AN ABSTRACT OF THE THESIS OF

CHRIS CORNWELL for the degree of MASTER OF SCIENCE

in FOOD SCIENCE presented on August 30, 1979

Title: CAUSES OF BROWNING IN PEAR JUICE CONCENTRATE DURING STORAGE

Abstract approved: _____
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The causes of browning in pear juice concentrate (PJC) during storage were investigated. Two experiments were devised to assess the importance of oxidative and Maillard browning reactions in samples of PJC stored at 37°C. In addition, volatile analysis of certain stored samples was performed instrumentally and through taste panel evaluation.

Color change in samples during storage was measured as increased absorbance at 420 nm, and as changes in Hunter L, a, b values.

Pear juice and PJC (72°Brix) produced commercially from d'Anjou pears were used to prepare samples.

Treatments included removal of polyphenols with polyvinylpolypyrrolidone (PVPP) and Amberlite XAD-4. Use of the Folin-Ciocalteu reagent showed a reduction in total polyphenolic content of 64% and 82%, respectively. Both treatments resulted in light colored PJC samples.

Amino acids were removed with Dowex-50 cation exchange resin. A ninhydrin reagent was used to show the absence of alpha-amino nitrogen containing compounds. Dowex treatment also resulted in the removal of polyphenolics (48%) and some decolorization.

The three samples described above, and an untreated PJC sample were stored for over 6 months. Results showed that PVPP treatment only slightly inhibited browning. Treatment with XAD-4 inhibited the browning rate more initially; however, both treatments did not prevent browning from ultimately approaching the rate of the untreated sample.

The Dowex treated sample was relatively stable during storage and did not undergo extensive browning as did PVPP and XAD-4 treated samples.

These results suggested additional sample modifications which were used in sample preparation for the second storage experiment.

The alpha-amino nitrogen content was restored (as glycine) to Dowex treated PJC. Pear juice was treated with Dowex, and then oxygenated for 2 hours, followed by XAD-4 treatment. The pH of the treated juice was adjusted to that of untreated pear juice (pH 4.1), and it was concentrated to 72°Brix.

Samples were also treated, separately, by oxygenation, nitrogenation, and reduction in pH (to 3.3 and 2.4). In addition, ascorbic acid was added to Dowex treated PJC, which was then oxygenated to

promote the formation of dehydroascorbic acid.

The samples above were stored for over 3 months. Results showed that after restoration of the alpha-amino nitrogen content to the Dowex treated sample, the rate of browning became similar to that of the untreated sample.

The Dowex-oxygenated-XAD-4 treated sample was the most stable. Reduction of pH did not reduce the rate of browning. Oxygenation increased the rate of browning when compared to the untreated sample; however, only for the first 20 days of storage, and afterwards, the rate was nearly identical.

The Dowex treated sample with added ascorbic acid, browned less than the sample treated with Dowex, alone.

Increased levels of ascorbic acid were measured during storage of PJC, while none was detected prior to storage. This effect was attributed to the formation of reductones during Maillard browning reactions.

Furfuraldehyde was identified tentatively, by GC-MS in stored PJC. Spectrophotometric evidence suggested that hydroxymethylfurfural may have been present in PJC during storage. Taste panel evaluation of stored PJC samples showed that off flavors were strong in the untreated sample, but not present in the Dowex treated sample.

The water activity of PJC was 0.73, which was conducive for the occurrence of Maillard browning reactions.

The evidence suggests that Maillard reactions predominated in browning of PJC during storage. Oxidative, or polyphenolic browning was of much less importance.

Causes of Browning in Pear Juice Concentrate During Storage

by

Chris Cornwell

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed August 1979

Commencement June 1980

APPROVED:

Associate Professor of Food Science and Technology
in charge of major

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Date Thesis is Presented August 30, 1979

Typed by Opal Grossnicklaus for Chris Cornwell

ACKNOWLEDGEMENTS

Sincere thanks are due to Dr. Ronald Wrolstad for providing assistance when needed, during this project. It was an enjoyable experience to work with him, as my major professor. I also appreciated the helpful suggestions provided by Dr. Morris Montgomery, Dr. W. D. Loomis, Mr. Darrell Beavers, Mr. Jeff Culbertson, and Mrs. Lois McGill.

Financial assistance provided from the Pacific Northwest Regional Commission "Study of Fruit and Berry Concentrate Processing in the Pacific Northwest" (grant no. 10790054) was very helpful. I hope my work may be of some benefit to those concerned with browning during the storage of pear juice concentrate.

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INTRODUCTION

Economic Value of Pear Juice Concentrate

During 1978, almost 40 million pounds of pear fruit was used to produce nearly 360 thousand gallons of pear juice concentrate (PJC) in the Oregon-Washington region. Production of one gallon of PJC required the use of 111 pounds of fruit.

At a price of 6.25 dollars per gallon the economic value of PJC from this area alone was determined to be over 2.23 million dollars.

Uses of Pear Juice Concentrate

PJC, produced from surplus and cull pears is a shelf stable (microbiologically) product and may be stored inexpensively until sold. Its uses include the production of wine, vinegar, beverages, and as a syrup base for canning fruits (Akavan, 1977). A bland PJC product is sought for such uses.

Color Deterioration

PJC is stored in 55 gallon drums at ambient temperatures. During storage it becomes very dark brown and off flavors develop.

These problems decrease its value and versatility for the uses above.

Pear juice (PJ), diluted from PJC, and added to pear halves during canning would increase the value of PJC; however improved color stability is needed. Some PJC is refrigerated during storage to control color degradation.

Composition of PJC

A. Sugars and Acids

Akavan (1977) analyzed the sugars and acids found in PJC produced commercially from Bosc and Comice pears He reported results in grams of sugar per 10 gram PJC sample as: fructose (4.36 g), glucose (0.88 g), sorbitol (1.20 g), and sucrose (0.85 g), for a total of 7.29 grams.

Acids were reported in milli-equivalents acid per 10 gram PJC sample as: phosphoric (0.43 meq), malic (1.50 meq), citric (0.05 meq), and quinic (0.04 meq), for a total of 2.02 milli-equivalents acidity.

B. Amino Acids

Chang (1978) reported results of amino acid analysis of cloudy-ripe Bartlett PJ from 1975, 1976, and 1977 samples. The total amino acid content (protein and free) ranged from 1.38-2.88 mg/ml PJ. The content of free amino acids ranged from 0.53-0.95 mg/ml PJ. In addition, some ammonia was included in these totals.

Major free amino acids were reported as threonine and serine;

although asparagine and/or glutamine co-chromatographed with these. Other quantitatively important free amino acids were aspartic acid, proline, glutamic acid, and phenylalanine.

Pribella (1964) determined that the free amino acid content of PJC was about 1/3 of the total nitrogen content, which is comparable to the totals above, found in PJ.

C. Polyphenols (Pear Puree)

Sioud and Luh (1966) determined the polyphenolic composition of Bartlett pears which were crushed whole to produce a puree, and then aseptically canned.

The relative amounts of polyphenolic compounds found were leucoanthocyanidins (32.40%), catechin and epicatechin (32.50%), chlorogenic acids (23.78%), quercitrin and isomers (10.00%), quercetin (0.68%), and p-coumaryl quinic acids (0.5%).

Chlorogenic acid and catechins are known to serve as substrates for polyphenoloxidase (Ranadive and Haard 1971).

Suspected Causes of Browning During Storage of Pear Juice Concentrate

A. Phenolic Browning Reactions

When polyphenols are oxidized to quinones (or semi-quinones) they may polymerize, or condense with functional groups of protein such as sulfhydryl and primary amino groups, resulting in the formation of brown color (Loomis 1974; and Synge 1975). It has been

reported that reactions between polyphenols and amino acids are the most important in non-enzymatic browning of food products. Furthermore, in neutral medium these reactions are stronger than those between glucose and amino acids, but a decrease in pH inhibits the browning reactions (Segal et al. 1971).

The brown color developed in freshly produced PJC samples is undoubtedly due to the action of pear polyphenoloxidase upon orthopolyphenols (Sioud and Luh, 1966). Skorikova (1970) found that pears lost much of their polyphenolic content during pressing for juice. Leucoanthocyanidins were reduced by 50% during the first 5 minutes, and chlorogenic acid and some flavanols were oxidized. Heating of the pressed juice caused additional loss of polyphenols.

Ranadive and Haard (1971) reported that the extent of enzymic browning in certain pear varieties may be a measure of polyphenolic content.

During storage of PJC, polyphenoloxidase may continue to oxidize remaining o-polyphenols, even though the water activity is reduced (Acker 1969). In addition, many of the polyphenolic compounds in PJC can readily oxidize when exposed to air, and form brown polymers, in the absence of enzyme activity (Robinson 1975).

Anderson (1970) discovered that when fruit juice concentrates were allowed to stand for 3-4 months; oxidation and polymerization of the phenolic fraction occurred (the storage temperature was not

given). When oxidation products were removed, the concentrates were more stable to further browning.

B. Non-Enzymic Browning Reactions

Non-enzymic-browning reactions include a diverse group of dehydration, fragmentation, and polymerization reactions involving sugars. The chemistry and kinetics of these reactions are not fully understood. When they occur in the absence of nitrogenous compounds, they are described as caramelization reactions. When they occur in the presence of nitrogenous compounds, especially primary and secondary amines, they are called carbonyl-amine or Maillard reactions (Schallenberger and Birch, 1975).

The initial stages of the Maillard reaction are colorless (Hodge 1953). During this time, an acid catalyzed condensation reaction occurs between aldehydes, ketones, or reducing sugars with amines, amino acids, peptides, or proteins, which leads to the formation of glycosylamines. A base catalyzed rearrangement follows and the resulting product may then undergo further sugar degradation reactions. These reactions may occur at moderate temperatures (Reynolds 1969). Degradation products such as reductones and furfurals are produced which can brown in the absence of amino compounds (above). When such compounds are present, browning is accelerated tremendously and nitrogen can be found covalently bonded within the brown

polymers, or melanoidins (Hodge and Osman 1976).

Maillard browning reactions are strongest between pH values of about 6 and 9. Below pH 6.5, the reaction is much weaker, and may occur through a different reaction mechanism (Ellis, 1959).

The rate of initial sugar-amine interactions increase as the water content of a system decreases, and are therefore most prevalent in dried and concentrated foods (Reynolds, 1968).

Evidence Supportive of Maillard Reactions as the Primary Cause of Browning in Pear Juice Concentrate During Storage

Akhavan (1977) measured a 6% loss in total sugars during storage of PJC at 37°C for 4 months. He also recorded an increase in browning. Hydrolysis of sucrose was suggested to account for the loss of 2.5% of the total sugar loss, while loss of fructose, glucose, and to a much lesser extent, sorbitol, may have accounted for the remaining 3.5% loss. He also analyzed a 3 year old PJC sample which had been stored at room temperature. Sucrose was absent, and high levels of sorbitol, which is non-reducing, and would not be utilized during Maillard browning reactions, was found.

In addition, Akhavan reported that decolorized samples of brown PJC revert to their original color after a few months. He suggested that Maillard reactions may have been responsible.

Pribella and Betusova (1964) discovered that while protein

suffered little change during the storage of apple, pear, and cherry fruit juice concentrates, a very large decrease in alpha-amino nitrogen concentration did occur.

They also prepared a model system consisting of an apple juice concentrate, an amino acid free concentrate (which had been derived from the former), and a 70% sugar solution (glucose-fructose, 1:1, with 3.4% "apple" acid added). Only the apple juice concentrate browned.

They concluded that it is possible to predict the color changes of juices from the results of a quantitative determination of alpha-amino nitrogen.

Anderson (1970) patented a process for improving the color and flavor stability of fruit juice concentrates. Nitrogenous components are removed using a base-exchange resin, followed by controlled oxidation of phenolic and other oxidizable compounds. The oxidized products are removed with activated carbon or other absorbent. Anderson found that removal of nitrogenous compounds alone was not sufficient to prevent considerable darkening during storage.

Brueinmer and Bowers (1977) reported that orange juice concentrate treated with Dowex 50 W-8x cation exchange resin (H+ form, 20-50 mesh) to remove amino acids and lower the pH to 2.0, browned less than untreated orange juice concentrate during storage for 15 weeks at 30°C. Dowex treated orange juice concentrate adjusted to

pH 4.5 browned considerably. It was concluded that low pH probably was more effective at reducing browning than removal of amino acids.

Honey is quite similar to PJC in moisture content, and pH range (Wootton 1976a). Honey also contains high levels of reducing sugars (Doner 1977), as does PJC. Bosi and Battagini (1977) found that the average total free amino acid content in 24 samples of nectar honey was 174.6±33.2 mg/100 g honey (the concentration of free amino acids in PJC is about 3 times higher). Major amino acids were proline and phenylalanine.

Ramsey and Milum (1933) added formaldehyde to honey to eliminate the effect of amino acids (Shiff's base formation). After heating to 121°C for 15 minutes, no change in color was observed. They concluded that Maillard reactions were important in the browning of honey.

Wootton (1976a) added sulfite (sodium metabisulfite) to honey and found that inhibition of browning during storage at 50°C occurred. The effectiveness of sulfite as an inhibitor of Maillard reactions, especially during the initial stages, has been intensively studied by McWeeny et al. (1974).

Wooton also found that addition of ascorbic acid to honey did not effect the rate of browning. Free amino acids were shown by Wootton (1976b) to decrease during storage of honey, although some amino acids increased. He attributed the increase to protein

hydrolysis.

Wootton (1978) discovered that storage of honey at 50°C for 44 days resulted in increased levels of hydroxymethylfurfural, furfural, and furandialdehyde.

Purpose and Experimental Design

The purpose of this work was to reveal the cause(s) of browning in PJC during storage. Various sample modifications, followed by storage of samples @ 37°C served as the basis of this investigation.

Phenolic absorbents such as polyvinylpolypyrrolidone (PVPP) and Amberlite XAD-4 were used to bind phenolic compounds and thus remove them from PJC samples.

PVPP binds phenolic compounds through the development of very stable hydrogen bonds with phenolic hydroxyl groups. XAD-4 is a styrene-divinylbenzene polymer with a surface area of 725 m^2/g and an average pore diameter of 40 Å. Phenolics are bound to XAD-4 through hydrophobic interactions, mainly (Loomis et al., 1979).

Dowex AG 50W-X4 cation exchange resin was used to remove amino acids; however it also has the capacity to remove phenolics (Gray 1978). The use of a phosphate based cation exchange cellulose to remove amino acids more selectively was tried; but amino acids were not adequately removed.

Browning was measured @ 420 nm with a spectrophotometer.

A Hunter D-25 colorimeter was used to provide a measure of changes in chromaticity, and sample "lightness."

McWeeny (1969) has emphasized that as browning progresses in a sample, the chromophoric functional group(s) may become modified, effecting a spectral shift. Such a shift could conceivably cause a large apparent increase (or decrease) in sample browning as measured by a single narrow wave band of light; although total brown pigment concentration may undergo little change.

The Hunter D-25 colorimeter was used to guard against this possibility, as monochromatic light is not used (Hunter 1975). In addition, sample dilutions which reduce the precision of measurements made with a spectrophotometer, and are laborious, were not necessary when the Hunter instrument was used.

Lea and Hannan (1949) measured the rate of a glucose-casein reaction between 0° and 90°C, and found that the reaction occurred uniformly with increasing temperature.

Waletzko (1976) has shown that non-enzymic browning (including Maillard reactions) increased with higher storage temperatures in a model system utilizing an intermediate moisture food product (a_w 0.85). He found that use of accelerated temperature conditions did not result in absolute straight lines on an Arrhenius plot. When browning rates at 35° and 45°C were used to predict browning which might occur at 25°C, higher rates were predicted than that which was

actually measured.

Waletzko attributed this effect to either a change in mode of deterioration or more likely, the problem of obtaining good data with a heterogenous food system. The rate of browning at 45°C was about 10 times that at 25°C.

Instrumental volatile analysis and taste panel evaluation of PJC aroma samples were performed to determine if off flavors were produced in PJC samples during storage. Non-enzymic browning reactions (including Maillard) are known to produce flavor compounds (Hodge 1967).

EXPERIMENTAL

Part I. Experiment 1

1.0 Origin of Pear Juice and Pear Juice Concentrate Used to Prepare Samples for Storage

Pear juice (PJ) and pear juice concentrate (PJC) samples processed from d'Anjou pears were obtained December 1977 from Sabroso Co., Medford, Oregon and frozen at -40°C until use.

Production of PJC at Sabroso Co. (Root 1977) began following removal of whole pears from cold storage (-1°C). These pears had been stored since harvesting on September 15, 1977. The pears were washed with cold tap water (2°C) and crushed in a Rietz Disintegrator. One % by weight wood pulp Kraft paper was added to facilitate grinding during disintegration.

This pulpy mixture was then pumped to a Rietz press, and 1.5% by weight rice hulls were added as a pressing aid. The temperature of the resulting press juice was 2°-5°C and the color was described at this stage as "white."

The PJ was next heated to 54°C using a scraped-surface heat exchanger, and pumped to 2000 gallon tanks. A high temperature pectinase, Pectinol 59L was added (12 fluid ounces per 2000 gallon PJ). The juice was depectinized for 1 hour and then filtered through a pressure leaf diatomaceous filter to remove cloudiness.

Filtered PJ was next transferred to holding tanks prior to blanching. This 12° Brix juice was described as "amber," and 2 gallons were collected for samples.

A Fran-Rica Rotary Coil hot break operated at 88°C with a residence time of 2000 gallons per hour was used to blanch the filtered PJ. Blanched juice was pumped to a single effect two stage Buffalo evaporator held at 24 in Hg vaccuum and 63°C.

The PJ was concentrated to 72° Brix, a process which took about 2 hours, and the resulting PJC was cooled to 38°C by a Votator heat exchanger. Two gallons of this PJC, described as "amber brown" were collected for samples.

1.1 Preparation of Pear Juice Concentrate Samples for Storage

A. Method Used for Estimation of Total Phenolic Content

Phenolics were measured using the Folin-Ciocalteu reagent. Preparation of the reagent and reaction conditions used were the same as suggested by Singleton and Rossi (1965). PJC samples were diluted 1:6 with distilled water. One ml of the resulting PJ was used for analysis, which was performed in duplicate. Total reaction volume was 100 ml.

A standard curve from zero to six-hundred mcg gallic acid (recrystallized twice) was prepared. The molar absorptivity of gallic acid used was 22,500. Results were calculated in gallic acid equivalents. Sample absorbance was determined at 765 nm using 1.0 cm pathlength cells and a Beckman DBG spectrophotometer.

B. Method Used for Detection of Amino Acids

The ninhydrin reagent of Moore (1968) was formulated and used to detect the presence of amino acids in PJ. Reaction conditions used were as proposed by Blackburn (1968).

A 1 ml sample of PJ was mixed gently in a sealed screw-cap test tube. The tubes were then heated for 15 minutes in a 100°C water bath. Afterwards, 5 ml of distilled water was added to each tube and they were cooled to below 30°C. The tubes were then shaken thoroughly on a Vortex mixer for 30 seconds to insure oxidation of hydrindantin by air. Samples were then diluted as necessary so that their absorption at 570 nm could be determined with a Beckman DBG spectrophotometer.

C. Method Used for Concentration of Pear Juice Samples After Treatment

Due to the viscous nature of PJC, it was diluted 1:6 with distilled water to facilitate sample treatments. A 16°Brix reconstituted PJ (RPJ) resulted.

Following treatment, RPJ samples were adjusted to pH 4.1 and then concentrated to 72°Brix using a Büchi rotary evaporator (water bath temperature, 40°C; vacuum, 29 in Hg).

D. Method Used for Measuring Refractive Index

Refractive index was measured as percent soluble-solids, using a Bausch and Lomb refractometer maintained at 20°C.

E. Sample Treatments

1. Removal of Phenolics and Brown Pigment with PVPP

PVPP (GAF Corp. Polyclar AT) was washed as described by Loomis (1974), collected by filtration, and used immediately.

A thick slurry of washed PVPP and distilled water was poured into a large Buchner funnel containing Whatman No. 1 filter paper. A PVPP-pad about 1/2 in thick was formed by careful suction to avoid cracking the pad, and the the water eluent was discarded. RPJ was filtered through the PVPP-pad which was then rinsed with 200 ml distilled water. The eluant (treated RPJ and water washings) was collected, and the process repeated with a fresh PVPP-pad until no further reduction in phenolics, as measured by the Folin-Ciocalteu reagent could be obtained. The PVPP treated RPJ was then concentrated to 72 °Brix, as previously described.

2. Removal of Phenolics and Brown Pigment with XAD-4

Amberlite XAD-4 (Rohm and Haas Co.) was purified by treatment in a large Soxhlet extractor containing acetone, for 4 days, according to the procedure of Loomis et al. (1979). It was then washed with distilled water and stored hydrated in a sealed container until use.

Purified XAD-4 was made into a slurry with distilled water and poured into a column plugged with glass wool. The column bed contained about 100 ml of XAD-4 after settling. Headspace water was drained, and PJ (rather than RPJ, see section 1.0) was added through

a reservoir which fed by gravity into the column. After the PJ had passed through the column, it was collected, and the process repeated until the XAD-4 could no longer effectively remove phenolics, as measured by the Folin-Ciocalteu reagent. At this point, the column was rinsed with about 100 ml distilled water. The eluant (treated PJ and water washings) was collected, and the used XAD-4 discarded. The column was then repacked with fresh XAD-4. When the phenolic content of treated PJ could not be reduced further using a fresh column of XAD-4, the treatment was ended, and the juice was concentrated to 72° Brix, as above.

3. Removal of Amino Acids with Cation Exchange Chromatography

The hydrogen-form of Dowex cation exchange resin (Biorad Co. AG 50 W-X4, 200-400 mesh) was used to remove amino acids from RPJ.

A slurry was made of the Dowex resin with distilled water and about 20 ml was poured into each of 4 columns, plugged with glass wool. Distilled water was used to rinse the resin in each of the columns, and the headspace volume drained before RPJ was carefully added.

No more than 200 ml RPJ eluant was collected from each column, as larger volumes increased the possibility of incomplete removal of amino acids. The ninhydrin reagent discussed above, was used to detect the presence of amino acids. The column was rinsed

with about 25 ml distilled water, and the eluant (treated RPJ and water washings) was collected. The Dowex treated RPJ sample was then concentrated to 72° Brix, as above. Total phenolics were measured with the Folin-Ciocalteu reagent.

E. Storage Conditions

Sixteen 10 ml screw-cap vials were used to store each of the PJC samples at 37°C in the dark. These samples included the three samples described above, and an untreated, or control sample. In addition, about 25 ml of each PJC sample was stored in 1.5 cm pathlength clear plastic rectangular cells, fitted with a tight fitting plastic lid to eliminate evaporation. These cells were used for Hunter determinations. Fifty ml of each sample was stored in Erlenmeyer flasks sealed with Parafilm and aluminum foil, at -40°C.

F. Measurement of Sample Color During Storage

1. Measurement of Absorbance @ 420 nm

PJC samples were diluted in duplicate and measurements of the absorbance @ 420 nm were taken, at weekly intervals. A Beckman DBG spectrophotometer was used with 1 cm pathlength cells, for this purpose. The average absorbance of the two readings was multiplied by the sample dilution factor to obtain the absorbance of samples, before dilution.

2. Measurement of Lightness, Chromaticity, and Haze

Hunter L, a, b and CIE-Y values were determined at weekly intervals using a Hunter D-25 colorimeter in the transmission mode, and 1.5 cm plastic cells (see E. above).

The Hunter L, a, b scales consist of a lightness scale, "L", and chromaticity scales, "a", and "b". The lightness scale is divided into 100 equivalent units, with white equal to 100, black equal to 0, and shades of gray in between. This scale is designed to simulate a human's perception of lightness (Hunter 1975).

The positive side of the "a" scale measures redness; while the negative side measures greeness. The positive side of the "b" scale measureness yellowness; while the negative side measures blueness.

Useful combinations of the above scales include the Scofield-Hunter equation, or $\triangle E = [(L_1 - L_2) + (a_1 - a_2) + (b_1 - b_2)]^{\frac{1}{2}}$, the hue angle, or $\tan^{-1} b/a$, and the saturation index, or $(a^2 + b^2)^{\frac{1}{2}}$.

$\triangle E$ is equal to one NBS unit of color difference which is about three times as large as a MacAdam unit. One MacAdam unit approximates the least perceptible color difference.

The hue angle defines the hue of the specimen, i.e., whether it is red, orange, green, etc., and the saturation index is an indicator of the vividness of that hue, or how far removed it is from the gray of the same lightness.

Since the Hunter system defines a uniform 3-dimensional color

solid, the lightness, hue angle, and saturation index must be known before colors can be compared.

The Hunter light source was kept in alignment with the light collecting sphere (position I) to obtain readings which included only diffuse transmitted light (a light trap was used at the sphere exit). Readings were also taken with the light source pivoted (position III), so that both diffuse and specular components would be included.

Haze was measured with the CIE-Y function. Percent haze was calculated as:

$$\% \text{ Haze} = \frac{\text{CIE-Y (position I)}}{\text{CIE-Y (position III)}} \times 100$$

The Hunter D-25 colorimeter was standardized weekly and standard transmission filters were used to establish a record of its stability.

1.2 Determination of Water Activity of Pear Juice Concentrate

Water activity of PJC was determined through the use of a Water Activity-Value Analyzer, Model 5803, built by G. Lufft Metallbarometer Fabrik Co. The meter was calibrated with barium sulfate in a controlled temperature room at 26°C. The water activity of PJC was determined by allowing the sample to equilibrate within the meter for 3 days.

1.3 Determination of Mineral Content of Untreated and Cation Exchange Treated Pear Juice Concentrates

A Jarrel Ash atomic absorption spectrophotometer was used to determine the content of Fe, Cu, Zn, Mg, and Ca in the untreated and Dowex treated PJC samples. Samples of both were taken from storage and digested overnight on a hot plate in 20 ml nitric acid and 5 ml perchloric acid. The samples were made up to 20 ml with distilled water before suction into the instrument.

1.4 Determination of Ascorbic Acid and Dehydroascorbic Acid Content in Pear Juice Concentrate

The ascorbic acid content of PJC was determined according to the method of Loeffler and Ponting (1942). A blue dye, 2, 6-dichloroindophenol, was decolorized when reduced by ascorbic acid, present in the sample, and the extent of decolorization was measured as loss of absorbance at 565 nm. A Bausch and Lomb Spectronic 20 was used to detect changes in absorption.

The dehydroascorbic acid (DAA) content in PJC was determined by the method of Pearson (1974). DAA was reduced to form ascorbic acid in the presence of H_2S.

The sample was purged with H_2S for 10 minutes, sealed, and placed in the refrigerator overnight. The following day, H_2S was removed by purging the sample with N_2, for 2 hours. The ascorbic acid content was determined as above.

The difference in ascorbic acid content before and after

reduction of the sample by H_2S, was determined as its DAA content.

1.5 Determination of the Ultraviolet Spectra of Untreated and Cation Exchange Treated Pear Juice Concentrate Samples

A Beckman Acta III recording spectrophotometer was used to provide ultraviolet spectra of cation exchange and untreated PJC samples. Samples kept in frozen storage were thawed, and diluted, so that spectra could be recorded. These spectra were compared with spectra from the same samples, but after storage at 37°C.

Part II. Experiment 2

2.0 Origin of Pear Juice Concentrate Used to Prepare Samples for Storage

All samples were prepared from the same PJC, as in Part I (see section 1.0).

2.1 Preparation of Pear Juice Concentrate Samples for Storage

A. Method Used for Detection of Amino Acids

The method used for detection of amino acids was the same as described in Part I (see section 1.1 B).

B. Method Used for Concentration of Pear Juice Samples After Treatment

The method used for concentration of RPJ samples, was the same as described in Part I (see section 1.1 C).

C. Determination of Alpha-Amino Nitrogen Content in Pear Juice Concentrate

Dowex treated PJC from Part I (see section 1.1 D.3) was removed from frozen storage, thawed, and diluted 1:5 with

water. Glycine was added to aliquots at levels of 0.1-1.0 mg/ml. Untreated PJC was also removed from frozen storage, thawed, and diluted 1:5.

These samples were then treated with ninhydrin (see A, above). A standard curve was prepared by plotting absorption @ 570 nm of each Dowex treated RPJ sample against its glycine concentration. A Beckman DBG spectrophotometer was used for these measurements. Absorbance of the untreated RPJ was compared to the standard curve and multiplied by 5 (dilution factor), to obtain the alpha-amino nitrogen content of PJC).

D. Sample Treatments

1. Removal of Amino Acids

The method used to remove amino acids was the same as described in Part I (see section 1.1 D.3).

2. Removal of Amino Acids, Oxidation, and Treatment with XAD-4

RPJ was treated with Dowex as in (1), above; however, before concentration, O_2 was bubbled into it at the rate of 8 liters/minute for 2 hours. The juice was then passed through 2 columns of fresh XAD-4 as described in Part I (see section 1.1 D.2), and concentrated to 72° Brix.

3. Removal of Amino Acids and Addition of Glycine

Fifty ml Dowex treated PJC from Part I (see section 1.1 D.3) was removed from frozen storage, thawed, and diluted 1:4 with distilled water. Three hundred mg glycine was added as a dry powder. The juice was thoroughly mixed and then concentrated.

The same procedure was followed with a 50 ml sample treated

as in (2) above, that had been stored for 1 month; however 150 mg glycine were added.

4. Removal of Amino Acids and Addition of Ascorbic Acid

RPJ was treated as in (1) above. In addition, 500 ppm ascorbic acid was added as a dry powder (so that the concentration would have been 0.5 mg/ml in the original pear juice) and bubbled into solution with O_2 so that dehydroascorbic acid would prevail.

5. Oxidation and Treatment with XAD-4

Oxygen was bubbled into RPJ at the rate of 8 liters/minute/2 hours. The juice was then treated with XAD-4 as described in Part I (see section 1.1 D.2).

6. Oxygen Gas Purge

Untreated PJC was purged with O_2 for 2 hours.

7. Nitrogen Gas Purge

Untreated PJC was purged with N_2 for 1 hour. It was then poured carefully (to avoid aeration) into a plastic cell. The cell's headspace was flushed with N_2 and it was sealed (see E, below).

8. Acidification

Two samples of untreated PJC were acidified using concentrated HCl to pH 2.39 and pH 3.26.

E. Storage Conditions

Storage conditions were the same as in Part I (see section 1.1 E); however, samples were stored in 1.0 cm pathlength cells. These were

covered with a dual layer of Parafilm and an outer layer of aluminum foil. Excess sample was stored in screw-cap test tubes.

F. Measurement of Sample Color During Storage

The Hunter D-25 colorimeter used in Part I was used again, to measure lightness and chromaticity of samples (see section 1.1 F.2).

Part III. Volatile Analysis of Pear Juice Concentrate

1.0 Instrumental Analysis of Volatiles from Pear Juice Concentrate Samples

Volatile content of two different samples of PJC were compared. One sample was the same as in Part I (see section 1.0) which remained frozen until use and the other sample had been stored in a laboratory at room temperature for 3 years.

Sample volatiles were collected using the technique of porous polymer entrainment (Miller et al. 1972). A 10 ml sample of PJC was added to a small flask containing granular anhydrous Na_2SO_4 (salt added to saturate the aqueous phase), and about 5 mg of 1-tetradecanol was added to prevent foaming.

The flask was first sealed with a cap containing a rubber septum, and then immersed in a water bath held at 60°C for 10 minutes. After 5 minutes had passed, the porous polymer (Poropak Q) attached to an entrainment apparatus was heated to 55°C. When the 10 minute interval had passed, the flask was attached to the entrainment apparatus and kept at 60°C. N_2 was bubbled through the sample at a rate of 33 ml/minute.

Entrainment was allowed to proceed for 30 minutes, after which the porous polymer containing the entrained volatiles was transferred to

another apparatus, held at 55°C, and N_2 was used to flush out residual water for 20 minutes. The porous polymer was next reversed to the other end. The trap was immersed in dry ice and N_2 was passed through the porous polymer, which was heated to 135°C, and volatiles were collected in the trap for 45 minutes. The trap was then sealed and frozen until analysis.

Analysis of volatiles was performed using gas chromatography coupled with flame ionization, alkali flame ionization, and mass spectral detection systems. The alkali flame ionization detector was optimized by carrier gas flow modifications so that it would be sensitive to nitrogen containing compounds. Mass spectral analysis was performed with the use of a Finnigan 1015C quadrapole mass spectrometer coupled with computer assisted data processing.

Stainless steel capillary columns (0.03" I.D., 500 feet) coated with SF-96 mixed with a small percentage of Igepal Co 888 (surfactant) were used with all detectors. The carrier gas flow rate was about 15 ml/minute, and temperature programming was performed beginning at 70°C and increasing 2°C/minute. When 160°C was reached, the sample was allowed to proceed isothermally until the peak thought to correspond to 1-tetradecanol was recorded. Identical conditions were used throughout the experiment.

Since identical conditions were used, retention times were used to aid in comparison of chromatograms produced with the various

detection systems.

1.1 Taste Panel Evaluation of Pear Juice Concentrate Aroma from Stored Samples

Two samples, both from the same PJC, one untreated and the other treated with Dowex cation exchange resin (see Part I, section 1.1 D) were stored for 83 days at 37°C.

After storage, samples were diluted 1:10 with distilled water, and untrained judges were asked to score each, according to total aroma intensity, pear-like character, and desirability. The scale used for scoring samples ranged from 1 to 10. Each judge was also asked to describe each sample aroma.

This work was performed using the OSU flavorium facilities. The samples were contained in ground glass stoppered Erlenmeyer flasks which were covered with aluminum foil. In addition, red overhead lighting was used in the booths to mask color differences.

RESULTS AND DISCUSSION

Part I. Experiment 1

1.0 Browning

A. Results of Instrumental Analysis

1. Variation in Sample Browning

The characteristics of PJC samples prior to storage are shown in Table 1. Treatment with XAD-4 resulted in the greatest reduction of polyphenolics, followed by PVPP and Dowex cation exchange treatments. The removal of alpha-amino nitrogen containing compounds was achieved by Dowex treatment; however, the partial removal of polyphenolics could not be avoided.

As shown by sample absorbance @ 420 nm, and Hunter "L" values, it is evident that XAD-4 treatment resulted in the most effective decolorization, followed by PVPP and Dowex treatments. The untreated sample began storage much darker than the other samples.

Browning during storage of samples was expressed as increasing absorbance @ 420 nm as shown in Figure 1. The rates of browning of untreated, XAD-4, and PVPP treated samples, as shown by their slopes, were very similar. The slope of the Dowex treated sample showed only a small increase which indicated that very little browning had occurred.

The Hunter "L" scale was also used to measure sample browning

Table 1. Characteristics of pear juice concentrate samples prior to storage

Sample	Alpha-Amino Nitrogen mg/ml	pH	RI*	Absorbance @ 420 nm	Hunter L-Value	%-Haze	Phenolics**	% Reduction in Phenolics from Untreated Sample
Untreated	6.0	4.17	71.8	5.5	27.9	42.3	2.80	0
Dowex	trace	4.14	71.8	2.7	55.8	15.8	1.47	48
PVPP	NT***	4.14	72.0	1.4	73.8	9.8	1.00	64
XAD-4	NT	4.15	72.0	0.8	77.8	7.1	0.50	82

*RI = Refractive Index (measured as percent soluble solids)

**Phenolics calculated as mcg Gallic Acid Equivalent/ml PJC (see Experimental, Part I, 1.1 A)

***NT = Not Tested

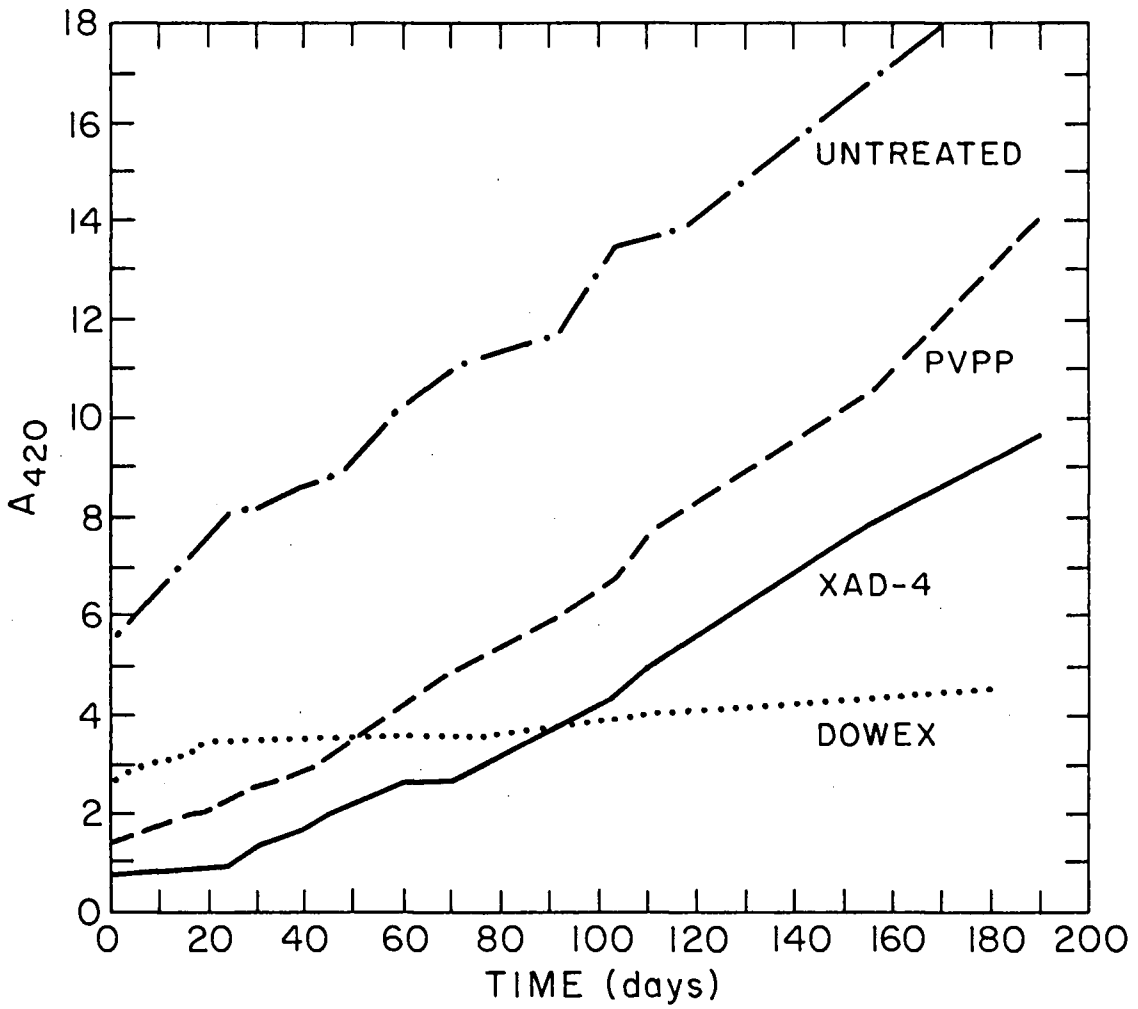

Figure 1. Increase in absorbance @ 420 nm during storage of pear juice concentrate samples

during storage (see Experimental, Part 1 section 1.1 G. 2). Browning was indicated by decreasing "L" values, and results are shown in Figure 2. The XAD-4 and PVPP treated samples seem to have changed most; however, the untreated sample became the darkest. The Dowex treated sample remained relatively light colored.

The Hunter "L" scale is closely related to the human visual response to sample "lightness." It is known that this response is by nature, logarithmic, and its effect is most apparent at both extremes of the "L" scale (Hunter 1975). To better represent browning during storage of samples, as measured with Hunter "L" scale, a standard curve was developed.

After the storage period had ended, all samples were serially diluted, i.e., 1:2, 1:4, 1:8, etc., and Hunter L, a, b values recorded for each dilution. "L" values of the untreated and Dowex treated samples were plotted against their dilution factor, which was defined "pigment concentration." Refer to Figure 15 (Appendix). Pigment concentrations were then plotted against time, as shown in Figure 3.

This method was assumed to be valid for XAD-4 and PVPP treated samples, because pigment concentration calculated from a given sample dilution could be used to predict additional "L" values at succeeding dilutions. Actual sample "L" values when plotted at their predicted points did not deviate significantly, which is shown

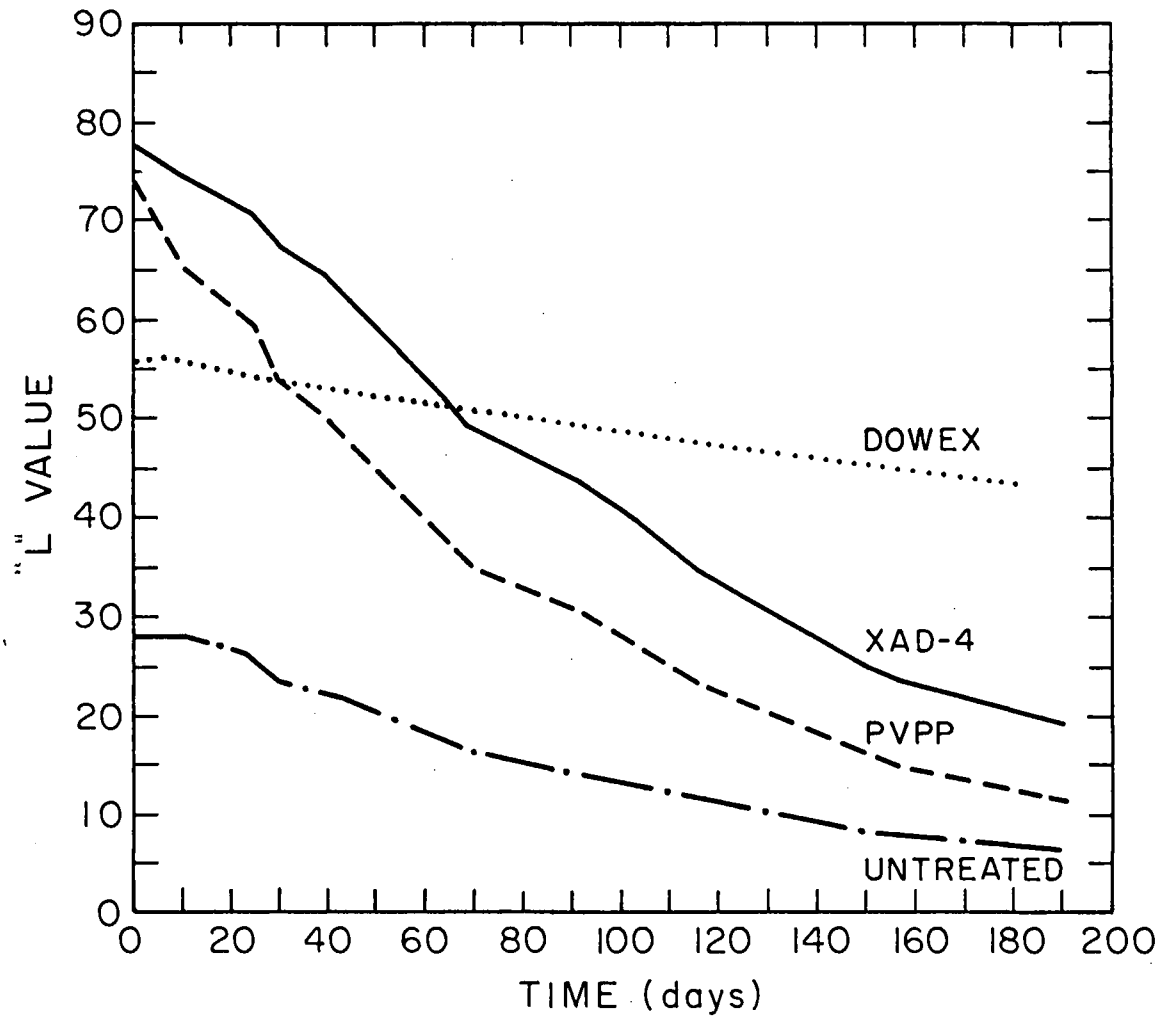

Figure 2. Decrease in "L" value during storage of pear juice concentrate samples

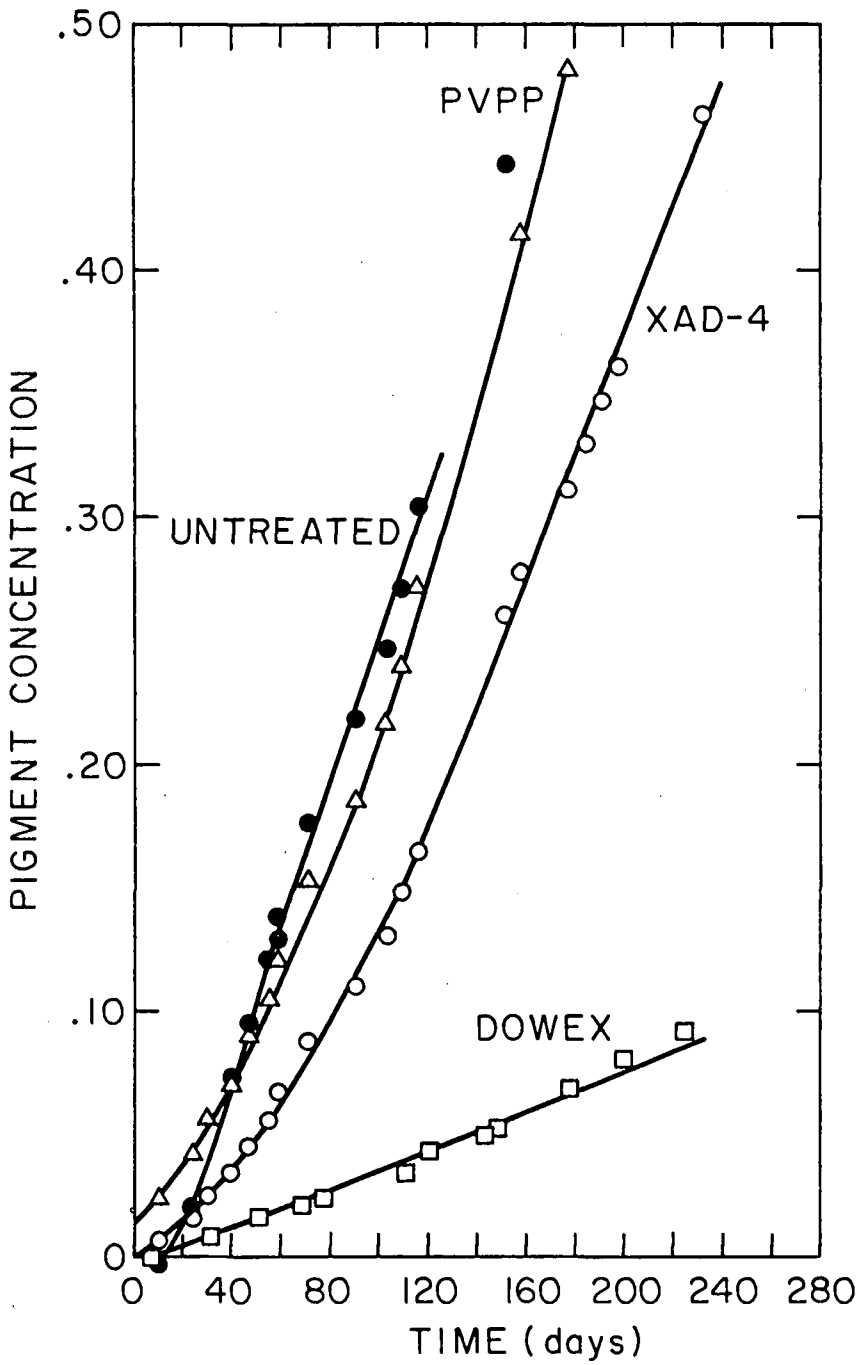

Figure 3. Change in "pigment concentration" (derived from "L" value; refer to Figure 15, Appendix). Pigment concentration at day zero was subtracted from later pigment concentrations of all samples, for comparative purposes.

in Figure 15.

Results as shown in Figure 3 confirm those described above for browning @ 420 nm. Again, the untreated, XAD-4, and PVPP treated samples appear to have browned at nearly the same rate, while the Dowex treated sample was relatively stable.

Treatment with XAD-4 seems to have inhibited initial browning slightly, while PVPP treatment resulted in very little change. It should be noted that the XAD-4 treated sample was not produced from reconstituted pear juice, as were the PVPP and Dowex treated samples, but from the original pear juice (see Experimental, Part I, section 1.1 C). The additional "treatment" of the PVPP and XAD-4 samples may have contributed to their faster rate of browning during the first 10 days of storage, when compared to the untreated sample.

2. Color Changes

The Scofield-Hunter equation was used to determine the difference in sample color which developed due to browning during storage, from sample color at day zero, at weekly intervals (see Experimental, Part I, section 1.1 G. 2). This difference was expressed as $\triangle E$. Increases in $\triangle E$ (or color difference) for each sample, were plotted against time as shown in Figure 4.

The PVPP and XAD-4 treated samples appeared to have undergone the most color change, while the untreated sample changed much less. Since browning rates for the untreated, PVPP, and XAD-4

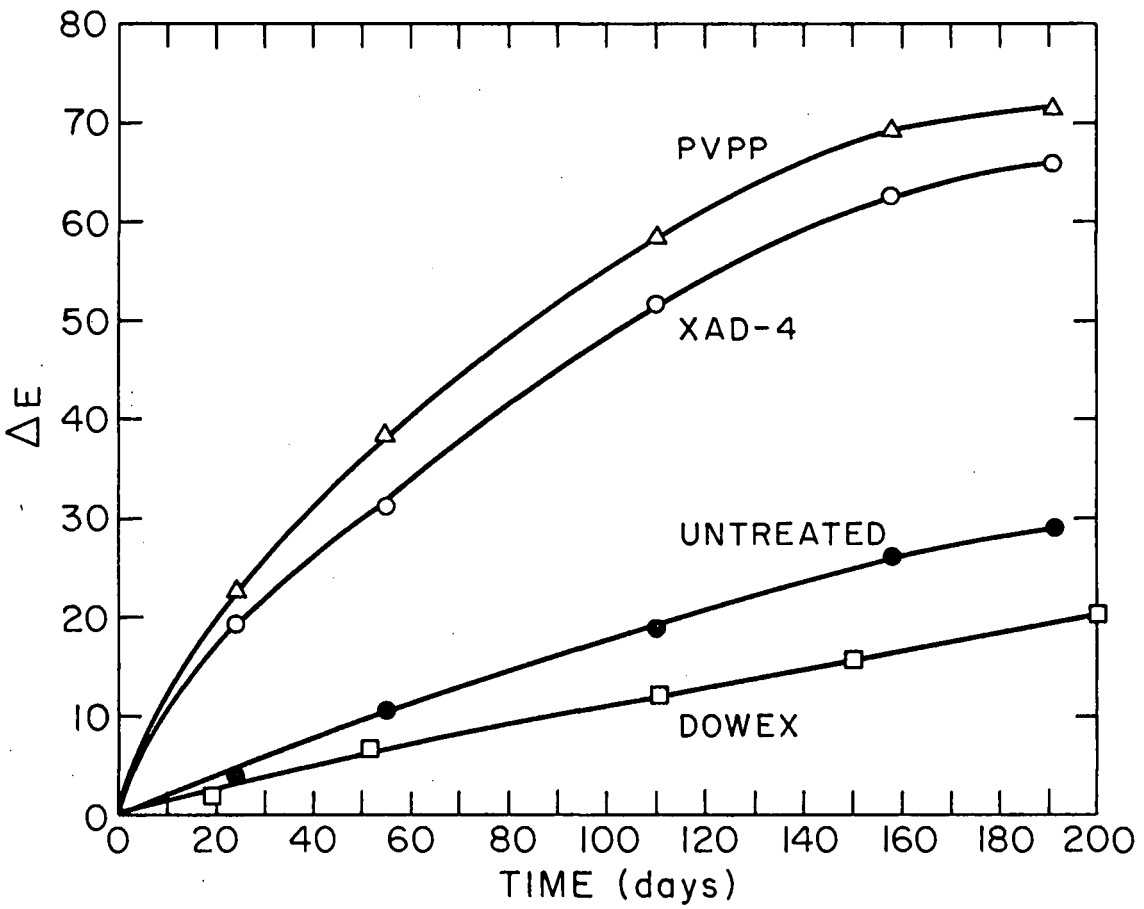

Figure 4. Change in Scofield-Hunter Index (Δ E) during storage of pear juice concentrate samples

treated samples were similar (as shown above) it seems that the untreated sample should have also shown similar color changes. Furthermore, as both PVPP and XAD-4 treated samples increased in browning at linear rates (Figures 1, 3), color change gradually declined in both samples.

The reasons for these apparent contradictions were based upon the close relationship between $\triangle E$ and the human visual response to color difference, as discussed previously (see Experimental, Part I, section 1.1 G. 2).. This response to color difference is probably similar to that discussed for sample lightness, above. Francis and Clydesdale (1975), have reported the relative insensitivity of the human eye to light within the shorter wavelength region of the visible spectrum (below 500 nm), where browning is most apparent.

The untreated sample began storage much darker than the other samples (Table 1), and the same color difference expressed as $\triangle E$ would not have been as large, as in the other, lighter samples.

The XAD-4 treated sample changed color more slowly than the PVPP treated sample, indicating greater color stability. The Dowex treated sample, which browned little during storage, also showed the least color change.

It was noticed in visual observation of the samples, that the XAD-4 and PVPP treated samples were initially light yellow, but during storage changed to red-brown, and then dark brown. The

Dowex treated sample was yellow-brown initially but became a darker shade of brown during storage. The untreated sample began storage red-brown and during storage became very dark brown. Hunter L,a,b data was used to verify these observations (see Experimental, Part I, section 1.1 G.2).

Changes in "a" value during storage of samples are shown in Figure 5. An increase in "a" value was indicative of increased redness. XAD-4 and PVPP treated samples increased in "a" value from near zero, until a maximum was reached, which then declined as samples became dark brown towards the end of storage. The untreated sample increased briefly in "a" value, which then declined during storage. The Dowex treated sample increased in "a" value throughout storage.

Changes in "b" value during storage of samples are shown in Figure 6. An increase in "b" value was indicative of increased yellowness. XAD-4 and PVPP treated samples increased in "b" value, which reached a maximum, and then declined rapidly in both samples during storage. The untreated and Dowex treated samples both declined in "b" value during storage.

All samples decreased in hue-angle during storage as shown in Figure 7. A hue-angle of ninety (degrees) would have represented a pure yellow hue, which would change as hue-angle decreased, to yellow-orange, orange, red-orange, and at zero, pure red. It should

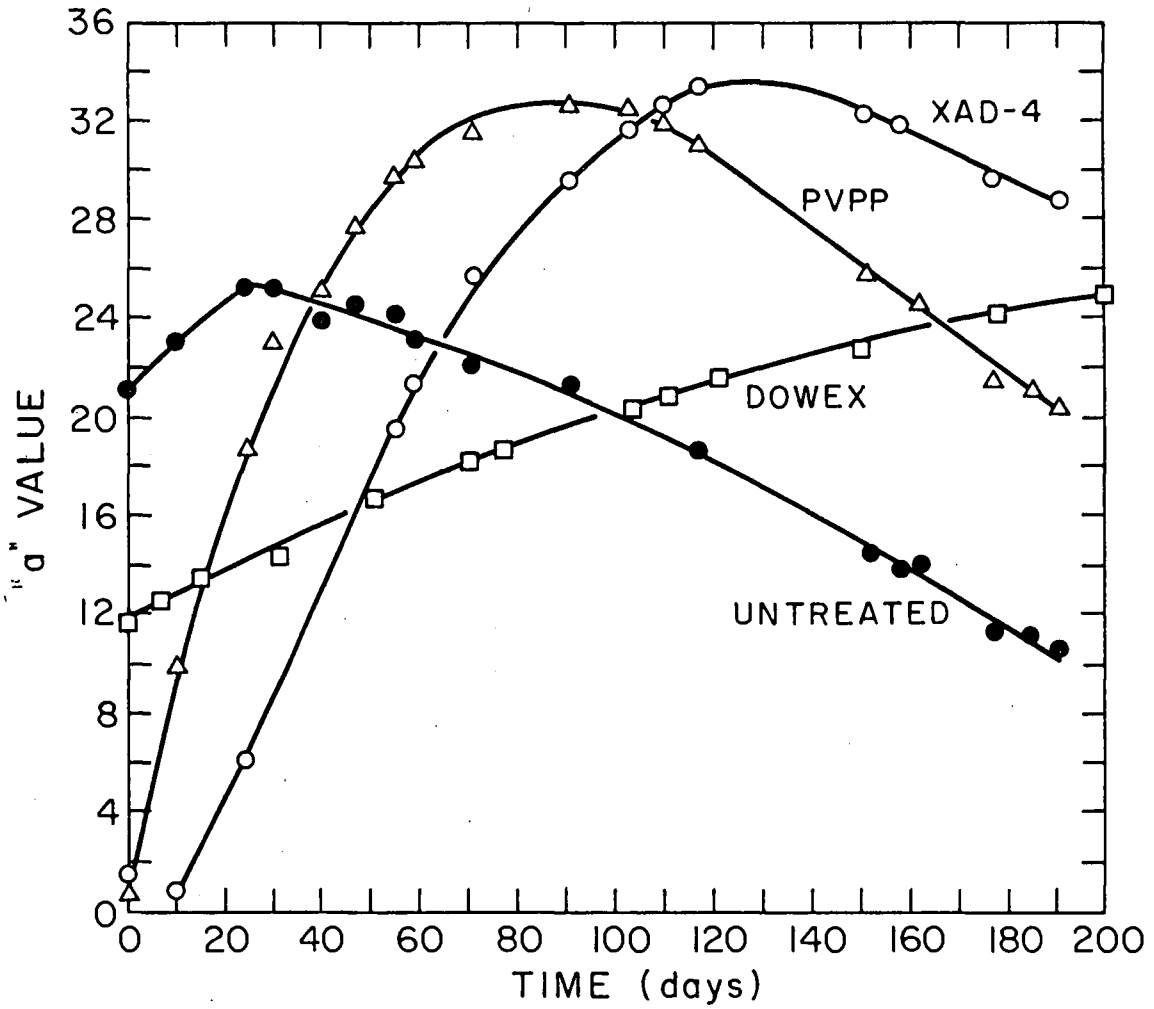

Figure 5. Change in "a" value during storage of pear juice concentrate samples

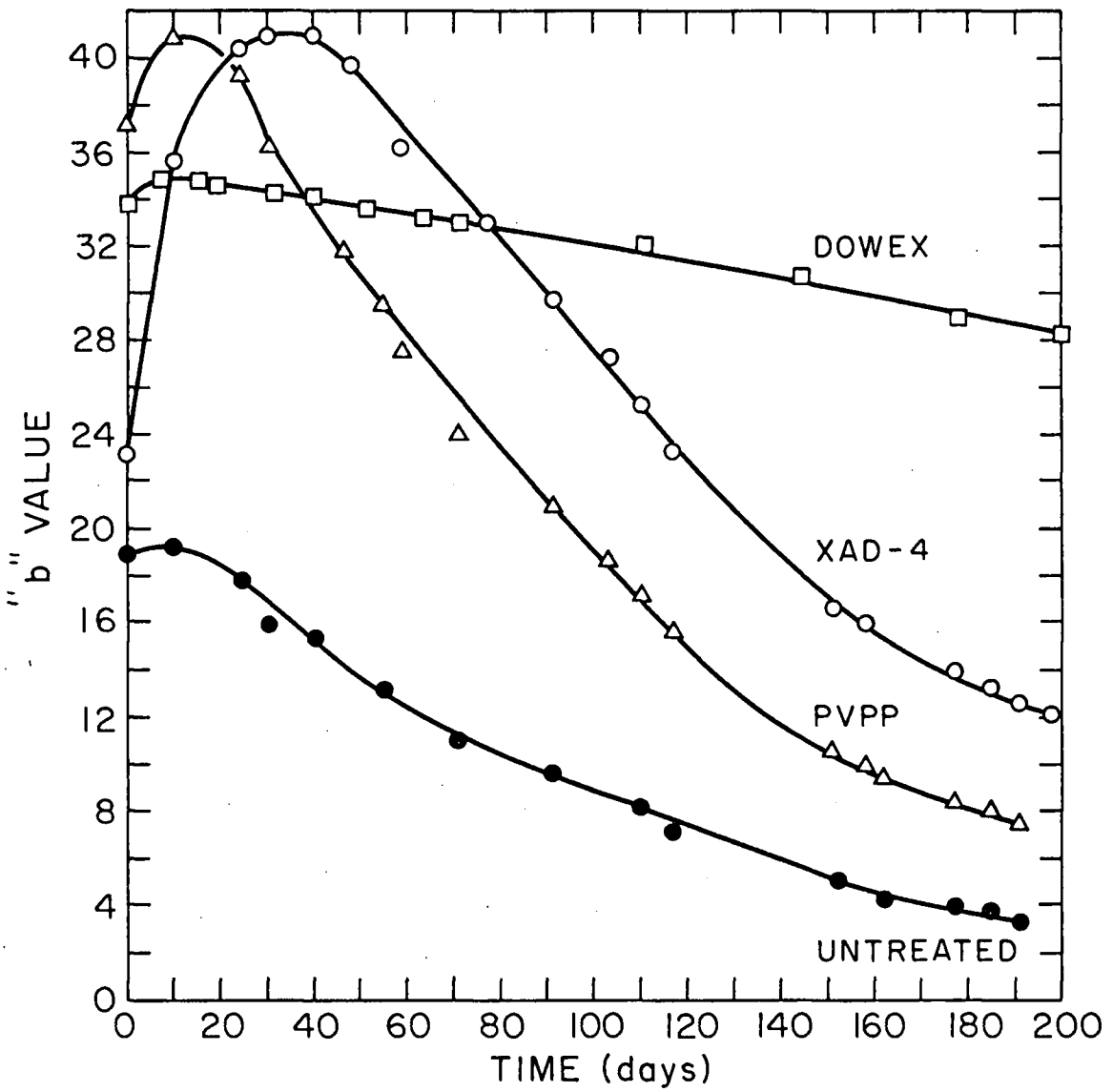

Figure 6. Change in "b" value during storage of pear juice concentrate samples

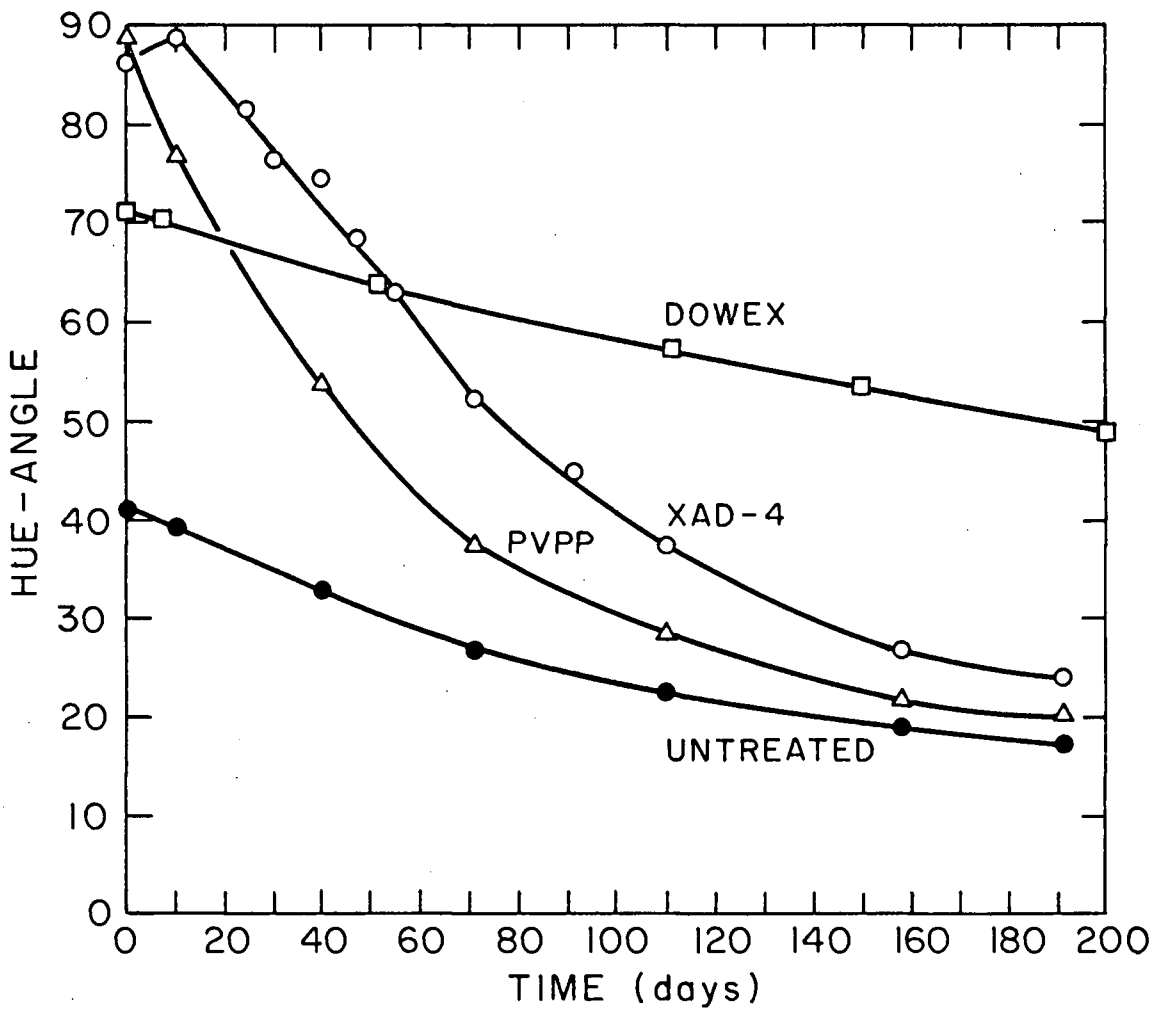

Figure 7. Change in hue-angle during storage of pear juice concentrate samples

be noted that hues of the untreated, XAD-4, and PVPP treated samples became very similar during storage. The Dowex treated sample changed relatively little in hue. Changes in hue angle nearly paralleled changes in "L" value (Figure 2).

When change in hue-angle was plotted against "L" value as in Figure 8, it was evident that PVPP and XAD-4 treated samples were nearly identical in hue. Change in hue-angle of the untreated sample occurred within a small range of the "L" scale. This was due to the much darker color of the untreated sample, as compared to the other samples, and to the logarithmic nature of the "L" scale, as previously discussed. The Dowex treated sample also showed changes in hue-angle within a small range of the "L" scale, but this was due to its greater color stability (see Figures 1 and 3).

Within the same range of the "L" scale, hue-angles for the untreated and Dowex treated samples were slightly higher than those of the XAD-4 and PVPP treated samples. The rate of change in hue-angle was nearly parallel for all samples.

Saturation index is a function which describes the vividness (or saturation) of sample hue. It was plotted against "L" value in Figure 9. Changes in saturation index for the PVPP and XAD-4 treated samples were nearly the same. Changes in saturation index for the untreated and Dowex treated samples occurred within small ranges of the "L" scale for the same reasons as mentioned above. Within the

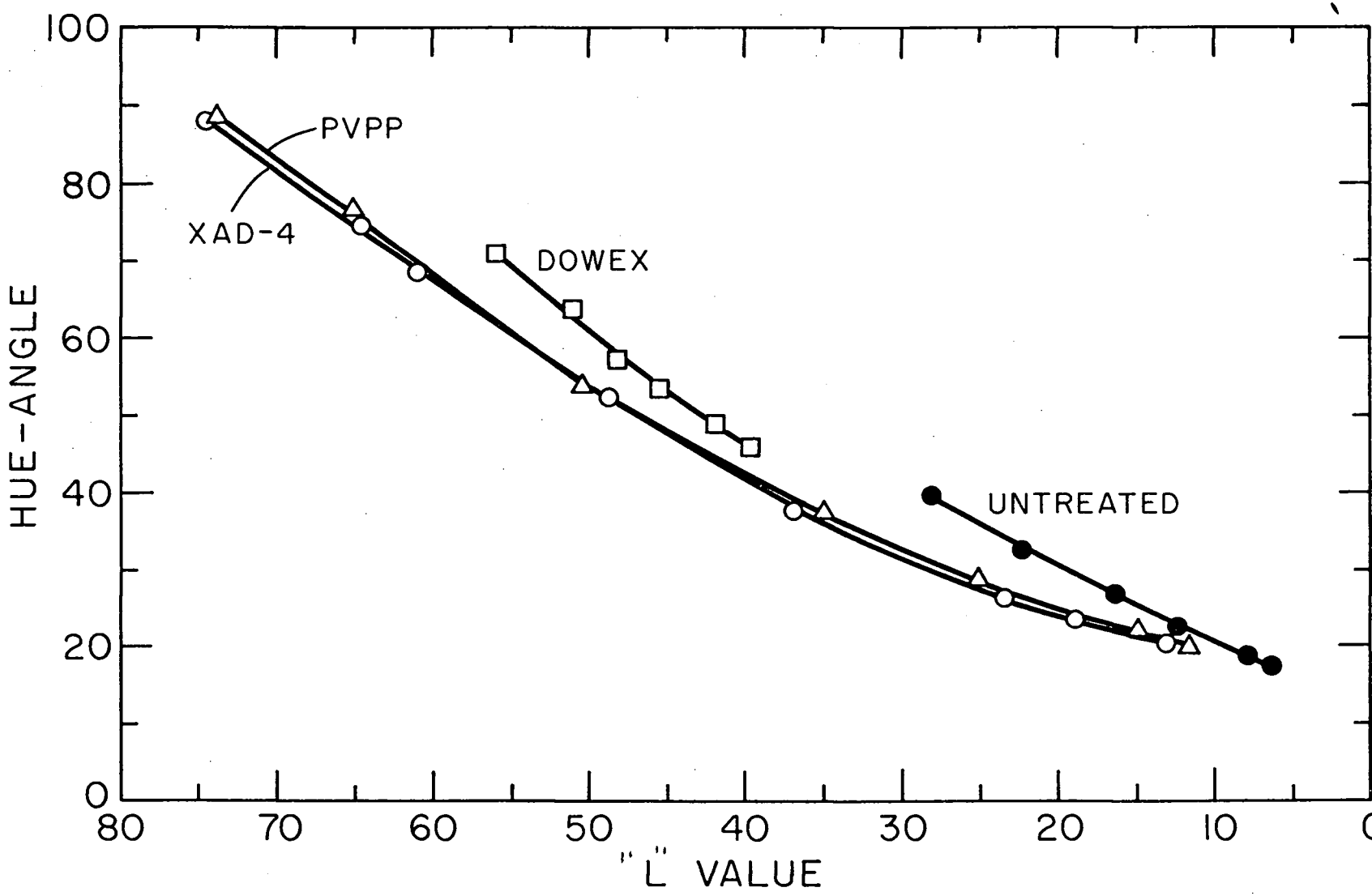

Figure 8. Change in hue-angle versus "L" value during storage of pear juice concentrate samples

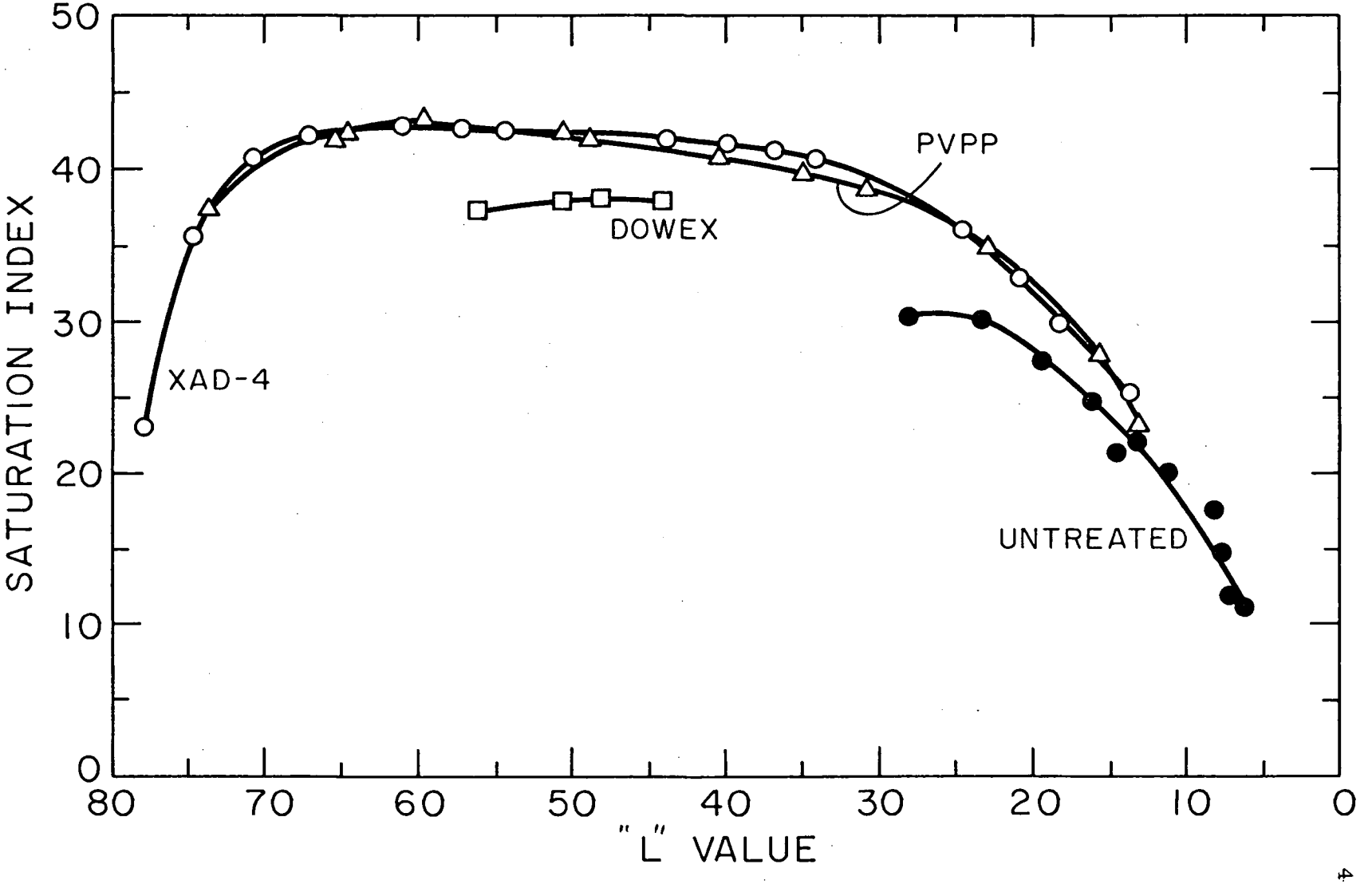

Figure 9. Change in saturation index versus "L" value during storage of pear juice concentrate samples

same range of the "L" scale, changes in saturation index for the untreated and Dowex treated samples were lower than for XAD-4 and PVPP treated samples.

The characteristics of color in the Hunter three dimensional color solid are lightness, hue, and saturation. The term, chromaticity, is an incorporation of hue and saturation. At identical "L" values, the chromaticity of different samples must match closely to produce similar colors.

The untreated and Dowex treated samples differed in chromaticity from the XAD-4 and PVPP treated samples. The latter two samples were very similar in chromaticity. Small color differences which resulted between these samples may have been due to variation in chromophore structure, or composition. Sample treatments, which altered the chemical composition of PJC, may have caused the browning mechanism (s) to change, which could result in different chromophores.

Serial dilutions of each sample were made following storage, and Hunter L, a, b values were recorded for each dilution. Change in hue-angle versus "L" value, based upon this Hunter data, was plotted in Figure 10. Results indicate that samples were very similar in hue.

The results shown in Figure 8, which displays change in hue-angle during storage of samples, were compared to results shown in Figure 10. It is evident that changes in hue-angle due to browning during storage, or sample dilution after storage, were very similar.

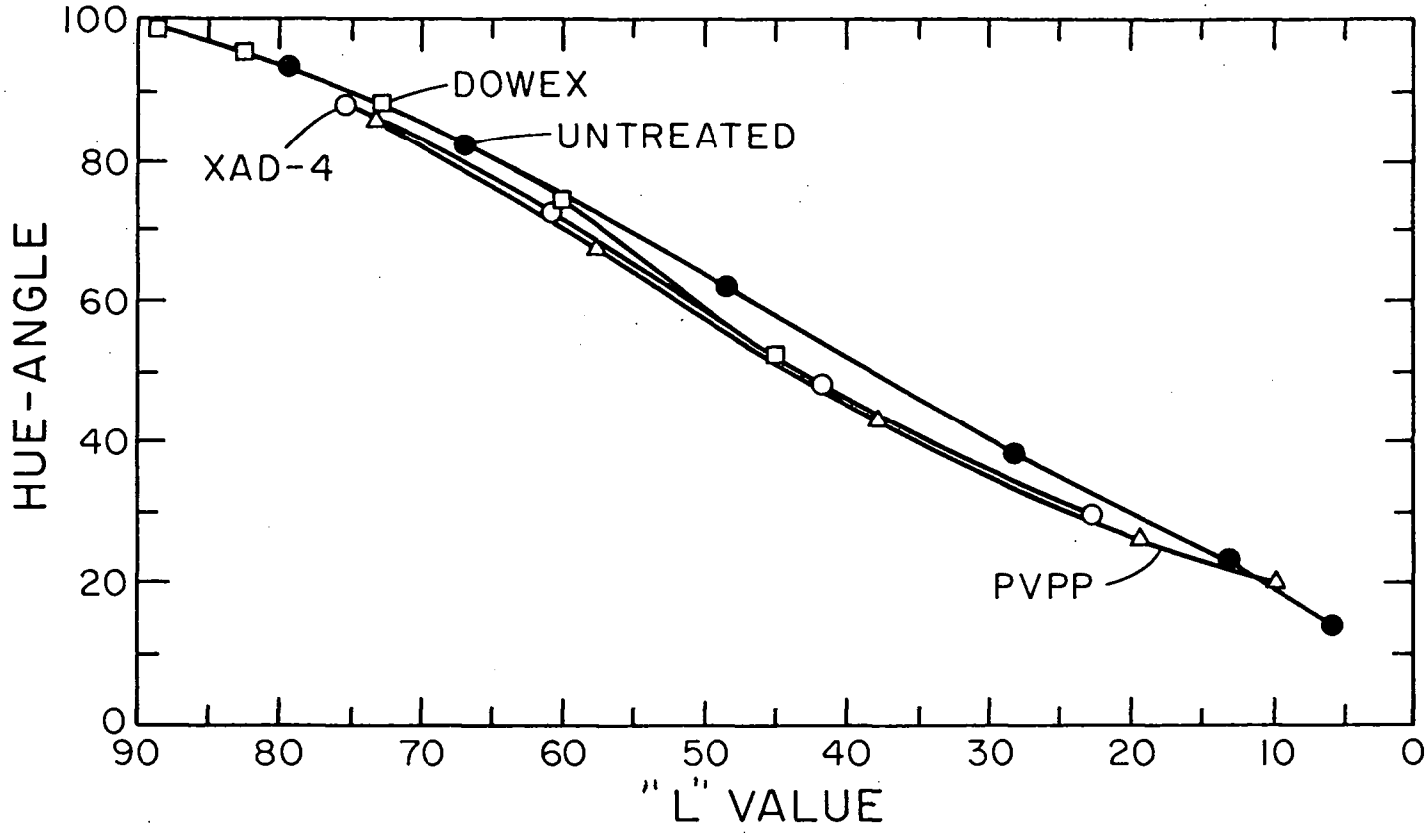

Figure 10. Change in hue-angle versus "L" value after dilution of pear juice concentrate samples

Change in saturation index versus "L" value, based on the sample dilution data from above, was plotted in Figure 11. Results show that samples were very similar in saturation. Results shown in Figure 9, which displays change in saturation index during storage of samples, were compared to results shown in Figure 11. It is apparent that changes in saturation index due to browning during storage, or sample dilution after storage, were quite similar.

Since it was possible to dilute stored samples back to their initial color prior to storage, as shown above, permanent color change did not occur, and seems to have been dependent upon "L" value. The dependence of chromaticity upon "L" value has been shown, by Van Buren (1974).

3. Increase in Haze During Storage of Pear Juice Concentrate Samples

A haze develops when light is scattered by the surface of particles rather than being absorbed. The initial haze values of all samples seemed related to initial "L" values (Table 1). For example, the untreated sample was much darker than other samples initially, and its haze was much greater. All samples increased in haze during storage, as shown in Figure 12.

The greatest change in haze was shown by the PVPP treated sample, which was followed by the untreated sample. The XAD-4 treated sample which was lowest in total phenolics changed relatively little. It did show a rapid increase in haze after 200 days of storage, but only one point represented this change. The Dowex treated sample

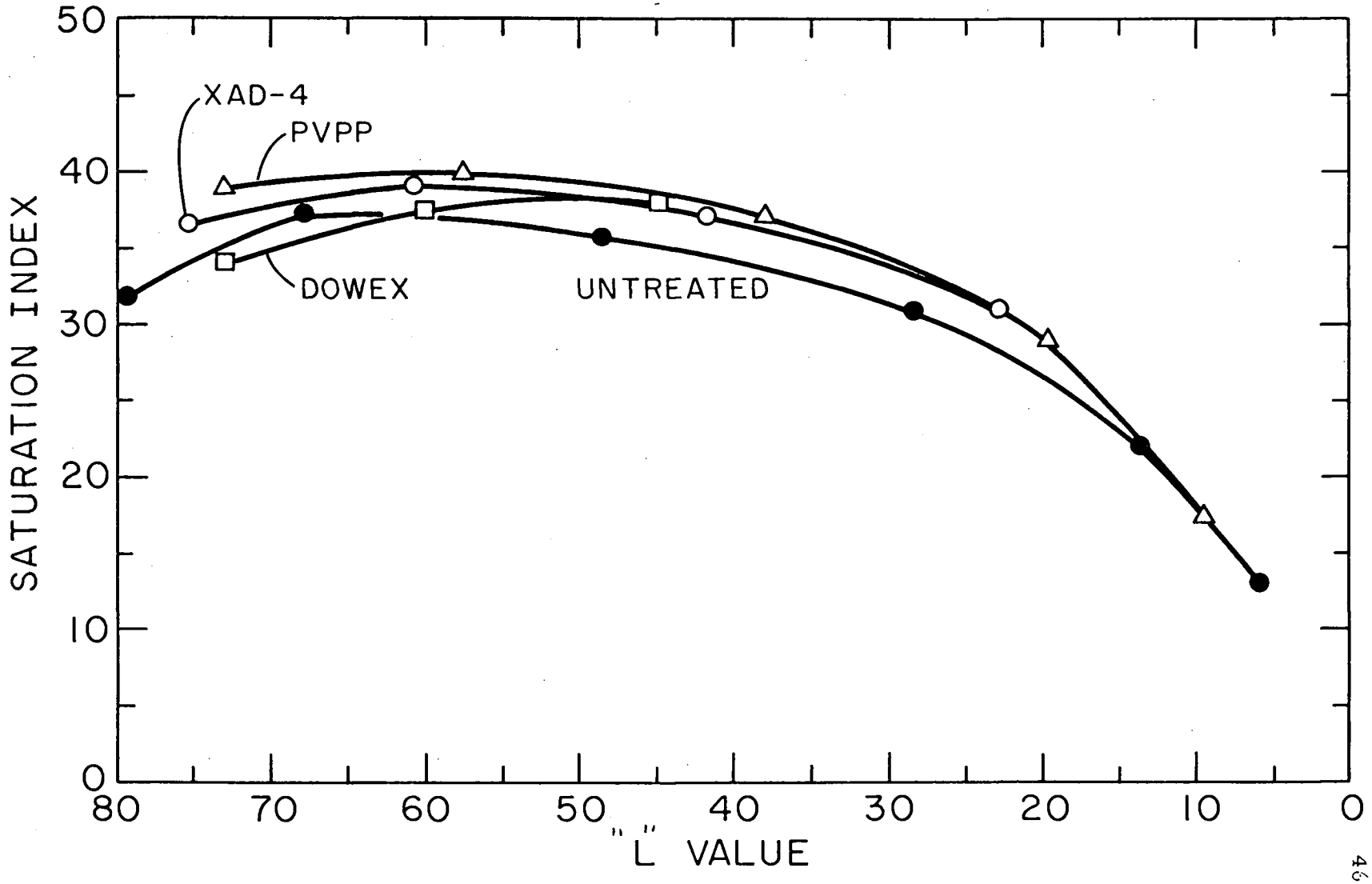

Figure 11. Change in saturation index versus "L" value after dilution of pear juice concentrate samples

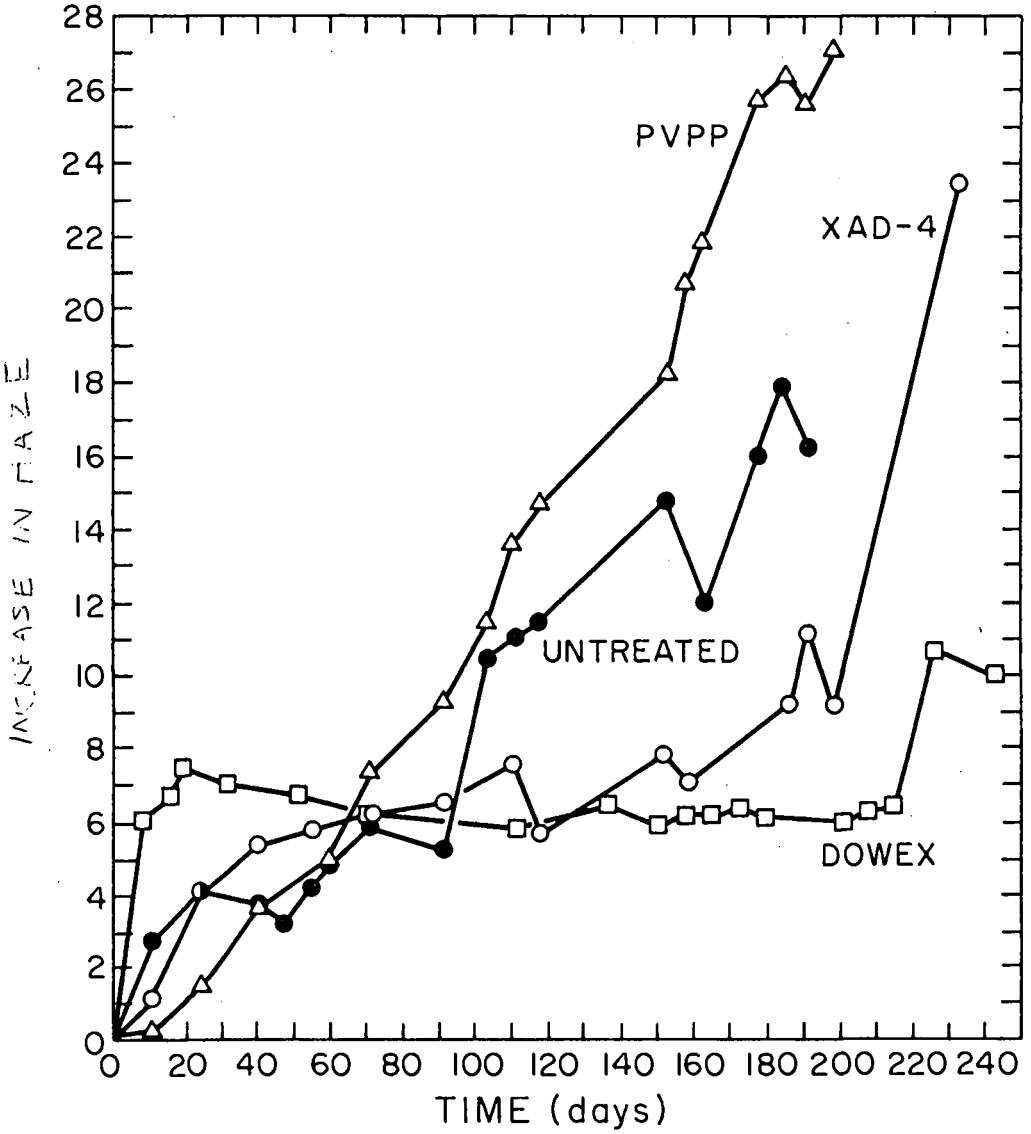

Figure 12. Increase in haze during storage of pear juice concentrate samples. Haze value at day zero was subtracted from later haze values of all samples, for comparative purposes (see Table 1).

increased suddenly in haze during the first 10 days of storage. Afterwards, it was very stable to changes in haze and only after 220 days of storage did another increase occur.

Increases in haze may have been due in part to the formation of colloidal protein-phenolic complexes (Chang 1979).

1.1 Water Activity of Pear Juice Concentrate

Eichner and Karel (1972) have reported that a maximum browning reaction (Maillard) occurs in most foods between water activities of 0.3-0.7. The position of this maximum depends on the food type, and therefore, water activity cannot be used to predict optimum browning conditions. The water activity of PJC was found to be 0.73 (@ 72% soluble solids), and while Maillard browning may not have been maximal, it would have been expected to occur.

Bruemmer and Bowers (1977) found that growth of osmophilic yeasts (usual contaminants in fruit concentrates) was inhibited in 75° Brix orange juice concentrate samples stored at 30°C. They suggested a minimum of between 70° and 75° Brix as necessary to prevent such growth in orange juice concentrates. PJC samples were stored @ 72°Brix and changes in this value did not occur.

1.2 Mineral Content of Untreated and Cation Exchange Treated Pear Juice Concentrate Samples

Dowex cation exchange treatment resulted in a substantial reduction in mineral content, when compared to the untreated sample, as shown in Table 2. Metal-complexes with organic acids, such as citric,

Table 2. Mineral content (ppm) of cation exchange treated and untreated pear juice concentrate samples

Sample	Fe	Cu	Zn	Mg	Ca
Untreated Control	39	7	21	420	140
Dowex	14	1	8	3	ND*

*ND = None Detected

may have prevented even further reduction (see Introduction, pg. 2). The quantity of Dowex resin found necessary for removal of amino acids from pear juice was much greater than calculated. This was probably due to the interference of minerals with the negatively charged sulfonic acid functional groups of the resin.

Small amounts of minerals, organic acids, and phosphates can reduce the activation energy needed to cause browning in the absence of amino compounds (Hodge 1953). Removal of metal ions to large extent by Dowex cation exchange treatment, would have reduced this effect.

1.3 Ascorbic Acid and Dehydroascorbic Acid Content of Pear Juice Concentrate During Storage

The ascorbic acid and dehydroascorbic acid contents of PJC samples were very low; however, they seemed to "increase" during storage as shown in Table 3. Ascorbic acid is degraded through both oxidative and non oxidative reactions in foods including concentrated orange and lemon juices (Kurata and Sakuri 1967a). Fischer and Friese (1967) determined that about 90% of the ascorbic acid in commercial pear juice had been destroyed by oxidation. Skorikova (1970) found that pear ascorbic acid was completely lost during the pressing of juice.

Motai (1972) used 2, 6-dichloroindophenol to measure reductone content of melanoidin pigments, estimated as ascorbic acid. Melanoidins have been shown by Kawashima (1977) to possess antitoxidant activity.

Table 3. Change in apparent ascorbic acid and dehydroascorbic acid contents (ppm) during storage of pear juice concentrate

Sample	Days Stored	Ascorbic Acid	Dehydroascorbic Acid
Filtered Pear Juice	0	11	13
Untreated PJC	0	0	75
" "	30	14	82
" "	212	92	128

For these reasons, it was concluded that the apparent "increase" in ascorbic acid content during storage was due to reductone formation.

1.4 Measurement of Phenolic Content After Storage of Pear Juice Concentrate Samples

Measurement of phenolics after storage showed an apparent increase in all samples, as shown in Table 4. The Folin-Ciocalteu reagent was used to measure phenolics (see Experimental, Part I, section 1.1 A). Singleton and Rossi (1966) found that ascorbic acid, a reductone, interfered with the analysis of phenolics as determined with this reagent.

The untreated sample showed the largest "increase" in phenolics, followed by the PVPP and XAD-4 treated samples. The Dowex treated sample showed a much smaller "increase;" however, it was stored for a shorter period. This same order was shown as samples increased in browning (see section 1.0 A.1).

Reductones are known to form during Maillard browning reactions, as previously discussed (see section 1.3). It is suggested that reductone formation was responsible for the observed "increases" in phenolics. Maillard reactions should not have occurred in the Dowex treated sample and this may be the reason why it showed the smallest "increase" in phenolics.

Table 4. Apparent "increase" in phenolics* after storage of pear juice concentrate samples

Sample	Days Stored	Phenolics after Storage (I)	Phenolics @ Day Zero (II)	Increase in Phenolics after Storage (I-II)
Untreated	202	4.67	2.80	1.87
Dowex	154	1.73	1.47	0.26
PVPP	202	2.60	1.00	1.60
XAD-4	202	1.72	0.50	1.22

*Phenolics calculated as mcg Gallic Acid Equivalent/ml PJC (see Experimental, Part I, 1.1 A)

1.5 Ultraviolet Spectra of Untreated and Cation Exchange Treated Pear Juice Concentrate Samples

Absorbance in the ultraviolet (UV) spectrum of the untreated sample at day zero, peaked @ 320 nm and @ 285 nm. After storage, the peak at 320 nm was no longer distinguishable; however, absorbance had greatly increased in both UV and visible regions of the untreated sample's spectrum.

No distinguishable peaks were present in the UV spectrum of the Dowex treated sample at day zero. After storage, little increase in absorbance had occurred, relative to the untreated sample, but a strong peak @ 285 nm had developed.

As no samples showed absorption peaks @ 405 nm, it was chosen as a reference point, so that the relative change in UV spectra could be compared between samples, as shown in Table 5. It is apparent that after storage, the peak @ 285 nm decreased substantially in the untreated sample. Although a peak @ 285 nm was not present in the Dowex treated sample at day zero, or for the untreated sample @ 320 nm after storage, peak height ratios were still included. In both cases, points on the two sample absorption spectra corresponding to these wavelengths, were used.

The peak which corresponded to 320 nm in the untreated sample's spectrum at day zero, but was absent in the spectrum of the Dowex treated sample, was probably due to chlorogenic acid, which absorbs in this region (Smith 1979). This polyphenol is prevalent in pears

Table 5. Ultraviolet spectra change during storage of cation exchange treated and untreated control pear juice concentrate samples

Sample	Days Stored	Peak Height Ratios* 285/405	320/405
Untreated Control	0	27.0	24.0
Untreated Control	192	8.9	5.4
Dowex	0	18.1 (NP)**	8.0 (NP)
Dowex	152	24.7	8.0 (NP)

*Peaks at 285 nm and 320 nm were probably due to hydroxymethylfurfural, and chlorogenic acid, respectively. Browning @ 405 nm was used as a reference point, for comparative purposes.

**NP = No Peak

(Ranadive and Haard, 1971). Its apparent absence in the Dowex treated sample may have been due to the strong attraction of polystyrene resins for chlorogenic acid as demonstrated by Gray (1978).

The peak @ 285 nm in spectra of both stored samples, and in the spectrum of the untreated sample, before storage, was probably due to 5-(hydroxymethyl)-2-furfuraldehyde (HMF). This compound absorbs strongly at 285 nm as shown by Tahler and Cates (1974), and was found to develop in apple juice concentrate during storage (Vasatko and Pribella, 1965). It should be noted, however, that other compounds, such as proteins and phenolics also absorb in the region near 285 nm (Loomis 1974). HMF is a degradation product of hexose sugars. It is known to be a product of non-enzymatic browning reactions (Hodge 1953).

Ketosamines formed during initial stages of the Maillard reaction may undergo 1,2-enolization to form osuloses, and HMF may form from these (Reynolds 1968). Burton (1964) reported that browning reactions leading to furfural derived melanoidins, are probably more important under conditions of low amino acid to sugar ratio and acidic pH (as found in PJC). McWeeny and Burton (1963) found that browning of aldose-amine systems via the formation and reaction of HMF was slow at 50°C and within a pH range of 5.5-6.0.

The results suggest that HMF was present in the Dowex treated sample and accumulated during storage. The presence of HMF would

be evidence of sugar degradation in the absence of amino acids. Above pH 3, the Lobry de Bruyn-van Ekenstein transformation is favored versus dehydration, as the mechanism of fructose degradation. This may also be true for glucose (Shallenberger and Birch, 1975). Fructose is the major sugar of PJC (see Introduction, pg. 2).

HMF may have also been present in the untreated sample, and if so, it may have been utilized in the formation of melanoidins during storage. Chlorogenic acid was probably present initially in the untreated sample, but during storage may have degraded to form brown polymers.

Part II. Experiment 2

1.0 Variation in Sample Browning

Change in pigment concentration (as derived from Hunter "L" values; refer to Figure 15, Appendix) is shown for Experiment 2 samples in Figures 13a and 13b. It is evident that Dowex treatment, as in Experiment 1, resulted in strong inhibition of the browning rate from that of the untreated sample (Figure 13a). Sample treatment designed to promote oxidation of phenolics and their subsequent polymerization, followed by XAD-4 treatment to remove oxidized products, resulted in slight inhibition of browning (Figure 13a). This result was similar to that obtained after sample treatment by XAD-4 alone, in Experiment 1 (see Part I, section 1.0A.1).

Dowex treatment has been shown to be an effective inhibitor of

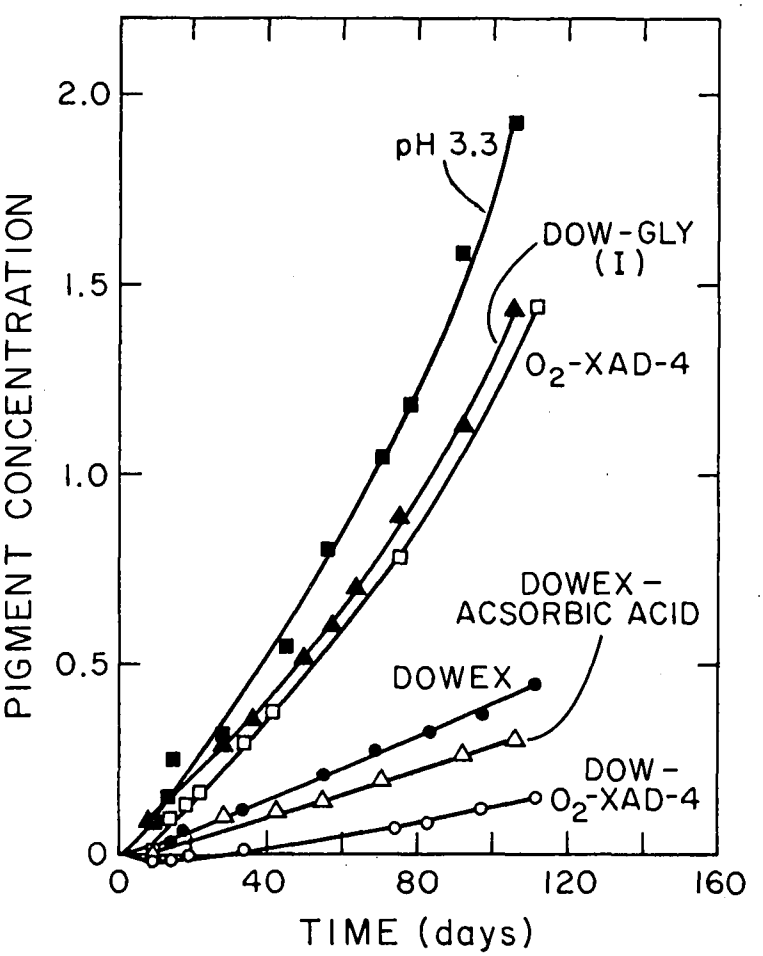

Figure 13a. Change in "pigment concentration" (derived from "L" value; refer to Figure 15, Appendix). Pigment concentration at day zero was subtracted from later pigment concentrations of all samples, for comparative purposes.

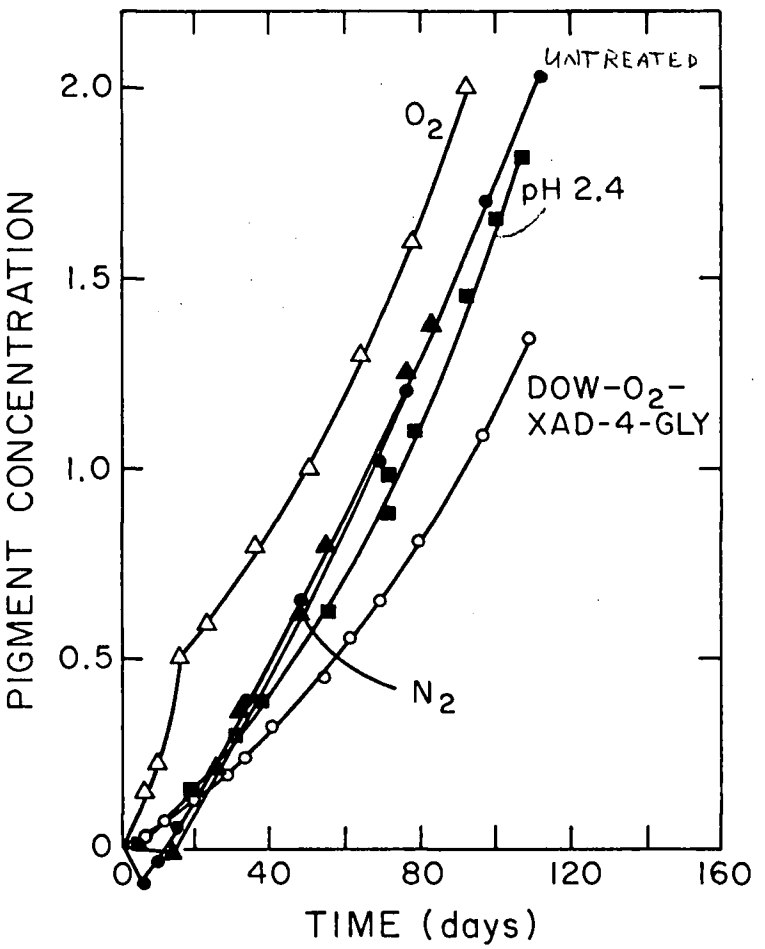

Figure 13b. Change in "pigment concentration" (derived from "L" value)

browning during storage; however, such treatment was not entirely selective in the removal of amino acids. The phenolic content of Dowex treated PJC from Experiment I was reduced by nearly 50% (see Part I, Table 1). Minerals were removed which are known to be capable of lowering the activation energy necessary for browning reactions to occur at lower temperatures (see Part I, section 1.2).

Dowex treatment could have removed a phenolic component not removed by either XAD-4 or PVPP treatments, both of which were not effective inhibitors of browning during storage of PJC (see Part I, section 1.0 A.1).

The alpha-amino nitrogen content in PJC was measured in terms of glycine (see Experimental, Part II, section 2.1.C). It was found equal to 6.0 mg glycine/ml PJC. Since PJC samples were essentially composed of 72% sugars, the ratio of amino acids to sugars was extremely low (1:120). Free amino acids have been shown to undergo a large decrease during storage of PJC (Pribella and Betusova 1964). Motai (1974) showed that melanoidins gave a positive ninhydrin response, although

no free amino acids could be detected as contaminants. Elemental analysis of these melanoidins was calculated to be $C_8H_{11}NO_6$.

For the reasons presented above, removal of amino acids and its effect on the rate of browning could not be certain. To determine if Maillard reactions were primarily responsible for browning in PJC during storage, the alpha-amino nitrogen content was restored, as glycine, to Dowex treated PJC. This caused the rate of browning to approach that of the untreated sample (Figure 13a). Therefore, it was concluded that Maillard reactions in PJC samples were the major cause of browning.

Motai (1973) showed that amino acids and peptides were responsible for the color tone of melanoidins. Melanoidins from peptides generally exhibited darker tones compared with those from amino acids. Among the many amino acids tested, glycine, aspartic acid, and histidine were found to produce the most brown color.

The use of glycine to restore the alpha-amino nitrogen content in Dowex treated PJC probably produced as much, or more color as any other amino acid would have. It is also possible that the original amino acid complement of PJC may have been less productive in this respect than glycine; however, peptides which probably were present could have produced more browning.

It is likely that amino acids were incorporated into melanoidins

formed during browning of PJC, and they may have continued to promote Maillard browning reactions.

Wootton (1976a) studied the loss of free amino acids during storage of honey. He suggested that decreases in amino acids caused by their incorporation into melanoidins, were compensated for by protein breakdown. It is conceivable that protein degradation in PJC during storage, may produce free amino acids, or peptides which could undergo Maillard reactions. The protein content of PJC has been shown to decrease slightly during storage (Pribella 1964).

Reduction of pH in PJC samples to inhibit possible Maillard reactions did not lower the rate of browning during storage, as compared to the rate of the untreated sample (Figures 13a, and 13b).

Hodge (1976) reported that inhibition of Maillard browning reactions is best accomplished by keeping the pH below the isoelectric points of the amino acids, peptides, and proteins in the sample. The Dowex treated sample was adjusted to pH 4.1, which is below the isoelectric point of glycine at pH 5.97; however, after addition of glycine, as described above, to the Dowex treated sample, browning occurred readily.

Dowex treatment, oxygenation, and treatment with XAD-4 (Dow-O_2-XAD-4) produced the most stable PJC (Figure 13a). The increased stability of this sample compared to the sample treated with Dowex, alone, was probably due to the oxidation of remaining

phenolics, followed by their subsequent polymerization, and removal with XAD-4.

The alpha-amino nitrogen content was restored, as glycine, to the Dow-O_2-XAD-4 treated sample; however, only to 1/2 concentration. The browning rate which resulted, was nearly identical to that of the Dowex treated sample with glycine added to full concentration (Figure 13b). It is evident that the concentration of glycine, in this case, did not affect the rate of browning.

PJC was treated with O_2 to promote the oxidation of phenolics. Results show that the rate of browning was increased, but only during the first 20 days of storage; otherwise it appeared to be identical to the rate of the untreated sample (Figure 13b). Oxygen is not essential for Maillard browning reactions to occur (Ellis 1959), as it is for polyphenolic browning (Loomis 1974; and Robinson 1975).

PJC was treated with N_2 to inhibit the browning of polyphenolics, by forcing the removal of O_2. Results show that the nitrogenated sample browned at a rate nearly identical to that of the untreated sample (Figure 13b). Marshall (1951) suggested that oxygen dissolved in apple juice exists in both "free" and "bound" states. Only the "free" oxygen could be removed by deaeration. It is probable that oxygen was still present in PJC, following nitrogenation.

The degradation of ascorbic acid may have contributed to

browning in PJC. Ascorbic acid was not found present in PJC prior to storage; however, the oxidized form, dehydroascorbic acid (DAA) was detected (see Part I, section 1.3).

Wedzicha and McWeeny (1974) discovered that glycine participates in the formation of colored products from ascorbic acid, but not in earlier stages of its degradation involving colorless intermediates. DAA has been implicated as the source of ascorbic acid browning, and it browns more rapidly in the presence of amino compounds (Hodge 1953).

The possible contribution of ascorbic acid and DAA to browning of PJC during storage was tested. Ascorbic acid was added to Dowex treated PJC (see Experimental, Part II, section 2.1 D.4). Results show that the rate of browning during storage was inhibited slightly from that of the sample treated with Dowex, alone (Figure 13a). Ascorbic acid has been used to inhibit polyphenolic browning (Joslyn and Ponting 1951), and such an inhibition may have occurred. It is likely that the presence of amino acids would have accelerated browning of DAA in PJC.

As the quantity of ascorbic acid and DAA detected in pear juice was very low, ascorbic acid browning is unlikely to have been a major contributor to browning of PJC (see Part I, section 1.e).

Part III. Volatile Analysis of Pear Juice Concentrate

1.0 Results of Instrumental Volatile Analysis of Pear Juice Concentrate

Flavor compounds are known to be produced during Maillard browning reactions. Furfurals, imidazoles, and pyrazines, such as 2, 5-dimethylpyrazine are among such compounds which have been identified (Hodge 1967). Volatile analysis of PJC was performed to determine if such compounds may have been generated during storage.

The use of gas chromatography coupled with an alkali flame ionization detector (AFID) indicated that nitrogen containing compounds may have been present in both PJC samples analyzed (see Experimental, Part III 1.0). Only the aroma sample of the stored PJC sample was analyzed with a mass spectrometer, and only a few of the peaks present in its chromatogram were investigated, as shown in Figure 14.

Among compounds tentatively identified by mass spectral analysis, were 2-furaldehyde (furfural), hexanal, 2-hexenal, limonene, and a primary alcohol with a m/e value of 88. Furfurladehyde had a retention time of 15.5 minutes, and its m/e value was 96. It was also the most prevalent volatile in the PJC aroma sample. Nitrogen containing compounds were not found.

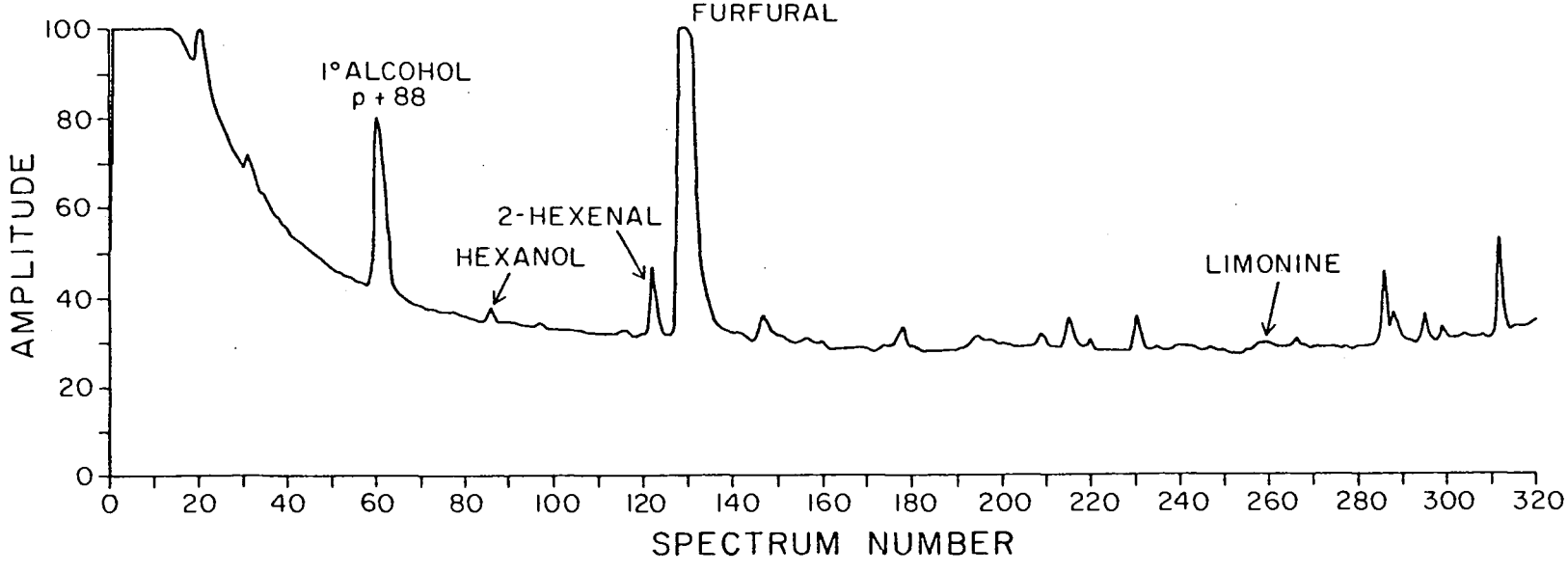

Figure 14. Gas chromatogram of an aroma sample from stored pear juice concentrate

1.1 Taste Panel Evaluation of Pear Juice Concentrate Aromas from Stored Samples

It was observed that all samples, except the Dowex treated sample, of Experiment 1, had developed strong odors during storage. A taste panel was used to evaluate these odors; however, stored PJC samples of Experiment 2 were used.

Statistical analysis showed that while the untreated sample aroma was scored more intense; the Dowex treated sample aroma was scored more desirable, and of a more pear-like character, as shown in Table 7 (see Experimental, Part III, 1.1).

Judges described the untreated PJC sample aroma as oxidized, burnt, and molasses-like. Aroma of the Dowex treated sample was described as weak and fruity-like.

Dowex treatment may have prevented formation of the flavor compounds. Another possibility is that Dowex treatment may have removed flavor compounds and their precursors.

Table 6. Mean scores* of taste panel evaluation of aromas from stored cation exchange treated and untreated pear juice concentrate samples

Sample	Total Aroma Intensity (1)	Pear-Like Character (2)	Desirability (3)
Untreated	6.22	2.19	2.19
Dowex	3.13	4.90	4.80

*Least Significant Difference at the 1% level for categories (1) = 1.92, (2) = 1.76, (3) = 1.93

CONCLUSION

Causes of Browning in Pear Juice Concentrate During Storage

Results of Parts I-III all support the conclusion that Maillard reactions were predominantly responsible for the browning of PJC during storage at 37°C. This conclusion was based upon the following observations:

1. Removal of amino acids from PJC samples by Dowex cation exchange treatment substantially reduced the rate of browning. Amino acids are critical to the formation of Maillard reaction intermediates and are also incorporated into melanoidins (see Part I, section 1.0 A.1, and Part II).

2. Restoration of the amino acid content, as glycine, in Dowex treated samples caused the rate of browning to approach that of the untreated sample (see Part II).

3. XAD-4 and PVPP treatments were effective for the decolorization and removal of phenolics from PJC; however, they were not effective inhibitors of browning (see Part I, section 1.0 A.1).

4. An increase in reductone reducing power was detected in PJC samples after storage, as shown by an apparent "increase" in ascorbic acid, a characteristic reductone. A similar "increase" in phenolic content was probably also due to the formation of reductones. The Dowex treated sample showed a much smaller "increase."

Reductones are known to form during Maillard browning reactions, and melanoidins show reductone reducing power (see Part I, sections 1.3, and 1.4).

5. The water activity of PJC was conducive to Maillard reactions (see Part I, section 1.1).

6. Furfurals were detected in PJC during storage, and are indicative of sugar degradation, which is accelerated in the presence of amino acids (see Part I, section 1.5, and Part III, section 1.0).

7. Off flavors developed during storage of PJC samples, except in the Dowex treated sample. Development of flavor compounds is characteristic of Maillard browning (see Part III, section 1.1).

8. Neither oxygenation or nitrogenation had a marked influence on the browning rate when compared to the untreated sample. Oxygen is not essential for Maillard reactions to occur (see Part II).

9. PJC samples appeared red-brown and dark brown during storage. A dark colored precipitate was noticed after a sample of severely browned PJC was diluted. As reported by Fennema (1976), colloidal and insoluble melanoidins formed during the final stages of Maillard browning reactions, are red-brown and dark brown in color (see Part I, section 1.0 A.2).

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APPENDIX

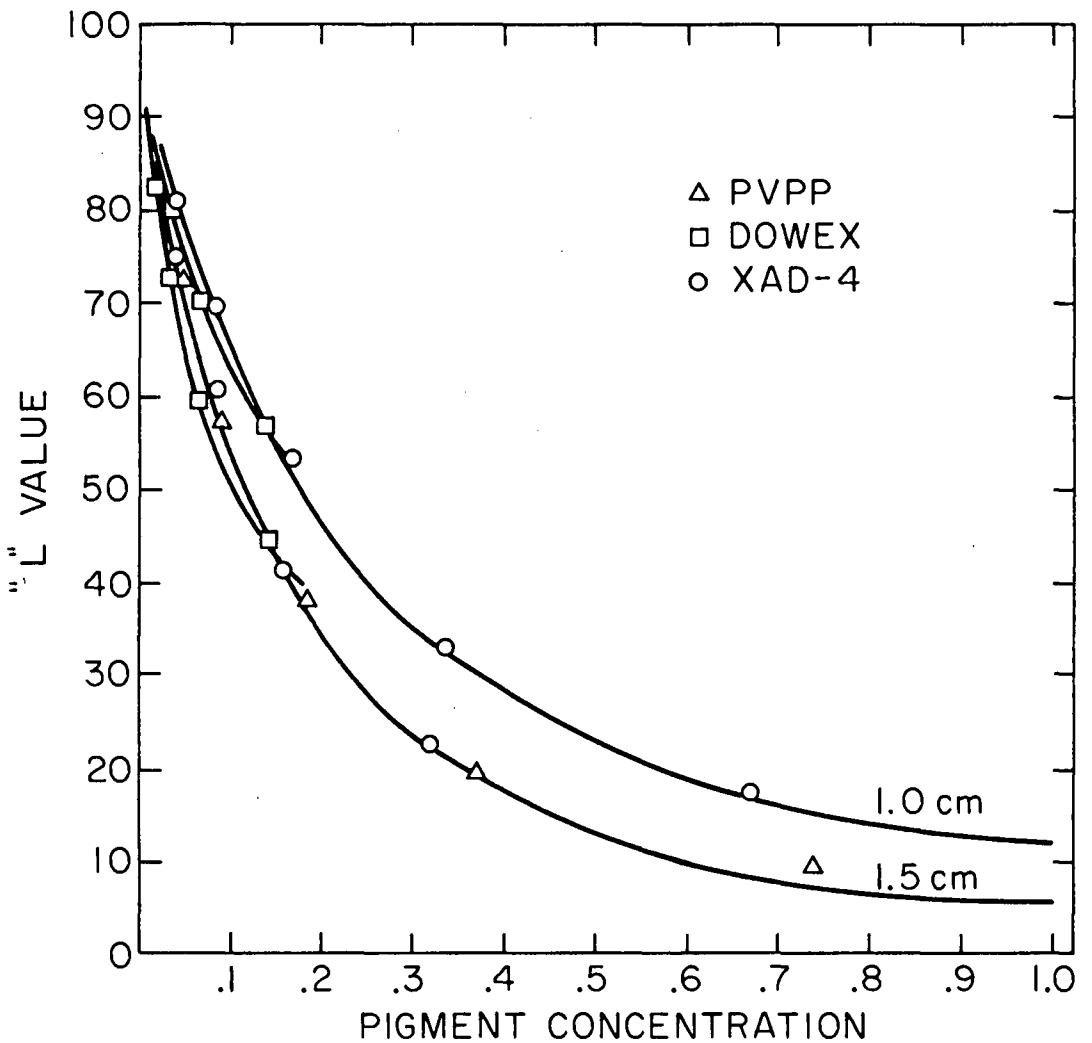

Figure 15. Standard curves used to determine "pigment concentrations" of pear juice concentrate samples. During Experiment 1, 1.5 cm plastic cells were used in the Hunter analysis of stored pear juice concentrate samples. During Experiment 2, 1.0 cm plastic cells were used. The Dowex standard curve was used to obtain pigment concentrations of the Dowex, Dow-O_2-XAD-4, and Dowex with added ascorbic acid treated samples, only, of Experiment 2.