
50
40
30
20
10
0
3
6
10
16

Fig. III.8

TABLE III.1 EFFECT OF DIFFERENT PROCESSING PROCEDURES AND ENZYME TREATMENTS ON ANTHOCYANIN PIGMENT CONTENT IN STRAWBERRY JUICE AND NEW WINE[a].

	UNTREATED PULP(-SO2)	UNTREATED PULP	PULP FERMENTED	PECTINASE D5s	PECTINASE B-20	ROHAPECT VR	D5s: CELLULASE	D5s: PROTEASE
JUICE	96.3	97.1	89.4	115.5	68.5	163.6	133.1	174.4
NEW WINE	15.1	13.3	16.3	13.9	5.4	12.0	12.8	12.2

[a] All results are average of at least two separate determinations, expressed as mg/L. Wines prepared by fermenting juices extracted by the different pre-press pulp treatments (refer part I, tables II.1 and II.2). All treatments contain 25 mg/L SO_2 unless otherwise specified.

TABLE III.2 STATISTICAL INTERPRETATION[a] OF THE EFFECT OF DIFFERENT PROCESSING PROCEDURES AND STORAGE TIME ON STRAWBERRY WINE COLOR PARAMETERS, ASCORBIC ACID AND SO_2 CONTENT.

a) Anthocyanin Pigment Content

TIME	N	$\bar{X}$ ± SE
0 WEEKS	8	14.65 ± 0.5470
3 WEEKS	8	12.69 ± 0.5470
6 WEEKS	8	9.94 ± 0.5470
10 WEEKS	8	5.34 ± 0.5470
16 WEEKS	8	4.06 ± 0.5470

4.1 5.3 9.9 12.7 14.7

b) Color Density

WINES	N	$\bar{X}$ ± SE
UNTREATED PULP ($-SO_2$)	10	0.93 ± 0.0316
UNTREATED PULP ($+SO_2$)	10	0.86 ± 0.0316
PECTINASE D5s TREATED PULP ($+SO_2$)	10	0.99 ± 0.0316
PULP FERMENTED ($+SO_2$)	10	1.24 ± 0.0316

0.86 0.93 0.99 1.24

c) Polymeric Color

TIME	N	$\bar{X}$ ± SE
0 WEEKS	8	0.58 ± 0.0689
3 WEEKS	8	0.78 ± 0.0689
6 WEEKS	8	0.60 ± 0.0689
10 WEEKS	8	0.74 ± 0.0689
16 WEEKS	8	0.85 ± 0.0689

0.58 0.60 0.74 0.78 0.85

WINE	N	$\bar{X}$ ± SE
UNTREATED PULP ($-SO_2$)	10	0.65 ± 0.0490
UNTREATED PULP ($+SO_2$)	10	0.58 ± 0.0490
PECTINASE D5s TREATED PULP ($+SO_2$)	10	0.75 ± 0.0490
PULP FERMENTED ($+SO_2$)	10	0.90 ± 0.0490

0.58 0.65 0.75 0.90

TABLE III.2 continued

d) Percent Polymeric Color

TIME	N	$\bar{X}$ ± SE
0 WEEKS	8	62.70 ± 2.7761
3 WEEKS	8	76.94 ± 2.7761
6 WEEKS	8	62.30 ± 2.7761
10 WEEKS	8	74.62 ± 2.7761
16 WEEKS	8	81.05 ± 2.7761

62.30 62.70 74.62 76.94 81.05

e) Hunter L value

TIME	N	$\bar{X}$ ± SE
0 WEEKS	8	22.61 ± 1.6575
3 WEEKS	8	17.04 ± 1.6575
6 WEEKS	8	22.66 ± 1.6575
10 WEEKS	8	21.94 ± 1.6575
16 WEEKS	8	21.09 ± 1.6575

17.04 21.09 21.94 22.61 22.66

WINE	N	$\bar{X}$ ± SE
UNTREATED PULP ($-SO_2$)	10	22.40 ± 1.2252
UNTREATED PULP ($+SO_2$)	10	23.30 ± 1.2252
PECTINASE D5s TREATED PULP ($+SO_2$)	10	19.84 ± 1.2252
PULP FERMENTED ($+SO_2$)	10	18.73 ± 1.2252

18.73 19.84 22.40 23.30

f) Hunter b value

TIME	N	$\bar{X}$ ± SE
0 WEEKS	8	8.29 ± 0.6209
3 WEEKS	8	5.91 ± 0.6209
6 WEEKS	8	9.33 ± 0.6209
10 WEEKS	8	9.61 ± 0.6209
16 WEEKS	8	9.70 ± 0.6209

5.91 8.29 9.33 9.61 9.70

WINE	N	$\bar{X}$ ± SE
UNTREATED PULP ($-SO_2$)	10	9.57 ± 0.7718
UNTREATED PULP ($+SO_2$)	10	8.42 ± 0.7718
PECTINASE D5s TREATED PULP ($+SO_2$)	10	7.32 ± 0.7718
PULP FERMENTED ($+SO_2$)	10	8.96 ± 0.7718

7.32 8.42 8.96 9.57

TABLE III.2 continued

g) Hunter a value

WINE	N	$\bar{X}$ ± SE
UNTREATED PULP ($-SO_2$)	10	2.80 ± 0.2502
UNTREATED PULP ($+SO_2$)	10	2.10 ± 0.2502
PECTINASE D5s TREATED PULP ($+SO_2$)	10	1.24 ± 0.2502
PULP FERMENTED ($+SO_2$)	10	3.68 ± 0.2502

1.24 2.10 2.80 3.68

h) Browning Index

TIME	N	$\bar{X}$ ± SE
0 WEEKS	8	0.47 ± 0.0500
3 WEEKS	8	0.61 ± 0.0500
6 WEEKS	8	0.50 ± 0.0500
10 WEEKS	8	0.57 ± 0.0500
16 WEEKS	8	0.65 ± 0.0500

0.47 0.50 0.57 0.61 0.65

WINE	N	$\bar{X}$ ± SE
UNTREATED PULP ($-SO_2$)	10	0.52 ± 0.0346
UNTREATED PULP ($+SO_2$)	10	0.46 ± 0.0346
PECTINASE D5s TREATED PULP ($+SO_2$)	10	0.57 ± 0.0346
PULP FERMENTED ($+SO_2$)	10	0.70 ± 0.0346

0.46 0.52 0.57 0.70

i) Ascorbic Acid

TIME	N	$\bar{X}$ ± SE
0 WEEKS	8	8.56 ± 0.6181
3 WEEKS	8	5.21 ± 0.6181
6 WEKKS	8	3.97 ± 0.6181
10 WEEKS	8	2.58 ± 0.6181
16 WEEKS	8	2.38 ± 0.6181

2.38 2.58 3.97 5.21 8.56

TABLE III.2 continued

j) Sulfur Dioxide

TIME	N	$\bar{X}$ ± SE
0 WEEKS	8	31.60 ± 1.7964
3 WEEKS	8	23.47 ± 1.7964
6 WEEKS	8	22.40 ± 1.7964
10 WEEKS	8	21.33 ± 1.7964
16 WEEKS	8	18.40 ± 1.7964

18.4 21.3 22.4 23.5 31.6

WINE	N	$\bar{X}$ ± SE
UNTREATED PULP ($-SO_2$)	10	0.00 ± 2.2163
UNTREATED PULP ($+SO_2$)	10	20.24 ± 2.2163
PECTINASE D5s TREATED PULP ($+SO_2$)	10	25.76 ± 2.2163
PULP FERMENTED ($+SO_2$)	10	24.32 ± 2.2163

00.0 20.2 24.3 25.8

[a] Scheffe's test for multiple comparisons, any two underscored by the same line are not significantly different.

TABLE III.3 STATISTICAL INTERPRETATION[a] OF THE EFFECT OF DIFFERENT ENZYME TREATMENTS AND STORAGE TIME ON STRAWBERRY WINE COLOR PARAMETERS, ASCORBIC ACID AND SO_2 CONTENT.

a) Anthocyanin Pigment Content

TIME	N	$\bar{X}$ ± SE
0 WEEKS	10	11.29 ± 0.9753
3 WEEKS	10	10.56 ± 0.9753
6 WEEKS	10	7.37 ± 0.9753
10 WEEKS	10	4.00 ± 0.9753
16 WEEKS	10	2.71 ± 0.9753

2.71 4.00 7.37 10.56 11.29

WINE	N	$\bar{X}$ ± SE
PECTINASE D5s	10	9.01 ± 1.6762
PECTINASE B-20	10	3.77 ± 1.6762
ROHAPECT VR	10	7.41 ± 1.6762
D5s:CELLULASE (1:1)	10	8.20 ± 1.6762
D5s:PROTEASE (1:1)	10	7.60 ± 1.6762

3.77 7.41 7.60 8.20 9.01

b) Color Density

WINE	N	$\bar{X}$ ± SE
PECTINASE D5s	10	0.99 ± 0.0246
PECTINASE B-20	10	0.77 ± 0.0246
ROHAPECT VR	10	0.91 ± 0.0246
D5s:CELLULASE (1:1)	10	1.01 ± 0.0246
D5s:PROTEASE (1:1)	10	1.13 ± 0.0246

0.77 0.91 0.99 1.01 1.13

c) Polymeric color

TIME	N	$\bar{X}$ ± SE
0 WEEKS	10	0.56 ± 0.0400
3 WEEKS	10	0.71 ± 0.0400
6 WEEKS	10	0.67 ± 0.0400
10 WEEKS	10	0.75 ± 0.0400
16 WEEKS	10	0.82 ± 0.0400

0.56 0.67 0.71 0.75 0.82

WINE	N	$\bar{X}$ ± SE
PECTINASE D5s	10	0.75 ± 0.0424
PECTINASE B-20	10	0.59 ± 0.0424
ROHAPECT VR	10	0.64 ± 0.0424
D5s:CELLULASE (1:1)	10	0.72 ± 0.0424
D5s;PROTEASE (1:1)	10	0.81 ± 0.0424

0.59 0.64 0.72 0.75 0.81

TABLE III.3 continued

d) Percent Polymeric Color

TIME	N	$\bar{X}$ ± SE
0 WEEKS	10	62.39 ± 2.1456
3 WEEKS	10	75.83 ± 2.1456
6 WEEKS	10	68.00 ± 2.1456
10 WEEKS	10	80.30 ± 2.1456
16 WEEKS	10	79.74 ± 2.1456

62.39 68.00 75.83 79.74 80.30

e) Hunter L value

TIME	N	$\bar{X}$ ± SE
0 WEEKS	10	18.46 ± 0.7405
3 WEEKS	10	14.80 ± 0.7405
6 WEEKS	10	16.46 ± 0.7405
10 WEEKS	10	17.94 ± 0.7405
16 WEEKS	10	17.73 ± 0.7405

14.80 16.46 17.73 17.94 18.46

WINE	N	$\bar{X}$ ± SE
PECTINASE D5s	10	19.84 ± 0.7064
PECTINASE B-20	10	16.67 ± 0.7064
ROHAPECT VR	10	16.14 ± 0.7064
D5s:CELLULASE (1:1)	10	16.17 ± 0.7064
D5s:PROTEASE (1:1)	10	16.57 ± 0.7064

16.14 16.17 16.57 16.67 19.84

f) Hunter b value

TIME	N	$\bar{X}$ ± SE
0 WEEKS	10	5.83 ± 0.4632
3 WEEKS	10	4.62 ± 0.4632
6 WEEKS	10	5.64 ± 0.4632
10 WEEKS	10	6.75 ± 0.4632
16 WEEKS	10	7.27 ± 0.4632

4.62 5.64 5.83 6.75 7.27

WINE	N	$\bar{X}$ ± SE
PECTINASE D5s	10	7.32 ± 0.4680
PECTINASE B-20	10	4.82 ± 0.4680
ROHAPECT VR	10	5.24 ± 0.4680
D5s:CELLULASE (1:1)	10	6.08 ± 0.4680
D5s:PROTEASE (1:1)	10	6.65 ± 0.4680

4.82 5.24 6.08 6.65 7.32

TABLE III.3 continued

g) Hunter a value

TIME	N	$\bar{X}$ ± SE
0 WEEKS	10	1.10 ± 0.1838
3 WEEKS	10	1.17 ± 0.1838
6 WEEKS	10	0.93 ± 0.1838
10 WEEKS	10	0.72 ± 0.1838
16 WEEKS	10	0.37 ± 0.1838

0.37 0.72 0.93 1.10 1.17

WINE	N	$\bar{X}$ ± SE
PECTINASE D5s	10	1.24 ± 0.1503
PECTINASE B-20	10	0.24 ± 0.1503
ROHAPECT VR	10	0.65 ± 0.1503
D5s:CELLULASE (1:1)	10	1.05 ± 0.1503
D5s:PROTEASE (1:1)	10	1.11 ± 0.1503

0.24 0.65 1.05 1.11 1.24

h) Browning Index

TIME	N	$\bar{X}$ ± SE
0 WEEKS	10	0.46 ± 0.0283
3 WEEKS	10	0.56 ± 0.0283
6 WEEKS	10	0.51 ± 0.0283
10 WEEKS	10	0.57 ± 0.0283
16 WEEKS	10	0.63 ± 0.0283

0.46 0.51 0.56 0.57 0.63

WINE	N	$\bar{X}$ ± SE
PECTINASE D5s	10	0.57 ± 0.0283
PECTINASE B-20	10	0.47 ± 0.0283
ROHAPECT VR	10	0.50 ± 0.0283
D5s:CELLULASE (1:1)	10	0.56 ± 0.0283
D5s:PROTEASE (1:1)	10	0.64 ± 0.0283

0.47 0.50 0.56 0.57 0.64

i) Ascorbic Acid

TIME	N	$\bar{X}$ ± SE
0 WEEKS	10	7.40 ± 0.3127
3 WEEKS	10	4.20 ± 0.3127
6 WEEKS	10	2.89 ± 0.3127
10 WEEKS	10	1.90 ± 0.3127
16 WEEKS	10	1.80 ± 0.3127

1.80 1.90 2.89 4.20 7.40

TABLE III.3 continued

j) Sulfur Dioxide

TIME	N	$\overline{X}$	±	SE
0 WEEKS	10	31.04	±	1.2961
3 WEEKS	10	26.40	±	1.2961
6 WEEKS	10	22.48	±	1.2961
10 WEEKS	10	23.44	±	1.2961
16 WEEKS	10	15.20	±	1.2961

15.20 <u>22.48 23.44 26.40</u> 31.04

[a] Scheffe's test for multiple comparisons, any two underscored by the same line are not significantly different.

TABLE III.4 SIMPLE CORRELATION ANALYSIS[a] OF THE WINE[b] PARAMETERS ANALYZED[c].

	UNTREATED PULP($-SO_2$)	UNTREATED PULP	PULP FERMENTED	PECTINASE D5s	PECTINASE B-20	ROHAPECT VR	D5s: CELLULASE	D5s: PROTEASE
SO_2-ANT	-------	+0.6975	+0.7642	+0.9575	+0.7188	+0.8759	+0.8588	-------
SO_2-CD	-------	-------	-0.8253	-0.7492	-0.9082	-0.8138	-0.7640	-------
SO_2-PC	-------	-0.6701	-0.8182	-0.8633	-0.6646	-0.7813	-0.9306	-0.9349
SO_2-PPC	-------	-------	-------	-0.7159	-------	-------	-0.8066	-0.8589
SO_2-8I	-------	-------	-0.7919	-0.8438	-------	-0.7865	-0.9377	-0.9170
SO_2-ASC	-------	+0.9279	+0.9324	-------	+0.6605	+0.8072	+0.8373	+0.9013
SO_2-HaV	-------	-------	-------	+0.8292	+0.7824	+0.8218	+0.9153	-------
SO_2-HbV	-------	-------	-------	-------	-------	-0.7063	-0.6893	-------
ANT-CD	-------	-------	-------	-0.7228	-0.6836	-------	-------	-------
ANT-PC	-0.6513	-------	-0.6607	-0.8621	-------	-0.7921	-0.8794	-0.7658
ANT-PPC	-------	-------	-------	-0.7744	-------	-0.7444	-0.9350	-0.7544
ANT-BI	-0.6567	-------	-------	-0.8514	-------	-0.7340	-0.8467	-------
ANT-ASC	+0.9619	+0.7755	+0.8965	+0.9290	+0.6513	+0.8510	+0.8641	+0.8244
ANT-HaV	-------	-------	-------	+0.8377	+0.8412	+0.9175	+0.9168	+0.9343
ANT-HbV	-------	-0.6879	-0.6644	-------	-0.8618	-0.8336	-0.8391	-0.8436
CD-PC	-------	-------	+0.7325	+0.6457	-------	+0.7571	+0.7337	-------
CD-BI	-------	-------	+0.7183	-------	-------	+0.8530	+0.7800	-------
CD-ASC	-------	-------	-0.6749	-0.7827	-------	-------	-------	-------
CD-HaV	-------	-------	-------	-0.6620	-0.7537	-------	-------	-------
PC-PPC	+0.9018	+0.8245	+0.9099	+0.9373	+0.7082	+0.8893	+0.9250	+0.9535
PC-BI	+0.9794	+0.9395	+0.9967	+0.9957	+0.9731	+0.9716	+0.9801	+0.9579
PC-ASC	-0.7524	-------	-0.7336	-0.9387	-0.7767	-0.8704	-0.8672	-0.9172
PC-HaV	-------	-------	-------	-0.8069	-------	-------	-0.8661	-------
PPC-8I	+0.9303	+0.8644	+0.9138	+0.9466	+0.7381	+0.7785	+0.8653	+0.9233
PPC-ASC	-------	-------	-------	-0.7986	-------	-0.8725	-0.8812	-0.8182
PPC-HaV	-------	-------	-------	-0.6908	-------	-------	-0.8052	-------
BI-ASC	-0.7240	-------	-0.7018	-0.9195	-0.6968	-0.7632	-0.7896	-0.8052
BI-HLV	-0.6577	-------	-------	-------	-------	-------	-------	-------
BI-HaV	-------	-------	-------	-0.7846	-------	-------	-0.9079	-------
8I-HbV	-------	-------	-------	-------	-------	-------	+0.6356	-------
ASC-HaV	-------	-------	-------	+0.7977	-------	+0.6536	+0.7113	+0.6477
HLV-HaV	+0.9698	+0.7007	+0.9328	-------	-------	-------	-------	-0.8030
HLV-HbV	+0.8594	+0.9107	+0.8753	+0.7099	-------	+0.7723	+0.8620	+0.9278
HaV-HbV	+0.9036	-------	+0.9296	-------	-0.9099	-0.9597	-0.8909	-0.8961

[a] Simple correlation, testing Ho:ρ=0; Ha:$\rho\neq$0, $r_{0.05,8}$=0.632.

[b] Wines prepared by fermenting juices extracted by the different pre-press pulp treatments (refer tables II.1 and II.2, part I). All treatments contain SO_2 unless otherwise specified.

[c] SO_2:Sulfur Dioxide; ANT:Anthocyanin; CD:Color Density; PC:Polymeric Color; PPC:Percent Polymeric Color; BI:Browning Index; ASC:Ascorbic Acid; HLV:Hunter L Value; HaV:Hunter a Value; HbV:Hunter b Value.

TABLE III.5 SIMPLE CORRELATION ANALYSIS[a] OF WINE PARAMETERS ANALYZED VS. SENSORY ANALYSIS DATA[b].

Sulfur Dioxide-Clarity	-0.7572	Polymeric Color-Browning	-0.7790	Hunter a-Browning	-0.8473
Ascorbic acid-OCA[c]	-0.7491	Polymeric Color-Hue	+0.7941	Hunter b-Clarity	-0.7218
Color Density-Browning	-0.7689	Browning Index-Browning	-0.7696	Hunter b-Browning	-0.7394
Color Density-Hue	+0.7631	Browning Index-Hue	+0.7870		

[a] Simple correlation, testing Ha:$\rho=0$; Ha:$\rho\neq0$, $r_{0.05,6}=0.707$.

[b] Quality evaluation scored on a desirability scale of from nine (extremely desirable) to one (extremely undesirable).

[c] Over-all color and appearance.

TABLE III.6 STRAWBERRY WINE COLOR AND APPEARANCE EVALUATION: EFFECT OF DIFFERENT PROCESSING PROCEDURES.

WINE[a]		MEAN SCORE[b]				
		COLOR INTENSITY	HUE	BROWNING	CLARITY	OVER-ALL COLOR AND APPEARANCE
UNTREATED PULP	($-SO_2$)	5.77	5.46	4.92	6.69	5.46
UNTREATED PULP	($+SO_2$)	5.69	5.31	5.00	6.31	5.31
PECTINASE D5s TREATED PULP	($+SO_2$)	6.31	5.77	5.23	7.15	6.00
PULP FERMENTED	($+SO_2$)	6.08	5.85	4.54	7.15	5.92
LSD[c] at 5%		0.74	0.76	0.72	0.57	0.76
LSD[c] at 1%		0.99	0.99	0.95	0.75	1.01

[a] Wines prepared by fermenting juices extracted by listed pre-press pulp treatment (refer part I, tables II.1 and II.2).
[b] Quality evaluation scored on a desirability scale of from nine (extremely desirable) to one (extremely undesirable).
[c] Least significant difference.

TABLE III.7 STRAWBERRY WINE COLOR AND APPEARANCE EVALUATION: EFFECT OF DIFFERENT ENZYME TREATMENTS.

WINE[a]	MEAN SCORE[b]				
	COLOR INTENSITY	HUE	BROWNING	CLARITY	OVER-ALL COLOR AND APPEARANCE
PECTINASE D5s ($+SO_2$)	6.31	5.77	5.23	7.15	6.00
PECTINASE B-20 ($+SO_2$)	5.46	4.92	5.08	7.69	5.62
ROHAPECT VR ($+SO_2$)	5.54	5.31	5.08	7.38	5.69
D5s:CELLULASE(1:1)($+SO_2$)	6.08	5.92	5.00	7.46	5.77
D5s:PROTEASE (1:1)($+SO_2$)	6.00	5.46	4.85	7.08	5.62
LSD[c] at 5%	0.74	0.75	0.72	0.57	0.76
LSD[c] at 1%	0.99	0.99	0.95	0.75	1.01

[a] Wines prepared by fermenting juices extracted by listed pre-press pulp treatment (refer part I, tables II.1 and II.2).
[b] Quality evaluation scored on a desirability scale of from nine (extremely desirable) to one (extremely undesirable).
[c] Least significant difference.

TABLE III.8 SENSORY ANALYSIS OF STRAWBERRY WINE: EFFECT OF DIFFERENT PROCESSING PROCEDURES.

WINE[a]		MEAN SCORE REFERENCE-DIFFERENCE[b] COLOR	AROMA	TASTE	HEDONIC SCALE[c]
UNTREATED PULP	($-SO_2$)	3.09	3.62	4.08	5.00
UNTREATED PULP	($+SO_2$)	3.68	3.38	3.54	4.85
PECTINASE D5s TREATED PULP[d]	($+SO_2$)	1.55	2.08	2.92	5.46
PULP FERMENTED	($+SO_2$)	2.50	2.81	3.88	5.15
LSD[e] at 5%		0.78	0.81	0.80	0.62
LSD[e] at 1%		1.04	1.09	1.06	0.83

[a] Wines prepared by fermenting juices extracted by listed pre-press pulp treatment (refer part I, tables II.1 and II.2).
[b] Scored on a intensity scale of from 1 (same as reference) to 9 (extremely different from the reference).
[c] Scored on a hedonic scale of form 9 (like extremely) to 1 (dislike extremely).
[d] Reference.
[e] Least significant difference.

TABLE III.9 SENSORY ANALYSIS OF STRAWBERRY WINE: EFFECT OF DIFFERENT ENZYME TREATMENTS.

WINE[a]	MEAN SCORE[b] REFERENCE-DIFFERENCE			HEDONIC SCALE[c]
	COLOR	AROMA	TASTE	
PECTINASE D5s[d] ($+SO_2$)	1.63	2.82	2.82	5.09
PECTINASE B-20 ($+SO_2$)	2.88	2.36	3.27	5.00
ROHAPECT VR ($+SO_2$)	1.88	1.82	2.82	5.91
D5s:CELLULASE(1:1)($+SO_2$)	1.38	2.27	2.91	6.36
D5s:PROTEASE (1:1)($+SO_2$)	1.63	2.82	2.91	4.91
LSD[e] at 5%	0.94	1.17	1.24	1.04
LSD[e] at 1%	1.27	1.56	1.66	1.40

[a] Wines prepared by fermenting juices extracted by listed pre-press pulp treatment (refer part I, tables II.1 and II.2).
[b] Scored on an intensity scale of form 1 (same as reference) to 9 (extremely different form reference).
[c] Scored on a hedonic scale of from 9 (like extremely) to 1 (dislike extremely).
[d] Reference.
[e] Least significant difference.

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A P P E N D I C E S

APPENDIX 1

Scoring System:
Superior: 9, 8, 7
Acceptable: 6, 5, 4
Unacceptable: 3, 2, 1

The Flavorium
Departament of Food Science and Technology
Oregon State University
Name:_____
Date:_____

Please write the sample number in the space following the statement which best describes your opinion of the sample.

STRAWBERRY WINE COLOR AND APPEARANCE EVALUATION

	COLOR INTENSITY	HUE	BROWNING	CLARITY	OVER - ALL COLOR AND APPEARANCE
9 - Extremely desirable					
8 - Very desirable					
7 - Moderately desirable					
6 - Slightly desirable					
5 - Neither					
4 - Slightly desirable					
3 - Moderately undesirable					
2 - Very undesirable					
1 - Extremely undesirable					

If unacceptable, Why?
Comments:
(Aroma, Flavor, etc.)

APPENDIX 2

The Flavorium
Departament of Food Science and Technology
Oregon State University

Product: STRAWBERRY WINE Name:_____

The side cup contains a reference "Ref" sample. Please score the degree of difference between the coded samples and the Ref sample then score how well you like the coded samples. Thank you.

REFERENCE DIFFERENCE			OVER-ALL
COLOR	AROMA	TASTE	DESIRABILITY
Same as "Ref"			Like Extremely
Very Slight Difference			Like Very Much
Slightly Different			Like Moderately
Slightly to Moderate			Like Slightly
Moderate			Neither
Moderate to Strong			Dislike Slightly
Strong Difference			Dislike Moderately
Strong to Extreme			Dislike Very Much
Extremely Different			Dislike Extremely

Which sample do you prefer?_____
Why? (Comment freely).

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