AN ABSTRACT OF THE THESIS OF

JEANNE HODGSON for the degree of MASTER OF SCIENCE

in Food Science and Technology presented on August 10, 1981

Title: ULTRAFILTRATION OF PEAR JUICE: CHEMICAL AND PHYSICAL ANALYSIS

Abstract approved: Dr. Morris Montgomery

Ultrafiltration of d'Anjou pear juice in the batch mode with a Romicon HFXS MXII pilot-scale ultrafilter produced a "sparkling clear", light-colored permeate which was sterile, essentially free of pectin, and lower in phenolics than the feed juice. Retention characteristics of PM-50, PM-30 and PM-10 hollow fiber membranes were tested at a volume concentration ratio of 1.14. No differences in retention characteristics were found; the membranes were completely permeable to sugars, malic acid and α-amino compounds and semipermeable to pectic substances and phenolics. Filtration temperatures of 15°C, 30°C and 50°C did not affect retention characteristics. The higher temperature did reduce the number of microorganisms in the retentate. Transmembrane pressures, varying from 103 to 153 kPa, also did not affect composition of the permeate.

The effects of filtration time (as volume concentration ratio) were tested with a PM-50 membrane at 50°C with a transmembrane pressure of 153 kPA. Flux declined as the log of volume concentration ratio. With increasing filtration time, retention efficiency for pectic substances and phenolics decreased. The permeates became more golden

in color but remained clear. Loss of efficiency was attributed to concentration polorization. Pectic substances were thought to be the major contributor to the formation of the gel layer.

Ultrafiltration of Pear Juice: Chemical and Physical Analysis

by

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A THESIS

submitted to

Oregon State University

in partial fullfillment of

the requirements for the

degree of

Master of Science

Completed August 10, 1981

Commencement June 1982

APPROVED:

Professor of Food Science and Technology in charge of major

Head of Food Science and Technology

Dean of Graduate School

Date thesis is presented August 10, 1981

Typed by Jane Tuor for Jeanne Hodgson

ACKNOWLEDGEMENTS

Dr. Morris Montgomery was responsible for much of what I have learned. He refused to let me avoid my own questions and actually, by forcing me to search for answers, provided the incentive that made this work possible. His Socratic question, "Why then, do you study?" brought all of my research into true perspective. I am thankful for his generosity and support.

Mr. Darrell Beavers also deserves (and has!) my greatest appreciation. Both this present work and my everpresent curosity on the subject of juice clarification are of his doing. His encouragement was immeasurable and his assistance invaluable.

I am extremely grateful to the entire Food Science Department at Oregon State University. They provided the educational background and the monetary assistance necessary for completion of this study.

To my husband, Gary, thank you for bringing Phillipians 4:8 to fulfillment in us.

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ULTRAFILTRATION OF PEAR JUICE: CHEMICAL AND PHYSICAL ANALYSIS

INTRODUCTION

During the 60 years in which winter pears have been grown in the Northwest, there has been a large increase in production of both fresh and processed fruit. Because of their bland flavor and high sugar content, pear juice also has become a source of "natural" sugar in juice blends, in syrup for canned fruits and as a fermentable base for pop wines. In most cases the juice is required to be mild in flavor, light colored, and clear. Efforts to produce such a juice more efficiently are now becoming economically significant.

Generally, pear juice has been clarified by a combination of pectinase and one or more clarifying agents. Because the mechanism of clarification is not fully understood, it is difficult to know how clarifying aids should be used. Incorrect use can result in stabilization of the cloudy juice rather than clarification. The application of ultrafiltration to pear juice clarification offered an opportunity for producing clear juice physically, avoiding use of chemical fining methods.

Ultrafiltration offers additional advantages; the energy requirements, primarily for pumping, are low, the process is relatively nondestructive, and the operation can be made continuous. Because pectin in the retentate has not been hydrolyzed, there is a possibility of recovering and purifying it. The retention of microorganisms by the membrane results in the further advantage of "cold sterilization". By

avoiding the usual heat pasteurization juices, can be preserved with minimal degradation.

In a separate study, Kortekaas (1980) determined the effects of processing parameters on the ultrafiltration of pear juice. The physical-chemical changes brought about in the pear juice by ultrafiltration are reported here.

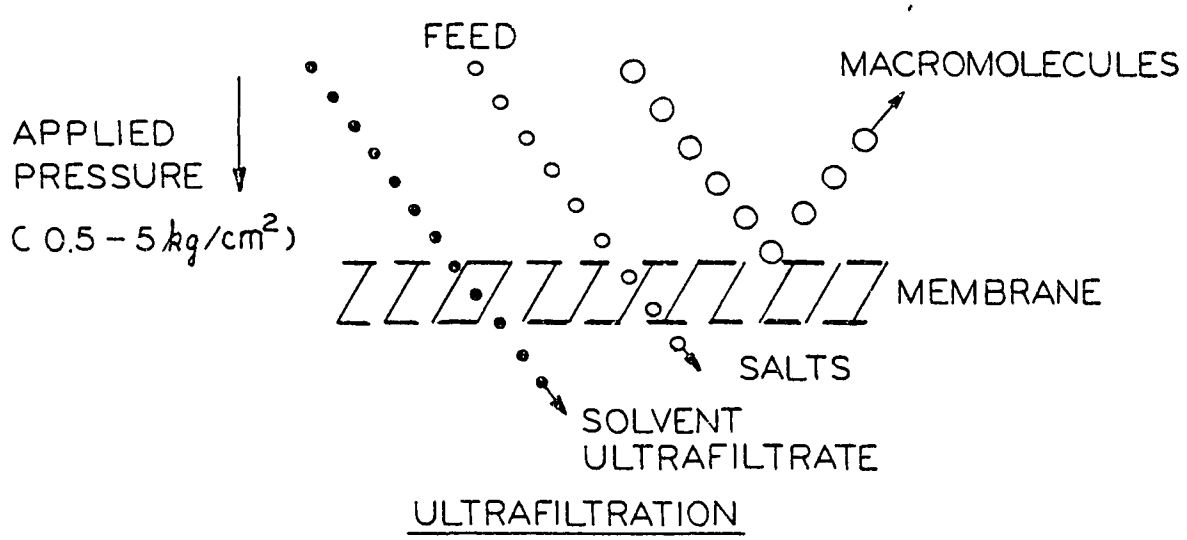
LITERATURE REVIEW

Ultrafiltration

Ultrafiltration is a pressure-activated membrane separation process. While the basic definition is applicable to reverse osmosis as well, some important differences exist. Because reverse osmosis operates largely by diffusion through a membrane, the effects of osmotic pressure are significant and must be overcome with high operating pressures (2450 to 6879 kPa); (A kilopascal equals 98.2 kg/cm^2). Ultrafiltration is primarily a sieving process similar to normal filtration. Operating pressures generally range from 50 to 500 kPa (Porter, 1975).

As shown in Figure 1, reverse osmosis has application in the separation of salts and other low molecular weight (less than 500) solutes from their solvents while ultrafiltration retains solutes, primarily colloids and macromolecules, with a molecular weight greater than 500. In Figure 2 the major filtration processes are categorized by their retention of particles of a specific average diameter. Often, however, retention characteristics are defined in terms of molecular weight cut-off. In both cases particle rejection is given as an average rather than an absolute value. Some of the factors found to influence separation efficiency are molecular configuration, pH and charge of the molecule, salt content, presence of other solutes, solvent, and membrane type (Melling, 1974).

Most ultrafiltration membranes are anisotropic, diffusive ultrafilters (Melling, 1974). The anisotropic or asymmetric membrane



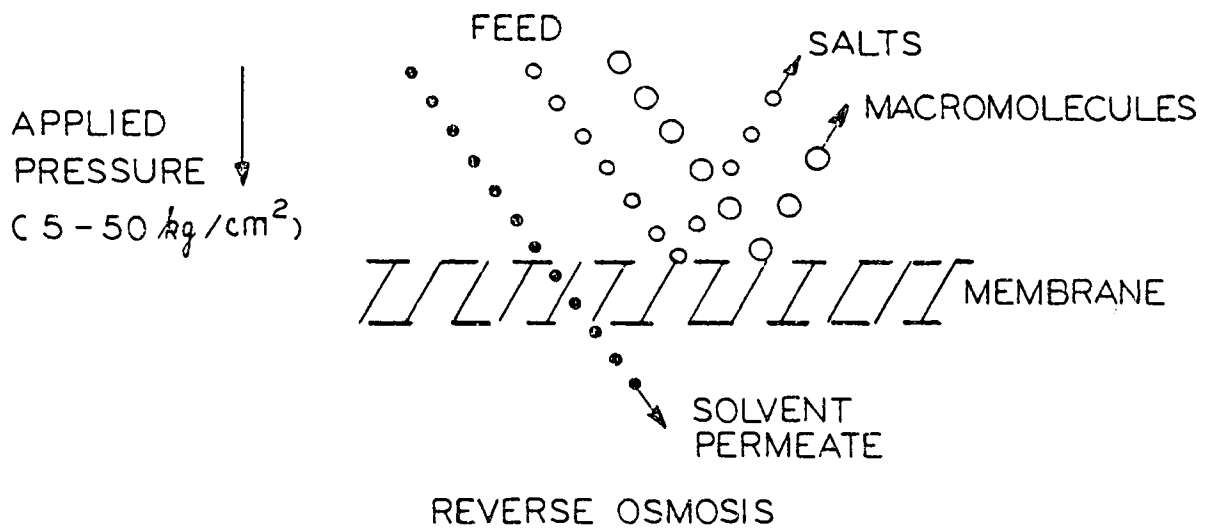


Figure 1. Solute rejection in ultrafiltration and in reverse osmosis

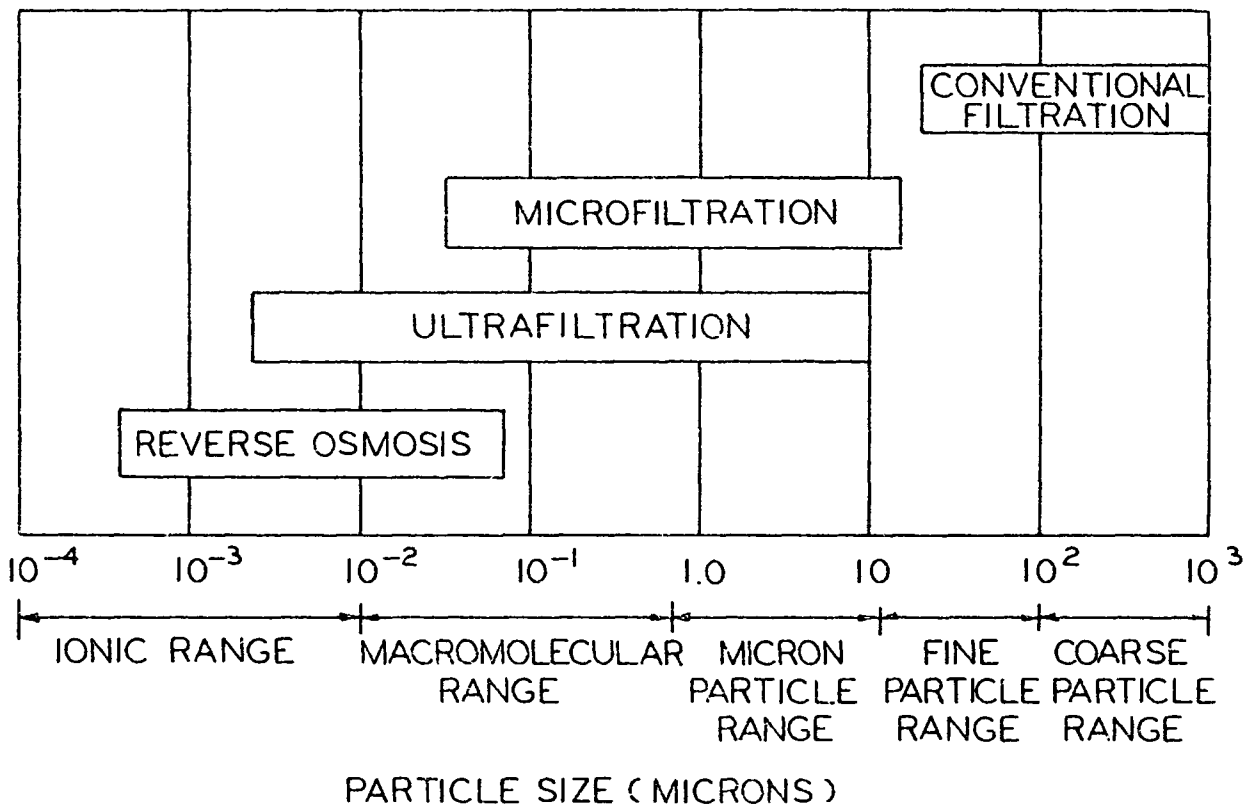


Figure 2. Particle retention in the major function processes

(Figure 3), developed in 1963 by Loeb and Sourirajan, came as a major transition point in membrane technology. Anisotropic membranes consist of a highly porous, relatively thick (0.05-0.25 mm) substructure supporting a dense but thin (0.1-5μ) skin (Porter and Michaels, 1971). Pores of the substructure are from 0.1 to 1.0μ in diameter, while those of the skin are in the order of $10^{-3}\mu$ (Porter, 1975). The advantage of such a membrane is that high solute retention can be coupled with high permeability. The thin skin offers little resistance to flow and minimizes fouling within the membrane. Molecules which penetrate the surface generally pass through the porous substructure as well.

Membranes must be able to retain their microporous structure when used with abrasive solutes, moderately corrosive solvents, and high pressures. Polymers which have demonstrated ability to withstand such stress include poly(methyl methacrylate), poly(vinyl chloride), polyester, polycarbonate, polypropylene, polystyrene, polyacrylonitrile, nylon, polytetrafluoroethylene, aromatic polysulfones, aromatic polyrthers, cellulose nitrate, and cellulose acetate (Porter, 1975; Michaels, 1974). The cellulose esters were among the first polymers used in ultrafiltration. Because of their tendency to "creep consolidate" or lose their porous structure, their limiting temperature requirement (not over 40-60°C), and their susceptibility to strongly acidic or weakly alkaline media and to microbial and enzymatic attack, the cellulose membranes have been replaced in many operations with the more resistant synthetic polymers (Michaels, 1974).

Ultrafiltration membranes are available in basically four configurations: tubular, flat sheet, spiral-wound, and hollow fiber. Horton

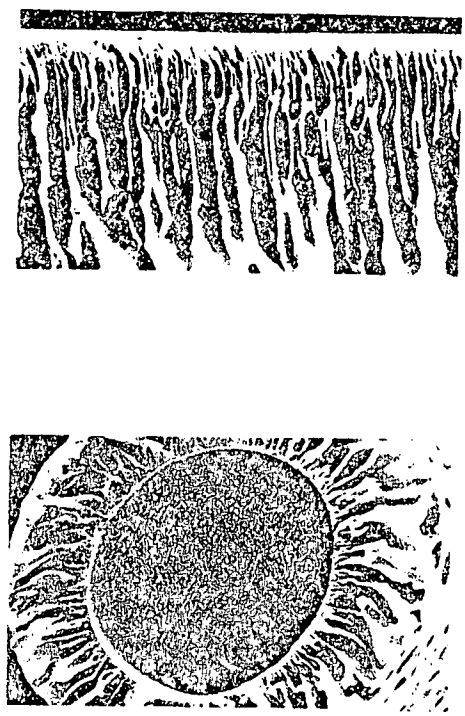


Figure 3. Electron photomicrograph of anisotropic membrance: conventional and hollow fiber

(1975) provides a summary of the advantages, disadvantages, and availability of each configuration. Hollow fiber ultrafilters have the advantages of being relatively inexpensive and the most compact of any configuration. The primary disadvantages are the susceptibility to plugging or fouling with occasional difficulty in cleaning and the need to replace the entire cartridge when a single fiber fails. While most configurations are readily available, the hollow fiber configuration appears to be manufactured by fewer companies. However, this situation may have changed since Horton's report in 1975. Manufacturers of ultrafilters include Dorr-Oliver, Amicon, Romicon, Nuclepore, Abcore, Kalle, Wafilin, DDS, and Paterson-Candy.

In ultrafiltration theory the description of mass transfer across the membrane usually is based on either of two models: the solution-diffusion model and the pore model (Setti, 1976). In the solution-diffusion model permeate flux, the volume of permeate per unit area of the membrane per unit time, is governed by the difference in applied pressure on the membrane. Flux (J) can be expressed as:

$$J = \frac{A(\Delta P - \Delta \pi)}{\Delta x}$$

where: ΔP = pressure difference across the membrane

π = osmotic pressure of feed and permeate

Δx = membrane thickness

A - coefficient characterizing membrane performance with respect to solvent and solute

In the pore model, permeate flux is controlled by porosity, pore size distribution and specific interactions within the membrane pores. Flux

is explained using Poiseuille's Law as:

$$J = \frac{K(\Delta P - \Delta \pi)}{\eta . \Delta x}$$

where: η = viscosity of the permeate

K = membrane permeability coefficient characteristic of a specific membrane

Generally, the pore model has been used to describe ultrafiltration. However, some workers consider mass transfer phenomena in ultrafiltration to be described most accurately by a combination of the two models (Michaels et al., 1965; Lonsdale, 1972; Evans and Glover, 1974).

In practice neither the solution-diffusion model nor the pore model adequately describe mass transport. Both models assume conditions of solute homogeneity up to the actual surface of the membrane. However, as solvent is preferentially removed through the membrane, the solute builds, forming a gel layer at the interface. Once the concentration polarization gel layer is formed, the flux is controlled as much or more by the permeability of the gel as by the permeability of the membrane. Flux often becomes independent of the transmembrane pressure drop.

In most ultrafiltration applications a concentration polarization model based on the concentration gradient of the solute (dC/dx) is commonly used (Porter and Michaels, 1971). Flux is dependent on the bulk solute concentration and on the hydrodynamic flow characteristics adjacent to the membrane. Mathematically:

$$J = k \log \frac{C_g}{C}$$

where: C_g = solute gel concentration

C = bulk solution concentration of solute

k = mass transfer coefficient which is dependent on the solute diffusivity and hydrodynamic conditions adjacent to the gel layer

A theoretical evaluation of the mass transfer coefficient, k, also is possible (Bellucci et al., 1975)

Reduction of the effects of concentration polarization can be accomplished by reducing solvent flux through the membrane, increasing the flow rate (shear rate) past the membrance, increasing solute diffusivity and decreasing solvent viscosity by an increase in operating temperature, decreasing the tube diameter, or using short tubes or channels (Setti, 1976). Hollow fiber ultrafiltration has been found to be especially effective in removing the gel concentration layer, particularly when high viscosity fluids are filtered (Madsen, 1974).

When ultrafiltration is considered as a unit operation in industrial processing, it is generally in areas where concentration, fractionation, and purification of a solute are desired. Bell et al., (1977) have reviewed many ultrafiltration applications. Examples of its application in the food industry include recovery of protein from cheese whey, production of milk concentrates, concentration of soy protein, purification and concentrations of enzymes, recovery of proteins, starch, and peel oil from waste water, concentration of egg white, glue and gelatin concentration, removal of proteins, starches, and gums from sugar extracts, and clarification of fruit juices. One of the most recent applications of ultrafiltration is the membrane enzymatic reactor. Enzymes are either anchored to the membrane or encapsulated in it while the substrate

is passed through the membrane.

Application of ultrafiltration in the clarification of juice was first studied by Heatherbell et al., in 1977. Ultrafiltration proved successful in the removal of pectins and starch and produced a "sparkling clear" juice. In 1979, Brokes et al., from Czechoslovakia patented an ultrafiltration process for removing pectins, proteins, and enzymes from juices. Kortekaas (1980) examined the processing parameters of pear juice ultrafiltered with membranes of three molecular weight cut-offs: 50,000, 30,000, and 10,000. Permeate flux for all three membranes was optimized at an average transmembrane pressure of 157 kPa with a flow rate of 0.15 meters per second. Flux was found to increase with increasing temperature.

Clarification of Fruit Juice

Measurement of Clarity

The measurement of clarity in juices has progressed from purely subjective evaluations to objective methods based on the interaction of light with colloidal particles. In a disperse system light may: 1) pass through the system, 2) be refracted by particles, 3) be reflected by particles, 4) be absorbed and thus converted to thermal energy, or 5) be scattered. Methods for quantifying the clarity of a juice take advantage of the exceptional ability of colloidal systems to scatter light.

Turbidimetric measurements are based on the decrease in intensity of light transmitted through the sample due to scattering of light. Often transmittance or absorbance are determined spectrophotometrically at

specific wavelengths; however, absorption of light by the particles may result in significant error.

Nephelometric methods avoid absorbance errors and measure the scattered light directly, generally at some predetermined angle other than 180°. Although light is scattered in all directions upon striking a particle, the distribution of scattered light depends upon the size and shape of the particle. Rayleigh in 1871 worked out the relationships between the properties of the scatterer (shape, size, refractive index) and the angular distribution of the scattered light. A complete description of the phenomenon is complex and takes into account such factors as wavelength, amplitude, phase, and polarization as well as effects caused by interferences, diffraction, multiple scattering, and by colored sols. In general, scattering is observed if particles are randomly distributed in a medium having a refractive index different from their own and if the particles have dimensions of approximately one order of magnitude or less than that of the incident wavelength (Voyutsky, 1978).

Industrial nephelometers measure the intensity of light relative to a standard, either internal or external to the instrument. Based on Rayleigh's equation the light intensity is directly proportional to the concentration of colloidal particles. A high correlation (0.81) between sol concentration and the visual perception of cloudiness has been noted by Pieczonka and Cwiekala (1974). Most nephelometers, in addition, determine a ratio of scattered light to transmitted plus forward-scattered light to correct for light absorbed by pigmented sols. The method provides a simple and accurate measurement of clarity even

in colored juices.

Mechanical Clarification

Voyutsky (1978) states that emulsions, including the colloidal suspensions of fruit juices, can be broken by centrifugation, filtration, and electrophoresis. Treatment with electrical current has not been used in clarification, although it has been found effective in juice extraction (Lazarenko et al., 1978), and centrifugation is a common method of removing, generally, the more coarse particles. Filtration, however, is almost always the method chosen for removing the suspended particles in juices.

Generally, the filters employed in juice clarification (principally rotary vacuum filters, filter presses, horizontal types of pre-coat filter and filter membranes) are used in conjunction with some type of filter aid. A common characteristic of all filter aids is the large surface area per unit weight. This is accomplished either by the media existing in a finely-divided physical state or as a result of it having an highly porous structure. Diatomaceous earth, the most common filter aid, is of the latter type.

Filtration theory can be explained most simply as the combined effect of two mechanisms: 1) transport of the particles to the filter surface and 2) adsorption or attachment of particles to the surface. In the first mechanism physical phenomena such as sieving, straining, sedimentation, inertial impingement, interception, and Brownian diffusion predominate and in the second mechanism electrokinetic and chemical phenomena are involved (Fiore and Babineau, 1979; Wnek, 1974). The realtionship between pore size and particle size determines which of

the two mechanisms predominates. For particles larger than the pore size, physical phenomena are most important; for particles smaller than the pore size, electrochemical phenomena predominate. When particles are of intermediate size, filtration is controlled by both mechanisms (Fiore and Babineau, 1979).

Recently, an appreciation has developed for the role of electrokinetic adsorption in filtration. Just as the zeta potential (the potential drop across the layer of ions surrounding a particle) influences the repulsion and attraction and thus the stabilization or coagulatoon of suspended particles, this potential drop also controls, to a large extent, the ability of particles to adsorb to the filtration medium. Several investigations have demonstrated the importance of electrokinetic phenomena. The effectiveness of asbestos as a filtration medium has been shown to be due as much to the high isoelectric point (pH 8.3) and the resultant positive charge in neutral or acidic solutions as it is to the large surface area (Riddick, 1968). Fiore and Baineau (1979) have reviewed similar studies. In addition they have found that by chemically modifying the surface charge of diatomite and replacing the native negative zeta potential with a positive zeta potential, the filtration efficiency was increased from 25% to 75%, depending on particle size.

Electrokinetically modified filters are a relatively recent development in juice filtration technology. Processes have been developed to commercially manufacture filter aids which are both highly porous and chemically modified to improve adsorption of colloidal particles. One example, the "Zeta Plus" filters designed by AMF Cuno, use a matrix of graded density to give even submicron filtration at high flow rates (AMF Cuno, 1979).

Color in Fruit Juices

Measurement of Color

The perceived color of a juice is a result of preferential absorption of specific wavelengths by pigments in the juice plus the ability of the human eye to measure at each wavelength the light that is not absorbed. In an effort to find objective methods for describing color, a number of color standards, color systems, and techniques for measuring color have been developed. Methods for measuring color include spectrophotometric and reflective techniques where color is quantitated in terms of absorption at a specific wavelength but without further interpretation of how it would be visually perceived. Two color systems, the Hunter and CIE systems, provide scales for describing color in terms of human perception. The Hunter system is summarized below.

The Hunter color solid consists of three cartesian coordinates: a lightness (L) scale which ranges from 0 (darkest) to 100 (lightest) and two chromaticity scales ("a" and "b"). A positive "a" describes redness and a negative "a" greeness while a positive "b" describes yellowness and a negative "b" blueness. Color also can be interpreted in terms of Hunter polar coordinates where hue angle locates the color on the a-b plane and saturation index indicates the saturation or "degree of departure from the gray of the same lightness" (Hunter, 1975).

To relate the Hunter color of one juice with that of another, the color difference (ΔE) must be calculated. According to the Scofield-Hunter equation:

$$\Delta E = [(L_1 - L_2) + (a_1 - a_2) + (b_1 - b_2)]\ ½$$

The color difference, ΔE, is approximately three times as large as the

MacAdam unit, the unit of least perceptible color difference (Hunter, 1975).

Methods of Color Removal

In pear juice the colored pigments are primarily phenolic compounds. Therefore, any phenolic adsorbant would be expected to reduce the color. Bentonite, PVP, and PVPP, and gelatin all have been found to remove pigments from juices (Amerine and Joslyn, 1951; Narziss and Bellmer, 1976; Singleton, 1967; Beavers et al., 1981). Activated carbon also has a high, non-specific adsorptive capacity. Kuhn-Abaunza (1981) found carbon to be superior to PVP, PVPP, gelatin, and bentonite in the removal of color in pear juice.

Composition of Pear Juice

Pectic Substances

In 1825 Braconnot (1825) coined the word pectin to describe the jellying compound of fruit preserces. The derivation is from the Greek "πηκος", meaning to congeal or solidify. As the body of chemical information on pectic substances grew, the American Chemical Society established a standard definition. The revised (1949) definition for pectic substances is "a group designation for those complex colloidal carbohydrate derivatives which occur in or are prepared from plants, and contain a large proportion of anhydrogalacturonic acids units which are thought to exist in a chain-like combination. The carboxyl groups of polygalacturonic acids can be partly esterfied by methyl groups and partly or completely neutralized by one or more bases (McCready, 197). Doesburg (1965) points out that the vague, general nature of the defin-

ition reflects the great heterogeneity of pectic substances. There may be variations in molecular weight, in the amount and distribution of methoxyl and acetyl groups, and in the quantity and distribution of other non-uronide materials. In addition, irregularities may exist within the chain itself.

Generally, pectic substances can be divided into three groups: pectic acids, pectinic acids, and protopectin. The following definitions are those presented by Doesburg (1965) and contain the most recent revisions.

"Pectic acid" is applied to pectic substances composed mostly of colloidal polygalacturonic acids and nearly free from methylester groups. Pectates are either normal or acid salts of pectic acid.

"Pectinic acids" is used for colloidal polygalacturonic acids containing more than a negligible portion of methyl ester groups. Pectinates are either normal or acid salts of pectinic acids.

"Protopectin" is applied to the water insoluble pectic substances in plants from which pectic substances can be produced.

The above definitions are based on the structure and composition of pectic substances. The term "pectin" is described both in terms of structure and of gelling property. "Pectin" designates those water-soluble pectic acids of varying methyl ester content and degree of neutralization which are capable of forming gels under suitable conditions. "High methoxyl" pectins form gels in the presence of a high sugar and acid concentration while "low methoxyl" pectins form gels without sugar and when certain metallic ions are present (Doesburg, 1965).

Pectic Substances in Pears

In pears the softening which occurs during storage and ripening is accompanied by changes in both the content and the composition of the pectic substances. These pectic changes have been the subject of several studies.

Weurman (1952) followed the changes in the pectic substances of Doyenne Boussock pears during growth. In the early stages of development, the total pectin content (soluble and protopectin) remained relatively constant at approximately 0.8% (fresh weight). Prior to harvest, however, there was a rapid decrease to less than 0.5% total pectin. The decrease was paralleled by a decrease in the protopectin concentration, although it does not appear that protopectin was hydrolyzed entirely to soluble pectin. Rather, there is probably some shifting of the equilibrium between the different pectic fractions.

Date and Hansen (1954) examined the pectic changes occurring during storage at -1.11° to 0.56°C of Bosc, Bartlett, and d'Anjou pears. In all three varieties both protopectin and total pectin concentrations increased during storage. In d'Anjou and Bartlett pears the increase was followed by a decrease during the last part of the storage period. d'Anjou pears were found to contain more pectin and protopectin throughout storage than did Bartlett or Bosc.

Emmett (1929) studied the effect of storage temperature on changes in pectic content of Conference pears and found that the decrease in total pectin was minimal at 1°C but more definite at 4° and 5°C. At 1°C protopectin decreased slightly and soluble pectin increased slightly. At 4°C protopectin, as well as soluble pectin, again decreased very slightly. At 5°C, however, protopectin content decreased rapidly to

0.01% (fresh weight) while soluble pectin showed a rapid initial increase followed by a slow decrease. Although changes in the texture of the stored fruit did correlate with changes in the concentration of protopectin, Emmett (1929) noted that the degree of esterification of the pectin also may be an important factor. His measurement of juice viscosity reflect the methoxyl content and show that the relationship between texture and pectin probably does involve both changes in concentration and changes in composition. Gee et al., (1959) have confirmed his hypothesis by showing in Bartlett pears a decrease in degree of esterification from up to 95% for hard, green pears to 64% for soft, ripe pears.

The relationships between pectin content and ripening have been studied by Emmett (1929), Tindale et al., (1938), Kidd et al., (1940), Ulrich et al., (1949), Weurman (1952), Date and Hansen (1954), Jermyn and Isherwood (1956), Esau et al., (1962), Luh et al., (1966), Wang et al., (1972), and Mizuno et al., (1975). Generally, ripening is accompanied by a decrease in protopectin due to hydrolysis and a resulting increase in soluble pectin. At later stages the soluble pectin concentration remains relatively constant or decreases, possibly due to degradation of the polygalacturonic chains. The decrease in total pectin during ripening may be caused by hydrolysis of the pectin to oligogalacturonides or it may be caused by utilization of galacturonic aicd as an energy source (Dame et al., 1956)

Methods for Measurement of Pectic Substances

Early methods of quantitation consisted of gravimetric analysis after alcoholic or cationic precipitation. Estimates of the impurities

in the precipitates ranged from 10 to 90% (Rouse and Atkins, 1955). In 1922 Carre and Haynes developed the more accurate calcium pectate method and Wichmann the pectic acid method. Improvements were made in these two procedures by Poore (1934), Hinton (1940), and Fellers and Rice (1932). Pectic substances also have been determined by titration (Owens et al., 1952), optical rotation (McCready et al., 1951), decarboxylation (Lefevre-Tollens, 1907; Whistler et al., 1940; McCready et al., 1946; Tracey, 1948), and by colorimetry based on meta-hydroxydiphenyl (Blumenkrantz and Aboe-Hansen,1973), anthrone, naptharesorcinol, dinitrobenzoic acid, and carbazole (Doesburg, 1965). The carbazole method of Dische (1947) has been applied to the determination of pectic substances by Stark (1950) and has since been modified (McComb and McCready, 1952; Dietz and Rouse, 1953; Bitter and Muir, 1962; Galambos, 1967; Bartholomae et al., 1977). More recent developments include an iodometric method (Meurens, 1978), a paper chromatographic method (McCready and Gee, 1960), a gas-liquid chromatographic method (Chang, 1979), and an high pressure liquid chromatographic method (Klink, 1980).

Since the pectic substances cover a range of solubilities, extraction methods have been developed to solubilize selectively the various fractions. Owens et al., (1952) noted three requirements to be met during the extraction: 1) tissues must be maintained in a swollen condition to allow diffusion of the pectin, 2) some combination of heat, acid or cation acceptors must be used to break cross links between pectin carboxyls and other macromolecules, and 3) hydrogen bridges between associated polymers and salt linkages with proteins must be dissociated by thermal energy or addition of hydrogen bond-breaking agents.

Generally, high-methoxyl pectic substances are extracted with weak acid and heat, while low-methoxyl pectinic acids require extraction at higher pH values plus the addition of chelating agents. To prevent degradation due to the higher pH, temperature must be kept low (below 60°C) compared to that for the acid extraction (Doesburg, 1965).

Pectic substances are further purified by precipitation with ethanol or acetone or by cationic precipitation with calcium, magnesium, iron, aluminum, copper, nickel, or other metallic polyvalent ions. Analysis of total pectic substances may follow direclty or the isolate may be further fractionated. Fractionation may occur prior to purification. Procedures using either ethanol of varying concentrations or combinations of water, ammonium oxalate, acid and alkali have been developed by Kertesz and McColloch (1950), Kertesz, (1951), Reynaud (1951), Owens et al., (1952), and Rouse and Atkins (1955).

Nitrogenous Compounds in Pears

In 1949, Joslyn and Stepka examined the free amino acids of apples, apricots, prunes, and pears and found that the amino acid content of Bartlett pears was lower than that of the other fruits. Principle amino acids were asparagine, serine, and glycine. Ulrich and Thaler in 1955 reported the presence of relatively large amounts of aspartic acid and asparagine throughout the growth of Williams' and Passe-Crassane pears. To a lesser extent they detected glutamic acid, serine, threonine, α-alanine, valine, leucine, and to a much lesser extent, phenylalanine. Lysine was present only at maturity. During growth of the fruit the proline content increased and at maturity proline became the major amino acid. Burrough (1957a) also confirmed that in juice pressed from 20

varieties of perry pears, proline was prominant in most and, in some, was the major amino acid.

More recently Fernandez-Flores et al., (1970) have used gas-liquid chromatography to develop a profile of the free amino acids in pears. Aspartic acid, proline, isoleucine, and serine were the major amino acids. In 1979, Chang analyzed both free and total amino acids in cloudy, ripe Bartlett pear juice over a three-year period. The predominant free amino acids were in the first year serine, proline, and aspartic acid and, in the second and third years, serine, asparagine and/or glutamine, and proline. In the analysis of total amino acids, aspartic acid was to a very great extent the predominant amino acid in all three samples.

In the past 25 years some of the less common amino acids have been isolated from pears. Methyl-hydroxyproline was identified as one of the principal amino acids in fermented perries (Burroughs, 1960). In 1956, Burroughs separated what appeared to be hydroxypiperidine-2-carboxylic acid from perry pear extracts and by 1957(b) had isolated 1-aminocyclopropane-1-carboxylic acid (ACPC) from 17 out of 20 perry pear samples. As of 1960, ACPC had not been detected in dessert pears. Again in 1977, Rossetti et al., examined the free amino acids of 20 pear juices. In addition to the usual protein amino acids, they confirmed the presence of ACPC and cis-4-hydroxymethyproline, identified ornithine in 14 of the samples, and found four unidentified ninhydrin-positive compounds.

In 1928, Chatfield et al., reported a total nitrogen content for Bartlett pears of 0.064 mg/100g. Widdowson and McCance (1946) found

that both "English Eating" and "English Cooking" pears contained 0.03 mg nitrogen/g. Based on a study of 15 of the amino acids in pear, Fernandez-Flores (1970) calculated 31.8 mg of free amino acids/100 g fresh fruit.

The free amino acid content of 20 perry pear juices was reported by Burroughs (1957a) as varying from less than 5 mg/100 ml to 13 mg/100 ml. Pribella et al., (1964) studied pear juice concentrate and found that the free amino acid content was approximately one third of the total nitrogen. Cornwell (1980) found 6.0 mg/ml of α-amino nitrogen in pear juice concentrate prior to storage. Chang (1979) analysed both free and total amino acids in filtered and unfiltered Bartlett pear juices from three successive years. Free amino acids ranged from 32.8% to 47.3% of the total nitrogen. From 79.6% to 91.3% of the total amino acids were retained in the clarified juice.

There has been considerable interest over a period of several decades in relating the protein/amino acid content of the pear to the development of the fruit, including ripening and storage. Wange et al., (1971) examined the protein nitrogen content of developing pears prior to picking. On a fresh weight basis there was a drop in protein nitrogen over a one-month period from approximately 27 mg/100 g to approximately 20 mg/100 g. The values for protein nitrogen, however, are of questionable accuracy due to their determination by the Lowry method. Loomis (1974) has noted that for plant proteins the values given by the method of Lowry et al., (1951) are often in error by orders of magnitude.

The data of Kidd et al., (1940) suggested that in Conference pears the respiration rate/unit of protein is constant over a large part of

their development. The net increase in protein during the respiration climacteric of detached fruit indicated a change in the balance between soluble and protein nitrogen. Ulrich (1958) also noted that outside of the respiration climacteric, changes in the nitrogen constituents of stored pears were small.

Method for Measuring Amino Acids

Moore and Stein (1954) developed the ninhydrin reagent for use in the quantitative analysis of α-amino acids. Ninhydrin causes decarboxylation and deamination of amino acids. Subsequent condensation of the amine with another molecule of ninhydrin gives a colored product called Ruhemann's purple (λ_{max}=570 nm). Proline reacts with ninhydrin to give a colored complex with maximum absorbance at 440 nm.

The ninhydrin reagent has been modified several times. Hydrindantin, the reduced form of ninhydrin, makes the reaction quantitative and therefore, reproducible. Since hydrindantin is almost completely insoluble in water, a solvent capable of dissolving both ninhydrin and hydrindantin had to be found. Moore (1968) has developed a solvent using dimethyl sulfoxide with a lithium acetate buffer.

Methods of Measuring Total Phenolics

Various techniques have been developed for the quantitative estimation of total phenols. Colored products are quantitatively formed when phenolics are reacted with oxidizing agents such as ceric sulfate (Spencer et al., 1954), permanganate (Smit et al., 1955), or phosphomolybdate (Singleton et al., 1965); coupling agents such as diazotized p-nitroaniline (Bray et al., 1952), diazotized p-amino-benzoic acid

(Woof et al., 1966), Gibbs reagent (King et al., 1957), or 4-aminophenazone (4-aminoantipyrine) (Bendelow, 1978); nitroso compounds such as nitrous acid (Mohler et al., 1957); acids such as butanol-hydrochloric acid (Dadic, 1971); and metals such as iron (Kursanov et al., 1946) or titanium (Eskin et al, 1978). Direct spectrophotometric methods also can be used to measure phenolic compounds. More detailed information can be obtained by measuring changes in absorptivity brought about by a change in pH or by the addition of metals or other modifiying agents (Swain et al., 1964).

Methods used to analyze unseparated mixtures of phenoloics are necessarily empirical due to differences in the selectivity of a reactant for various phenolic compounds. Swain et al., (1964) reviewed the advantages and disadvantages of several methods and for total phenols recommended the Folin-Ciocalteau method. The recommendation was based on evaluations of sensitivity, precision, accuracy for non-specific mixtures of phenolics, interferences, and time requirements for each method.

Phenolic Compounds in Pears

In pear juice the phenolic substances are responsible for much of the color, haze, and flavor. Phenolics are the pigments primarily responsible for color due to their contribution to polyphenol oxidase (PPO) browning (discussed more fully in the section on browning). Kieser et al., (1953) were first to show that the formation of hazes in perries was accompanied by a reduction of the soluble "tannin". The insoluble precipitate appeared to be complex leucoanthocyanin of high molecular weight. While phenolic acids and dihydrochalcones contribute to flavor, the major contribution is from the flavan-3-ols ((+)-catechin and (-)

-epicatechin) and the flavan-3, 4-diols (leucoanthocyanidins). Lea et al., (1978) have assessed the role of procyanidin fractions in flavor. Their findings showed that bitterness was associated with oligomeric procyanidins, and that no one procyanidin could be uniquely identified with either bitterness or astringency.

* In the older literature the word "tannin" designated a large mixture of phenolic compounds. Williams (1957) found the "tannin" content of pears based on permanganate titration to range from 0.1% (for dessert pears) to 1.2% (for perry pears). Jacquin and Travernier (1954) also used a permanganate titration to show that the "tannin" content of perry pears increased during the early stages of growth, reached a maximum and then declined to half the maximum. Ryugo (1969) used both the Lowenthal method (permanganate titration) and the Pro method (Folin-Denis) to document the same trends in the Oriental pear. Changes in differences between the results of the two methods were explained as changes in the "make-up of the tannin complex" as the fruits matured. In 1955, Jacquin adopted the more exact definition for tannins as those compounds having the "general properties of tannins and precipitated by gelatin in 1% aqueous solution. After examining 16 varieties of pears, the "true tannins" were found to make up from 48-88% of the total "tannin".

Williams (1957) found that some perry pears contained large quantities of leucoanthocyanins. Kieser et al., (1953) in their analysis of perry pear precipitate by paper chromatography, found only a small part of the leucoanthocyanin to be mobile. The majority of the material was found to be polymeric. In 1960 Luh et al., isolated a pigment from Bartlett pears that resembled cyanidin. Using sulfurous acid to control oxidation during hydrolysis, Nortje (1965) was able to isolate two pro-

anthocyanidins from Bartlett pears. The first appeared to be a dimer consisting of a 5,7,3',4'-tetrahydroxyflavan-3,4-diol unit and an (-)-epicatechin unit. The second proanthocyanidin seemed to consist of three flavan units which with further hydrolysis yielded equal amounts of (+)-catechin and (-)-epicatechin. Sioud et al., (1966) also isolated two "leucoanthocyanidins" (proanthocyanidins) from Bartlett pear puree. They estimated that these proanthocyanidins contributed 32.50% of the polyphenol material.

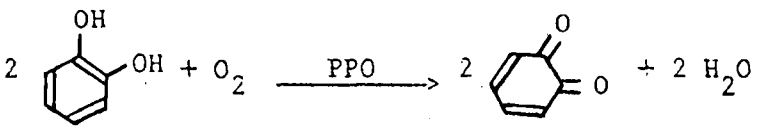
Sioud et al., (1966) listed catechin/epicatechin (32.50% and chlorogenic acids (23.78%) as the two other major phenolic components of pear puree. Ranadive et al., (1971) quantitated the major phenolics in four pear varieties and found that in mature fruit the chlorogenic acids varied from 2.5 to 5.5 mg (of (+)-catechin/100 g pulp) and catechin/epicatechin from 2.50 to 2.72 mg. In ripe fruit the chlorogenic acids varied from 2.25 to 22.00 mg and catechin/epicatechin from 1.87 to 11.00 mg. Other workers studying the catechins and hydroxycinnamic acid derivatives of pears have reported the presence of chlorogenic acid (Bradfield et al., 1952; Herrmann, 1958; Sondheimer, 1958), neochlorogenic acid (Herrmann, 1958; Sondheimer, 1958), cryptochlorogenic acid (Sondheimer, 1958), isochlorogenic acid (Herrmann, 1958; Sondheimer, 1958), p-coumaric acid (Mosel et al., 1974b), p-coumarylquinic acid (Cartwright et. al., 1955; Sioud et al., 1966), esters of ferulic acid (Herrmann, 1958), (+)-catechin (Herrmann, 1958; Siegelman, 1955; Nortje, 1966; Mosel et al., 1974a,b), (-)-epicatechin (Herrmann, 1958; Siegelman, 1955; Nortje, 1966), and caffeic acid (Sioud et al., 1966; Mosel et al., 1974a,b). Isochlorogenic acid later was found to be a mixture of

dicaffeoylquinic acids (Scarpati et al., 1964; Corse et al., 1965).

Phenolic compounds from other groups have been identified recently. In 1965 Nortje et al., isolated isorhamnetin-3-rhamnogalactoside and a derivative of isorhamnetin-3-glucoside from the fruit of Pyrus communis L. cultivar Bon Chretien. Quercetin glycosides and isomers were found in Bartlett pear puree (Sioud et al., 1966) and in the fruit of several new pear varieties (Ranadive et al., 1971). Also Durkee et al., (1968) and possibly Ranadive et al., (1971) have found arbutin (hydroquinone β-D-glucoside) and a related glucoside in the immature fruit of Kieffer and Phileson pears and in the mature fruit of Beurre d'Anjou and Bartlett pears.

Polyphenoloxidase Browning

The browning of pear juice, especially that browning which occurs during production of the juice, is primarily the result of oxidation of specific phenolic compounds by polyphenoloxidase (PPO). PPO (EC 1.14.81 monophenol monooxygenase) is categorized by two distinct types of activity: 1) the hydroxylation of monophenols to o-diphenols and 2) the dehydrogenation of o-diphenols to o-quinones. In pears only catecholase activity has been reported (Tate et al., 1964; Sioud and Luh, 1966). The reaction requires the presence of oxygen and of a copper co-factor:



When pears are crushed and pressed, PPO, as well as both of the required substrates, oxygen and o-diphenols, are present together. The quinones produced are highly reactive and, therefore, polymerize or condense with amino acids and proteins to form the brown melanin pigment.

Both catechins and chlorogenic acid are well known PPO substrates and their contribution to browning in the pear has been well documented (Siegleman, 1954; Weurman et al., 1953; Walker, 1964; Tate et al., 1964; Ranadive et al., 1971; Stelzig et al., 1972). Chlorogenic acid was shown to be the major substrate in pear tissue, while catechin and epicatechin were the principal substrates in the peel. Arbutin, a compound recently detected in pears (Durkee et al., 1968), would also be a substrate for PPO browning.

During the development of the pear, both the susceptibility to friction discoloration (caused by PPO) and the phenolic content have been shown to decrease, reaching a constant level prior to harvest (Hulme and Rhodes, 1971; Mellenthin et al., 1974). Ranadive et al., (1971) found that post-harvest ripening was accompanied by an increase in total phenolics, and also indicated that the browning tendency of four pear varieties correlated well with the total phenolic content. Mellenthin et al., (1974) related decreases in susceptibility to discoloration during storage of pears to declining levels of total phenolics. However, PPO activities also decreased during storage and may have caused an accumulation of phenolic compounds.

Methods for Measuring Sugars

Early methods for sugar quantitation often entailed use of a combination of proximate analyses. Total reducing sugars could be determined by alkaline copper reduction. With iodine oxidation of the glucose, the contribution of fructose and glucose could be estimated (Lothrop, 1931), and by measuring the increase in reducing power after mild hydrolysis the sucrose content also could be measured (Strain, 1937). Sorbitol

analysis was performed by reaction with benzaldehyde to dibenzal-sorbitol (Strain, 1937). Paper or thin-layer chromatography were used to measure minor sugars (Ash et al., 1955).

More recently, carbohydrates have been separated by gas-liquid chromatography of their trimethysilyl derivatives (Seeley et al., 1963). Fitelson in 1970 applied this method to the detection of adulteration in fruit juices. Initially, sugars were removed by lead precipitation in ethanol of pectin and fruit acids. Akhavan (1977) found that a significant percentage of sugars precipitate with the lead salts. However, gas-liquid chromatographic analysis of sugars separated by ion exchange resulted in complete recovery. With the use of both cation and anion exchangers, sugars were purified very efficiently.

Because of the ease of analysis, enzymatic quantitation of major sugars has gained in acceptance. Youtz (1980) found enzymatic analysis of the major sugars in Bartlett pear juice to be comparable to gas-liquid chromatographic analysis. However, to quantitate gulcose, fructose, sucrose, and sorbitol three separate enzyme tests are required thus reducing the time-saving advantage of enzyme analysis.

Within the last ten years, applications of high pressure liquid chromatography (HPLC) to the determination of sugars have increased dramatically. HPLC offers several advantages over GLC. Volatization is not required, eliminating the derivatization step and reducing thermal degradation caused by high temperatures. The technique is rapid and efficient. For sugar analysis there is the added advantage of detecting both α and β anomers as a single peak.

Monosaccharides generally have been separated on columns containing a polar bonded stationary phase. Examples of supports are Partisil-10

PAC (Whatman), MicroPak-NH_2 (Varian), Bondapak Carbohydrate (Waters), and Lichrosorb-NH_2 (E. Merck). Acetonitrile-H_2O mixtures have been used as the mobile phase or less frequently due to decreased selectivity, methanol-H_2O mixtures. A second method involves cation-exchange columns prepared with calcium or other metal ions with water as the mobile phase. The mechanism of separation, although not well understood, probably is caused less by ion-exchange interactions than by size interactions. Sugars elute in order of decreasing molecular size.

For sugar analysis, a differential refractive index (RI) detector is preferred over the ultraviolet (UV) detector. Analysis with the RI detector is less sensitive and non-specific but interferences are minimal and quantitation is simplified. Both sensitivity and specificity can be increased with the use of the UV detector. However, at the common UV wavelengths derivatization is required. Detection at 192 nm, a region where sugar carbonyls absorb, has been reported but with little increase in sensitivity (Binder, 1980). Also, in this region impurities may cause appreciable absorption.

Sugar Content of Pears

Several workers have contributed data on the sugar concentrations of fresh pears. Reducing sugars were reported by Magness (1920) as 8% (fresh weight) for Bartlett pears and by Hulme (1958) as 8.00%, 7.71%, and 7.61% for Bartlett, Bosc, and d'Anjou pears, respectively. Kline *et al.*, (1970), comparing two different analytical methods, found reducing sugar concentrations of 7.0% using the Munson-Walker procedure and of 6.8% using the GLC method. For total sugar concentrations, Hulme (1958) listed the percentage fresh weight for five varieties: Bartlett,

Table 1. Contents of major sugars of whole, fresh pears.

	Sugar (g/100 g fruit)			
	Fructose	Glucose	Sucrose	Sorbitol
Bartlett[a]	4.32	0.71	5.60	---
Bartlett[d]	---	---	1.49	---
Bartlett[f]	7.88	1.00	1.28	---
Bartlett[g]	6.5	1.4	1.8	2.6
unripe Bartlett[i]	7.98	1.80	0.54	1.77
half-ripe Bartlett[i]	9.32	1.52	1.05	1.56
ripe Bartlett[i]	8.89	1.23	0.95	1.30
Bosc[a]	4.09	1.24	3.33	---
unripe Bosc[c]	5.26	1.60	4.15	2.90
ripe Bosc[c]	7.94	3.23	1.39	1.85
Bosc[d]	---	---	3.39	---
Bosc[f]	6.22	1.08	2.46	---
Bosc[g]	5.1	1.7	3.1	2.6
Clapp's Favorite[f]	6.20	0.76	1.10	---
Comice[a]	4.39	1.19	2.05	---
d'Anjou[d]	---	---	1.89	---
Empire dessert[b]	6.28	3.47	1.04	---
English culinary[b]	6.00	2.18	1.12	---
English dessert[b]	7.00	2.44	0.98	---
European varieties[c]	5.1	1.7	3.7	2.8
"fresh pears"[j]	4.57	3.58	0.45	---
Korean varieties[h]	5.4	1.6	2.9	2.5

a) Gerhardt et al., 1934, b) Widdowson et al., 1935, c) Martin, 1937, d) Hulme, 1958, e) Buchloch et al., 1969, f) Lee et al., 1970, g) Kline et al., 1970, H) Lee et al., 1972, i) Akhavan, 1977, j) Hurst et al., 1979.

9.27 and 8.33; Bosc, 11.09 and 10.10; d'Anjou, 9.72; "English Eating", 10.4; and "English Cooking", 9.3. At peak ripeness, Akhavan found that Bartlett pears contained 13.5% sugar (fresh weight). The percentages of each of the major sugars and of sorbitol have been reported by numerous workers. These are compiled in Table 2. In addition, Ash and Reynolds (1955) have found trace quantities of xylose, galactose, and mesoinositol and Mohler et al., (1971) have detected trace amounts of arabinose.

It has been known since 1988, when Kelhofer analyzed Siebenmannsbienen pears, that the sugar content increases with ripening. A few years later, Cruess and Stone (1916) found that fruit picked later in the season was higher in soluble solids. Magness (1920) found that the increase in total sugars was, during the early part of the season, a result of an increase in reducing sugars and, during the later part of the season, a result of an increase in sucrose.

Since the work of the early 1900s, numerous workers have studied the changes occurring during ripening. Martin (1937) confirmed earlier work with his finding that during ripening of early Bosc pears total sugars rose from 9.35% (fresh weight) to 11.15%. In a later picking, sugars increased to 12.68%. Tindale et al., (1938), Kidd et al., (1940) and Ulrich (1938) also have determined changes in sugars during ripening. In general, both sucrose, after an initial rise, and sorbitol concentrations declined. Fructose and glucose concentrations increased, fructose with a slight decline toward the end of ripening. In 1977, Akhavan analyzed Bartlett pear sugars using GLC and found that during ripening sucrose and fructose contents rose and then decreased. Sucrose

rose more rapidly than fructose. Concentrations of glucose and sorbitol decreased with ripening.

The changes in sugars during storage of pears also have been the subject of considerable research. Emmett (1929), in his investigation of chemical changes in Conference pears during storage, comprehensively reviewed work up to that date. In addition, he noted that in pears stored at 1°, 4°, and 5°C there were reductions in total sugars and increases in reducing sugars (based on fresh weight). Leonard et al., (1954) again reviewed progress in the area of changes in sugars during storage.

Relatively little information has been published on the sugars of pear juice. Akhavan (1977) compared the sugar content of Bosc and Comice pear juice before and after depectinization. Fructose increased from 5.60 to 5.91 g/100g juice, glucose increased from 1.59 to 1.79 g/100g, sucrose decreased from 1.65 to 1.52 g/100g, and sorbitol dropped insignificantly from 1.90 to 1.79 g/100g. The changes reflect inversion of sucrose to fructose and glucose. According to Akhavan (1977), the change in total sugar from 10.74 to 11.00 g/100g juice also was insignificant. Youtz (1980) noted similar increases in reducing sugar levels during depectinization of Bartlett pear juice. In heated juice, the effects were more evident; fructose increased from 6.22 to 6.89 g/100g juice, glucose increased from 1.66 to 1.90 g/100g, and sucrose decreased from 0.94 to 081 g/100g.

Chang (1979) investigated the acid precipitated colloid of ripe Bartlett pear juice. Analysis of precipitates in juices pressed from pears harvested over a three year period revealed the presence of three

sugars: fructose ranging from 3.4 to 5.1% (dry basis), galactose ranging from 2.2 to 2.9%, and glucose ranging from 0.9 to 1.5%. Kuhn (1981) determined the sugar contents of aerated d'Anjou pear juice after several decolorizing and clarifying treatments. Changes in sugars after the treatments were small: fructose varied from 5.8 to 6.1 g/100 ml juice, glucose varied from 1.5 to 1.6 g/100 ml, sorbitol varied from 2.5 to 2.6 g/100 ml, and sucrose varied from 0.25 to 0.32 g/100 ml.

Methods for Measuring Acids

Methods for the analysis of individual organic acids include separation by paper, thin layer, or ion exchange chromatography and detection either by titration or by quantitative colorimetric reaction. By 1967, the anion exchange titration method of Busch et al., (1952), modified by Hulme and Wooltorton (1957), was the preferred method for analysis of acids in a mixture (Kiksis and Prioreschi, 1967). Later, GLC separation of the TMS esters of acids became possible (Martin and Swinehart, 1968) and soon was widely accepted. The rapid advances in HPLC technology have only recently led to the preference of HPLC analysis of organic acids. Originally, the methodology called for separation on an anion exchange resin and detection with a differential refractometer (Palmer and List, 1973). Coppola et al., (1978) have developed a reverse phase HPLC method using a Bondapack/C_{18} column allowing the separation of acids at ambient temperature rather than at the elevated temperatures required by the ion exchange method.

Organic Acids in Pears

In pears, the acid concentration is relatively low with citric

and malic acids contributing most of the acidity. In early work, acid content was reported primarily as pH and titratable acidity (TA). Emmett (1929) calculated the acidity of juice made from Conference pears stored at different temperatures. For up to 27 days of storage, the acidity ranged from 0.053 to 0.086 g/100 ml for pears at 12°C and from 0.070 to 0.086 g/100 ml for pears at 1°C. Neither storage temperature nor time in storage correlated with acidity of the juice. Later studies, however, showed a decrease in acidity during storage (Chen, 1981).

Gerhardt and Ezell (1934) determined both the pH and TA (ml 0.1N NaOH/25 ml juice) of several pear juices:

	Bartlett	Bosc	Comice	d'Anjou
pH	4.12	4.65	4.38	4.19
TA	9.8	5.2	8.1	9/7

In 1964, Li and Hansen reported the concentrations of major acids (meq/100 g fruit) in d'Anjou and Bartlett pears:

	Malic Acid	Citric Acid	Quinic Acid
d'Anjou	4.90	0.13	0.08
Bartlett	2.40	2.71	0.14

In Bartlett pears, Fernandez-Flores et al., (1970) found 3.90 meq malic acid/100 g, 1.60 meq citric acid/100 g, and 0.20 meq quinic/100 g. Akhavan (1977) also using GLC, reported the variation in the levels of four major acids of a mixture of Boxc and Comice pear juice before and after depectinization and after concentration:

	Juice[a]	Depectinized Juice[a]	Conc.[b]
Phosphoric Acid	0.43	0.52	0.43
Malic Acid	1.67	1.61	1.50
Citric Acid	0.08	0.07	0.05
Quinic Acid	0.05	0.05	0.04
Total	2.23	2.35	2.02

a-meq acid/67.2 g juice, b=meq acid/10.0 g concentrate

In Switzerland, Blumenthal and Helbling (1977) analyzed eight varieties of pears. In the juice, the average total titratable acidity was 8.45 g/l (as malic acid). Average citric, isocitric, and malic acid concentrations were 0.075 g/l, 0.032 g/l, and 10.0 g/l respectively.

The major organic acids in pears are malate and citrate; however, a number of minor acids have been identified. In whole fruit, in pulp, or in juice acids identified include malic, citric, quinic, glycolic, shikimic, glyceric, mucic, succinic, lactic, galacturonic, citramalic, tartaric, and syringic (Hulme and Rhodes, 1971; Li and Hansen, 1964; Fernandez-Flores et al., 1970). The metabolic changes of the major acids and to some extent the minor acids taking place during storage and ripening have been reviewed by Hulme and Rhodes (1971) and Akhavan (1977).

Microorganisms in Pears

Fruit, because of its acidic nature, generally is host to a distinct group of organisms. The microbiology of pears and especially of pear juice has not been of very great interest although the microbiology of apples, also a pome fruit, has been investigated extensively. Sound apples were found to carry a relatively small load comprised almost

entirely of weakly fermentative, non-sporing yeasts (Forgacs, 1942). The following yeasts have been identified: Candida malicola, C. pulcherrima, C. krusei, Torulopsis spp., Rhodotorula spp., Crypotococcus spp., Sporobolomyces roseus, Debaryomyces kloeckera, Kloeckera apiculata, Saccharomyces bisporus, S. ovarum, and S. delbrueckii (Ayres et al., 1980).

Molds also have been shown to be principle contributors in the spoilage of apple juice. In sound fruit, mold counts varied from 10^3 to 10^5 per apple compared to yeast which averaged from 10^2 to 10^6 per apple (Ayres et al., 1980). The molds found most commonly in apple juice were members of the Penicilia, Aspergilli, and Mucorales (Fields, 1962). Penicillium expansum, the mold responsible for patulin, was the primary microorganism in 75% of the moldy fruit.

Forgacs (1942) has determined that of the relatively few bacteria in apples most were mesophilic and a very small number were either thermophilic or facultative thermophilic. Townsend (1939) has reported that butyric acid producing, spore forming anerobes were of importance in the spoilage of low-acid canned fruits.

Forgacs (1942) has shown that in the production of juices, the apple is not the major source of microorganisms. Although contamination occurs during processing, he as well as Marshall and Walkely (1951), found that filtration significantly reduced the microbial load. Filtration through diatomaceous earth brought a 99.9% reduction in the number of microorganisms. Berry and Diehl (1934) were among the first to study the effects of cold storage on the microbiology of juice. They noted that in apple juice (2,000,000 microorganism/ml) stored at -5°C or below, over 90% of the bacteria were destroyed in one month. With longer

storage, the microbial content was lowered even further. Forgacs (1942) found a reduction of 76% for apple juice frozen for 7 days and of 99.9% for juice held for 27 days.

In the production of juices, heat pasteurization is the most common method of preservation. The pasteurizing time is determined primarily by the microbial load and by the acidity of the juice (Fabian and Marshall, 1935). Fabian and Marshall (1935) found that in cider, the vegetative yeast were killed at a temperature of 65.5°C in 10 minutes, but that the most resistant mold spores required 79°C for 20 minutes and certain bacteria either 10°C for 30 minutes or 80°C for 15 minutes. Seitz germ-proof filtration has been shown to be an effective alternative to heat pasteurization (Carpenter et al., 1932). More recently, ultrafiltration was used to produce a sterile juice (Heatherbell et al., 1977).

EXPERIMENTAL

Source of Pear Juice

d'Anjou pears were taken out of controlled atmosphere storage in May, 1980, and ripened. As measured on a Baullauf Pressure Tester (5/16 inch diameter shaft), five bins from Diamond Fruit (Hood River, Oregon) were ripened to an average of 73.8 Newtons (N). A single bin from the Mid-Columbia Experiment Station (Hood River, Oregon) was ripened to an average of 43.7 N. The pears were washed, mixed, and ground in a Fitzpatrick hammermill at 1800 RPM with a 5/18 inch screen. One half of one percent ground paper press aid was added and the pulp was pressed in an hydraulic rack-and-cloth press for approximately 15 minutes at 1.45×10^3 kPa (210 psi). The juice was mixed with diatomaceous earth and filtered through a Shenk plate filter (grades DB and 532 paper, Scott Laboratories, San Raphael, Calif.) which had been precoated with diatomaceous earth. After filtration the juice was pumped into two 550 l water-jacketed kettles and blended. Samples were frozen immediately in 10 l batches and stored at -23°C until used. Individual batches were thawed in a steam kettle with gentle stirring to keep the juice at less than 35°C.

Ultrafiltration

Ten l batches of juice were ultrafiltered with a Romicon pilot-scale hollow fiber ultrafilter (model HFXS MXII) using PM-50, PM-30, and PM-10 cartridges. Molecular weight cutoffs of the three membranes were

50,000, 30,000 and 10,000, respectively. The ultrafilter was operated in the batch mode with the permeate stream collected and the concentrate stream recirculated back into the feedtank (Figure 4). Permeate flow in ml per minute was measured with a stopwatch and graduated cylinder. Figures were converted to flux using a membrane area of 0.1 m^2 per cartridge. Each batch was filtered at either 20°C or 50°C. Temperature was maintained by heating juice in a steam kettle or by cooling it in an ice bath.

Ultrafiltration cartridges were cleaned according to the following procedures: a solution of Terg-A-Zyme detergent (20 g per 10 l) was circulated for 15 minutes, the memberane was rinsed for 30 minutes with water, a 200 ppm sodium hypochlorite solution was circulated for 15 minutes, the membrane was rinsed with water for 30 minutes. The temperature of the cleaning colutions was 50°C. Solutions were maintained at an average transmembrane pressure of 157 kPa.

Methods of Analysis

pH

The pH of the pear juice was measured with a Corning model 125 pH meter at 20-24°C.

Titratable Acidity

Total titratable acidity was determined in duplicate according to the AOAC (1975) procedure. Results were expressed as ml 0.1 N NaOH per 100 ml juice.

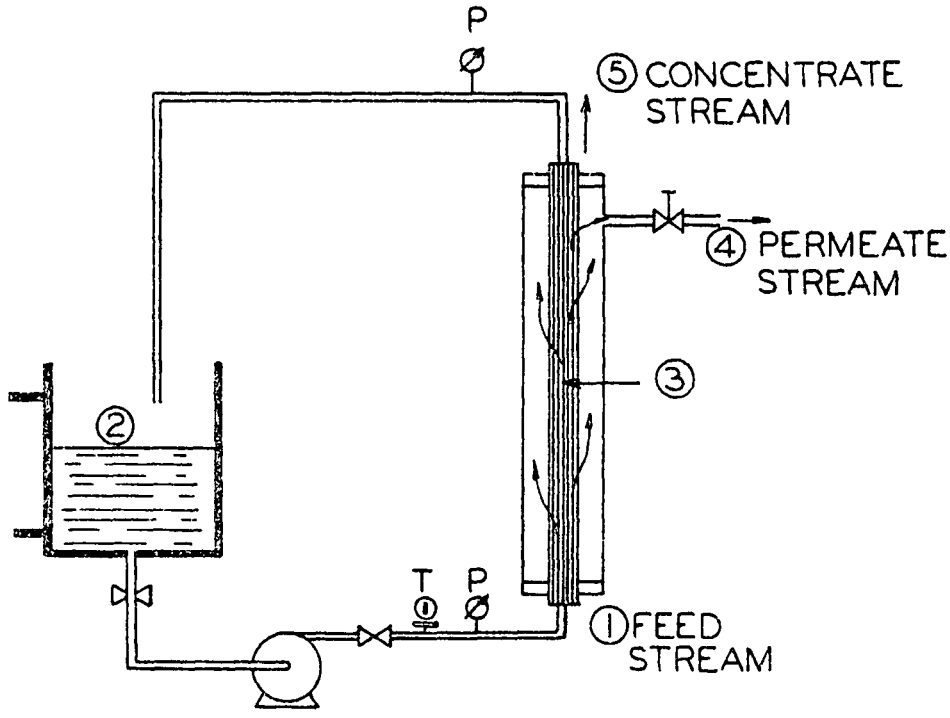


Figure 4. Ultrafiltration in the batch mode

Color and Clarity

The Hunter Color Difference Meter (model D-25, Hunter Associates Laboratory, Inc.) was used to measure lightness (L value), color (a and b values), and haze (%haze = $\frac{\text{CIE Y (position 1)}}{\text{CIE Y (position 3)}}$ X 100) of the juice. Measurements were made in the transmittance mode using a 1 cm glass cell.

Sugars and Acids

Juice was prepared for analysis according to the following procedure. Juice samples of 20 ml were mixed with 80 ml of 95% ethanol and held at 2°C for 1 hour. The precipitates, as well as some pigment, were removed by filtering the juice with suction through approximately 10 g PVPP (washed according to Loomis (1974)) and rinsing three times with 25 ml each of 80% ethanol. The filtrate was brought to volume in a 250 ml volumetric flask. After thorough mixing 100 ml were removed with a volumetric pipette for sugar analysis, leaving 150 ml for acid analysis. Both sugar and acid samples were evaporated on a Buchi rotary evaporator at 35°C and -3.3 kPa (29 inches Hg vacuum) to approximately 5 ml.

Samples for sugar determination were passed in series through 6 ml of cation exchange resin (Bio-Rad AG 50W-X4, 200-400 mesh, in the hydrogen form) and 6 ml of anion exchange resin (Bio-Rad AG 1-X8, 200-400 mesh, in the acetate form) and rinsed with deionized water into a 50 ml volumetric flask containing 5 ml of 10% D-mannitol and 1 ml of 0.5% $CaCl_2$. For analysis of acids the samples were passed through 6 ml of the cation exchange resin, rinsed with deionized water, and collected in a 100 ml volumetric flask. Both sugar and acid samples were filtered through a 0.45 µm Millipore filter and held at -10°C until analysis.

Sugar and acid analysis was performed with the aid of a Varian model 5000 high performance liquid chromatograph. Sugars were separated on a 7.8 X 300 mm Aminex HPX-87 carbohydrate column (Bio-Rad Laboratories) with column temperature of 85°C and a mobile phase of 0.01% $CaCl_2 \cdot 2H_2O$ in degassed, deionized water (filtered through a 0.45 µm Millipore filter). Column flow rate was 1 ml per minute and pressure 68 atmospheres. Injection volume was 10 µl (Glenco micro-syringe, 10 µl capacity). Peak areas from the Varian refractive index detector were recorded with a Hewlett Packard model 3380A recording integrator and integrated by hand as one half the height times the width. Sugar concentrations were calculated as:

$$\text{Sugar (g/100 ml)} = \frac{A_s}{A_{is}} \times \frac{W_{is}}{K} \times \frac{DF}{\%R} \times 5$$

where A_s is the peak area of the sample, A_{is} the peak area of the mannitol internal standard, W_{is} is the weight of the internal standard, K the detector response factor, DF the dilution factor, and %R the percent recovery. Three to five replicates of each sample were injected and the results averaged.

Acids were separated on a 7.8 X 300 mm Aminex HPX-87 organic acid column (Bio-Rad Laboratories) with column temperature at 65°C, mobile phase of 0.0123 N H_2SO_4 in deionized, filtered water, flow rate at 0.8 ml per minute, and pressure at 66 atmospheres. The detector, a Varian liquid chromatography variable wavelength detector (model UV-50), was set at 210 nm. Injection volume was 20 µl (Glenco micro-syringe, 50 µl capacity). Peak areas were recorded and integrated with a Hewlett Packard model 3380A recording integrator. An external standard was used

to quantitate malic acid. The standard curve was calculated using linear regression of peak area vs. concentration. Concentration was corrected for percent recovery and dilution effects as with the sugars. In addition, conversion factors were applied so that final results could be expressed as a meq/100 ml. Results of two to three replicates were averaged.

Total Phenolics

Total phenolics were determined according to the micro-method of Singleton and Rossi (1965) using Folin-Ciocalteau reagent obtained from Fisher Scientific Co. Samples were read on a Perkin-Elmer model 550 spectrophotometer with 1 cm cells. Standard curves were prepared from twice crystallized gallic acid (Mallinckrodt).

Alpha-Amino Nitrogen

Alpha-amino nitrogen was determined by mixing 1 ml juice diluted 1:100 with distilled water and 1 ml ninhydrin reagent (Sigma Chemical Co.) in a sealed screw-capped test tube and mixing gently. The tube was heated for 15 minutes in a boiling water bath which had been covered with aluminum foil to exclude light. The mixture was cooled to room temperature, 5 ml distilled water were added and this was mixed thoroughly on a Buchler Evapo-Mix Shaker. Absorbance was read at 570 nm on a Perkin-Elmer model 550 spectrophotometer.

Standard curves were prepared with glycine. Glycine was dissolved in amino acid-free pear juice prepared by running a 50 ml juice sample through approximately 20 ml of cation exchange resin (Bio-Rad AG 50W-X4, 200-400 mesh, hydrogen form) and collecting the middle fraction.

Pectic Substances

Pectic substances were extracted initially according to the method described by Rouse and Atkins (1955). For routine analysis the method was simplified by combining Rouse and Atkins' procedure with that of Owens et al., (1952).

An aliquot of juice, the amount depending on pectin concentration, was weighed into a 50 ml graduated centrifuge tube. Hot (75°C) 95% ethanol was added to a volume of 40 ml and the mixture heated for 10 minutes in a water bath at 85°C with occasional stirring. The glass rod used for stirring was rinsed with 95% ethanol and the contents of the tube made to a final volume of 50 ml. The tube was centrifuged at 1725 X G for 15 minutes and the supernatent decanted and discarded. The leaching procedure was repeated twice more and after the final decanting the precipitate was transfered with distilled water to a 100 ml volumetric flask. Five ml of 1 N NaOH were added and the contents of the flask brought to volume with distilled water. The extract was allowed to stand for 15 minutes and the pectic substances were determined according to the modified carbazole method of Galambos (1967).

A standard curve was prepared using galacturonic acid monohydrate (Sigma Chemical Co.) dried over P_2O_5 for a minimum of 24 hours. The galacturonic acid standards were prepared as suggested by Rouse and Atkins (1955).

Microbial Analysis

Juice for microbial analysis was collected in sterilized 50 ml volumetric flasks. Samples were taken from the unfiltered juice prior to filtration, from the permeate stream of the ultrafilter at intervals

during filtration, and from the retentate when filtration was completed. Juice was plated in quadruplicate onto Orange Serum Agar. After incubation for 48 hours at 30°C, the plates were counted.

RESULTS AND DISCUSSION

Effect of Time of Ultrafiltration on the Composition of Pear Juice

In the batch mode changes in retention characteristics with time are best measured in terms of volume concentration ratio (VCR). As permeate was drawn out of the system over the period of filtration the VCR, defined as initial juice volume divided by retentate volume, increased (Table 2).

It was expected that changes in the retention characteristics of the membrane with time would result in changes in permeate composition. Theoretically, as VCR increases the effects of concentration polarization become significant. Not only does permeate flux drop, the efficiency with which a particular solute is retained also is subject to changes. If the characteristics of the solute (size, shape, and degree of hydration) and of the membrane are such that the membrane is essentially impermeable to the solute, the formation of a gel layer will not affect retention. However, if the solute is only partially retained, concentration polarization in the case of a viscous but relatively fluid gel layer will cause a decrease in retention. On the other hand, in the case of a strong, coherent gel layer, the efficienty with which more permeable solutes are retained will increase (Blatt et al., 1970).

Mathematically (Blatt et al., 1979), retentivity of the membrane for a specific macromolecular solute is expressed as:

$$\sigma = 1 - \frac{C_f}{C_b}$$

TABLE 2.--Effect of filtration time(VCR) on the color and haze of ultrafiltered pear juice[a]

Time[b]	VCR[c]	Hunter haze	Hunter L	ΔE
3.5	1.02	1.5	89.9	0
12.0	1.08	1.4	89.1	1.3
20.5	1.14	1.3	88.8	2.6
30.5	1.21	1.5	88.6	3.1
41.5	1.29	1.4	88.1	3.8
55.0	1.38	1.3	87.8	5.2
71.0	1.48	1.6	87.3	5.6
92.0	1.60	1.5	86.6	6.8
125.0	1.74	1.4	86.4	7.8
191.0	1.90	1.5	86.1	8.4
	Final Retentate	23.8	62.2	33.4
	Juice	14.2	69.5	25.9

[a](ΔP=153 kPa, PM-50 membrane, 50°C)

[b]minutes

[c]Volume Concentration Ratio

where: σ = rejection coefficient of the membrane (fraction of solute in the upstream solution which is retained by the membrane)

C_f = solute concentration in the ultrafiltrate

C_b = concentration of solute in the solution upstream of the membrane

In the case of a solute which is small enough to pass through some pores but too large for others, the rejection coefficient is given as:

$$\sigma = 1 - \frac{(1-a)\ C_b}{C_b}$$

where a is the fraction of the total solvent flux passing through the solute rejecting pores and 1-a is the fraction carrying solute through the membrane. As the gel layer builds and the concentration of solute at the surface of the membrane increases the rejection coefficient becomes:

$$\sigma = 1 - \frac{(1-a)\ C_{pore}}{C_b}$$

As the concentration of solute carried through the membrane, C_{pore}, increases, the rejection coefficient and also the retention efficiency of the membrane decrease.

It is the view of Blatt et al., (1970) that with colloidal solutes the mechanism of concentration polarization is quite different from that of macrosolutes. The gel layer has been found to accumulate rapidly, build to a relatively large thickness (depending on fluid velocity and channel height), and to flow, either in part or as a whole, laterally over the membrane surface by fluid shear. The theory of colloid ultrafiltration has not been studied extensively; however, it is probable that solute rejection would best fit the case of a viscous but fluid

gel layer where solute mobility was high enough to cause a decrease in rejection of the solute.

The relationship between flux and VCR is logarithmic and can be described (Breslau and Kilcullen, 1977) as:

$$J = k \ln(VCR)$$

In the initial study of Kortekaas (1980), the ultrafiltration of Bartlett pear juice was found to fit this equation. In the present work the flux of d'Anjou pear juice also decreased as the logarithm of VCR (Table 2); however, the slope was steeper and the initial flux of the PM-50 membrane was lower. Since the PM-50 membrane had been used extensively in previous tests, it was likely that loss of efficiency may have been caused by irreversible fouling or membrane decay. Dejmek (1975) noted that cleaning did not always restore a membrane to the original permeability and, even in cases where full flux was restored, the cleaned membrane sometimes experienced a faster flux decline than that of a new membrane.

To study the effect of VCR on the composition of pear juice, the juice was ultrafiltered under the conditions shown by Kortekaas (1980) to give maximum permeate flux. Pear juice was filtered through a PM-50 membrane at 50°C with a transmembrane pressure of 157 kPa.

Clarity and Color

The average haze value of the permeates was 1.4 (Table 2); visually all the permeates were "sparkling clear". A Hunter haze value of 2 or less has been found to correspond to pear juice of sufficient clarity that no light scattering can be observed when a high intensity beam is passed through a cylinder of juice 2.5 cm in diameter (Beavers et al.,

1981). The high haze values for the juice (14.2) and for the retentate (23.8) indicate that the colloidal particles responsible for cloudiness were being retained by the membrane. Permeate haze values did not change as the VCR increased, indicating that concentration polarization of the membrane did not effect retention of the colloidal particles.

Color was expressed as Hunter L, "a", and "b" values. L values decreased with time (Table 2), resulting in slightly darker, more pigmented permeates. The decrease, however, was slight and at a VCR of 1.9 the L value of 86.1 did not approach that of the juice (69.5). The retentate, with an L value of 62.2, was darker than the juice.

In Table 2 the overall color change (ΔE) is shown with increasing VCR. The increase in ΔE was due primarily to changes in the Hunter "b" value of the permeate. The "a" value remained constant an an average of -3.9 and, while the L value did decrease, the changes were relatively small. The relatively large changes in "b" value are shown in Figure 5. Generally, the "b" value of a product is an indication of yellowness; visually, the increasing golden color of the permeates supported the instrumental results. The relationship between "b" and VCR was approximately linear (Figure 5).

Refractive Index and Sugar Concentration

HPLC detector response factors were calculated as 1.03, 1.04, and 1.07 for glucose, fructose, and sorbitol, respectively. Percent recoveries were 99.8% for glucose, 97.1% for fructose, and 98.3% for sorbitol. The data in Table 3 show that no significant difference exists between either individual sugar concentrations or concentrations of total

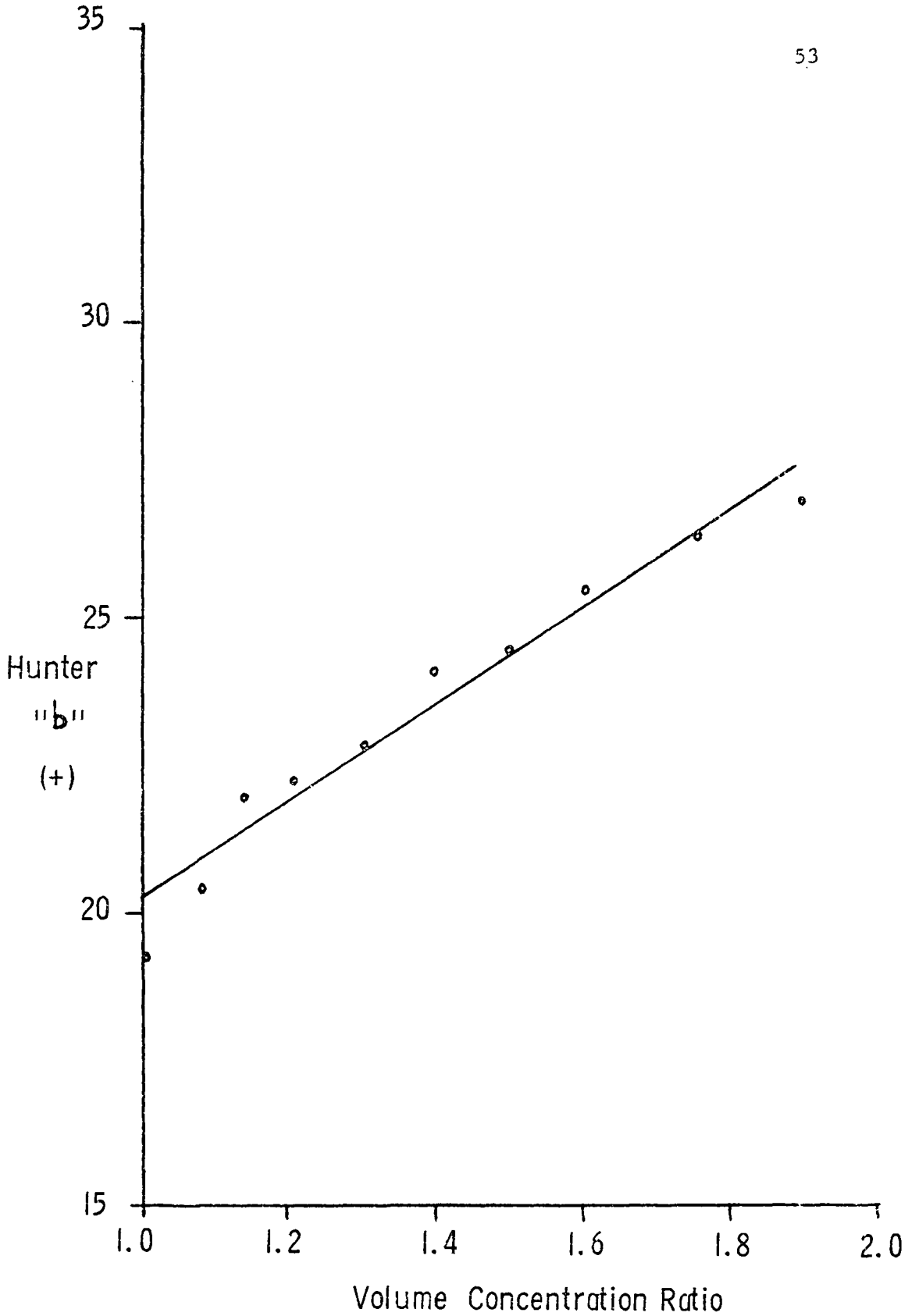


Figure 5. Ultrafiltration of pear juice: Hunter "b" value vs. volume concentration ratio

TABLE 3.--Effect of filtration time (VCR) on the sugar concentrations of ultrafiltered pear juice[a]

Time[b]	VCR[c]	Refractive Index	Glucose[d]	SD[e]	Fructose[d]	SD[e]	Sorbitol[d]	SD[e]	Total[f]
3.5	1.02	1.350	1.89	.16	7.19	.18	2.92	.35	12.00
12.0	1.08	1.350	2.02	.09	7.01	.24	2.73	.08	11.76
20.5	1.14	1.350	1.75	.23	6.29	.82	1.76	.02	9.80
30.5	1.21	1.350	2.09	.31	7.41	.80	2.60	.35	12.10
41.5	1.29	1.350	2.05	.46	6.66	.77	2.67	.21	11.38
55.0	1.38	1.350	1.91	.30	6.96	.63	2.80	.16	11.67
71.0	1.48	1.350	2.26	.31	7.20	.08	1.98	.35	11.44
92.0	1.60	1.350	2.07	.19	6.83	.29	2.07	.19	10.97
125.0	1.74	1.350	2.28	.08	7.02	.13	2.70	.13	12.00
191.0	1.90	1.350	1.96	.28	6.98	.25	2.58	.13	11.52
	Final Retentate	1.352	1.93	.09	6.25	.28	2.63	.23	10.81
	Juice	1.350	2.10	.12	7.32	.59	2.20	.12	11.62

[a] (ΔP=1053 kPa, PM-50 membrane, 50°C)

[b] minutes

[c] volume contration ratio

[d] g/100 ml juice

[e] standard deviation

[f] excluding sucrose

sugars of the juice, the retentate, or the permeates. The membrane, therefore, was assumed to be totally permeable to free sugars. High variation in the results is evidence of degradation of the HPLC cation exchange column. Although the loss of column resolution made accurate calculation of sucrose impossible, the sucrose content could be estimated as 0.25% for the juice, the retentate, and all the permeates.

Soluble solids, measured as refractive index (Table 3) did not change with time but remained at 1.350, the refractive index of the unfiltered juice. This constancy reflected the results of the sugar analysis. The increase in refractive index of the retentate (1.352) does not appear as an increase in sugar content and, therefore, must be attributed to other compounds. The almost total retention of pectic substances by the membrane (Table 5) in comparison to the high permeability of most other compounds make pectic substances a probable cause for the increase in refractive index.

Titratable Acidity, pH, and Acid Concentration

Malic, citric, phosphoric, and quinic acids were detected in the pear juice; however, only malic acid was measured. Quinic acid was found in a very small quantity which, because of its close proximity to the malic acid peak, was difficult to integrate accurately. Phosphoric acid does not produce a separate chromatographic peak with the system used. It can be detected as an increase in the general acid-solvent peak but again, it would be impossible to measure with accuracy. Citric acid was found in all samples in concentrations of approximately 0.1 meq/100 ml juice. At such low concentrations error of measurement was too great to have established differences between samples. Akhavan (1977)

reported that of the total acid concentration of pear juice, 75% was contributed by malic acid; this acid, therefore, was used as a measure of the permeability of the membrane to pear juice acids.

Malic acid concentration was related to peak area through an external standard. The correlation coefficient was 1.00. Percent recovery of the malic acid was 98.4%. Malic acid concentrations of the permeates, retentate and juice are shown in Table 4. No differences were found at the 95% significance level. Average malic acid concentration was 9.06 meq/100 ml of ultrafiltered pear juice.

Table 4 also shows that pH remained constant throughout the period of filtration. While pH measures hydrogen ion concentration alone, titrable acidity takes into account the buffering action of acids in their salt form. While changes in titratable acidity (Table 4) are relatively small and statistically insignificant, it is possible that if a higher VCR could have been achieved changes in titratable acidity might have been significant. Titratable acidity of the permeate increased proportionally to VCR. The highest titratable acidity was that of the retentate.

Alpha-Amino Nitrogen Concentration

The α-amino nitrogen content of the samples was measured in terms of glycine with the standard curve having a regression coefficient of 0.999. As shown in Table 5, the concentration of α-amino compounds in the permeate did not change with time and was equal to that of the retentate. The juice, however, did show a slightly higher level of α-amino compounds. The difference was just significant at the 95% level. A general drop in α-amino nitrogen of both permeate and retentate could be

TABLE 4.--Effect of filtration time (VCR) on the pH, titratable acidity, and malic acid concentration of ultrafiltered pear juice[a]

Time[b]	VCR[c]	pH	Titratable[d] Acidity	Malic Acid[e]	SD[f]
3.5	1.02	4.42	30.2	9.05	0
12.0	1.08	4.42	30.5	9.35	.01
20.5	1.14	4.42	30.8	8.42	.02
30.5	1.21	4.41	30.5	8.38	.01
41.5	1.29	4.42	30.7	9.09	.01
55.0	1.38	4.42	31.0	9.76	.10
71.0	1.48	4.42	31.1	8.55	.04
92.0	1.60	4.42	31.2	9.00	.02
125.0	1.74	4.42	31.3	9.58	.03
191.0	1.90	4.42	31.3	9.37	.01
	Final Retentate	4.41	32.0	8.88	0
	Juice	4.41	31.2	9.34	.08

[a](ΔP-153 kPA, PM-50 membrane, 50°C)

[b]minutes

[c]Volume Concentration Ratio

[d]0.1 N NaOH/100 ml juice

[e]meq/100 ml juice

[f]0.1 N NaOH/100 ml juice

TABLE 5.--Effect of filtration time on the concentrations of pectic substances, phenolics, and α-amino nitrogen in pear juice ultrafiltrate[a]

Time[b]	VCR[g]	Pectin[c]	SD[d]	Total Phenolics[e]	SD[d]	α-amino N[f]	SD[d]
3.5	1.02	<.1	-	256.5	3.5	10.2	.4
12.0	1.08	<.1	-	259.2	.4	9.9	.2
20.5	1.14	<.1	-	259.2	.4	10.2	.3
30.5	1.21	<.1	-	257.2	4.6	10.2	.1
41.5	1.29	.1	.1	261.0	0	10.4	.1
55.0	1.38	.1	.1	261.2	.4	10.0	.1
71.0	1.48	.1	.1	261.2	.4	10.0	.1
92.0	1.60	.1	.1	262.5	.7	10.2	.2
125.0	1.74	.2	.1	262.2	.4	10.1	.3
191.0	1.90	.2	.09	263.5	0	10.1	.3
	Final Retentate	5.0	1.0	278.5	0	10.0	.3
	Juice	2.9	.87	267.3	.4	10.6	.3

[a] (ΔP=153 kPa, PM-50 membrane, 50°C)

[b] minutes

[c] as mg/ml anhydrogalacturonic Acid

[d] standard deviation

[e] as μg/ml gallic acid

[f] mM glycine

[g] Volume Concentration Ratio

explained as the result of Maillard reactions. Heatherbell et al., (1977) attributed an increase in the amber color of pasteurized apple juice permeate to Maillard browning. However, the apple juice showing the increased browning had been ultrafiltered to a very high VCR (11.9). In this case the reduction in water activity of the retentate would be significantly greater than what was obtained in the present study. Since pear juice remained relatively dilute, it is less likely that Maillard browning would have been observed in this study.

Concentration of Pectic Substances

As shown in Table 5 most pectic substances were retained by the membrane. Even at a VCR of 1.9, when concentration polarization severely limited flux, only 4% of the pectin in the feed stream passed through the membrane. Early in the run, while flux was high, the amount of pectin detected in the ultrafiltrate was negligible. Retention was essentially total.

The high retention of pectic substances stands in sharp contrast to the permeability of the membrane to most other juice components. Because of this retention the pectic substances were deemed primarily responsible for gradual build up of the concentration polarization layer with time and for the resulting decrease in flux. In their studies on concentration of orange juice by reverse osmosis, Watanabe et al., (1978) also reported that pectin was the main fouling component. In later work Watanabe et al., (1979) used a model system with purified "solutions" of pectin and cellulose to show that reduction of permeate flux was directly proportional to the concentration of pectin deposited on the membrane. In orange juice, as in pear juice, the presence of sugars, acids, and

polyvalent cations was expected to aid in the formation of a pectin gel rather than a typical macromolecular deposit. The effect on flux would be complicated by this additional factor.

The pectic substances of two ultrafiltrates (VCR 1.1 and VCR 1.8) have been fractionated according to solubility and the pectin in each fraction quantitated (Table 5). Although separation is not very definitive, it is generally accepted that those pectic substances soluble in water are primarily high methoxyl pectins; those soluble in ammonium oxalate are salts of pectic acids and low methoxyl pectinic acids, and those soluble in sodium hydroxide are protopectin (Rouse and Atkins, 1955). The observed overall increase in pectic substances in the ultrafiltrate with time (Table 5) was shown (Table 6) to be due to an increase in the protopectin fraction; water soluble and ammonium oxalate soluble fractions shows no change. The explanation for this leaves two possibilities to be considered: either the membrane was selectively permeable to the protopectin fraction or compaction of the gel layer with increasing concentration polarization was causing a loss of solubility of the pectin.

The nature of the pectin gel might, itself, indicate that the latter phenomenon was occurring. O'Beirne (1980) has suggested that in apples both the cementing action and the insolubility of the middle lamella may be explained by a special type of gelation, described by Rees (1969), where the gel is compacted and evacuated of solvent. Doesburg (1965) has summarized the possible causes for the insolubility of protopectin as: 1) covalent bonding of pectic substances to other cell wall constituents by secondary bonds, 2) the presence of cations with

Table 6. Effect of time (VCR)[a] on the pectic compounds in pear juice ultrafiltrate[b].

VCR	water extract	ammonium oxalate extract	sodium hydroxide extract
1.1	17 µg/ml	16 µg/ml	15 µg/ml
1.8	17 µg/ml	15 µg/ml	22 µg/ml

a. volume concentration ratio, b. P=138 kPa, PM-50 membrane, 50°C.

the resulting loss in solubility of low methoxyl pectic substances and with reduction of swelling of high methoxyl pectins, and 3) mechanical enmeshing of filamentous macromolecules of pectic substances with each other and with other polymers. The work of Pilnik (reported in a note to Joslyn, 1962) supports the role of mechanical enmeshing in the solubility of pectic substances. The pectin content of juice expressed from acid-treated apple pomace was measured at intervals during the pressing. "Free run" juice had a high protein content while juice collected at the end of the pressing, when pressure was highest, contained little pectin. Watanabe et al., (1979) also found that under conditions of high permeation flux, when pressure was high, the typically viscous pectin gel was converted to a pectin film which was insoluble in water. A similar situation may be occurring during ultrafiltration of pear juice. The expected drop in efficiency of retention with increasing concentration polarization would result in leakage of the compressed pectic material into the permeate.

From another perspective the pectin concentration can be related in terms of its effect on viscosity to changes in permeate flux. Kortekaas (1980) has shown that the viscosity of Bartlett pear juice increased exponentially with increasing VCR (Figure 6). The concentration of pectic substances in d'Anjou pear juice likewise increased with VCR (Table 5). The difference in the pear juices used in the two studies precludes the possibility of directly relating viscosity and pectic content. However, it is well known that in juices the overall pectin content as well as the degree of esterification contribute to the viscosity of the juice (McCready and McComb, 1954). The fact that pectin is

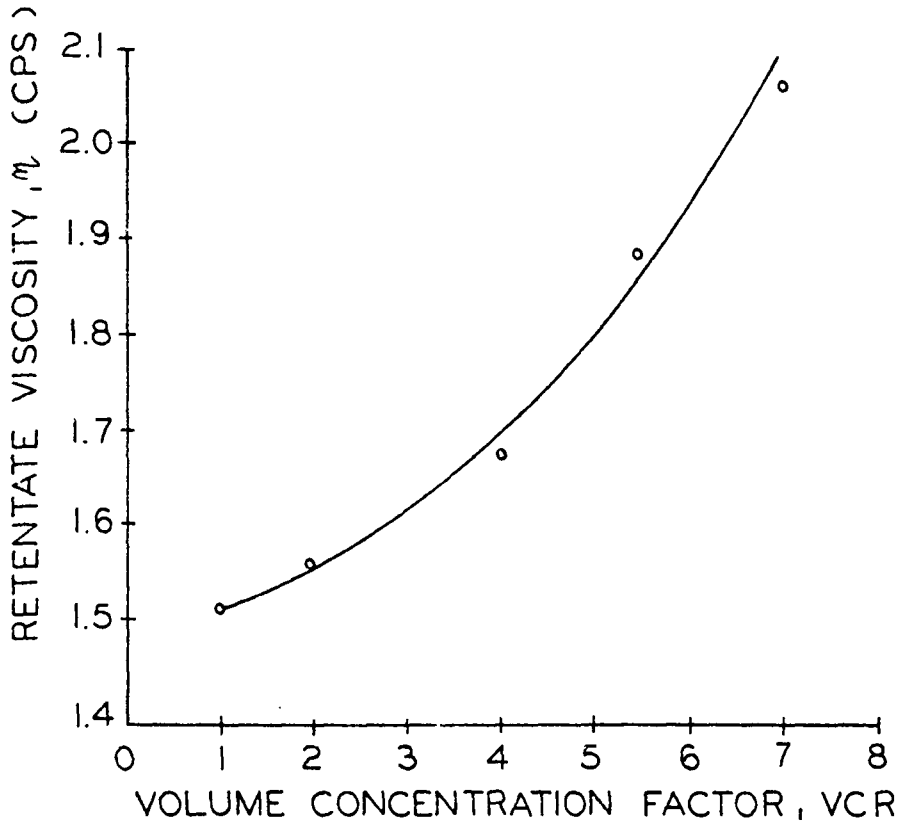


Figure 6. Relationship between viscosity and volume concentration ratio

retained by the membrane to a far greater extent than other compounds also is evidence that the pectic substances are primarily responsible for increases in viscosity with time.

The equation, $J = k \log \frac{C_g}{C}$, which was discussed earlier, illustrates the effect of viscosity on permeate flux. The mass transfer coefficient, k, can be expressed (Bellucci et al., 1975) as: $k = \frac{D_s}{\lambda}$, where D_s is the solute diffusivity coefficient and λ is the thickness of the gel layer. The viscosity increases in pear juice result in a decrease in the diffusivity coefficient which then is reflected as a decrease in the mass transfer coefficient. The above equation shows that decreases in the mass transfer coefficient are directly proportional to decreases in permeate flux.

Total Phenolic Concentration

The total phenolic concentration of the permeate increased with increasing VCR (Table 5). The retentate contained 32 µg/ml more phenolic compounds than the initial permeate. However, at a VCR of 1.9 the total phenolic concentration of the permeate (263.5 µg/ml) approached that of the original juice (267.3 µg/ml). The higher concentration of total phenolics in the retentate (278.5 µg/ml) shows that phenolic compounds were retained to a slight degree. The decrease in the efficiency of retention probably was a result of concentration polarization.

It was expected that to some extent polyphenoloxidase browning would occur during ultrafiltration of the juice. The constant recirculation of retentate offered conditions especially favorable for oxidation of phenolics; oxygen was present, temperatures were fairly warm, the pH of the pear juice was relatively high and remained so, and presumably the

enzyme was retained by the membrane and, therefore, increased in concentration. In spite of these factors, there is little evidence that polyphenoloxidase browning was taking place.

The extent of polyphenoloxidase oxidation during ultrafiltration of the pear juice probably is fairly limited. Other workers have shown that most of the easily oxidizable phenolics (o-dihydroxyphenols) have been oxidized prior to filtration. Skorikova and Lyashenko (1970) have found that pears lost much of their phenolic content during pressing. The leucoanthocyanidin concentration was reduced by 50% during the first 5 minutes. Chlorogenic acid and some flavonols also were oxidized. Heating was found to cause additional losses. Kuhn (1981) worked with d'Anjou pear juice from the same pressing as in the present studey. She found that even extreme aeration of the juice did not reduce or change the concentration of phenolics. This was attributed to extensive oxidation which had occurred during the juice processing. If significant oxidation could not be induced by forced aeration of the juice, it is reasonable that the effects of ultrafiltration would be even less significant.

If the role of polyphenoloxidase browning is assumed to be minor, the changes in color and lightness of the permeate with increasing VCR must be explained. The simplest explanation is that increases in color and decreases in lightness represent an increase in the actual pigment concentration. Polymerized o-dihydroxyphenols, such as caffeic acids, chlorogenic acids, catechins, and leucoanthocyanins, may be contributing to the measured Hunter values although oxidized phenolics would be expected to contribute less to the measured total phenolic concentration

than the monomeric phenolics (Singleton and Rossi, 1965). Other flavonoids, flavones and flavonols especially, are known for the yellow color of the pigment itself; recently, quercetins have been isolated from several varieties of pear (Sioud et al., 1966; Ranadive et al., 1971). The relationship between the measured phenolic content and the total color difference (ΔE) of the ultrafiltrates, juice, and retentate is shown in Figure 7. The two measurements are directly proportional with a correlation coefficient of 0.944. Total phenolic concentration also can be shown to be proportional to Hunter "b" value and inversely proportional to Hunter L value. Correlation coefficients are 0.88 for Hunter "b" and 0.93 for Hunter L.

Effect of Temperature on the Composition of Ultrafiltered Pear Juice

Pear juice was ultrafiltered through a PM-50 membrane at an average transmembrane pressure of 153 kPa. Three batches were run at temperatures of 15°C, 30°C, and 50°C with samples taken from each at a VCR of 1.14. The effect of temperature on permeate flux and on the viscosity of the juice has been reported by Kortekaas (1980) and will not be discussed here. The pectin concentration of the juice (Table 5) probably was sufficient to account for the changes in viscosity.

A comparison of the composition of permeates from each temperature is presented in Table 7. Titrable acidity, pH, and malic acid concentration did not change with temperature. The difference between the malic acid concentrations at 15° and 50°C is significant at the 95% level. Since 8.42 meq/100 ml juice is lower than the acid concentrations of other permeates filtered at the same temperature (Table 4), the appar-

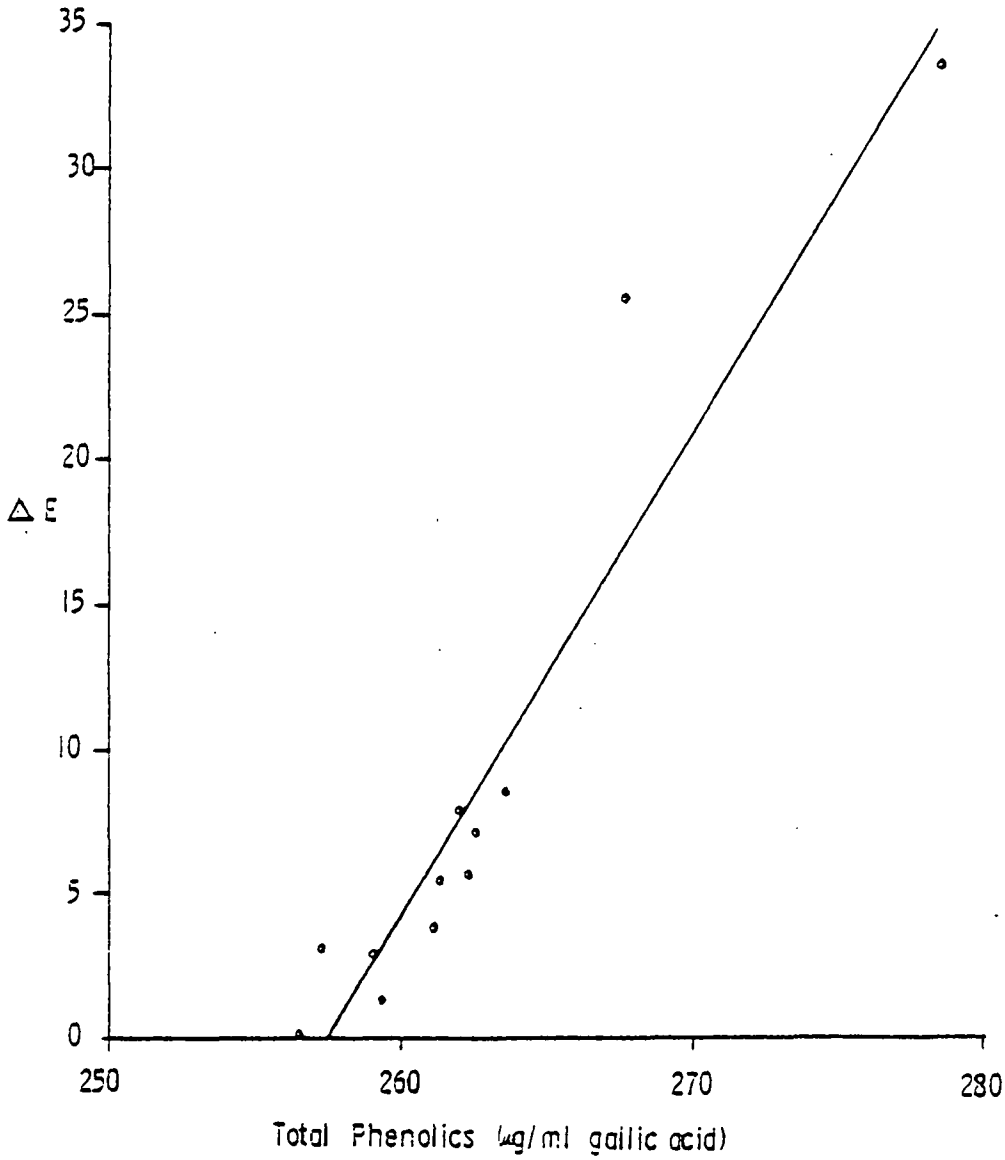


Figure 7. Pear juice ultrafiltrate: ΔE vs. total phenolics

TABLE 7.--Composition of pear juice permeates ultrafiltered at different temperatures[a]

	15°C	30°C	50°C
pH	4.41	4.42	4.42
Titratable Acidity[b]	30.0	30.3	30.8
Malic Acid[c]	9.84	9.22	8.42
Refractive Index	1.350	1.350	1.350
Glucose[d]	2.62	2.63	1.75
Fructose[d]	6.97	8.40	6.29
Sorbitol[d]	2.94	2.08	1.76
Total Sugar[d]	12.53	13.11	9.80
Phenolics[e]	259.8	258.5	259.2
Pectin[f]	<.1	<.1	<.1
α-amino N[g]	10.0	10.3	10.2
Hunter haze	1.8	1.6	1.3
Hunter L	98.3	97.9	88.8
Hunter a	-4.0	-4.7	-4.0
Hunter b	+20.6	+25.3	+21.8

[a] (ΔP=153 kPA, PM-50 membrane, VCR=1.14)

[b] ml 0.1 N NaOH/100 ml juice

[c] mq/100 ml juice

[d] g/100 ml juice

[e] mq/ml gallic acid

[f] mg/ml anhydrogalacturonic Acid

[g] mM glycine

apparent differences with temperature probably were not significant.

Refractive indices and sugar concentrations, as shown by glucose, fructose, and sorbitol concentrations and by total sugar concentrations, also were similar. The permeate at 50°C again showed a somewhat lower sugar concentration. This also is within experimental error, especially in light of permeates taken at VCRs directly before and after the VCR of 1.14 used for the comparison (Table 3). Essentially all pectic substances were retained at each of the temperatures. The concentrations of α-amino compounds and total phenolics also remained constant at all temperatures. Accordingly, no changes were noted in Hunter L, "a", "b" values. This appears to contrast with the results of Heatherbell et al., (1977) which showed an increase in color at high (50°C) temperatures. However, the higher color was found only at a high VCR (greater than 11.9). Such high VCRs were not achieved in the present study. All permeates were "sparkling clear" as shown by Hunter haze values of less than two.

Effect of Transmembrane Pressure on the Composition of Ultrafiltered Pear Juice

Juice was ultrafiltered at four transmembrane pressures, all with a maximum inlet pressure of 172 kPa. Most transmembrane pressures involving lower inlet pressures did not give sufficient flux to make ultrafiltration practical. The membrane used was a PM-50 and temperature was maintained at 50°C. Samples were taken at a VCR of 1.14.

Theoretically, higher transmembrane pressures could be expected to increase the rate of formation of the gel layer and, therefore, to bring an earlier reduction in retention efficiency. However, the effects of

pressure on permeate composition (Table 8) do not support this. No significant differences were observed in the composition (sugar, acids, phenolics, pectic substances, α-amino compounds) or in the Hunter readings of each of the permeates. This agrees with the findings of Peri and Setti (1976) that variation in operating pressure has very little effect on the retention of whey proteins.

Effect of Membrane Pore Size on the Composition of Ultrafiltered Pear Juice

Pear juice at 50°C was ultrafiltered at 153 kPa through PM-50, PM-30, and PM-10 membranes (molecular weight cutoffs of 50,000, 30,000, and 10,000, respectively). Samples from each batch were collected at a VCR of 1.14.

Compositions of the permeates from each membrane are reported in Table 9. Concentrations of sugars, acids, phenolics, pectic substances, and α-amino compounds showed no change. Hunter haze, L. "a", and "b" values also were constant. It appears that the PM-10 membrane is as permeable to low molecular weight compounds such as sugars, acids, α-amino compounds, and some phenolics as is the PM-50 membrane. Likewise, high molecular weight phenolics and pectic substances were equally retained by both membranes. The effects of time (VCR) might have revealed a difference; however, data were not sufficient to determine this.

Microbial Analysis of Ultrafiltered Pear Juice

Samples for microbial analysis were taken aseptically from the unfiltered juice, from the permeate at three different VCRs, and from the retentate. Dilutions were not required and 0.1 ml aliquots were plated

directly. The results, reported in Table 10, were an average of at least four plate counts.

Results of the plate counts showed that the permeates at all stages of filtration were essentially free of microorganisms. Retention was as complete at 15°C as at 50°C. The fact that the retentate colony count was higher at 15°C than at 50°C demonstrates the effectiveness of heat in reducing the microbial load. Not only were the ultrafiltrates at 50°C free of microorganisms but the retentates at the temperature were as well. A more dramatic reduction in plate count was found by Heatherbell et al., (1977) when apple juice was ultrafiltered at 45°C instead of at 22°C. The microbial load of the unfiltered juice in their study was much higher than what was found in the present study. In the pear juice the decrease in the number of viable organisms can be attributed to the time (two to seven months) in which the juice was held at -10°C. The work of Berry and Diehl (1934) and of Forgacs (1942) demonstrated that freezing can more than account for the reduction in microbial load. Prefiltration of the pear juice through diatomaceous earth also would be expected to reduce significantly the number of microorganisms (Berry and Diehl, 1934; Marshall, 1951).

Storage of Ultrafiltered Pear Juice

Ultrafiltrate had been aseptically filled into sterile glass bottles and stored at 38°C and 52°C for up to one year. Examination of the juice revealed evidence of microbial contamination (fermentation and mold mycelia) in some of the bottles. However, most of the juice showed no evidence of spoilage. It is likely that contamination occurred

TABLE 8.--Composition of pear juice permeates ultrafiltered at varying transmembrane pressures.[a]

	Transmembrane Pressure (kPa)			
	153	138	121	103
pH	4.42	4.40	4.41	4.40
Titratable Acidity[b]	30.8	31.0	30.9	30.7
Malic Acid[c]	8.42	9.76	9.05	9.09
Refractive Index	1.350	1.350	1.350	1.350
Glucose[d]	1.75	2.48	2.51	2.39
Fructose[d]	6.29	7.22	6.84	7.01
Sorbitol[d]	1.76	2.33	2.45	2.53
Total Sugar[d]	9.80	12.03	11.80	11.93
Phenolics[e]	259.2	260.0	257.5	259.2
Pectin[f]	<.1	<.1	<.1	<.1
α-amino N[g]	10.2	10.6	10.2	10.4
Hunter haze	1.3	1.9	1.6	1.5
Hunter L	88.8	89.1	89.1	89.0
Hunter a	-4.0	-4.1	-3.6	-4.1
Hunter b	+21.8	+21.8	+21.3	+22.4

[a](inlet pressure = 172 kPA, PM-50 membrane, 50°C, VCR=1.14)

[b]ml 0.1 N NaOH/100 ml juice

[c]meq/100 ml juice

[d]g/100 ml juice

[e]μg/ml gallic acid

[f]mg/ml anhydrogalacturonic acid

[g]mM glycine

TABLE 9.---Composition of pear[a] juice permeates ultrafiltered through membranes of varying pore size

	PM-10	PM-30	PM-50
pH	4.42	4.41	4.42
Titratable Acidity[b]	30.0	30.0	30.0
Malic Acid[c]	8.42	9.34	8.42
Refractive Index	1.350	1.350	1.350
Glucose[d]	2.07	2.45	1.75
Fructose[d]	7.13	6.81	6.29
Sorbitol[d]	2.13	2.30	1.76
Total Sugar[d]	11.33	11.56	9.80
Phenolics[e]	259.8	258.2	259.2
Pectin[f]	<.1	<.1	<.1
α-amino N[g]	10.8	10.4	10.2
Hunter haze	1.6	1.6	1.3
Hunter L	86.6	87.9	88.8
Hnnter a	-4.0	-4.7	-4.0
Hunter b	+26.0	+25.3	+21.8

[a] (ΔP=153 kPA, 50°C, VCR=1.14)

[b] ml 0.1 N NaOH/100 ml juice

[c] meq/100 ml juice

[d] g/100 ml juice

[e] µg/ml gallic acid

[f] mg/ml anhydrogalacturonic Acid

[g] mM glycine

TABLE 10.--Colony counts of pear juice, permeates, and retentates ultrafiltered under different condition.

Process Temperature	Membrane	ΔP(kPa)	Juice	Colony Count per ml juice VF_1	VF_2	VF_3	Retentate
50°C	PM-50	138	80	<1	<1	<1	1
50°C	PM-50	121	90	<1	3	-	<1
50°C	PM-50	103	90	<1	<1	<1	<1
15°C	PM-10	103	60	<1	<1	<1	25

while bottles were being filled. Even at a high flux bottles often took up to 10 minutes to fill. Contamination caused by leakage through or around the membrane may have been a problem as well. In two samples which had been stored the longest, brown precipitates has formed. Since the juice appeared clear and uncontaminated, it is possible that the precipitate was caused by polymerization of phenolics. However, an analysis of the precipitate was not conducted to confirm this.

Hunter readings for some of the stored juices are reported in Table 11. Juices stored at 38°C, even those stored for up to one year, remained clear and light colored. Hunter readings were comparable to freshly filtered juice. Juice stored at 52°C remained clear but was much darker and more golden in color. Hunter L values were lower and Hunter "b" values higher than for juices stored at the lower temperature. The increase in color was probably the result of phenolic polymerization.

TABLE 11.--Hunter haze and color values of ultrafiltrates stored at 38°C and 52°C.

Storage Temperature	Storage Time	Membrane	Filtration Temperature	VCR	Hunter L	Hunter a	Hunter b	Hunter Haze
38°C	12 mo.	PM-30	50°C	1.90	86.5	-3.3	+23.4	2.0
38°C	8 mo.	PM-50	50°C	1.02	88.2	-3.6	+20.0	2.0
38°C	8 mo.	PM-50	50°C	1.48	89.4	-4.8	+20.3	2.1
38°C	8 mo.	PM-10	50°C	1.60	88.0	-3.8	+21.8	2.1
38°C	11 mo.	PM-50	30°C	1.29	87.9	-4.3	+23.1	2.1
52°C	12 mo.	PM-50	50°C	1.90	81.7	-2.5	+33.7	2.1
52°C	8 mo.	PM-50	50°C	1.14	82.6	-3.0	+31.2	2.1

CONCLUSIONS

Ultrafiltered d'Anjou pear juice was "sparkling clear", light colored, essentially free of pectin, and lower in phenolics than the feed juice. In addition, the ultrafiltrate contained sugar, acid, and α-amino compound concentrations equal to those of the unfiltered juice and was microbiologically sterile. Retention characteristics of the three membranes, Romicon PM-10, PM-30, and PM-50, were identical at the VCR tested. Changes in filtration temperature and transmembrane pressure showed no evidence of causing changes in juice eomposition. However, in the retentate there was a greater reduction in the number of microorganisms at 50°C than that at the lower temperature (15°C).

The effects of time, or VCR, on permeate composition were more pronounced. At higher VCRs the membrane became increasingly permeable to pectic substances and to phenolics. The decrease in the lightness and increase in yellowness of the juice at high VCRs probably was indicative of the higher phenolic concentration. No evidence of Maillard browning was found and the dilute nature of the juice made it unlikely that Maillard browning was occurring. The decrease with time in retention efficiciency of the membrane for pectic substances and phenolics means that membrane pore sizes were sufficiently large to have been at least semipermeable to the two compounds. Concentration polarization probably was responsible for the loss of retention efficiency. In addition, the fact that flux was so much lower than expected was due to the rapid build-up of the concentration polarization gel layer. Pectic substances probably play a major role in formation of the gel layer.

Several factors should be examined if ultrafiltration is to become commercially feasible. Cost considerations include a relatively high initial cost, membrane replacement costs, and fairly low energy costs. Studies would need to be done to determine the lifetime of the membranes under normal processing conditions. Porter and Michaels (1971) have commented that membrane life is determined primarily by the fouling characteristics of the membrane rather than its mechanical durability. The present study demonstrates the significant reduction in flux that can occur when a membrane approaches the end of its lifetime. A decrease in flux would make filtration to a high VCR impossible; thus, it would be difficult to recover much of the clarified juice from the retentate. An economic analysis of juice ultrafiltration should include such losses. In addition, if ultrafiltration is to be used for "cold sterilization" the reliability of the retention characteristics must be studied. Formation of sediments in bottled permeates, especially those stored at high temperatures, point out the necessity of further study.

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