

AN ABSTRACT OF THE THESIS OF

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Title Phenolic Compounds in Bartlett Pears and Their Relation
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The purpose of this study was to determine the phenolic constituents of Bartlett pears and to evaluate the processed samples for extent of discoloration and to relate the two. Pears were obtained from three orchards in the Medford area and from three orchards at each of the three elevations, 500, 1700, and 2300 feet, in the Hood River area. Four replications were made.

Values for the total phenol content of the pears ranged from 54.3 to 120.8 milligrams per 100 grams of fresh pear tissue. The leuco-anthocyanin content of the pears ranged from 6.4 to 21.0 milligrams per 100 grams of pear tissue and the flavanol content ranged from 11.3 to 44.8 milligrams per 100 grams of pear tissue. The pH of the pears ranged from 3.70 to 4.09. Color of the pears processed for twenty-five, thirty-five and forty-five minutes was measured.

Those pears with the highest concentration of total phenols, leuco-anthocyanin and flavanols and the lowest pH were the pinkest when overprocessed. Pears which were least pink were among the lowest in total phenols, leuco-anthocyanin and flavanols and had the highest pH values. However, among the remaining fruits, the pinker pears were not necessarily those with the higher total phenolic content or the lower pH values.

PHENOLIC COMPOUNDS IN BARTLETT PEARS AND THEIR
RELATION TO DISCOLORATION OF THE CANNED PRODUCT

by

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PHENOLIC COMPOUNDS IN BARTLETT PEARS AND THEIR RELATION TO DISCOLORATION OF THE CANNED PRODUCT

INTRODUCTION

Color of canned Bartlett pears is of major importance to the commercial canner and to the consumer. A high quality product that possesses a typical white or light yellow color is desired by the consumer and the producer can demand a higher price for this product than one which is discolored. Canned Bartlett pears occasionally develop a pink or brown discoloration, particularly if the product is overprocessed.

Brown discoloration of fresh pears is due to the enzymatic oxidation of polyphenols which are present in the fruit. The oxidation is rapid in the fresh fruit; however, the polyphenoloxidase enzyme which catalyzes the oxidation is denatured during canning and browning of the canned product is much slower.

Pink discoloration of canned Bartlett pears is thought to be due to the conversion of a colorless leuco-anthocyanin pigment to the pink cyanidin form. (19, p. 308)

The purpose of this study was to determine the polyphenolic constituents in fresh Bartlett pears, to evaluate the processed samples for extent of discoloration and to relate the two.

REVIEW OF LITERATURE

History of Pears. The origin of the pear (Pyrus communis) has been traced to prehistoric times. Leaves of the genus Pyrus have been found in tertiary remains in Western Europe and in the Caucasian countries, but no fossil remains have been discovered in North America. (30, p. 358) After prehistoric times the pear became a native of Europe, Asia and Northern Africa. (32, p. 31)

The cultivation of pears began in the early 1700's at Mons, Belgium. There Abbé Hardenport planted pear seeds and thirty years later he introduced a number of varieties. Jan Baptiste Van Mons of Louvain also propagated pears and by 1825 had sent several hundred trees to America. (6, P. 432)

The Bartlett pear was discovered in a woods near Aldermaston, Berkshire, England by a Mr. Stair in the late 18th century. From him plants were obtained by a Mr. Williams, a nurseryman of Turham, Middlesex, who propagated and distributed them under the name of Williams Bon Chrétien. In 1797 or 1799, James Carter brought this variety to the United States for Thomas Brewer who planted the trees in his orchard. The Brewer estate was bought in 1817 by Enoch Bartlett of Dorchester, Massachusetts, and not knowing the true identity of the pear, he propagated it under his own

name. (14, p. 46)

Production of Pears. The Bartlett pear bears heavily and regularly in different soils and climates. The fruit ships well, keeps well and is unexcelled for canning. (6, p. 454) It is now the leading variety in the major pear producing regions of the United States and Canada. (17, p. 33)

Approximately eighty per cent of the pear crop of the United States is produced in the three Pacific coast states. Nearly two-thirds of the pear crop is canned and about one-third is sold fresh. (24, p. 26) Small amounts of the fruit are pickled, dried or used in speciality items.

Harvesting and Pear Quality. Many investigations have been carried out to study the factors that contribute to high quality canned pears. Among other factors that affect the canned pear are the maturity at harvest and the temperature to which the fruit is subjected during transit, storage and ripening. (9, p. 375)

For a superior product, pears are not allowed to ripen fully on the tree. Maturity is assessed by the color and more recently by the firmness of the fruit. Evaluation of color was carried out using color scales that were prepared for this purpose. Tests for firmness were originally done by applying pressure to the fruit with the thumb. A mechanical device was then developed by Morris of the

Washington Agricultural Experiment Station in connection with studies on the rate of ripening of apples in storage. A similar but improved tester was then developed by Magness and Taylor of the United States Department of Agriculture and is in common use at the present time. (1, p. 21)

Ezell and Diehl (12, p. 17) found that pears which had ripened to the stage where they could be penetrated with a pressure of fifteen to seventeen pounds with the Magness-Taylor tester gave a higher quality ripened fruit than did pears harvested at higher or lower pressures. Furthermore, pears are damaged less during transportation and handling at this ripeness. From a practical standpoint most growers harvest fruit when it gives a pressure test of fifteen to twenty pounds.

Pears that are picked at a pressure much above twenty pounds will fail to ripen or, if they ripen, will have poor texture and flavor. Such fruit when canned tends to have a pale orange-yellow color in contrast to the translucent cream color of the fruit picked at the right stage. (12, p. 17)

As soon as possible or within twenty-four hours after harvesting, the pears should be cooled to 31° F. (21, p. 4780) Fruit placed in cold storage has a better color and texture when it is subsequently ripened and canned than does fruit that is ripened and

canned immediately after harvest. The pears can be kept for two months at 29° to 30° F.; however, longer storage is accompanied by internal decomposition of the fruit and gradual loss of ripening ability. Investigations have shown that a ripening temperature of between 70° to 75° F. gives the most rapid and uniform ripening and produces a superior canned product. (12, p. 21) At this temperature the firmness of the fruit drops to two to three pounds within four or five days. The fruit is at its maximum dessert quality at this pressure.

Changes during Ripening of Pears. The pear is a complex biological system and many changes occur during the ripening process. Quite apparent changes in texture begin considerably in advance of any color change. The softening of the fruit is due to alteration of the pectic substances in the walls of the parenchyma cells, protopectin being changed to soluble pectin as ripening progresses. (11, p. 31) During ripening the color of the fruit changes gradually from green, in the unripe stage, to a yellow in the ripened stage. The amount and rate of softening as well as the changes in color appear to be influenced by the temperature and by the available soil moisture of the growing area. Pears from hot, dry districts tend to be softer and more yellow than those from cooler, moister districts. (2, p. 106)

During ripening there is also a marked and uniform increase in total sugars. In the earlier stages of ripening the increase is due mainly to reducing sugar while during the later part of the season it is mainly due to an accumulation of sucrose. (24, p. 498) Citric and malic are the major acids in Bartlett pears and the ripening of the fruit is accompanied by a rapid decrease of malic acid and a slower decrease in citric acid. (11, p. 32) A gradual increase in volatile reducing substances such as methyl alcohol, total carbonyl compounds, acetyl methyl carbinol, diacetyl and ester content occurs during ripening. (22, p. 642) Accompanying maturation is a gradual increase in soluble solids and a decrease in titratable acidity. (21, p. 479)

The ascorbic acid content of pears also decreases during ripening. Tressler and Moyer found that approximately one-third of the ascorbic acid content of Bartlett pears stored at 30° F. was lost during the first month of storage, after which relatively little loss occurred. (35, p. 375)

Phenols and Discoloration of Canned Bartlett Pears. During the heat processing of Bartlett pears a brown or pink discoloration which masks the translucent cream color of the high quality product may appear. Phenolic compounds are implicated in the oxidative browning and/or pink discoloration of the canned product. Three

such phenolic compounds that have been isolated from Bartlett pears are catechin (31, p. 101), a leuco-anthocyanin (18, p. 318), and chlorogenic acid (7, p. 169).

Catechin (Figure 1) and leuco-anthocyanins, (a sugar free form of which is shown in Figure 2), are members of the flavonoid

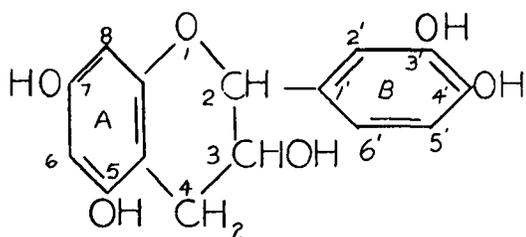


Figure 1

Catechin

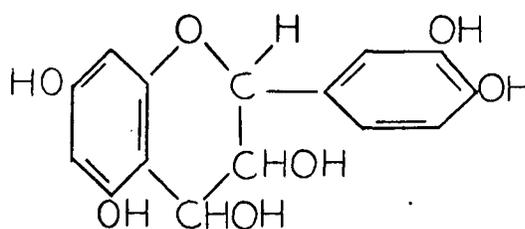


Figure 2

Leuco-anthocyanin

group of compounds. These pigments are characterized by their $C_6-C_3-C_6$ carbon skeleton which consists of two aromatic rings linked by a three carbon fragment. Leuco-anthocyanins and catechin are distinguished from other members of the flavonoid group, such as the flavonols, flavanonols, flavanones and isoflavones, on the basis of state of oxidation of the three carbon section. (37, p. 386) These latter compounds are collectively known as anthoxanthins. Many of the flavonoid compounds have sugar residues attached at one or more hydroxyl groups by glycosidic linkages. Sugars which commonly combine with phenolic hydroxyl groups include galactose, arabinose, xylose and particularly glucose and rhamnose. The sugar can occur not only attached as single sugar residues to particular

hydroxyl groups but at di- or trisaccharides. More than one position on the same molecule can be glycosylated; however, the three position is most commonly glycosylated, the five position less frequently and the seven position only rarely. (5, p. 267)

Catechin is a flavan-3-ol and is essentially a reduced flavone. This compound is never glycosylated. (5, p. 267) Catechin and leuco-anthocyanins are closely related structurally and resemble each other in their astringent quality and in their distribution in food.

Leuco-anthocyanins are colorless or nearly colorless flavan-3:4-diols, which when heated with hot concentrated hydrochloric acid yield anthocyanidins (Figure 3). (33, p. 109) Robert Boyle (26, p. 4)

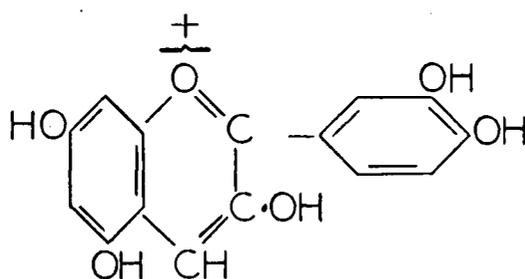


Figure 3 Anthocyanidin

and Otto Rosenheim (29, p. 178) were two of the first to observe and record the existence of a leuco-anthocyanin. In the early 1930's the Robinsons suggested a tentative structure for such a compound. (27, p. 207)

Leuco-anthocyanins appear to be present in plants in the

primitive members of a phyletic series and some investigators feel that "the production of leuco-anthocyanins is a primitive metabolic pattern associated with, but not essential to, a tree-like or woody habit of growth." (4, p. 130) Bate-Smith found that leuco-anthocyanins occurred in thirty-six per cent of all species he examined whereas in woody families sixty-one per cent contained leuco-anthocyanins. (4, p. 130) Until recently those chemical compounds which caused discoloration and astringency in many fruits and vegetables were called "tannins". Most of these compounds have since been shown to be leuco-anthocyanins.

Chlorogenic acid (Figure 4) is the cinnamic acid derivative

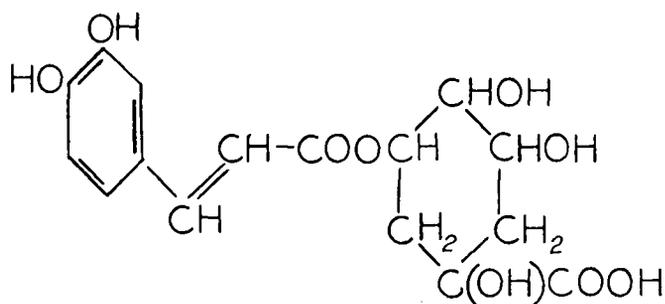


Figure 4

Chlorogenic Acid

most widely distributed in nature. The cinnamic acid derivatives are distinguished by their C_6-C_3 carbon structure which consists of a phenolic benzene ring with a three carbon chain attached. Like catechin and leuco-anthocyanin, chlorogenic acid has hydroxyl groups in the ortho position on the B ring.

Weurman and Swain (36, p. 678) and Bradfield et al (7, p. 169) have indicated that chlorogenic acid and isochlorogenic acid are the chief substrates for enzymatic discoloration of raw pears. Also, chlorogenic acid may contribute to the discoloration of canned pears as it may be autoxidized. The oxygen present in the jar containing the fruit will react with the chlorogenic acid and the flavanols present to form brown colored pigments similar to that formed by enzymatic action. (19, p. 308)

The pink discoloration of canned pears has been attributed to the presence of leuco-anthocyanin by several investigators. Bate-Smith has suggested that the discoloration of stewed pears is due to the presence of leuco-anthocyanin in the fresh tissues. (5, p. 272) Joslyn and Peterson (18, p. 318) have reported the presence of leuco-anthocyanin in the seeds, tissue and core of Bartlett pears and Joslyn (19, p. 309) suggested that the reddening in tinned pears that are stacked before they are completely cooled is due to the presence of leuco-anthocyanin in the raw fruit. Luh et al (23, p. 55) in their investigations of pink discoloration of canned Bartlett pears indicated that the pink pigment of the discolored pear was very similar or possibly even identical to cyanidin. These investigators found that a low pH, high titratable acidity and the presence of high "tannin" content were all associated with pink discoloration in

canned pears. They also indicated that there is a positive relationship between pink discoloration and overprocessed or slowly cooled pears.

Factors Affecting Polyphenol Content of Pears. The polyphenol content of fruit is known to vary with variety, location, season and climate and with the growth status of the tree. Environmental conditions which favor an exceptionally high accumulation of carbohydrates are generally considered to produce fruit with a high "tannin" content. (10, p. 119) Other factors such as soil type, cultural practices and application of fertilizers may also have an effect on the amount of polyphenols in fruit. (13, p. 59)

EXPERIMENTAL PROCEDURE

Bartlett pears were obtained from three orchards in the Medford area and from three orchards at each of three elevations, 500, 1700, and 2300 feet, in the Hood River area. From each orchard forty pears were taken at random from each of eight lugs. Thus eight lots of forty pears each or a total of three hundred twenty pears made up the sample from each orchard.

Handling of Pears before Analysis. To check the maturity of the pears at harvest, ten pears were withdrawn from each lot of forty. Using a United States Department of Agriculture fruit tester with a plunger 5/16 inches in diameter, the force required to insert the plunger 5/16 inches into the pared fruit was measured at three different locations in the mid-section of the fruit, avoiding sunburned and blemished areas. The pressure tests ranged from 17.9 to 20.4 pounds. These values are within the range normally used for harvesting Bartlett pears. The thirty pears remaining in each lot were put in storage at 30^o F. at a relative humidity of 85 per cent.

After the fruit had been in cold storage approximately one month, it was removed with the intent of assigning the thirty pears in each of the eight lots from each orchard to six sublots of five

pears each. Due to malfunctioning of the thermostat during preliminary storage some of the pears were frozen. Pears from two orchards were completely lost. The pears from each of the lots from the other ten orchards that appeared undamaged were assigned to four sublots of five pears each. In those lots in which the cold damage was slight, the extra pears were packaged separately. This meant that the number of replications was cut from the six, as planned, to four. Loss of pears due to freezing might have been due to location of the fruit in the cold storage room but was more likely due to differences in the concentration of sugar in the pears. If the latter is true, this introduced a bias in the samples.

Analysis of the pears began on November 3. For each analysis, one subplot of five pears from each of the eight lots from an orchard was ripened at a temperature of 70^o F. and a relative humidity of 85 per cent. The fruit was allowed to ripen until it gave a pressure test of not less than two and not more than three pounds on the United States Department of Agriculture pressure tester. When the pears were checked to see if they had reached the desired pressure, it was apparent that some of the fruit had been frozen even though the damage was not visible when they were placed in the ripening room. Cold damaged pears gave an abnormally high pressure test. Only those pears which gave a pressure test

between two and three pounds were used.

Processing of Pears to Develop Discoloration. Originally it was planned to use twenty-four pears for each analysis but due to the freezing damage this number was reduced to twelve. Each pear was cut in half using a stainless steel knife. One half of each pear was set aside for chemical analysis. The other half was pared and cored with stainless steel utensils and placed in a one per cent salt solution to retard enzymatic discoloration. The twelve pared halves were packed, four to a pint, into three wide-mouth fruit jars and covered with a boiling sirup made of one cup sugar to two cups of water. The three jars were sealed with metal disk closures and placed in a boiling water bath. One jar was processed for twenty-five minutes and, to test the color-forming potential of the fruit, the second and third jars were processed for thirty-five and forty-five minutes, respectively. At the end of the process, the jars were removed from the boiling water bath and placed on a rack to cool.

Extraction of Phenolic Pigments

To obtain a representative sample of the fruit a radial section weighing approximately two grams was cut from each of the

twelve pear halves and immediately immersed in thirty milliliters of 70 per cent alcohol (368.5 ml. of 95 per cent ethanol made to 500 milliliters with distilled water). The total weight of the composite sample was twenty-four grams. Sections were cut and handled so as to include only fresh, unoxidized tissue and the entire sample was heated to the boiling point and held for five minutes to inactivate enzymes. The composite sample was transferred to a blender with an additional twenty-five milliliters of 70 per cent alcohol and blended at high speed for three minutes. The slurry was transferred to fifty milliliter centrifuge cups and centrifuged at 3200 RPM for ten minutes. The supernatant was decanted through glass wool into a 100 milliliter volumetric flask. The blender was rinsed with twenty milliliters of 70 per cent alcohol and this was poured into the centrifuge cups onto the pear tissue with which it was mixed thoroughly. This slurry was centrifuged at 3200 RPM for six minutes and the supernatant was decanted through glass wool into the 100 milliliter volumetric flask. The blender was again rinsed with twenty milliliters of 70 per cent alcohol and this was poured into the centrifuge cups and mixed thoroughly with the pear tissue. This slurry was centrifuged at 3200 RPM for six minutes and the supernatant was decanted

through glass wool into the 100 milliliter volumetric flask. The extract was made to volume with distilled water. This alcoholic extract was used for determining the total phenol, leuco-anthocyanin and flavanol content of the pears. Colorimetric methods were used for determining each pigment and the optical density was read with a spectrophotometer.¹ All determinations were made in triplicate.

Extraction of Ascorbic Acid

A radial section weighing approximately two grams was cut from each of the twelve pears and immediately immersed in approximately thirty-five milliliters of 5 per cent metaphosphoric acid. The total weight of the composite sample was twenty-five grams. Sections were cut and handled in such a way as to minimize oxidation of ascorbic acid. The composite sample was then placed in a blender with an additional 140 milliliters of metaphosphoric acid and blended at high speed for three minutes. The slurry was

¹ Appreciation is extended to Dr. Clara A. Storvick, Professor of Foods and Nutrition, for the use of the Coleman Junior Spectrophotometer, Model 6A.

filtered through number one Whatman filter paper and the filtrate was divided equally between erlenmeyer flasks. Each flask was sealed with parafilm to prevent evaporation and one flask was refrigerated while the other was frozen. The ascorbic acid was determined on the aliquots that had been refrigerated because preliminary runs showed no difference between the ascorbic acid content of the refrigerated and the frozen samples.

Determination of pH

The fruit remaining after the radial sections were removed was diced and placed in a blender. The diced pears were macerated for approximately two minutes until a homogeneous slurry was obtained. The pH of this slurry was then measured with a Beckman pH meter.

Determination of Total Phenols

The Folin-Denis colorimetric method (3, p. 111) was used for measuring the total phenolic content of the pears. With this method, the phosphomolybditungstic acid is thought to be reduced by the phenolic compounds in an acid solution. These reduced compounds then give blue salts on the addition of alkali. Any substance possessing the oxy-phenyl bond, which includes ascorbic

acid, reacts with the Folin-Denis reagent. (8, p. 1159) To arrive at a true value for the total phenols the concentration of ascorbic acid must be determined and its phenol equivalent subtracted from the total phenols as measured to get a true value. For calculation purposes one milligram of ascorbic acid is equivalent to 0.80 milligrams of total phenols. (21, p. 479)

For the determination of total phenols, one milliliter of the pear extract was added to seventy-five milliliters of distilled water in a 100 milliliter volumetric flask. To this was added five milliliters of Folin-Denis reagent (375 milliliters of distilled water, 50 grams of sodium tungstate, 10 grams of phosphomolybdic acid and 25 milliliters of phosphoric acid refluxed for two hours, cooled and made to 500 milliliters with distilled water) with a syringe pipette. Ten milliliters of saturated sodium carbonate were added and the contents of the flask made to volume with distilled water. After one hour, the optical density was measured using a spectrophotometer set at a wave length of 700 $m\mu$. The blank was made as outlined omitting the test solution.

The concentration of total phenols in the pear extract was read from a standard curve. Data for this were obtained from solutions of tannic acid prepared in the following way. A stock

solution of tannic acid was made by placing 100 milligrams of tannic acid in a one liter volumetric flask and making it to volume with distilled water. From this a dilution was prepared that contained one milligram of tannic acid per ten milliliters. From the latter, dilutions were made to give solutions containing 100, 300, 500 and 1000 micrograms of tannic acid per milliliter. The optical density was read after thirty minutes and was plotted against the concentration of tannic acid to give the standard curve.

Determination of Leuco-anthocyanin

The leuco-anthocyanin content of the pears was determined by the method of Swain and Hillis. (34, p. 64) The estimation of leuco-anthocyanin is based on the transformation of this substance to anthocyanidin by heating in an acid solution. The proportion of anthocyanidin produced depends on the solvent used. Early methods used aqueous acid as the solvent; however, not more than ten per cent conversion is obtained. More recent investigations have indicated that alcoholic solvents yield approximately twenty-five per cent anthocyanidin. (34, p. 65) Swain and Hillis have indicated that butanol is the best solvent for this conversion as it is less volatile than other alcoholic solvents such as propanol.

For the determination of leuco-anthocyanin ten milliliters of leuco-anthocyanin reagent (25 milliliters of concentrated hydrochloric acid made to 500 milliliters with n-butanol) was placed in each of four ground-glass stoppered test tubes (7" x 1"). One milliliter of pear extract was added to each of the four tubes. After shaking, three of the tubes were placed unstoppered in a water-bath at 96° C. After three minutes the stoppers were placed firmly in position, the water-bath covered with aluminum foil to exclude light and the contents of the tubes heated for a total of forty minutes. The stoppers were then removed and the tubes cooled in tap water for five minutes. The optical density of the solution of anthocyanidin was determined at 540 $m\mu$ using the contents of the unheated tube as a blank.

The concentration of anthocyanin pigment developed in the pear extract was read from a standard curve. Data for this were obtained from solutions of cyanidin chloride prepared in the following way. A stock solution was made by diluting ten milligrams of cyanidin chloride to ten milliliters with 95 per cent ethanol. From this stock solution working standards were prepared containing 4, 10, 20, 40 and 60 micrograms per milliliter. These dilutions were made to volume with leuco-anthocyanin reagent. After mixing, the optical density of each was read at a wavelength of 540 $m\mu$.

Determination of Flavanols

The flavanol content of the pears was determined by the method developed by Swain and Hillis. In the presence of concentrated sulfuric acid compounds such as phloroglucinol and catechin which contain the 1:3:5-trihydroxybenzene nucleus form a carbonium ion which reacts with vanillin to give a red adduct with a maximum absorption at 500 m μ . (34, p. 64)

For the determination of flavanols two milliliters of pear extract were placed in each of two twenty-five milliliter erlenmeyer flasks. Four milliliters of vanillin reagent were added by means of a syringe pipette to one of the flasks containing pear extract and to one of the flasks containing water and four milliliters of 70 per cent sulfuric acid were added to the two remaining flasks. The reagent was made by dissolving one gram of recrystallized vanillin in 100 milliliters of 70 per cent sulfuric acid. This reagent was prepared freshly every three days. Before adding either the reagent or the sulfuric acid the flasks were placed in an ice water bath and were shaken as the acid was added in order to prevent overheating.

The flask containing water and sulfuric acid was used as the blank. The flask containing vanillin and water, that containing sulfuric acid and pear extract and the one containing pear extract

and vanillin were read against the blank at a wavelength of 500 m μ at exactly fifteen minutes after the addition of the sulfuric acid or the vanillin reagent. The reading of the contents of the flask containing pear extract and vanillin was corrected by subtracting that of the flask containing water and vanillin and that of the flask containing pear extract and sulfuric acid.

To convert the optical density into the quantity of catechin, a standard curve was used. Data for this was obtained from solutions of catechin prepared in the following manner. Fifty milligrams of catechin were dissolved in two milliliters of 95 per cent ethanol and made to a volume of fifty milliliters with distilled water. A series of dilutions was made containing the following concentrations in micrograms per milliliter; 1, 2, 3, 5, 10, and 15. The optical density was read at a wavelength of 500 m μ and was plotted against the concentration of catechin to give the standard curve.

Determination of Dehydroascorbic Acid

The dehydroascorbic acid content of the fresh pears was determined by the 2,4-dinitrophenylhydrazine method of Roe, Mills, Oesterling and Damron. (28, p. 201-206) In this method the l-ascorbic acid is oxidized to dehydroascorbic acid with bromine.

The dehydroascorbic acid is then coupled with 2,4-dinitrophenylhydrazine and the resulting osazone is treated with sulfuric acid to produce a red color which is measured with a spectrophotometer. The 2,4-dinitrophenylhydrazine reagent was made in the following manner. Two grams of 2,4-dinitrophenylhydrazine and 0.25 grams of thiourea were transferred to a 100 milliliter volumetric flask. They were dissolved and made to volume with approximately 9N sulfuric acid. To clarify this solution it was centrifuged for fifteen minutes at 3200 RPM and then filtered through glass wool. The reagent was refrigerated.

For the determination of dehydroascorbic acid a suitable aliquot of pear filtrate was poured into a test tube, a drop of bromine was added and this mixture was stirred. In order to remove excess bromine, the filtrate was decanted into another flask. This filtrate was aerated for fifteen minutes using a water trap. The pear filtrate was diluted with 5% metaphosphoric acid 1:1 by volume. Four milliliters of the diluted, bromine-oxidized pear filtrate were pipetted into each of four erlenmeyer flasks. One flask was reserved as a blank. One milliliter of 2,4-dinitrophenylhydrazine reagent was added with a syringe pipette to each of the other three flasks. The flasks were mixed well, capped with

parafilm and incubated in a water bath at 37° C. for six hours. The blank was not incubated. After removal from the water bath the flasks were uncapped, placed in ice water and five milliliters of 85 per cent sulfuric acid were dropped dropwise from a buret in not less than one minute. One milliliter of 2,4-dinitrophenylhydrazine was added to the flask containing the blank. One-half hour was allowed for the final development of the color, after which the optical density of the solution was read at 540 $m\mu$.

The concentration of ascorbic acid was read from a standard curve. Data for this were obtained from solutions of ascorbic acid prepared in the following way. One hundred milligrams of ascorbic acid were dissolved and made to volume with 0.5 per cent oxalic acid in a 100 milliliter volumetric flask. Five milliliters of this stock solution were placed in a 250 milliliter volumetric flask and made to volume with 5% metaphosphoric acid. A suitable aliquot of the diluted ascorbic acid solution was brominated. This was decanted and aerated for fifteen minutes. From this dilutions were made giving the following concentrations in micrograms per milliliter: 0.625, 1.25, 2.5 and 5.0. Each dilution was made to volume with metaphosphoric acid.

Measuring Color of Canned Pears

The color of the canned Bartlett pears was determined with the Hunter Color and Color-Difference Meter.² This instrument is a tristimulus colorimeter measuring color on three scales. The "L" scale measures visual lightness, the "a_L" scale measures redness when plus, gray when zero and greenness when minus and the "b_L" scale measures yellowness when plus, gray when zero and blueness when minus. The instrument compares unknown specimens with a standard of predetermined color characteristics. It approximates the eye of a skilled observer under ideal conditions and can measure a large number of samples without the fatigue which a judge would experience.

For the measurement of color each jar of canned pear halves was opened and the juice drained from the fruit. The fruit was then macerated with a stainless steel food mincer, avoiding incorporation of air. Two hundred strokes were used on each sample in order to

² Appreciation is extended to Dr. William A. Sistrunk, Assistant Professor of Food Science and Technology, for the use of the Hunter Color and Color-Difference Meter, No. 106.

obtain a uniform mixture. The macerated tissue was then poured into a polystyrene container and placed on the illuminated area of the instrument. The area of the sample illuminated was an oval approximately $2 \frac{1}{4} \times 1 \frac{1}{2}$ inches. "L", "a_L" and "b_L" readings were then made. The instrument was standardized against a National Bureau of Standards ivory porcelain tile SKC 31 having the following "L" scale values: "L", 75.1; "a_L", -1.2; and "b_L", 23.1.

RESULTS AND DISCUSSION

Hydrogen Ion Concentration. The pH of the pears from each orchard is given in Table 1. Fruit from the three orchards in the Medford area had the lowest pH with means of 3.70, 3.78 and 3.82

TABLE 1
pH of Fresh Bartlett Pears ¹

Area	Orchard	1	2	3	4	Mean
Medford	1	3.70	3.68	3.68	3.76	3.70
	2	3.70	3.72	3.89	3.81	3.78
	3	3.82	3.75	3.84	3.90	3.82
Hood River 500 Foot	1	3.77	3.80	3.90	3.90	3.84
	2	4.00	4.02	4.05	4.10	4.04
Hood River 1700 Foot	1	3.82	3.90	3.90	3.86	3.87
	2	3.92	3.84	3.90	3.90	3.89
Hood River 2300 Foot	1	3.96	3.95	4.00	3.90	3.95
	2	3.97	3.90	3.89	3.97	3.93
	3	4.10	4.05	4.02	4.20	4.09

¹ Composite sample of 12 pears

for orchards 1, 2 and 3, respectively. The pears from the three orchards at the 2300 foot level and orchard 2 at the 500 foot level of the Hood River area had the highest pH values. The mean pH of the

fruit from the 2300 foot level was 3.95, 3.93 and 4.09 for orchards 1, 2 and 3, respectively and the fruit from orchard 2 of the 500 foot level had a pH of 4.04. Fruit from the remaining orchards had pH values between 3.84 and 3.89. The mean pH of the fruit from all orchards was 3.91. Luh, Leonard and Patel (23, p. 55) reported that the majority of pears which they had investigated had a pH ranging from 3.9 to 4.2 and Hulme (16, p. 301) stated that Bartlett pears had an average pH of 3.73.

Total Phenols. Table 2 gives the total phenols as measured, ascorbic acid and its phenolic equivalent, and the total phenols as corrected by orchard for each of the four replications. The phenolic equivalent of ascorbic acid was subtracted from the total phenols as measured, to give the total phenols as corrected. The values given for total phenols as corrected, hereafter referred to simply as total phenols, include leuco-anthocyanin, catechin, chlorogenic acid and other compounds possessing the oxyphenyl bond which might be present in Bartlett pears.

The amount of ascorbic acid in the pears ranged from 5.3 to 7.4 milligrams per 100 grams of fresh pear tissue. Tressler and Moyer (35, p. 374), using the dichlorophenol indophenol titration method, reported an ascorbic acid content of 9.0 milligrams per one hundred grams in freshly picked Bartlett pears and a value of

TABLE 2

 Phenolic Compounds in Bartlett Pears ¹
 (mg/100 gms fresh pears)

Area	Orchard	Replication	Total Phenols (as measured)	Ascorbic Acid	Phenol Equivalent	Total Phenols (corrected)
Medford	1	1	95.8	6.3	5.0	90.8
		2	144.5	5.5	4.4	140.1
		3	108.3	6.3	5.0	103.3
		4	154.1	6.6	5.3	148.8
		Mean	125.7	6.2	5.0	120.8
	2	1	70.8	5.9	4.7	66.1
		2	80.0	4.9	3.9	76.1
		3	117.5	6.4	5.1	112.4
		4	95.8	6.0	4.8	91.0
		Mean	91.0	5.8	4.6	86.4
	3	1	70.8	5.8	4.6	66.2
		2	81.3	5.8	4.6	76.7
3		113.3	5.9	4.7	108.6	
4		94.5	6.9	5.5	89.0	
Mean		90.0	6.1	4.9	85.1	
Hood River 500 Foot	1	1	100.0	8.2	6.6	93.4
		2	89.5	8.0	6.4	83.1
		3	93.8	7.5	6.0	87.8
		4	106.3	5.8	4.6	101.7
		Mean	97.4	7.4	5.9	91.5
	2	1	59.1	5.1	4.1	55.0
		2	52.0	5.5	4.4	47.6
		3	54.1	5.4	4.3	49.8
		4	68.8	5.2	4.2	64.6
		Mean	58.5	5.3	4.2	54.3
Hood River 1700 Foot	1	1	72.9	6.2	5.0	67.9
		2	65.4	5.8	4.6	60.8
		3	52.0	6.7	5.4	46.6
		4	58.3	6.8	5.4	52.9
		Mean	62.1	6.4	5.1	57.0
	2	1	52.9	5.3	4.2	48.7
		2	65.4	5.4	4.3	61.1
		3	75.0	5.7	4.6	70.4
		4	84.1	5.2	4.2	79.9
		Mean	69.4	5.4	4.3	65.0
Hood River 2300 Foot	1	1	62.5	6.6	5.3	57.1
		2	75.0	7.4	5.9	69.1
		3	95.8	7.7	6.2	89.6
		4	88.3	7.8	6.2	82.1
		Mean	80.4	7.4	5.9	74.5
	2	1	68.7	7.1	5.7	63.0
		2	73.7	6.8	5.4	68.4
		3	92.5	7.3	5.8	86.7
		4	107.0	7.8	6.2	100.8
		Mean	85.5	7.2	5.8	79.7
	3	1	65.4	5.9	4.7	60.7
		2	54.1	5.8	4.6	49.5
		3	75.8	7.2	5.8	70.0
		4	67.5	6.5	5.2	62.3
		Mean	65.7	6.3	5.0	60.7

¹ Expressed as tannic acid

6.4 milligrams per one hundred grams of pear tissue in pears stored for two months.

The total phenol content of the fruit ranged from 54.3 to 120.8 milligrams per 100 grams of pear tissue. The fruit from orchards 1, 2 and 3 at Medford and orchard 1 at the 500 foot level of the Hood River area contained the largest amounts of phenolic compounds with 120.8, 86.4, 85.1 and 91.5 milligrams per 100 grams pear tissue, respectively. Fruit from orchard 2 at the 500 foot level, orchard 1 at the 1700 foot level and orchard 3 at the 2300 foot level of the Hood River area contained the smallest amounts of phenolic compounds with values of 54.3, 57.0 and 60.7 milligrams per 100 grams of pear tissue, respectively. Pears from orchard 2 at the 1700 foot level and orchard 1 and 2 at the 2300 foot level of the Hood River area were intermediate with values of 65.0, 74.5 and 79.7 milligrams per 100 grams pear tissue, respectively. Luh et al (23, p. 55) and Leonard et al (21, p. 480) reported a range of 36.5 to 125.0 milligrams of total phenols per 100 grams of fresh Bartlett pear tissue.

Leuco-anthocyanin Content. The leuco-anthocyanin content of the fresh pears is given in Table 3. The values ranged from 6.4 to 21.0 milligrams per 100 grams of fresh pear. Fruit from orchard 1 of the Medford area contained the highest concentration of

TABLE 3

Leuco-anthocyanin Content of Bartlett Pears ¹
(mg/100 gm fresh pear)

Area	Orchard	1	2	3	4	Mean
Medford	1	17.2	26.3	24.5	16.0	21.0
	2	10.2	11.0	19.8	12.5	13.4
	3	9.4	11.4	21.0	15.1	14.2
Hood River 500 Foot	1	15.3	16.8	10.0	11.6	13.4
	2	5.5	7.0	5.8	7.1	6.4
Hood River 1700 Foot	1	8.5	8.8	8.1	9.0	8.6
	2	6.2	8.9	10.6	10.5	9.0
Hood River 2300 Foot	1	7.5	10.8	14.4	16.3	12.2
	2	8.2	12.5	16.2	13.6	12.6
	3	7.5	6.8	7.9	9.7	8.0

¹ Expressed as cyanidin

leuco-anthocyanin with a value of 21.0 milligrams per 100 grams pear tissue. Fruit from orchards 1 and 2 at the 2300 foot level and orchard 1 at the 500 foot level of Hood River area and orchards 2 and 3 in the Medford area contained intermediate amounts of leuco-anthocyanin with values of 12.2, 12.6, 13.4, 13.4 and 14.2 milligrams per 100 grams pear tissue, respectively. Pears from orchard 2 at the 500 foot level, orchard 3 at the 2300

foot level and orchard 1 and 2 at the 1700 foot level of the Hood River area contained the smallest amounts of leuco-anthocyanin with values of 6.4, 8.0, 8.6 and 9.0 milligrams per 100 grams of pear tissue, respectively.

Flavanol Content. The flavanol content of the pears, given in Table 4, varied from 11.3 to 44.8 milligrams per 100 grams of pear tissue. The fruit from orchard 1 of the Medford area was especially high in flavanols with a value of 44.8 and that from orchard 1 at the 500 foot level of the Hood River area contained the second largest concentration, with 25.1 milligrams per 100 grams of pear. The fruit from the remaining orchards contained considerably less flavanols. Fruit from orchard 2 at the 500 foot level of the Hood River area contained the smallest amount with a value of 11.3 milligrams per 100 grams pear tissue. Pears from orchard 2 and 3 of the Medford area contained 13.4 and 13.5 milligrams flavanols per 100 grams of pear tissue, respectively, while orchard 3 at the 2300 foot level of the Hood River area contained 13.0 milligrams flavanols per 100 grams of pear tissue. Fruit from orchards 1 and 2 at the 1700 foot level of Hood River contained 15.8 and 15.5 milligrams flavanols per 100 grams pear tissue and orchards 1 and 2 of the 2300 foot level of the Hood River area contained 18.8 and 19.3 milligrams flavanols per 100 grams of pear tissue, respectively.

TABLE 4

Flavanol Content of Bartlett Pears ¹
(mg/100 gm fresh pear)

Area	Orchard	1	2	3	4	Mean
Medford	1	32.8	54.7	42.4	49.5	44.8
	2	16.0	12.7	13.6	11.1	13.4
	3	15.6	9.6	18.1	10.9	13.5
Hood River 500 Foot	1	36.3	25.0	19.0	20.2	25.1
	2	11.9	9.4	10.8	13.1	11.3
Hood River 1700 Foot	1	12.7	20.4	14.8	15.5	15.8
	2	9.7	18.5	16.8	16.9	15.5
Hood River 2300 Foot	1	13.5	18.4	22.1	21.1	18.8
	2	12.5	22.5	21.6	20.6	19.3
	3	11.1	15.2	12.3	13.3	13.0

¹ Expressed as catechin

Relation between Phenolic Compounds in Pears. Fruit from orchard 1 of the Medford area contained the largest amount of total phenols, leuco-anthocyanin and flavanols and fruit from orchard 2 at the 500 foot level of the Hood River area contained the smallest amount of total phenols, leuco-anthocyanin and flavanols. Pears from orchard 1 at the 500 foot level at the Hood River area

contained the second largest amount of total phenols and flavanols and the third largest amount of leuco-anthocyanin. Fruit from orchard 2 of the Medford area contained the third largest concentration of total phenols and leuco-anthocyanin, but the flavanol content of this fruit was low. Pears from orchard 3 of the Medford area contained the fourth largest concentration of total phenols, was second in concentration of leuco-anthocyanin and was low in flavanols. Fruit from orchard 1 at the 1700 foot level and orchard 3 of the 2300 level of the Hood River area was low in total phenols, leuco-anthocyanin and flavanols. Fruit from orchard 2 at the 1700 foot level and orchards 1 and 2 at the 2300 foot level of the Hood River area was intermediate in total phenols, leuco-anthocyanin and flavanols.

In all the fruit except that from orchards 2 and 3 of the Medford area, the flavanol content was greater by fifty to almost one hundred per cent than the leuco-anthocyanin content. Reportedly (34, p. 65), the method used in this study for determining the leuco-anthocyanin content of the pears measures approximately twenty-five per cent of the leuco-anthocyanin present. In this case the values for leuco-anthocyanin reported in this study should be multiplied by a factor of four. This means that leuco-anthocyanin makes up a high proportion of the total phenolic content of Bartlett pears,

as suggested by Kiesser, Pollard and Williams. (20, p. 1260)

Color of Canned Pears. The mean of the "L", the "a_L" and "b_L" values of the canned pears as measured by the Hunter Color and Color-Difference Meter are given in Table 5. At the twenty-five minute processing time those pears from the three orchards at the 2300 foot level, orchard 1 at the 500 foot level and orchard 2 at the 1700 foot level of the Hood River area were lighter, as indicated by high "L" values, than the pears from the other five orchards. In this latter group pears from orchard 2 at the 500 foot level and orchard 1 at the 1700 foot level of the Hood River area were the darkest, that is, had the lowest "L" values.

Lightness decreased with an extension of the processing time for fruit from all orchards except orchard 2 at the 500 foot level and orchard 1 at the 1700 foot level of the Hood River area. Four out of the five orchards with the highest "L" values at the twenty-five minute processing time also had the highest "L" values at the forty-five minute processing time. Fruit from orchard 2 of the Medford area was the darkest at the forty-five minute processing time. Pears from orchard 2 at the 2300 foot level of the Hood River area were the lightest at all three processing times.

In terms of "a_L" values at the twenty-five minute processing time, pears from orchard 2 at the 1700 foot level and orchard 3 at

TABLE 5

Hunter Values ¹ for Color of Canned Bartlett Pears
(processing times in minutes)

Area	Orchard	25	35 "L"	45	25	35 "a _L "	45	25	35 "b _L "	45
Medford	1	50.97	50.30	49.50	1.70	4.30	5.40	13.07	13.40	13.30
	2	51.25	50.30	47.10	0.67	1.82	1.27	14.55	13.77	13.20
	3	51.97	50.95	49.37	-0.35	1.90	3.10	14.70	14.25	14.25
Hood River 500 Foot	1	53.80	51.12	50.17	0.07	2.15	4.65	14.82	13.27	13.17
	2	49.70	49.30	49.80	1.55	0.72	0.80	13.67	13.97	13.97
Hood River 1700 Foot	1	49.92	50.95	50.12	1.15	0.90	3.20	13.65	14.45	13.75
	2	52.32	50.45	49.67	-1.37	0.20	2.90	14.10	13.87	13.52
Hood River 2300 Foot	1	53.35	51.85	50.27	1.12	1.25	5.20	13.67	14.07	14.25
	2	55.30	52.70	51.47	-0.15	1.50	4.82	15.12	14.15	13.55
	3	53.02	51.82	51.07	-2.00	0.55	0.17	14.80	13.15	14.32

1. Mean of 4 replications.

the 2300 foot level of the Hood River area were somewhat on the greenish side as indicated by negative " a_L " values. Fruit from orchard 1 of the Medford area and orchard 2 at the 500 foot level and orchards 1 at both the 1700 and the 2300 foot levels were slightly pink at the twenty-five minute processing time.

Fruit from all the orchards increased in pinkness as the processing time was increased with the exception of orchard 2 at the 500 foot level of the Hood River area which was slightly pink at the twenty-five minute processing time but decreased in pinkness at the forty-five minute processing time. Prolonging the processing time brought about the greatest increase in pinkness in those pears from orchards 1 and 2 at the 2300 foot level, orchard 1 at the 500 foot level and orchard 2 at the 1700 foot level.

At the forty-five minute processing time those pears from orchard 1 of the Medford area, orchard 1 at the 500 foot level and orchards 1 and 2 at the 2300 foot level of the Hood River area were most pink. Fruit from orchard 3 at the 2300 foot level, orchard 2 at the 500 foot level and orchard 2 of the Medford area was least pink at the forty-five minute processing time.

In general those pears that decreased most in lightness with increased processing time also became the pinkest. One exception to this was the fruit from orchard 2 of the Medford area which

decreased in lightness the most yet increased in pinkness only slightly. Fruit from the two orchards that showed no change in lightness with increased processing time (orchard 2 at the 500 foot level and orchard 1 at the 1700 foot level of the Hood River area) were similar in pinkness at the twenty-five minute processing time yet orchard 2 decreased in pinkness slightly and orchard 1 increased in pinkness at the forty-five minute processing time.

Luh et al (23, p. 56) reported that the pink pigment usually was present on the surface of the pears only, but in the work here reported the fruit was discolored throughout. These pears were allowed to stand for approximately four months after processing which would allow time for diffusion of the soluble cyanidin throughout the pear. Luh et al also indicated that only some of the pear halves turned pink after canning. Hillis and Swain (15, p. 587) found that leaves on the shady side of a tree were lower in leuco-anthocyanin and total phenol content than those on the sunny side. Luh et al suggested that pears exposed to sun might be higher in leuco-anthocyanin content than those in the shade and that this might be a reason for the differences between pear halves in their tendency to turn pink.

The Hunter "b_L" values for the fruit was similar for all ten orchards and for all three processing times.

Phenolic Content and pH of Raw Pears and Color of Processed Fruit. A summary of the phenolic content and pH of the fresh Bartlett pears and color of the canned pears is given in Table 6. Fruit from orchard 1 of the Medford area was highest in total phenols, leuco-anthocyanin and flavanols. The canned product had low "L" values and the highest "a_L" values at both processing times. In addition these pears had the lowest pH. Pears from orchard 1 at the 500 foot level of the Hood River area had the second highest concentration of total phenols and flavanols and were third in leuco-anthocyanin content. These pears, which also had a low pH, had a large decrease in lightness and increase in pinkness with increased processing time.

Pears from orchard 2 at the 500 foot level of the Hood River area were lowest in total phenols, leuco-anthocyanin and flavanols. They were slightly pink at the twenty-five minute processing time, less so at the forty-five minute processing time and were equally light at the twenty-five and forty-five minute processing times. In addition, these pears had a high pH.

Pears from orchards 2 and 3 of the Medford area were comparable in total phenols, leuco-anthocyanin and flavanol content. At the twenty-five minute processing time lightness values were similar but at the forty-five minute processing time the fruit from orchard

TABLE 6

Phenolic Content of Fresh and Color of Canned¹ Bartlett Pears

Area	Orchard	Total Phenols ²	Leuco-anthocyanin ²	Flavanols ² pH		25		25	
						L ³	45	a _L ³	45
Medford	1	120.8	21.0	44.8	3.70	50.97	49.50	1.70	5.40
	2	86.4	13.4	13.4	3.78	51.25	47.10	0.67	1.27
	3	85.1	14.2	13.5	3.82	51.97	49.37	-0.35	3.10
Hood River 500 Foot	1	91.5	13.4	25.1	3.84	53.80	50.17	0.07	4.65
	2	54.3	6.4	11.3	4.04	49.70	49.80	1.55	0.80
Hood River 1700 Foot	1	57.0	8.6	15.8	3.87	49.92	50.12	1.15	3.20
	2	65.0	9.0	15.5	3.89	52.32	49.67	-1.37	2.90
Hood River 2300 Foot	1	74.5	12.2	18.8	3.95	53.35	50.27	1.12	5.20
	2	79.7	12.6	19.3	3.93	55.30	51.47	-0.15	4.82
	3	60.7	8.0	13.0	4.09	53.02	51.07	-2.00	0.17

1. Processing times given in minutes
2. mg/100 gms. fresh pear tissue
3. Hunter Values

3 was pinker. The slight difference in pH of the pears should have favored the formation of a pink color in the fruit from orchard 2 but did not.

In total phenol, leuco-anthocyanin and flavanol content fruits from orchards 1 and 2 of the 1700 foot level and from orchard 3 at the 2300 foot level of the Hood River area were similar. Fruits from the latter orchard were the lightest at both processing times. Of these three orchards, pears from orchard 1 were the pinkest at the forty-five minute processing time while those from orchard 3 had a negative " a_L " value at the twenty-five minute processing time and no detectable pinkness at the forty-five minute processing time. The pears from orchards 1 and 2 were similar in pH values and those from orchard 3 were the highest of all ten orchards.

Fruits from orchard 1 and 2 at the 2300 foot level of the Hood River area were similar in total phenols, leuco-anthocyanin and flavanol content. These pears were among the lightest and also pinkest at the forty-five minute processing time. In pH these pears ranked third and fourth highest, respectively.

In this study pears from the orchard which was highest in total phenol, leuco-anthocyanin and flavanol content and lowest in pH were the pinkest when overprocessed. The fruits from the two orchards which were least pink when overprocessed were among the

lowest in total phenol, leuco-anthocyanin and flavanol content and highest in pH values. However, among the fruits from the other orchards the relationship between phenolic content, pH and pinkness in the pears was not clear cut. Among these orchards the pinker pears were not necessarily those with the higher phenolic content or the lower pH values. Thus it appears that other factors not investigated in this study also influence the extent to which pears discolor when processed.

SUMMARY

1. Total phenols, leuco-anthocyanin and flavanols were determined in Bartlett pears grown in Medford and at three elevations in the Hood River area.
2. Values for the total phenol content of the pears ranged from 54.3 to 120.8 milligrams per 100 grams of fresh pear tissue. The leuco-anthocyanin content of the pears ranged from 6.4 to 21.0 milligrams per 100 grams of pear tissue and the flavanol content ranged from 11.3 to 44.8 milligrams per 100 grams of pear tissue.
3. The pH of the pears ranged from 3.70 to 4.09.
4. Color of the pears processed for twenty-five, thirty-five and forty-five minutes was measured. Lightness in Hunter "L" values ranged from 49.70 to 55.30 for pears processed for twenty-five minutes and from 47.10 to 51.47 for pears processed for forty-five minutes. Pinkness of the canned fruit, as indicated by the "a_L" values ranged from -2.00 to 1.70 for the pears processed for twenty-five minutes and from 0.17 to 5.40 for the pears processed for forty-five minutes.
5. Fruit from eight out of the ten orchards decreased in lightness and nine out of the ten orchards increased in pinkness with prolonged processing time.

6. Pears that were pinkest when overprocessed were highest in total phenols, leuco-anthocyanin and flavanols and lowest in pH and pears that were least pink when overprocessed were lowest in total phenols, leuco-anthocyanin, and flavanols and highest in pH. However, among the fruits from the remaining orchards, the pinker pears were not necessarily those with the higher total phenolic content or the lower pH values. Other factors not investigated in this study appear to influence the extent to which pears discolor when processed.

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APPENDIX

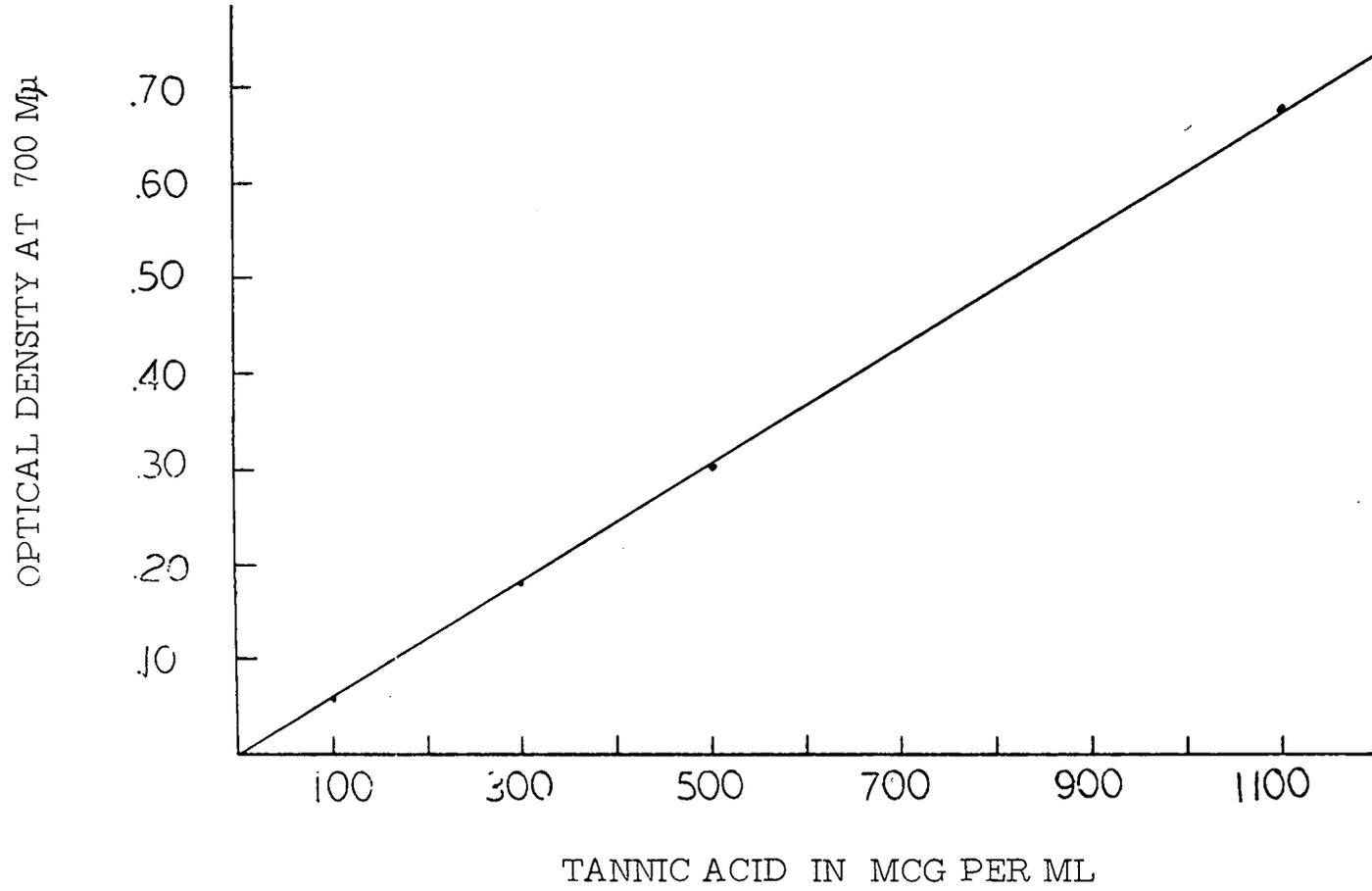
APPENDIX 1

Hunter Values for Color of Canned Bartlett Pears
(processing times in minutes)

Area	Orchard	Replication	25			35			45		
			L	a	b	L	a	b	L	a	b
Medford	1	1	49.8	2.9	12.3	51.0	2.4	13.7	49.6	2.9	14.8
		2	53.1	2.2	14.1	45.2	7.4	11.6	51.7	7.6	13.6
		3	50.4	0.2	14.1	50.6	4.4	13.6	46.0	6.7	11.5
		4	50.6	1.5	11.8	54.4	2.0	14.7	50.7	4.4	13.3
	2	1	48.7	-0.1	14.1	48.3	1.6	13.6	45.9	3.5	11.9
		2	48.4	0.3	13.5	52.0	2.8	13.4	46.4	0.4	13.5
		3	56.8	0.8	15.9	52.5	2.2	14.7	48.3	0.3	13.6
		4	51.1	1.7	14.7	48.4	0.7	13.4	47.8	0.9	13.8
	3	1	50.8	-0.3	14.6	51.7	2.5	14.0	48.7	4.5	14.0
		2	50.7	0.0	14.9	48.7	1.6	13.6	48.7	2.0	14.1
		3	52.8	-0.5	13.7	53.1	3.5	14.6	51.7	3.9	14.4
		4	53.6	-0.6	15.6	50.3	0.0	14.8	48.4	2.0	14.5
Hood River 500 Foot	1	1	54.5	1.0	14.4	52.4	1.5	12.9	49.4	8.7	12.5
		2	54.3	-0.2	15.2	50.4	1.4	13.5	51.4	2.8	14.0
		3	52.9	-0.2	14.5	50.1	3.8	12.9	49.5	2.6	12.9
		4	54.4	-0.3	15.2	51.6	1.9	13.8	50.4	4.5	13.3
	2	1	49.0	-1.6	13.3	50.4	-0.5	14.8	49.6	0.7	14.2
		2	50.3	-1.6	13.5	47.9	0.1	13.4	49.9	0.3	13.9
		3	48.1	-0.8	13.9	49.2	-0.9	13.7	49.9	2.0	13.7
		4	51.4	-2.2	14.0	49.7	-1.4	14.0	49.8	-0.2	14.1
Hood River 1700 Foot	1	1	52.5	0.3	14.4	50.8	0.9	13.9	50.4	5.0	13.4
		2	45.1	5.5	11.3	50.2	1.9	14.2	50.2	2.3	14.2
		3	49.6	0.7	14.2	50.7	0.1	14.9	49.2	3.1	13.2
		4	52.5	-1.9	14.7	52.1	0.7	14.8	50.7	2.4	14.2
	2	1	50.9	-2.3	13.3	50.9	-0.3	14.3	49.4	1.6	14.2
		2	51.2	-1.1	14.0	49.9	1.9	13.6	48.3	1.8	13.3
		3	53.8	-0.8	14.9	50.1	0.0	14.1	50.4	5.3	12.7
		4	53.4	-1.3	14.2	50.9	1.6	13.5	50.6	2.9	13.9
Hood River 2300 Foot	1	1	53.0	0.7	13.5	51.5	0.2	13.6	49.4	2.6	13.7
		2	53.8	0.0	14.9	51.7	2.9	13.7	49.9	7.7	12.8
		3	52.6	2.6	12.2	52.6	0.4	14.9	51.1	3.6	13.3
		4	54.0	1.2	14.1	51.6	1.5	14.1	50.7	6.9	13.2
	2	1	54.4	-0.9	14.3	54.2	-0.4	14.3	51.7	3.2	13.6
		2	55.3	0.3	15.7	52.1	3.6	14.0	51.6	4.3	13.4
		3	55.0	0.7	15.1	52.1	3.7	14.1	49.6	6.9	13.2
		4	56.5	-0.7	15.4	52.4	-0.9	14.2	53.0	5.8	14.0
	3	1	53.6	-1.8	14.7	52.7	1.0	13.6	52.1	0.3	14.7
		2	51.5	-2.4	14.4	52.3	-0.1	13.8	51.2	1.0	14.4
		3	54.4	-1.6	15.1	52.8	-1.1	14.6	50.8	-0.5	14.5
		4	52.6	-2.2	15.0	49.5	2.4	10.6	50.2	-0.1	13.7

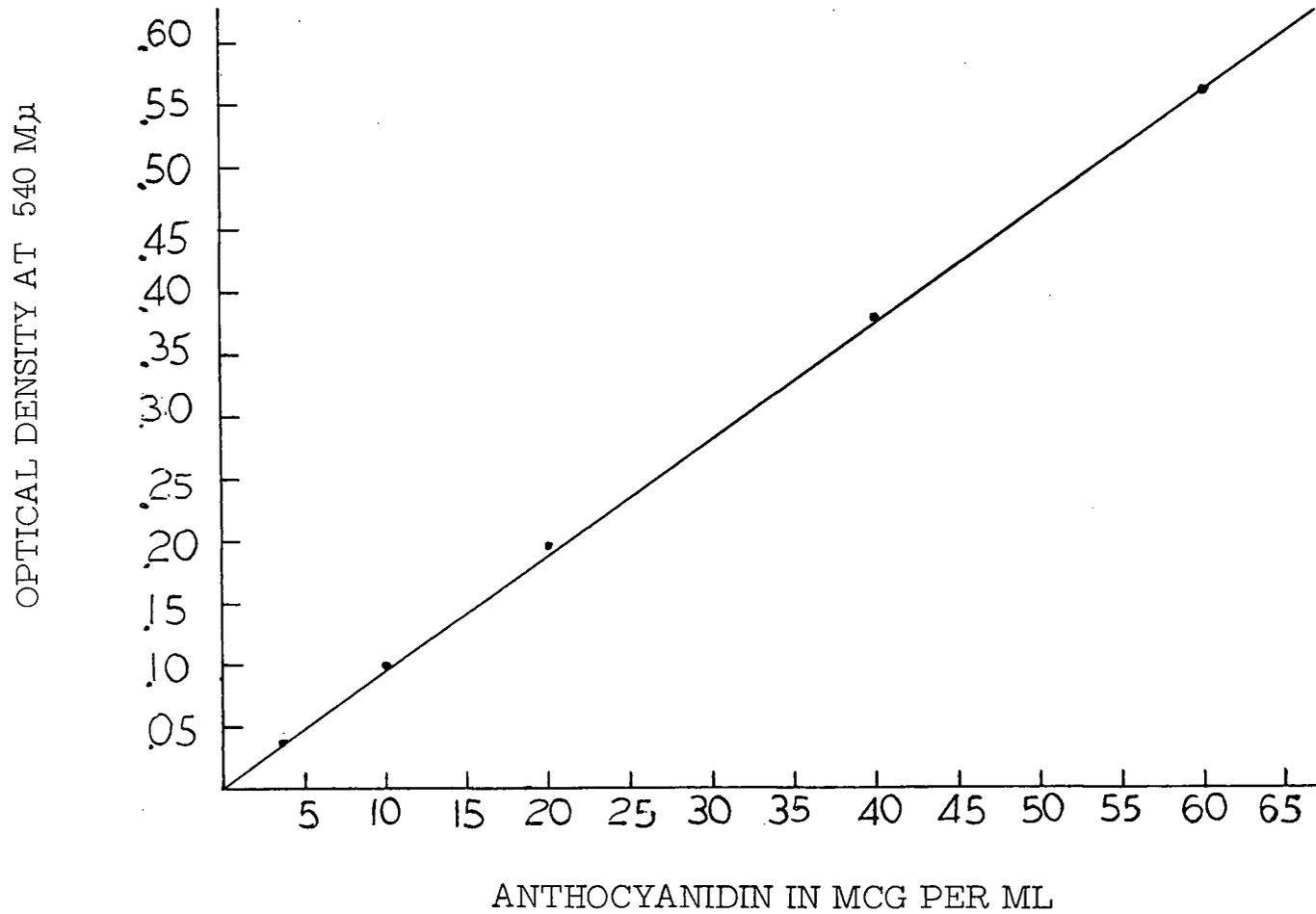
APPENDIX 2

STANDARD CURVE FOR TOTAL PHENOLS



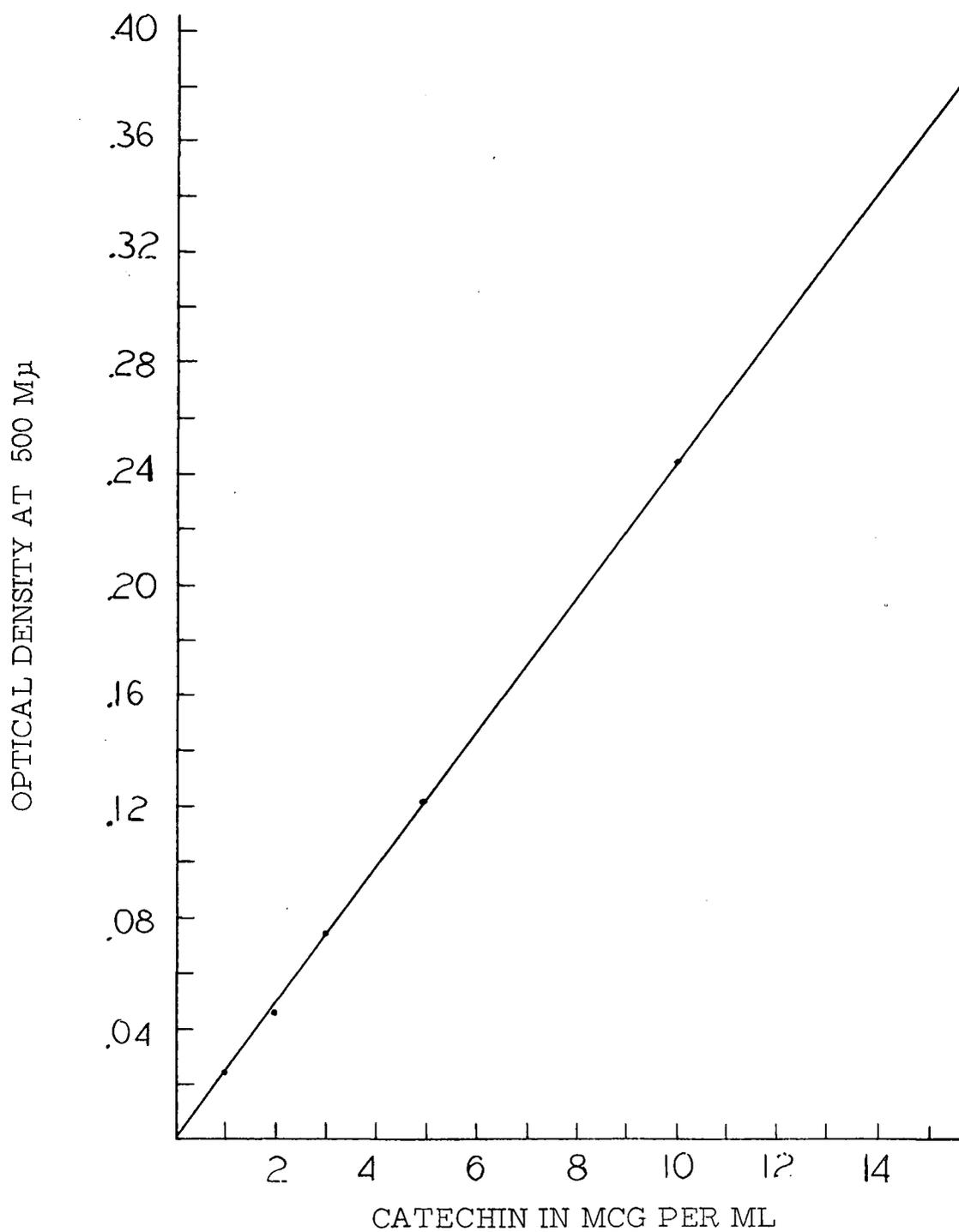
APPENDIX 3

STANDARD CURVE FOR (LEUCO)ANTHOCYANIDIN



APPENDIX 4

STANDARD CURVE FOR FLAVANOLS



APPENDIX 5

STANDARD CURVE FOR 1-ASCORBIC ACID

