

Abstract of Thesis

Marjorie Mae Mayer

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ABSTRACT OF THESIS

ISOLATION AND CHARACTERIZATION
OF POLYPHENOLIC
SUBSTANCES FROM PEACHES

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FORT COLLINS, COLORADO

Submitted by
Marjorie Mae Mayer

In partial fulfillment of the requirements
for the Degree of Master of Science
Colorado
Agricultural and Mechanical College
Fort Collins, Colorado

June 1949

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ABSTRACT

A balance between polyphenolic substances, fruit essences, organic acids and sugars has been suggested by Caldwell (2), 1928, as the determining factor in fruit flavor. Polyphenolic substances are also important in enzymatic discoloration. The need for their isolation and characterization to aid in further work on fruits prompted this study.

A method using a synthetic anion exchange resin was used to isolate polyphenols in crude form from peaches. This method and a general characterization of this polyphenolic material has been described in this paper.

Problem

To what extent and under what conditions is it possible to isolate polyphenolic substances from peaches by the use of a synthetic ion exchange resin?

Problem analysis.--1. To what extent and under what conditions can natural polyphenolic substances extracted from peaches by 80% ethyl alcohol be adsorbed on an anion exchanger?

2. To what extent and under what conditions can adsorbed polyphenolic substances be eluted from an anion exchanger?

3. How may polyphenolic substance be separated from peach organic acids which are also adsorbed on an anion exchanger?

4. What are the general characteristics of the polyphenolic substances obtained by the employed method?

Delimitations.--This problem has been limited to the isolation of crude polyphenolic substances from Elberta peaches by means of synthetic ion exchange resin. The isolated phenolic substances have been characterized only as to type.

Polyphenolic materials were extracted from Elberta peaches with 80% ethyl alcohol. They were separated from other acids of the peach at a pH of 7.0 by adsorption on Duolite A-4, an anion exchange resin. The adsorbed material was eluted with 2% sulfuric acid in 80% ethyl alcohol. The amount of polyphenolic material in the original extract, the amount not adsorbed and the amount eluted were determined with Folin-Denis reagent and expressed as milligrams of tannic acid.

The aliquots eluted before the effluent became acid were dried and both qualitative and quantitative tests made on the resulting material.

Ultraviolet absorption determinations on the original peach extract gave similar results to those obtained by Johnson (3), 1949. Two types of phenolic

substances were shown to be present, the one occurring in the larger quantity gave a maximum ultraviolet absorption at 278-280 millimicrons, and the other type gave a maximum at 322-324 millimicrons. Paper partition chromatography using ascending technique was used to separate and identify two types of tannins from the crude material isolated. After treating the chromatogram with ammonia fumes a blue fluorescent band of phenolic material matched the spot of d-catechin in color and Rf, and a yellow fluorescent band matched the spot of chlorogenic acid. When material extracted from these bands was examined for ultraviolet adsorption with the Beckman Spectrophotometer each was found to possess only one maximum, the blue fluorescent band having a peak at 278 millimicrons and the yellow band having one at 322 millimicrons. From this evidence it was assumed that a caffetannin similar to chlorogenic acid and a catechol tannin similar to d-catechin had been identified.

Suggestions for further study

A much higher percent of yield would be possible if a way could be devised to prevent a loss of polyphenolic substances while removing the acid from the last aliquot eluted. More study is needed here.

In a few chromatograms of some samples a light brown non-fluorescent area was noted at an Rf of

approximately 0.52. This material had a maximum ultraviolet absorption of 280 millimicrons. More research should be done to better determine its character. Possibly it is present in the original extract or it might be formed later. Since most of the chromatograms did not show this material no conclusions could be made concerning it.

A better purification of the crude polyphenolic material is desirable.

Ion exchange resins themselves, especially the anion resins, are the basis of much needed experimentation. Many of their properties are undetermined.

At present the measurements of polyphenolic substances in fruits have to be calculated by comparing with a standard tannic acid solution and hence reported as equivalent to a certain weight of tannic acid. By a more complete isolation of these substances a better method of analysis can be determined.

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T H E S I S

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR
SUPERVISION BY MARJORIE MAE MAYER
ENTITLED ISOLATION AND CHARACTERIZATION OF POLYPHENOLIC
SUBSTANCES FROM PEACHES

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE.

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Chapter I

INTRODUCTION

A balance between polyphenolic substances, fruit essences, organic acids and sugars has been suggested by Caldwell (2), 1928, as the determining factor in fruit flavor. Polyphenolic substances are also important in enzymatic discoloration. The need for their isolation and characterization to aid in further work on fruits prompted this study.

A method using a synthetic anion exchange resin was used to isolate polyphenols in crude form from peaches. This method and a general characterization of this polyphenolic material have been described in this paper.

The problem

To what extent and under what conditions is it possible to isolate polyphenolic substances from peaches by the use of a synthetic ion exchange resin?

Problem analysis.--1. To what extent and under what conditions can natural polyphenolic substances extracted from peaches by 80% ethyl alcohol be adsorbed on an anion exchanger?

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4. What are the general characteristics of the polyphenolic substances obtained by the employed method?

Delimitations.--This problem has been limited to the isolation of crude polyphenolic substances from Elberta peaches by means of synthetic ion exchange resin. The isolated phenolic substances have been characterized only as to type.

Chapter II

REVIEW OF LITERATURE

Polyphenolic substances have been reported by Caldwell (2), 1928, as playing an important part in fruit flavors and by Kertesz (4), 1934, as significant in enzymatic discoloration.

The presence of catechol-tannins in peaches was shown by Onslow (10), in 1920, and Lavollay (6), in 1944, reported the presence of d-catechin in peaches. No attempt was made to isolate the material in either study. Polyphenols were isolated in low yields from Japanese tea by Bradfield (1), 1946, but they were not fully identified. Johnson (3), 1949, using an ultraviolet adsorption technique has shown that two types of phenolic substances are present in peaches and other fruits. The type which occurs in the larger quantity gave a maximum ultraviolet adsorption at 280 millimicrons and the other type gave a maximum at 324 millimicrons.

Ion exchange resins have been successfully employed in the recovery of tartrates from grape wastes according to Matchett (8), 1944, and have been used in various food industries as reported by Mindler (9), 1948, and Stadtman (12), 1948. McColloch (7), 1945, reported

the removal of pectin-methylesterase from commercial pectinases using a synthetic ion exchange resin. Kunin (5), 1947, has reported that the anion exchange abilities of many anions have been shown to be dependent upon their structure, size, valence and the type of salt formed.

Rosenblatt (11), 1949, has described a test using Folin-Denis reagent by which phenolic materials can be determined on a quantitative basis. Wendler and Gage (13), 1949, have described a paper partition chromatographic method for the qualitative determination of flavonoid pigments.

Chapter III

METHODS AND MATERIALS

The methods used for the isolation of the crude polyphenolic substances from Elberta peaches involved extraction with ethyl alcohol, removal of ethyl alcohol, adsorption on and elution from a synthetic anion exchange resin and drying at a low temperature with reduced pressure. The details of the isolation are described in the following paragraphs.

Pitted peaches, 100 grams at a time were blended with 400 ml. of 80% ethyl alcohol in a Waring Blender for five minutes. The resulting material was centrifuged to remove pulp and precipitates. The decantate was distilled with reduced pressure using a water bath at a temperature between 40 and 50 degrees C^o to remove the alcohol. The extract was then filtered with reduced pressure using CELITE FILTER-AID. A 50 ml. aliquot was frozen and saved for tests to be performed later. The remaining extract, yellow in color, with a pH of approximately 3.8 was adjusted with concentrated NaOH to a pH of 7.0 using a Beckman pH meter. The color of the solution changed to brown as it became neutral.

The extract, then ready for adsorption, was

tested with Folin-Denis reagent 1/ to determine the amount of polyphenolic substances present. A blue color resulted, the density of which was determined in an Evelyn Colorimeter. By calculation the amount of polyphenolic material was obtained and expressed as milligrams of tannic acid. The apparatus used is shown in Figure 1.

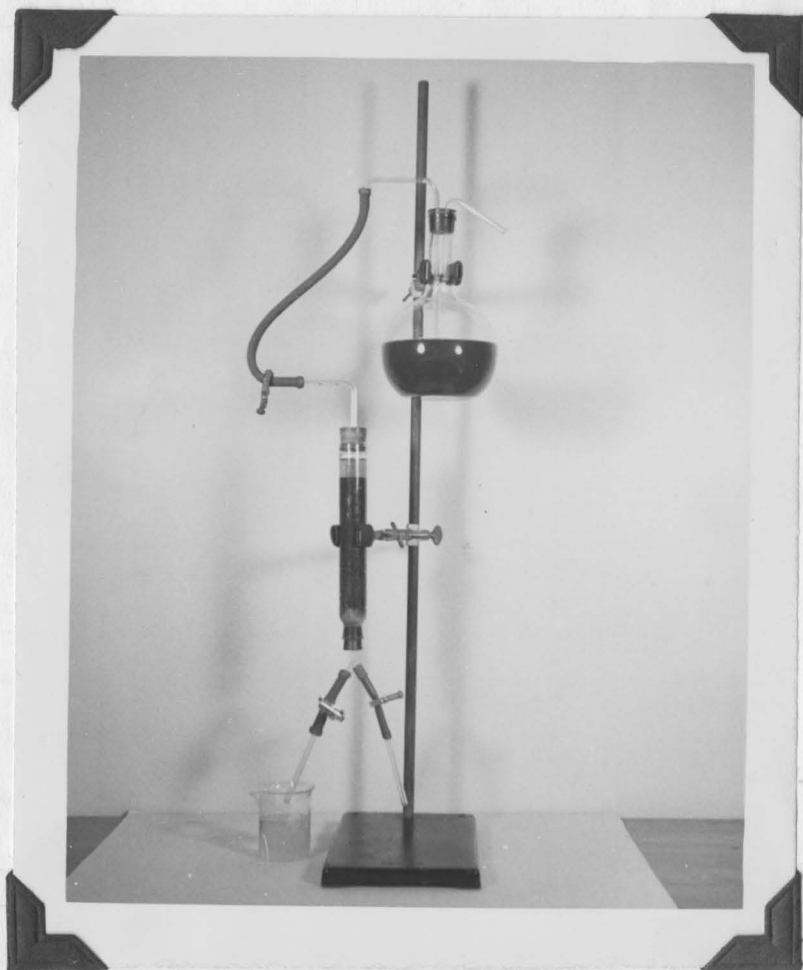


Figure 1.--Apparatus used for adsorption and elution of polyphenolic substances.

1/ The Folin-Denis test with instruction for the preparation of the reagent was described by Rosenblatt (11), 1941.

The extract was placed in a liter Florence flask. Part of the air was removed by suction to minimize oxidation. The glass cylinder was made by cutting off the bottom of a stoppered graduated cylinder and turning it upside down. Glass wool was placed in the bottom of the column and glass beads added. Thirty grams of anion exchange resin which had been soaked for several days were then packed into the cylinder.

Adsorption technique

Duolite A-2 2/ was used for the first experiments but it was later replaced by Duolite A-4 2/. The resin was put through several cycles of exhaustion with 5% sulfuric acid and regeneration with 5% ammonium hydroxide before it was used. It was then thoroughly back-washed until the wash water maintained a neutral pH as tested with pH paper. The wash water was drained from the column and the flask containing the peach extract connected. The clamps were adjusted so the extract dripped at approximately one drop per second. As the brown extract moved through the resin bed polyphenolic substances were adsorbed on the resin. The resulting light yellow effluent was collected. Folin-Denis tests were made frequently to follow the adsorption of polyphenols. All of the material was put through the column, care

2/ These resins were obtained from the Chemical Process Company, San Francisco 4, California.

having been taken that the column was not allowed to become dry at any time. The volume of the effluent was measured and a test made with Folin-Denis reagent to determine the exact amount of polyphenolic material not adsorbed by the resin. By subtracting this amount from the amount ascertained in the original extract before adsorption, the amount adsorbed was obtained. Fifty ml. of the material which passed through the column were frozen and saved for later tests.

Elution technique

The resin column was thoroughly backwashed with water and finally rinsed with 80% ethyl alcohol. A one liter Florence flask containing sulfuric acid in 80% ethyl alcohol was connected to the column and replaced the flask which had contained the peach extract. The acid solution was allowed to drip through the resin bed at approximately one drop every two seconds. At each noticeable change in eluted material as detected, by Folin-Denis tests, change in color, or change in pH, aliquots of the material were segregated. When no more tannin material was being eluted the volumes of various aliquots were determined, pH measurements taken with a Beckman pH meter and tannin tests made. The sum of the amounts of tannin-like material eluted was obtained to determine the efficiency of the elution process.

Those portions of eluted crude polyphenolic materials above a pH of 4.5 were dried with reduced pressure. The material with pH lower than 4.5 was not dried, because of possible chemical changes when heating in acid. It was neutralized with concentrated NaOH and the resulting salt filtered out. The alcohol was removed by distillation under reduced pressure and the pH adjusted to 7.0. The salt formed was centrifuged out. The decantate was taken to dryness with a water bath at a temperature of 40°-50° C. under reduced pressure of approximately 1 1/2 inches of mercury and finally in a vacuum oven. The powdered material was treated with several portions of 80% ethyl alcohol at a temperature of approximately -25° C. to extract polyphenolic material from any salt present.

Total acidity determination

To test the assumption that the organic acids of the peach were not adsorbed on the anion resin at a pH of 7.0, 40 ml. aliquots of original peach extract and the total effluent that had passed through the resin column were each treated with batches of excess Duolite C-3 ^{3/} a cation exchanger. The extracts were placed in two beakers and recently regenerated wet Duolite C-3 added and stirred until the pH became constant. This

^{3/} This resin was secured from Chemical Process Company, San Francisco 4, California.

resin was filtered off and aliquots of the liquid were titrated with standard NaOH to a pH of 8.0. The milliequivalents of acid in each extract were figured and compared.

Characterization tests

Tannin analyses of the various eluates and of the final dried samples were made with Folin-Denis reagent.

Paper partition chromatography as described by Wendler and Gage (13), 1949, using an ascending technique was employed to separate two types of tannin. A band of the isolated crude polyphenolic material and spots of concentrated solutions of chlorogenic acid 4/ and d-catechin 5/ were placed on Whatman filter paper No. 1, and chromatographed in 77% ethyl alcohol for 24 hours. The chromatogram before and after being exposed to ammonia fumes was examined under an ultraviolet light having a maximum emission at 3650A. The fluorescent areas were outlined, cut out and placed in test tubes and covered with water and ultraviolet absorption of the resulting solutions were determined.

Ultraviolet light absorption was determined

4/ Obtained from Central Laboratories, General Foods Corporation. Hoboken, New Jersey.

5/ Obtained from S. B. Penick and Company, Research Division 999W. Side Avenue, Jersey City 6, New York.

with a Beckman spectrophotometer on aliquots of the original extract, those collected at regular intervals during the elution, and upon the material separated by paper partition chromatography.

Chapter IV

ANALYSIS OF DATA

The following data have been arranged in the same order as the questions in the problem analysis, i.e. (1) the extent and conditions under which polyphenolic substances extracted from peaches were adsorbed and (2) eluted from a synthetic anion exchange resin, (3) the determination of total acidity of the peach extract before and after anion treatment showing a separation of polyphenolic material from other organic acids, and (4) type characteristics of the polyphenolic substances isolated in this study.

Adsorption and elution

Several experiments using different samples of peaches were performed to determine the best conditions of adsorption and elution. The process was essentially the same in each case and was described in the previous chapter. The only differences were in the resin and the elutrient used. Table 1 shows results of these experiments. The highest yield of crude polyphenolic material was obtained with sample 33 using Doulite A-4, and 2% sulfuric acid in 80% ethyl alcohol as an elutrient. The peaches had a very high tannin content and there was

sufficient material to completely exhaust the resin.

Because of the complex properties of the anion exchange resin there are many variables, and thus no exact significance can be attached to the different amounts adsorbed and eluted in the various experiments tabulated in Table 1. However the data showed that under conditions as described it is possible to adsorb and elute a large percent of the polyphenolic substances from peaches thus answering the first two questions of the problem analysis.

Separation of polyphenolic
substances from organic
acids of the peach

Total acidity determinations on aliquots of three samples before and after anion exchange treatment, showing that organic acids of the peach were not adsorbed to any appreciable extent or the anion exchange resin at a pH of 7.0, have been tabulated in Table 2. In all trials the original extract had a pH of approximately 3.8 and the extract which had undergone anion exchange had a pH of 8.9. Both reached equilibrium at a pH of 2.5 when treated with excess Doullite C-3. Aliquots of both titrated with NaOH were nearly the same in acidity as shown by milliequivalents. A good separation of polyphenols and other organic acids was effected giving an affirmative answer to the third question of the problem analysis.

Table 1.--COMPILATION OF DATA OBTAINED ON ADSORPTION AND ELUTION OF POLYPHENOLIC SUBSTANCES FROM PEACHES SHOWING RESULTS OF TESTS USING TWO DIFFERENT ANION RESINS AND SIX DIFFERENT ELUTRIENTS

Sample Number	Weight Grams	Resin Duolite	Elutrient	Milligrams of Tannin (Expressed as tannic acid)					
				Original	After	Total	Eluted		
				Extract	Adsorption	Adsorbed	Non acid	Acid	Total
7	800	A-2	5% HCl in 80% ethyl alcohol	882	131	751	111	168	279
9	1000	A-2	5% HCl in 80% ethyl alcohol	1231	377	854	199	358	557
11	1000	A-2	5% HCl in 80% ethyl alcohol	1259	753	506	16	357	373
15	1000	A-2	2% HCl in 80% ethyl alcohol	585	109	476	166	171	337
17	1000	A-2	5% CCl_3COOH in 30% ethyl alcohol	736	136	600	90	159	249
19	1400	A-2	5% CCl_3COOH in 85% ethyl alcohol	1038	250	788	141	362	503
21	1000	A-2	5% H_2SO_4 in 80% ethyl alcohol	626	217	409	33	200	233
23	1400	A-4	2% H_2SO_4 in 80% ethyl alcohol	1133	128	1005	47	681	728
25	1400	A-4	2% H_2SO_4 in 80% ethyl alcohol	974	135	839	68	274	342
27	1350	A-4	2% H_2SO_4 in 80% ethyl alcohol	932	125	807	260	244	504
29	2900	A-4	2% H_2SO_4 in 80% ethyl alcohol	1820	628	1192	270	733	1003
33	2000	A-4	2% H_2SO_4 in 80% ethyl alcohol	3056	748	2308	602	951	1553
39	2400	A-4	2% H_2SO_4 in 80% ethyl alcohol	1660	326	1334	186	469	655

Table 2.--TOTAL ACIDITY DETERMINATIONS ON PEACH EXTRACT BEFORE AND AFTER ANION EXCHANGE

	Original Extract			Extract After Anion Exchange		
	Sample 33	Sample 29	Sample 17	Sample 33	Sample 29	Sample 17
pH before cation exchange	3.9	3.7	3.8	8.9	9.0	8.9
pH after cation exchange	2.5	2.5	2.5	2.5	2.5	2.5
Milliliters extract titrated	40.0	40.0	40.0	40.0	40.0	40.0
Milliliters standard NaOH to adjust to pH of 8.0	(0.1208N) 34.8	(0.1104N) 36.8	(0.1104N) 38.0	(0.1208N) 31.6	(0.1104N) 33.2	(0.1104N) 34.8
Total acidity in milliequivalents per milliliter	1.05.	1.016	1.051	0.954	0.920	0.954
Difference in acidity before and after anion adsorption in milliequivalents per milliliter	0.097	0.096	0.097			

Characterization of the
isolated polyphenolic
material

Ultraviolet absorption determinations made on polyphenolic substances precipitated from original peach extracts have been shown in Table 3 and graphed in Figure 2.

The results of ultraviolet absorption determinations made on two drop aliquots collected at 15 minute intervals during the elution process showed the relative amounts of two polyphenolic materials being eluted throughout the process. These data have been given in Table 4 and graphed in Figure 3.

Ultraviolet absorption data for a sample of crude isolated material (33-2) have been shown in Table 5 and graphed in Figure 4. When this crude material was chromatographed (Figure 5) two separate types of polyphenolic material resulted. The data for these ultraviolet absorption determinations have been given in Table 6 and graphed in Figures 6 and 7.

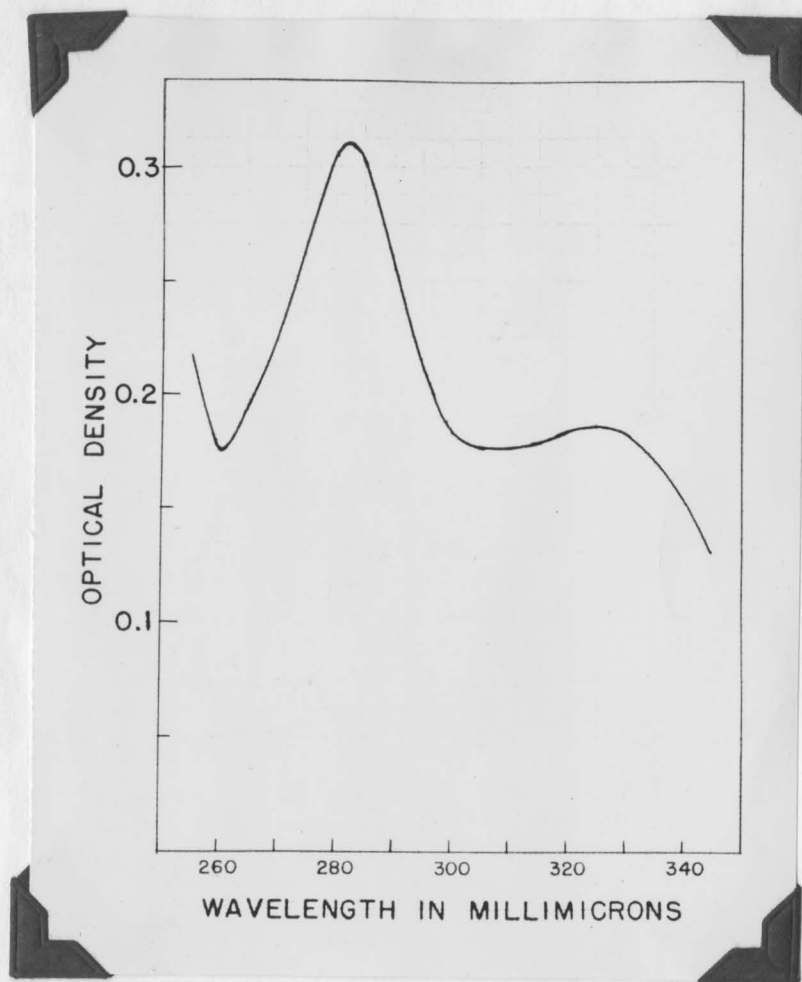


Figure 2.--Ultraviolet absorption determinations on polyphenolic material precipitated from original peach extract at a pH of 8.0 by lead acetate, Johnson (3), 1949. (Data given in Table 3.)

Two types of polyphenolic materials are shown in the above figure, one with maximum absorption at 280 millimicrons and the other at 322 millimicrons.

Table 3.--ULTRAVIOLET ABSORPTION DATA ON POLYPHENOLIC SUBSTANCES PRECIPITATED BY LEAD ACETATE AT A pH OF 8.0 FROM THE ORIGINAL PEACH EXTRACT, (JOHNSON (3), 1949). GRAPHED IN FIGURE 2.

Wave Length in Millimicrons	Optical Density
250	0.258
260	0.175
270	0.225
278	0.290
280	0.300
282	0.310
284	0.300
290	0.255
300	0.177
315	0.176
320	0.180
322	0.185
325	0.185
330	0.179
340	0.150

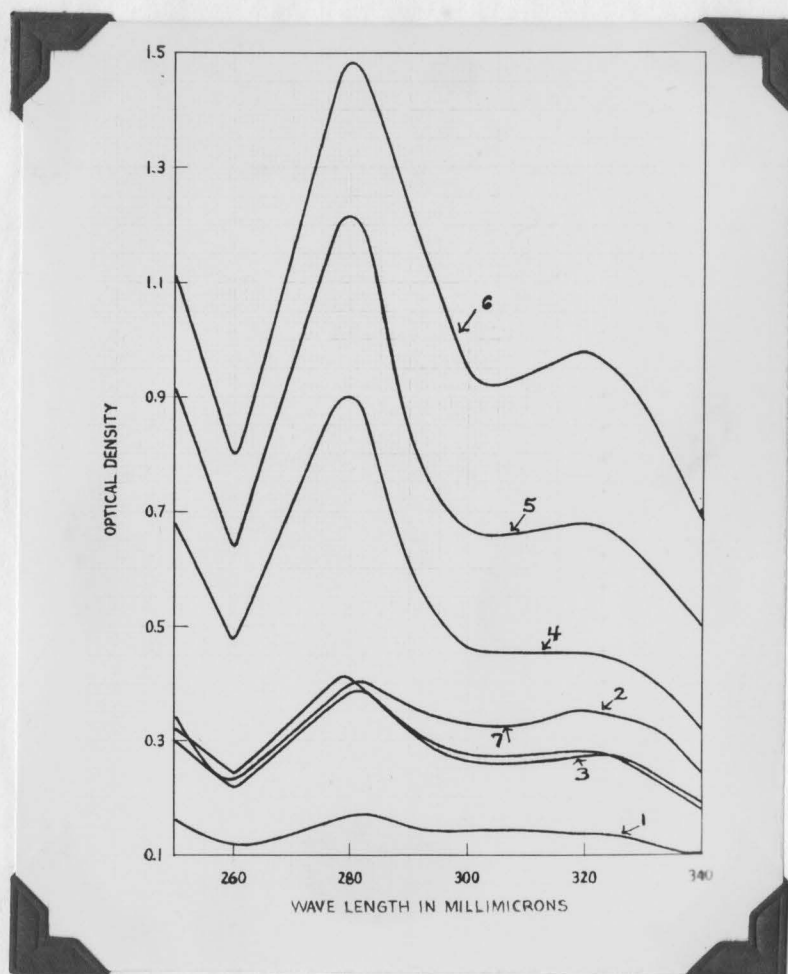


Figure 3.--Ultraviolet absorption determinations (Sample 33) on two drop aliquots of eluted material collected at 15 minute intervals. Elutrient 2% sulfuric acid in 80% ethyl alcohol. (Data in Table 4.)

Aliquot 1 showed the presence of a small amount of material with a maximum at 280 millimicrons. Aliquots 2, 3 and 7 showed more of that material and also a second material with a maximum at 324 millimicrons. Numbers 4, 5, 6 each had increasing amounts of both materials present.

Table 4.--ULTRAVIOLET ABSORPTION DETERMINATIONS (SAMPLE 33) ON TWO DROP ALIQUOTS OF ELUTED MATERIAL COLLECTED AT 15 MINUTE INTERVALS. ELUTRIENT 2% SULFURIC ACID IN 80% ETHYL ALCOHOL. GRAPHED IN FIGURE 3.

Wave Length in Millimicrons	Optical Density						
	Aliquot Numbers						
	1	2	3	4	5	6	7
250	0.154	0.320	0.297	0.680	0.920	1.164	0.336
260	0.113	0.235	0.210	0.462	0.628	0.784	0.220
270	0.132	0.307	0.300	0.705	0.942	1.136	0.320
278	0.152	---	0.377	0.895	1.200	1.450	0.405
280	0.162	0.392	0.384	0.900	1.216	1.480	0.410
282	0.165	0.397	0.384	0.880	1.200	1.472	0.408
284	0.162	0.389	0.375	---	---	---	---
290	0.140	0.350	0.303	0.590	1.150	1.120	0.306
300	0.134	0.329	0.267	0.452	0.656	0.930	0.254
305	0.132	0.330	0.262	0.450	0.654	0.924	0.252
310	0.135	0.336	0.270	0.450	0.660	0.940	0.255
315	0.138	0.342	0.272	0.450	0.670	0.960	0.260
318	---	0.344	0.272	0.450	0.672	0.972	---
320	0.139	0.345	0.271	0.450	0.672	0.976	0.264
322	0.138	0.341	0.269	0.445	0.678	0.978	0.264
325	0.131	0.334	0.262	0.439	0.654	0.960	0.260
330	0.127	0.328	0.243	0.410	0.612	0.900	0.245
340	0.093	0.230	0.177	0.307	0.450	0.680	0.139

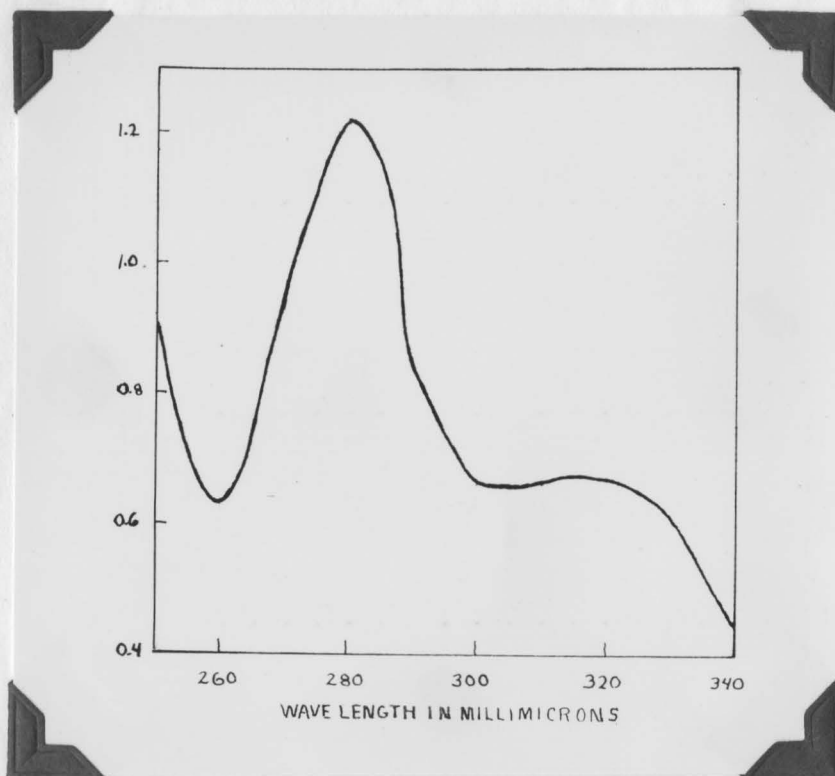


Figure 4.--Ultraviolet absorption determinations on crude polyphenolic material (Sample 33-2) eluted with 2% sulfuric acid in 80% ethyl alcohol at a pH of 4.5. (Data in Table 5.)

The crude polyphenolic material isolated by the described method showed the presence of the two characteristic peaks, one at 280 and the other at 320 millimicrons, just as in the determination on the original material.

Table 5.--ULTRAVIOLET ABSORPTION DETERMINATIONS ON CRUDE POLYPHENOLIC MATERIAL (SAMPLE 33-2) ELUTED WITH 2% SULFURIC ACID IN 80% ETHYL ALCOHOL AT A pH OF 4.5. GRAPHED IN FIGURE 4.

Wave Length in Millimicrons	Optical Density
250	0.920
260	0.628
270	0.924
278	1.200
280	1.216
282	1.200
284	1.150
290	0.836
300	0.656
305	0.654
310	0.660
315	0.670
318	0.671
320	0.672
322	0.668
325	0.654
330	0.612
340	0.450

Paper partition chromatography was employed to separate the crude tannin material isolated into two types. An illustration follows:

CHROMATOGRAM

(Copy)

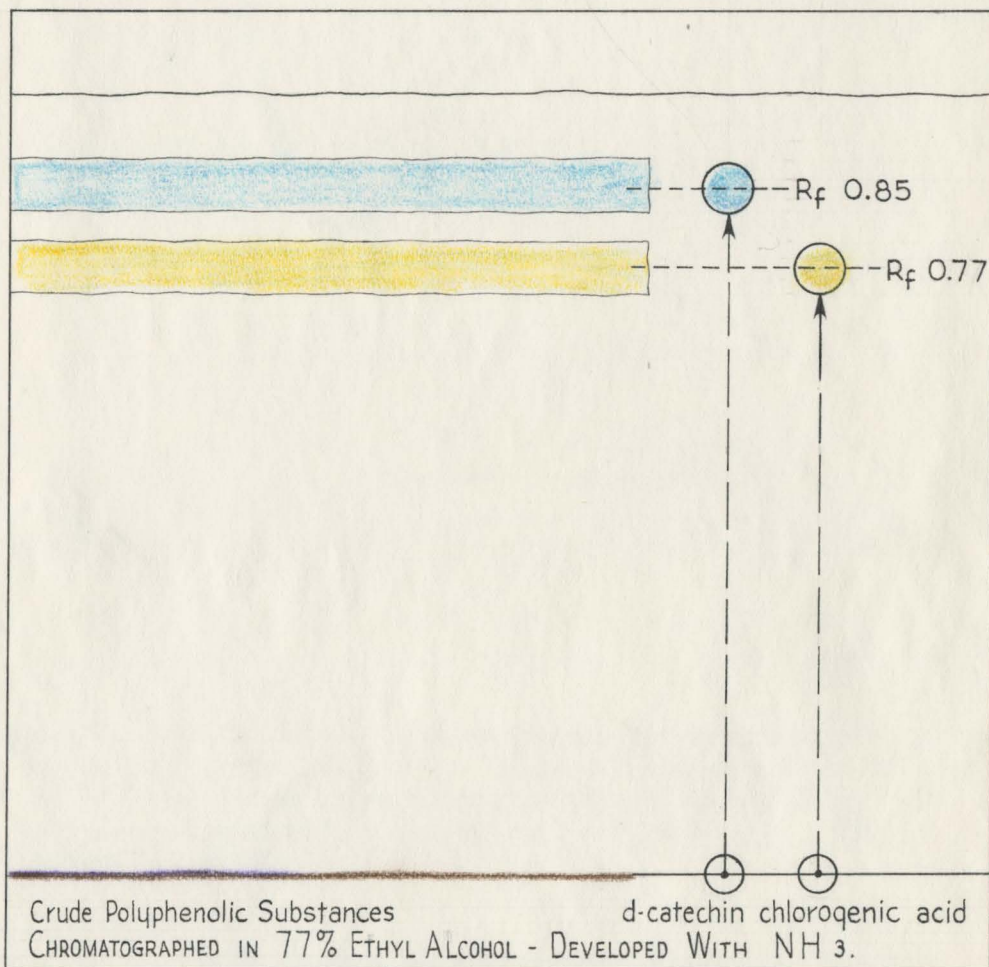


Figure 5.--Results obtained when a band of crude polyphenolic material and spots of d-catechin and chlorogenic acid were chromatographed in 77% ethyl alcohol 24 hours, and developed with ammonia.

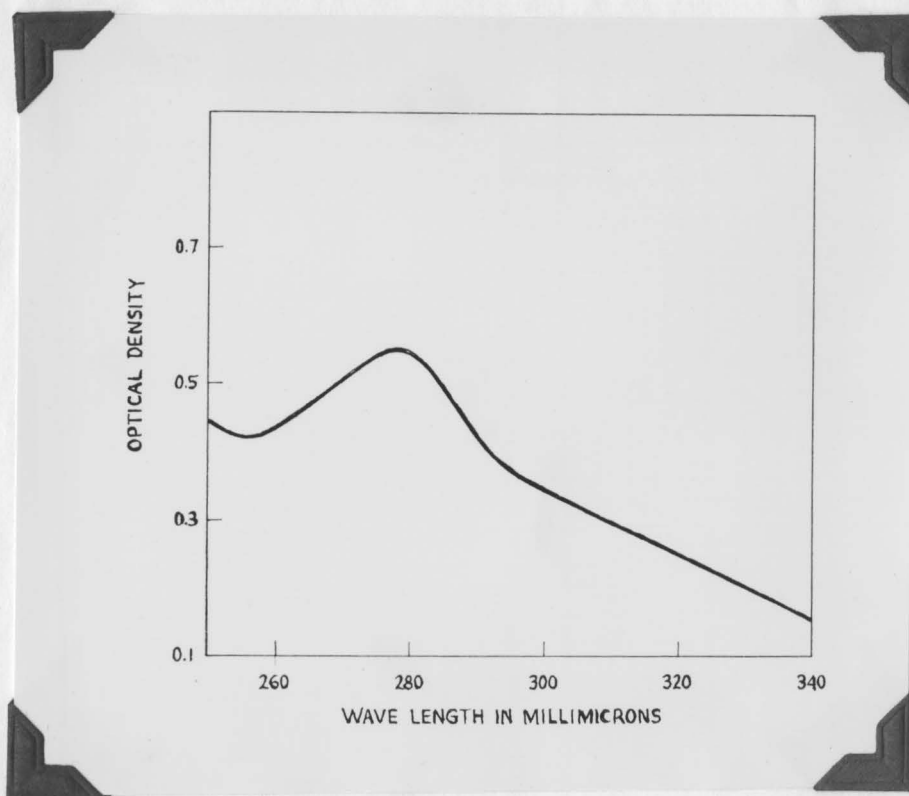


Figure 6.--Ultraviolet absorption determinations on material extracted from blue fluorescent band, Rf 0.77, cut from chromatogram which separated the crude polyphenolic material. (Figure 5) (Data shown in Table 6.)

The material extracted from the blue fluorescent band had only one peak showing a maximum adsorption at 278 millimicrons.

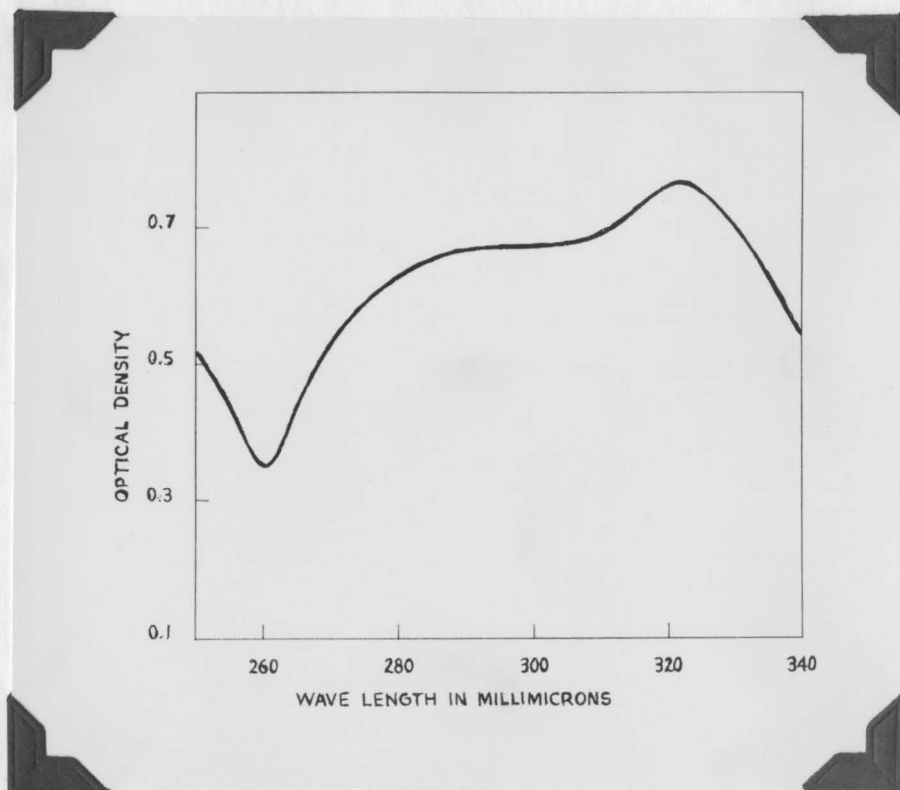


Figure 7.--Ultraviolet absorption determinations on material extracted from yellow fluorescent band Rf, 0.85, cut from chromatogram which separated the crude polyphenolic material. (Figure 5) (Data shown in Table 6.)

The material extracted from the yellow fluorescent band had only one peak showing maximum absorption at 322 millimicrons.

Table 6.--ULTRAVIOLET ABSORPTION DATA FOR TWO TANNINS
SEPARATED BY PAPER CHROMATOGRAPHY FROM 10 MILLIGRAMS
OF CRUDE POLYPHENOLIC MATERIAL (SAMPLE 33-2)
DEVELOPED WITH AMMONIA. (GRAPHS SHOWN IN FIGURES
6 AND 7.)

Wave Length in Millimicrons	Optical Density	
	Blue Fluorescent Band Rf 0.85	Yellow Fluorescent Band Rf 0.77
250	0.441	0.570
255	0.424	0.460
260	0.434	0.390
270	0.514	0.490
276	0.536	0.605
278	0.540	0.640
280	0.534	0.670
282	0.520	0.700
285	0.500	0.710
290	0.410	0.722
300	0.340	0.734
310	0.314	0.750
315	0.280	0.780
318	0.270	0.800
322	---	0.805
324	0.250	0.800
326	0.234	0.795
330	0.214	0.760
340	0.160	0.570
350	0.120	0.330

Summary of data presented

Adsorption, elution and separation.--As evidenced by the foregoing tables polyphenolic material was extracted from Elberta peaches and adsorbed at a pH of 7.0 on Duolite A-2 and Duolite A-4, synthetic anion exchange resins, and thus separated from other acids of the peach.

The adsorbed polyphenolic material was eluted with acidified alcohol.

Characterization.--Ultraviolet absorption determinations showed that the crude material eluted contained varying amounts of polyphenolic materials of the two types having maximum absorption at approximately 280 and 324 millimicrons.

Two tannins were separated from the crude material eluted by the use of paper partition chromatography. Ultraviolet absorption of these isolated substances showed one to have a maximum similar to that of d-catechin, a catechol tannin, at 278 millimicrons, and the other a maximum of 322 similar to that of chlorogenic acid, a caffetannin. The Rf values and color of chromatographed materials gave further evidence of the similarity of these isolated materials to catechol and caffe-tannins.

Chapter V

DISCUSSION

Adsorption

It was found by experimentation that natural polyphenolic substances extracted from peaches could be adsorbed on synthetic anion exchange resin. Duolite A-4 proved better than Duolite A-2 for this purpose. It had a greater capacity for polyphenolic substances than did Duolite A-2. Its characteristic color changes with exhaustion and regeneration were easy to follow. Many factors affected the adsorption. For a maximum adsorption all of the alcohol had to be removed from the extract. The temperature of the extract was important. If lower than room temperature poor adsorption resulted. The rate of flow of the extract had to be controlled to allow plenty of time for the polyphenolic substances to be adsorbed on the resin. In preliminary experiments 15 minutes contact with the resin seemed best as most of the polyphenolic material was adsorbed by this time and no appreciable increase in adsorption took place for several hours. The column itself had to be recently regenerated for best results. A column that remained in a regenerated condition for several days did not give as

good results as one completely regenerated just previous to its use. The total amount of material adsorbed decreased as the column was used from time to time, the greatest being adsorbed when the column was first used. Since little is known about the exact properties of the resin an assumption was made that some of the phenolic material combined chemically with some of the amino groups of the resin. If some of these groups are tied up chemically the adsorption will decrease with use of the resin. This assumption is in accord with reports of Kunin (5), 1948.

Elution

The elution of the adsorbed polyphenolic substances necessitated a great deal of experimentation. A column previously treated with the same polyphenolic material gave up more of that material than one being used for the first time. The column was as nearly exhausted as possible with the material to be eluted. Possibly some of the amino groups of the resin first reacted chemically with some of the polyphenols and held them in such close chemical combination as to prohibit ion exchange. For this reason only part of the adsorbed material could be recovered by elution. But as is also shown by Kunin (5), 1948, a greater percent of material adsorbed will be eluted after the column has been used

several times because when the groups on the resin which react chemically are once tied up the other groups act as ion exchangers and continue to adsorb and give up the material which they have adsorbed. Another evidence of a chemical reaction with the resin was the change in color of the resin itself. The more it was used the darker it became even when exhausted with 5% sulfuric acid.

Hydrochloric, sulfuric and trichloroacetic acids in varying strengths with different concentrations of ethyl alcohol were tried as elutrients. It was decided that 2% sulfuric acid in 80% ethyl alcohol was very effective. All gave a fair elution but sulfuric acid proved easiest to remove later when trying to isolate the polyphenolic material from the acid aliquot.

The first aliquot eluted with a high pH was light yellow and contained very little phenolic material. The next aliquot was neutral according to test paper and light red in color. It contained the highest percent of tannin like material. However, a high percent of tannin material also came off in the last portion which was dark red and very low in pH. All of the aliquots but the acid one were dried under reduced pressure at low temperatures. These dried polyphenolic substances were highly astringent and reddish brown in color. To recover the polyphenolic material in the last aliquot the

acid had to be removed by precipitation or neutralization. Part of the polyphenolic material was isolated from this aliquot but the loss was high. No method has been found to remove the acid which does not also remove a large amount of the polyphenols.

Separation from organic acids

In order to isolate the polyphenolic material it was necessary to separate it from the other acids of the peach. Preliminary experiments using a solution containing 0.5% citric acid and 0.1% tannic acid instead of peach extract were performed to see if a way could be devised to separate these two acids. Both batch and column methods using anion exchange resins were tried. It was found that at a pH of 7.0 there was a good separation of these two acids, the tannic acid being adsorbed and the citric acid going through the resin bed in the effluent.

When the peach extract was adjusted to a pH of 7.0 a similar result was obtained as was shown by total acidity determinations. The polyphenolic material was adsorbed and thus separated from the other acids of the peach.

Characterization

The densities of blue color produced in solutions containing 0.1 mg. per 100 ml. of polyphenolic

material by Folin-Denis reagent were determined with an Evelyn Colorimeter. Tannic acid solution had a density of 0.173, d-cathechin 0.164, chlorogenic acid 0.132 and samples of the crude material isolated ranged from 0.081 to 0.094. These figures for the crude material can not be taken as absolute criteria of purity because not enough is known about their structure, however there is an indication that these crude materials contain a high concentration of polyphenolic substances.

Ultraviolet absorption determinations on the original peach extract gave similar results to those obtained by Johnson (3), 1949. Two types of phenolic substances were shown to be present, the one occurring in the larger quantity gave a maximum ultraviolet absorption at 278-280 millimicrons, and the other type gave a maximum at 322-324 millimicrons. Paper partition chromatography using ascending technique was used to separate and identify two types of tannins from the crude material isolated. After treating the chromatogram with ammonia fumes a blue fluorescent band of phenolic material matched the spot of d-catechin in color and Rf, and a yellow fluorescent band matched the spot of chlorogenic acid. When material extracted from these bands was examined for ultraviolet absorption with the Beckman Spectrophotometer each was found to possess only one maximum, the blue fluorescent band having a peak at

278 millimicrons and the yellow band having one at 322 millimicrons. From this evidence it was assumed that a caffetannin similar to chlorogenic acid and a catechol tannin similar to d-catechin had been identified.

Suggestions for
further study

A much higher percent of yield would be possible if a way could be devised to prevent a loss of polyphenolic substances while removing the acid from the last aliquot eluted. More study is needed here.

In a few chromatograms of some samples a light brown non-fluorescent area was noted at an Rf of approximately 0.52. This material had a maximum ultraviolet absorption of 280 millimicrons. More research should be done to better determine its character. Possibly it is present in the original extract or it might be formed later. Since most of the chromatograms did not show this material no conclusions could be made concerning it.

A better purification of the crude polyphenolic material is desirable.

Ion exchange resins themselves, especially the anion resins, are the basis of much needed experimentation. Many of their properties are undetermined.

At present the measurements of polyphenolic substances in fruits have to be calculated by comparing with a standard tannic acid solution and hence reported

as equivalent to a certain weight of tannic acid. By a more complete isolation of these substances a better method of analysis can be determined.

Chapter VI

SUMMARY

Polyphenolic substances extracted from Elberta peaches with 80% ethyl alcohol were adsorbed on Duolite A-4, synthetic anion exchange resin at a pH of 7.0, and thus separated from other organic acids of the peach.

The adsorbed polyphenolic substances were eluted with 2% sulfuric acid in 80% ethyl alcohol and dried at a low temperature with reduced pressure.

The isolated material contained polyphenolic substances, which exhibited properties similar to d-catechin and chlorogenic acid, as evidenced by chromatography, fluorescence, and ultraviolet absorption determinations.

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