was caused to spread itself in a film over the inside of the dish and a drop of a sulphuric acid solution of diphenylamine allowed to trail over the film.

The solution of diphenylamine was made by dissolving it in concentrated sulphuric acid and then diluting with water almost to the point of precipitation.

In each case where the flour was reported to have been artificially bleached the drop left a blue path, while no coloration was perceptible in the cases of the unbleached flour. In some instances the blue was not so sharp as might be wished for but was in all cases distinguishable.

A flour bleached by a process using ozone alone as a bleaching agent would probably not respond to the diphenylamine test. Such a process, however, is not used in the West to the writer's knowledge. It is also possible but highly improbable that a flour might be found which, fresh from the wheat, would yield the blue color when tested. To decide these points a much larger number of samples will be examined.

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[CONTRIBUTION FROM THE DIVISION OF FOODS, BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE. SENT BY H. W. WILEY.]

GROWTH AND RIPENING OF PERSIMMONS.[1]

BY W. D. BIGELOW, H. C. GORE AND B. J. HOWARD.

Received April 3, 1906.

THE present work is a part of the systematic study of the ripening of fruits which we have undertaken, and regarding which two reports have already been made.[2] It has been found impossible to make as complete a study of the composition of a given fruit at various stages of its growth as is desirable, owing to the fact that some of the important constituents are present in many fruits in such a small amount that the limits of error of their estimation effectually preclude the possibility of determining the rate of their increase and decrease.

[1] Presented at the New Orleans Meeting of the American Chemical Society, December 30, 1905.

[2] U. S. Dept. Agr., Bur. Chem., Bull. 94, Studies on Apples, and U. S. Dept. Agri., Bur. Chem., Bull. 97, Studies on Peaches.

For this reason we have thought it advisable to select certain fruits because of their large percentage of constituents which in other fruits are present to a very small extent. It is not suggested that we can assume necessarily that a given constituent is subject to the same changes in all fruits. At the same time it is believed that the information gained by the study of the fruit in which a given constituent occurs in considerable quantity will be of value in affording a working hypothesis for the study of other fruits.

The persimmon was selected largely because of its content of a relatively large amount of tannin. This substance occurs in the apple and peach, regarding which studies have already been reported,[1] in such small amounts that the error of its determination is relatively large. Notwithstanding this, however, the tannin content is an important consideration with many fruits,[2] and its changes during the growth and ripening of the fruit are of considerable theoretical and practical importance.

The literature relating to the composition of persimmons is very meagre. The articles with which we are familiar are given in Table I. Besides the work on the fruit two articles[3] on persimmon products are included, and three articles[4] deal with the ripening of persimmons.

In the work here reported both wild (*Diospyros Virginiana L.*) and Japanese (*Diospyros Kaki L.*) persimmons were studied. Of the Japanese persimmons only the later stages of their ripening were considered. For the study of the growth of wild persimmons successive samples of fruit were taken from two trees during the growing season, and in addition examinations were made of fruit from a third tree at two periods of growth. The trees were located in Tacoma Park, Md. Tree No. 1 was located in the woods, tree No. 2 was located by a road-side, and tree No. 3 was on the edge of the woods. The fruit was gathered by one

[1] Loc. cit.

[2] E. g. in grapes to be used for wine-making, and in apples to be used for cider manufacture, see U. S. Dept. Agri., Bur. Chem., Bull. 71, p. 28.

[3] Ishii (Bull. Coll. Agri., Tokyo, 2, 101 (1894)) obtained mannose from persimmon seed. Tsukamoto: Ibid. 4, 329 (1900).

[4] Gerber: Annales des sciences naturelles, 4, 1 (8) (1896). Also, Botanical Magazine, Tokyo, 14, 179 (1900). Sawamura: Bull. Coll. Agric. Tokyo, 5, 237 (1902).

of the writers on the morning of the day on which the analyses were made, care being taken at each picking to secure as representative a sample as possible by taking fruit from different sides of the tree, and from the upper and lower branches.

The sampling presents perhaps the greatest source of error in connection with a study of this nature. Since the results are to be calculated to the weight of each ingredient in a single fruit, they can only be correct when the average weight of the fruits of each sample is the same as the average weight of fruits on the tree at the time of picking. The difficulty of approximating the average sized fruit in making a sample is obvious. We have attempted to minimize this difficulty as far as possible by selecting a large number of fruits at each date of sampling.

The Japanese persimmons were secured through the Office of the Pomologist of the Department from the Experiment Station at Auburn, Alabama.

METHODS OF ANALYSIS.

The sample was weighed, calyx removed and weighed, and the pulp and seeds separated and weighed. In case of the wild, ripe persimmons the separation of the pulp and seeds was a matter of some difficulty as the seeds were enclosed by a slippery envelope, which must be cut (best with a dull knife edge) in order to separate the seed cleanly. In this study it was essential that the separation be sharply made. This was attained in all cases. The sample was passed through a food chopper, and received in a tightly closed sample jar from which the various portions were weighed out. The methods of the Association of Official Agricultural Chemists were followed in the main in the analysis of the pulp, but as some changes were introduced, the methods employed are given here in detail.

Determination of Total Solids.—Ten grams were weighed into a tared flat-bottomed lead dish, stirred to an even paste with a little water, and evaporated to nearly constant weight in a vacuum oven at a temperature not higher than 70°, and under a pressure of 100 to 200 mm.

Determination of Total Acid.—Ten grams were weighed in a counterpoised sugar dish, and washed into a beaker of about 400 cc. capacity with about 300 cc. of water, brought to a boil, cooled somewhat (while the beaker remained covered with a watch-glass), titrated with tenth-normal sodium hydroxide, using phenolphthalein as indicator, and the result expressed as sulphuric acid.

Determination of Sugars.—The sugars were determined essentially as in our work with apples,[1] save that one-half normal solutions were employed, and any large excess of basic lead acetate was avoided.

Determination of Tannin.—Ten grams were weighed in a counterpoised sugar dish, transferred to a 200 cc. flask, and made up to the mark with water. After standing with frequent mixing for at least one hour, the mixture was filtered, and tannin determined in the filtrate by the Löwenthal-Proctor method as given by Allen.[2] The tannin was calculated as gallotannic acid.

Determination of Marc.—The term is used in this study to designate the material of the flesh of the persimmon which is insoluble in 95 per cent. alcohol. Water is generally employed in the estimations of marc, but in case of ripe persimmons, great difficulty is found in determining marc by water extraction methods because of the soft nature of the cell tissue, and its consequent tendency to pass through or to clog the pores of the filter. This difficulty is avoided by the use of alcohol instead of water. The details employed in the analysis of all samples follow.

Fifty grams of pulp were weighed in a counterpoised sugar dish, and washed into an Erlenmeyer flask with several volumes of strong alcohol (95 per cent.). It was then digested for several hours, or over night, on a steam-bath, at about the boiling-point of the alcohol, cooled, the material transferred to a mortar and thoroughly broken up with a pestle and thrown on a Büchner funnel, using a cloth filter and suction. The partially digested product was then returned to the flask, again digested with 95 per cent. alcohol, cooled, and again thrown on a filter as before, and this process repeated once or twice more. By this method all the sugar and other soluble matter, except mere traces, are removed. The marc is then transferred to a weighed aluminum dish, dried *in vacuo* at 70°, cooled and weighed. The temperature of 70° and vacuum drying were employed in order that in

[1] U. S. Dept. Agric., Bur. Chem., Bull. 94, p. 66.

[2] "Commercial Organic Analysis," Vol. III, Part 1, p. 74.

our analysis, the total determined matter without any correction could be added together for comparison with total solids.

The analyses of the persimmons are shown in Table II. The samples marked "Stored" were held in heavy card board boxes (mailing boxes) for the time indicated.

GROWTH AND RIPENING OF WILD PERSIMMONS.

In case of the fruit of trees No. 1 and No. 2, the trees from which five pickings of fruit were received, it will be noticed that the ratio of pulp to seeds gradually increases. The proportion of calyx rapidly falls from about 4 per cent. to about 1.5 per cent. of the weight of the fruit. The fruit from tree No. 3, of which only two pickings were obtained, was about twice as large as the fruit of tree No. 1 and nearly three times as large as that of tree No. 2. The first-mentioned also possessed a considerably larger proportion of pulp than that of the other two trees.

Review of Literature.—Practically no chemical work has been done on the study of the chemical changes that take place during the ripening of persimmons, although several writers have advanced theories regarding them. The theories of Gerber[1] have practically no chemical foundation, and merit the term "speculations" rather than "theories." Concerning the tannin change in the varieties of persimmons studied by him (*D. Kaki Zendi, D. Costata and D. Kaki Ochirakaki*), the author considers that the tannin does not give rise to sugars, but disappears by complete oxidation, forming carbon dioxide and water. Aso[2] considers it most probable that the tannin is gradually changed, perhaps by oxidation brought about by an oxidizing enzyme, and note that it accumulates in special cells. Oxidase was reported by Aso in the unripe fruit, and peroxidase and catalase in the ripe fruit.

Sawamura[3] gives three methods of curing persimmons, *viz.*: (1) keeping fruits for twelve hours in a barrel containing vapors of alcohol (generally freshly emptied sake barrels are used); (2) keeping fruits for twelve hours in warm water (30° to 40°); and (3) drying the peeled fruits in the sun. The tannin is believed by him to be changed to a tasteless substance by partial oxidation brought about by the enzymes in the fruit.

[1] Annales des sciences naturelles, 4, (8), 203.

[2] Botanical Magazine, Tokyo, 14, 179 (1900).

[3] Bull. Coll. Agric., Tokyo (1902).

By calculating to grams per persimmon the data given in Table II, we secure data regarding the actual gain and loss of the various ingredients in persimmons during the various stages of their life history.

In Fig. 1 is given the composition of wild persimmons grown on the tree in the woods, which we have designated as tree No. 1. This table is calculated on grams per fruit. It is especially noticeable that the weight of the pulp is less in the sample picked September 6th than in that picked a month previous. The total weights of solids and tannin are also lower in the sample picked September 6th, and the weight of sugar but slightly greater than in the sample picked on August 7th. The percentage of marc was not determined on September 2nd.

With the single exception of the sample noted, the weight of the pulp steadily increased until November 4th. A marked increase is also noted in the case of total determined solids, sugar and marc. In all the constituents above mentioned, the increase in weight was considerably more rapid between September 21st and October 13th than between October 13th and November 4th. The increase in the amount of marc, however, was greater in the first period mentioned than in the last. The weight of tannin increased very gradually from August 7th, the date of the first sample taken, to October 13th. From August 14th until November 4th the weight of tannin decreased somewhat rapidly, and the fact is somewhat striking and suggestive that tannin decreases during the period mentioned in proportion to the increase of marc. This will be discussed in greater detail later.

The changes in composition noted in tree No. 1 (Fig. 1) are identical with those in the fruit from tree No. 2 (Fig. 2) with the exception of the probable error previously noted in the size of the fruits in the sample taken from tree No. 1 on September 6th, and with the further exception that in the fruit from tree No. 2 the weight of the pulp, determined solids, and reducing sugar decreased, and the weight of total solids increased but slightly after October 13th. It appears that the fruit from tree No. 2 was in a more mature state on October 13th than the fruit from tree No. 1. It separated much more readily from the tree, and the loss in the weight of soluble solids and reducing sugar in the fruit from tree No. 2 after October 13th indicates that the fruit had reached its full maturity on the date mentioned. It is to be

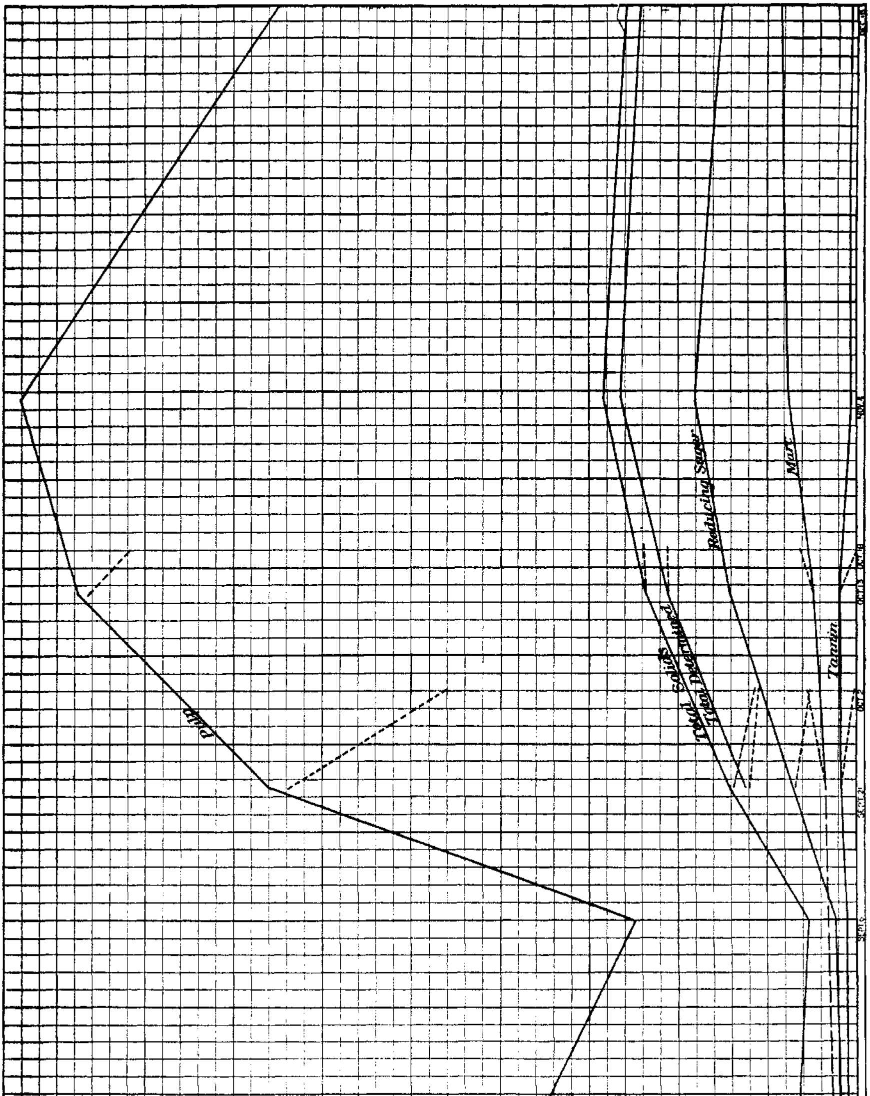

Fig. 1.—Chart showing chemical changes during growth and storage of wild persimmons (*D. Virginiana*) from tree No. 1, in grams per per-

TABLE II.—COMPOSITION OF PERSIMMONS AT DIFFERENT PERIODS OF GROWTH.

		Composition of whole fruit.					Analysis of pulp.				Polarizations.							
Serial number.	Variety.	Date.	Weight per fruit. Grams.	Pulp. Per cent.	Seeds. Per cent.	Calyx. Per cent.	Solids. Per cent.	Marc. Per cent.	Acid as H_2SO_4. Per cent.	Reducing sugar invert. Per cent.	Direct.	Invert.	Soluble tannin. Per cent.	Total nitrogen. Per cent.	Soluble nitrogen. Per cent.	Ash. Per cent.	Determined solids.	Undetermined solids.
14474	Wild persimmon, Tree No. 1	Aug. 7, 1905	4.97	75.4	21.1	3.5	18.38	6.81	.....	4.45	—0.9	—1.1 @28°	2.60	......	......	.....	......	.....
15176	Wild persimmon, Tree No. 1	Sept. 6, 1905	3.43	72.3	25.7	2.0	21.39	......	0.49	8.51	—2.35	—2.5 @27°	3.38	......	......	.....	......	.....
15301	Wild persimmon, Tree No. 1	Sept. 21, 1905	8.58	76.5	21.9	1.6	21.43	5.24	0.27	10.78	—3.6	—3.9 @25°	2.50	......	......	.....	18.79	2.64
15318	Wild persimmon, Tree No. 1, stored 11 days	Oct. 2, 1905	6.48	71.4	27.4	1.2	24.91	12.03	0.29	11.45	...	....	traces	......	......	.....	23.77	1.14
15334	Wild persimmon, Tree No. 1	Oct. 13, 1905	11.15	77.8	21.0	1.2	27.26	5.45	0.36	16.31	...	....	2.14	0.173	0.087	0.77	24.26	3.00
15348	Wild persimmon, Tree No. 1, stored 5 days	Oct. 18, 1905	10.37	78.8	20.0	1.2	29.32	7.74	0.33	17.72	—4.6	—4.6 @24°	traces	0.177	0.098	.....	25.79	3.53
15434	Wild persimmon, Tree No. 1	Nov. 4, 1905	11.75	79.9	18.5	1.6	30.24	7.88	0.37	19.29	—5.4	—6.0 @21°	0.54	......	......	.....	28.08	2.16
15532	Wild persimmon, Tree No. 1	Dec. 18, 1905	8.68	75.0	22.9	2.1	40.12	13.20	0.39	23.42	—7.2	—7.4 @24°	0.82	......	......	.....	37.83	2.29
14475	Wild persimmon, Tree No. 2	Aug. 7, 1905	4.58	62.1	33.1	4.8	22.41	8.28	.....	5.30	—1.0	—0.9 @28°	3.91	......	......	.....	......	.....
15177	Wild persimmon, Tree No. 2	Sept. 6, 1905	5.90	69.8	27.9	2.3	25.13	......	0.44	9.77	—2.4	—2.75@27°	3.98	......	......	.....	......	.....
15302	Wild persimmon, Tree No. 2	Sept. 21, 1905	7.32	76.9	21.3	1.8	24.66	5.86	0.26	13.16	—4.0	—4.4 @25°	3.00	......	......	.....	22.28	2.38
15317	Wild persimmon, Tree No. 2, stored 9 days	Sept. 30, 1905	6.40	75.1	23.4	1.5	28.82	12.59	0.12	13.84	...	....	traces	......	......	.....	26.55	2.27
15335	Wild persimmon, Tree No. 2	Sept. 13, 1905	7.94	78.4	20.4	1.2	29.61	6.48	0.27	18.05	...	....	1.98	0.135	0.074	0.65	26.78	2.83
15349	Wild persimmon, Tree No. 2, stored 5 days	Sept. 18, 1905	7.18	79.5	19.5	1.0	32.56	10.42	0.24	19.18	—5.0	—5.0 @24°	traces	0.155	0.079	.....	29.84	2.72
15440	Wild persimmon, Tree No. 2	Nov. 7, 1905	6.63	79.5	18.9	1.5	35.86	9.14	0.22	21.02	—5.8	—6.2 @24°	traces	......	......	.....	30.38	5.48
15327	Wild persimmon, Tree No. 3	Oct. 4, 1905	20.51	89.3	9.0	1.7	23.62	4.38	0.24	14.67	—4.6	—4.18@24°	2.47	0.074	0.028	0.62	21.76	1.86
15332	Wild persimmon, Tree No. 3, stored 8 days	Oct. 12, 1905	17.21	87.6	11.0	1.4	27.54	8.09	0.23	16.84	...	....	traces	0.100	0.051	.....	25.16	2.38
15375	Wild persimmon, Tree No. 3	Oct. 23, 1905	19.61	87.9[1]	10.8[1]	1.3[1]	27.16	6.86	0.36	17.09	—4.5	—4.6 @21°	2.01	0.126	0.052	0.82	26.32	0.84
15405	Wild persimmon, Tree No. 3, stored 7 days	Oct. 30, 1905	18.00	87.9	10.8	1.3	28.77	6.67	0.40	17.91	...	—4.84@22°	1.14	......	......	.....	26.12	2.65
15391	Japanese persimmons,[2] "Hachiya"	Oct. 26, 1905	137.7	97.6	1.0	1.4	20.75	4.40	0.11	14.57	—3.9	—3.7 @20°	0.68	0.100	0.045	.....	19.76	0.99
15445	Japanese persimmons, stored 25 days	Nov. 20, 1905	114.6	97.7	0.9	1.4	23.61	5.70	0.14	17.28	—2.8	—3.08@22°	traces	......	.... .	.....	23.12	0.49
15392	Japanese persimmons,[2] "Tsurunoko"	Oct. 26, 1905	86.15	96.0	2.9	1.1	22.71	3.52	0.10	17.26	—4.3	—4.6 @20°	0.34	0.086	0.027	.....	21.22	1.49
15442	Japanese persimmons, stored 14 days	Nov. 9, 1905	75.00	96.0	2.5	1.5	25.38	5.09	0.06	19.44	—3.8	—4.0 @24°	traces	......	......	.....	24.59	0.79
			sp. gr.															
15179	Persimmon must from unripe wild persimmons	Sept. 8, 1905	1.0805	.....	...	...	21.05	......	0.51	9.95	—2.4	—3.6 @27°	6.17	......	......	.....	16.63	4.42

[1] Error, used proportion of pulp, seed and calyx found in stored fruit.

[2] Quotation marks indicate that these fruits were from scions imported under the variety named, and grafted on American stock, but were not true to name.

TABLE I.—ANALYSES OF PERSIMMONS.

Composition of persimmon pulp expressed as per cent. of pulp.

Date.	Variety.	Author.	Weight per persimmon. Grams.	Pulp. Per cent.	Seeds. Per cent.	Solids. Per cent.	Reducing sugar. Per cent.	Acid. Per cent.	Ether extract. Per cent.	Nitrogen-free extract. Per cent.	Crude fiber. Per cent.	Ash. Per cent.	Nitrogenous matter. Per cent.	Albuminoids. Per cent.	Heat of combustion per gram. Calories.
1888	*Diospyros Virginiana*..............................	Parsons[1]	......	......	......	33.88	13.54[7]	none	0.70	29.71	1.78	0.86	0.83	.....	......
1895	*Diospyros Kaki Hiyakume*.......................	Colby[2]	175.0	......	......	......	18.20	.....	.....	......	.....	.....	.....	.....	......
1896	*Diospyros Virginiana*, Sample No. 1[8]........	Huston-Barrett[3]	14.1	86.15	13.85	29.10	......	.....	0.42	25.54	1.30	0.63	1.21	0.98	......
1896	*Diospyros Virginiana*, Sample No. 5.........	Huston-Barrett	19.3	82.58	17.42	28.92	......	.....	0.23	26.30	1.02	0.75	0.62	0.53	......
1896	*Diospyros Virginiana*, Sample No. 6.........	Huston-Barrett	14.1	83.33	16.67	35.70	......	.....	0.33	32.18	1.56	0.70	0.93	0.78	......
1896	*Diospyros Virginiana*, Sample No. 7.........	Huston-Barrett	6.3	85.24	14.76	34.99	......	.....	0.36	31.34	1.60	0.83	0.86	0.81	......
1896	*Diospyros Virginiana*, Sample No. 8.........	Huston-Barrett	5.9	82.00	18.00	48.21	......	.....	0.42	43.88	1.83	1.04	1.04	0.94	......
1896	*Diospyros Virginiana*, Sample No. 9[9]........	Huston-Barrett	14.0	88.50	11.50	38.14	......	.....	0.16	31.33	1.26	0.73	0.66	0.48	......
									Fats and carbohydrates.						
1898	*Diospyros Kaki Hachiya*........................	McBryde[4]	180	seedless		28.23	......	0.13[10]	26.42		.....	0.88	0.93	.....	......
1898	*Diospyros Kaki Tsuru*............................	McBryde	194	seedless		26.54	......	0.16	25.16		.....	0.64	0.74	.....	......
1898	*Diospyros Kaki Hiyakume*.......................	McBryde	175	......	1.71	29.83	......	0.13	27.58		.....	1.15	1.10	.....	......
1898	*Diospyros Kaki Yemon*............................	McBryde	150	seedless		23.74	......	0.10	22.69		.....	0.60	0.45	.....	......
1898	*Diospyros Kaki* Japanese graft on native....	McBryde	103	seedless		25.00	......	0.11	24.13		.....	0.45	0.42	.....	......
1898	*Diospyros Virginiana*..............................	McBryde	6.6	......	15.5	33.22	......	0.26	31.32		.....	1.26	0.64	.....	......
1898	*Diospyros Virginiana*..............................	McBryde	8.9	......	17.5	42.76	......	0.15	41.34		.....	0.84	0.58	.....	......
1898	*Diospyros Virginiana*..............................	McBryde	9.2	......	16.3	42.75	......	0.17	40.99		.....	1.28	0.48	.....	......
1902	*Diospyros Kaki*, large seedling..................	Jaffa[5]	......	......	22.4[11]	22.96	19.39	.....	0.31	......	0.93	0.72	1.61	.....	0.429
1902	*Diospyros Kaki Tane nashi*.......................	Jaffa	.....	......	18.8	18.07	12.81	.....	0.55	......	2.93	0.62	1.16	.....	0.346
1902	*Diospyros Kaki Yemon*	Jaffa	......	......	30.7	18.34	13.19	.....	0.85	......	2.37	0.61	1.32	.....	0.376
	Composition of persimmon pulp expressed as per cent. of total fruit.														
	Diospyros Kaki, sweet variety..................	Jaffa[6]	......	......	3.29[12]	17.97	......	.....	0.03	13.62[7]	.....	0.43	0.61	.....	......
	Diospyros Kaki, astringent variety............	Jaffa	......	......	2.76	16.35	......	.....	0.02	12.56	.....	0.43	0.58	.....	......

[1] Am. Ch. J. **10,** 488 (1888).

[2] Cal. Sta. Rept., 1895, p. 183.

[3] Indiana Expt. Sta., 1896, Bull. 60, p. 51.

[4] Tenn. Sta. **11,** 220 (1898).

[5] U. S. Dept. Agric. O. E. S. Bull. 132, p. 9.

[6] Tokyo Sanitary Expt. Sta. through Samamura Bull. Coll. of Agric., Tokyo, **5,** 237 (1902).

[7] Sucrose also reported 1.03 per cent.

[8] Of this sample, the ash of the pulp and of the seed was analyzed, the analysis of seed given and the results of special methods applied to the pulp are reported.

Analysis of ash.	SiO_2. Per cent.	Fe_2O_3. Per cent.	Mn_3O_4. Per cent.	CaO. Per cent.	MgO. Per cent.	P_2O_5. Per cent.	SO_3. Per cent.	K_2O. Per cent.	Na_2O. Per cent.	CO_2. Per cent.	Cl. Per cent.	H_2O. Per cent.	Total. Per cent.
Pulp............	1.52	0.44	0.06	4.74	2.23	7.26	6.84	53.45	2.36	15.94	0.30	4.32	99.46
Seed........	20.12	1.19	0.10	6.76	6.96	13.36	10.53	37.62	0.82	0.00	0.11	2.52	100.09

Analysis of seed.	Solids.	Ether extract.	Crude fiber.	Ash.	Nitrogen-free extract.	Nitrogenous matter.	Albumoids.
	90.14	3.04	17.60	2.07	56 83	10.60	8.10

Results of special methods applied to air-dry pulp (containing 3.84 per cent. H_2O):

Treated successively with:	Per cent. dissolved.
Chloroform	2.76
80 per cent. alcohol...	47.22
Cold water..	14.10
Boiling water, 2 hours..	4.99
Boiling 1¼ per cent. H_2SO_4, 1 hour............................	6.26
Boiling 1¼ per cent. NaOH, 1 hour..................	18.61

[9] Cultivated.

[10] Presumably calculated as malic.

[11] Refuse.

[12] Fiber and seeds.

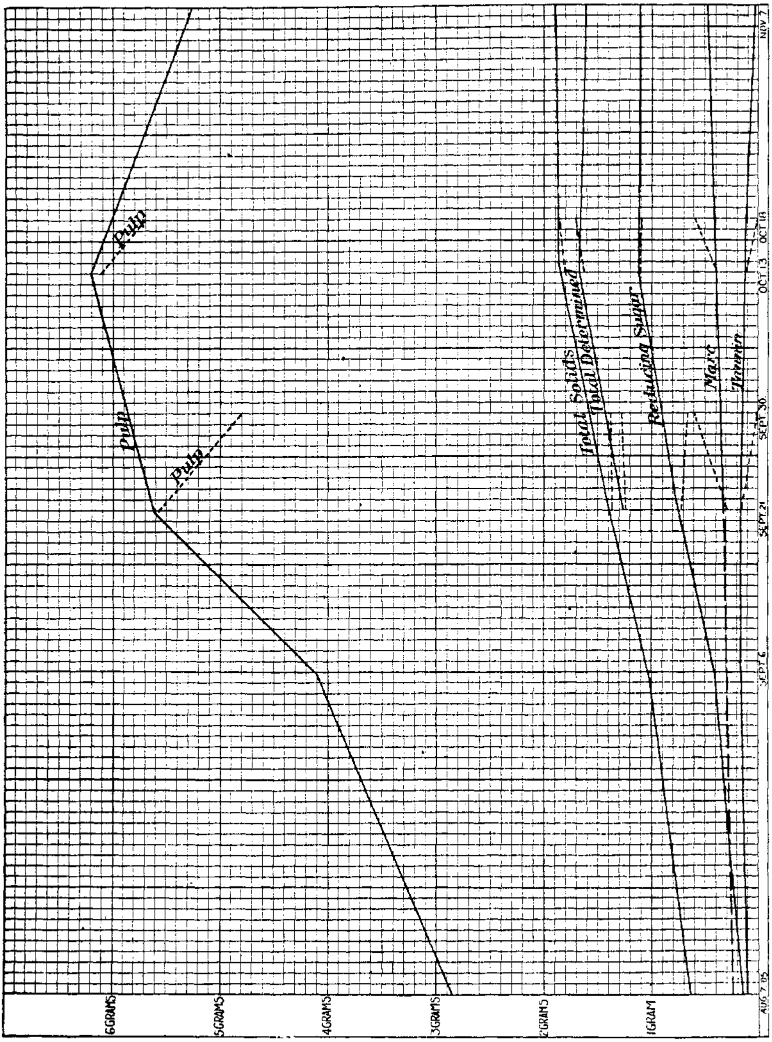

Fig. 2.—Chart showing chemical changes during growth and storage of wild persimmons (*D. Virginiana*) from tree No. 2, in grams per persimmon. Stored samples.

expected that after full maturity was attained a loss in reducing sugars and soluble solids would occur as this was found to be the case in apples and peaches.[1] In Fig. 2 as in Fig. 1, we note a corresponding increase in marc and decrease in tannin.

The results from the examination of the fruit of tree No. 3

[1] Loc. cit., see p. 689.

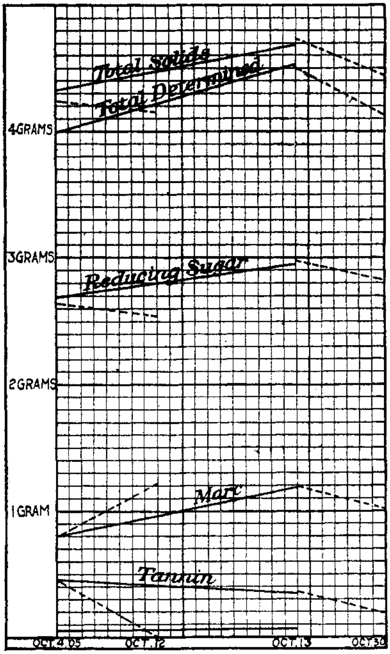

Fig. 3.—Chart showing chemical changes during growth and storage of wild persimmons (*D. Virginiana*) from tree No. 3, in grams per persimmon. Stored samples.

are given on the basis of grams per fruit in Fig. 3. As stated above the study of this fruit was begun late in the season, but the results are of interest as they confirm the results obtained from the fruit of the other two trees.

DISCUSSION OF RESULTS ON STORAGE.

As noted before (see p. 692) sub-samples of wild persimmons were stored in mailing boxes at laboratory temperature, at several times of picking. In all cases, a very marked acceleration of ripening took place. Such acceleration of ripening due to picking is a matter of common experience with fruits.[1] The weight and number of the fruits before storage were noted. As soon as the fruit had undergone the characteristic softening accompanied by the disappearance or marked lessening of the tannin the sample was again weighed and analyzed. The results are shown in Table II, and are plotted in Figs. 1, 2 and 3 in dotted

[1] See e. g. the acceleration of the ripening noted in case of unripe apples. U. S. Dept. Agric., Bur. Chem., Bull. 94, p. 51.

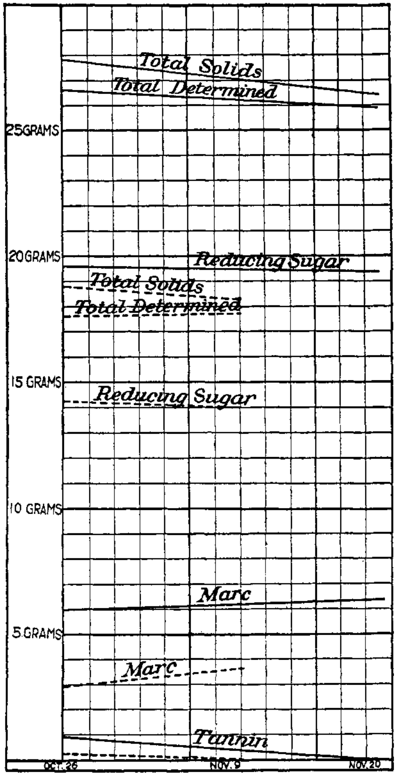

Fig. 4.—Chart showing the chemical changes during storage of two varieties of Japanese persimmons, in grams per persimmon. "Hachiga," "Tsurunoko."

lines on the basis of grams of constituent per fruit. During the storage marked desiccation took place. This accounts largely for the loss in the weight of the pulp.

Six experiments of this kind were carried on in the case of wild persimmons, and two experiments in case of Japanese persimmons (see Fig. 4). While the wild persimmons, in four cases out of six, the analyses showed a distinct loss of total solids, in two cases a slight gain of total solids appeared. The determined solids practically paralleled the total solids in five cases out of six. In one case, however (Fig. 2, analyses of September 21st and October 2nd), the two lines converge. The reducing sugars decreased in five cases out of six. This loss is probably due to consumption of sugars in respiration. To this loss in sugar is probably due the loss in total solids and in determined solids.

The marc increases sharply in five cases out of six, while the tannin decreases notably in all cases. Very similar results were obtained with Japanese persimmons (see Fig. 4), save that the changes were much slower. In Fig. 3, analyses on October 23rd and October 30th, the usual increase of marc was not observed. The tannin, however, did not fall to its usual minimum. The complete change of the tannin had not ensued, and it is possible that the tannin which was incompletely converted into the insoluble form, was partially soluble in the alcohol employed in the method for marc. The results on the marc would therefore be too low. This view is in part supported by the fact that the amount of determined solids has decreased somewhat faster than the loss in total solids or total sugar.

The chemical changes during ripening in storage are therefore as follows. There is a distinct loss in total solids, determined solids, and sugar. There is a notable increase in marc (in several cases the marc is more than doubled) and a corresponding decrease in tannin.

In comparison with the changes which take place in the normal growth of the persimmon, those which take place on storage of the picked fruit at room temperature are very different in some particulars, and very similar in others. While in the normally growing fruit an increase in solids and sugar is noted, decreases are found in the solids and sugar of stored fruit. This is not different from the usual experience with stored fruits.[1] The changes in marc and tannin, however, are very similar in the stored fruit to those which take place in the growing fruit.

The tannin of unripe persimmons possesses very peculiar properties. Some of these are described by Tsukamoto.[2] The product Kaki-shibu is made from a small astringent variety of Japanese persimmon by crushing in water and allowing the product to ferment. Owing to the property of the tannin of forming insoluble films on drying, the product is applied to fish lines, to packing paper for tea, and to various other articles. The tannin of the fermented product was found by Tsukamoto to give no precipitate with alcohol, but is precipitated by a

[1] See e. g. in case of apples, U. S. Dept. Agri., Bur. Chem., Bull. 94, pp. 57-58.

[2] On Kaki-shibu, a fruit juice in technical application in Japan. Bull. Coll. Agric. Tokyo, 4, 329 (1900-1902).

mixture of alcohol and ether. It is also precipitated by strong mineral acids and by acetic acid. We have not extended the observations of this writer, but have confirmed them. The insoluble tannin in the marc readily becomes soluble on warming with dilute mineral acid.

GENERAL DISCUSSION.

The normal ripening may be considered to begin about September 21st, in the case of the fruit on trees No. 1 and 2, the color change from green to golden yellow having taken place just before this date. Ripening had already started in the fruit of tree No. 3 on October 4th, the time of the first examination.

The two features in connection with the data presented herewith regarding the ripening of fruit which occur to us as most noteworthy, and perhaps least expected, are first the increase in the actual weight of sugar, total solids, and determined solids in each fruit after the growing season was supposed to be over, and second, the relation between the marc and the tannin. The first killing frosts in the locality where these persimmons were grown occurred on October 22nd, and freezing temperatures are recorded on four dates between October 13th and November 7th. On November 3rd a temperature of 30° is recorded. Notwithstanding this fact, it appears that the circulation in the persimmon tree during the period mentioned continued.

Duplicates on our determinations were steadily concordant. The ratios of sugar to determined solids, and of determined solids to total solids are uniformly consistent, and we see no reason to doubt the correctness of the results here presented. The only possibility of their incorrectness is a possible error in sampling, such as we have suggested probably occurred in the case of the sample from tree No. 1, taken September 6th. When we consider, however, that samples were taken from three trees, and that the results are all consistent with each other, we are forced to the conclusion that an actual increase in the weight of sugar and soluble solids occurred after freezing weather had begun. In the case of the fruit from tree No. 1 (Fig. 1) this increase continued up to the time of the sampling on November 4th. In the fruit from tree No. 2, however, there was no further increase after October 13th.

We find nothing in our results to explain the formation of sugar. The absence of sucrose and the occurrence of all of the sugar in the form of reducing sugar is interesting. The percentage of acid is very small in the persimmon, and there was no apparent increase or decrease during the life history of the fruit. This last may be partly due to the small amount of acid, and the consequent relatively large error in its determination.

It is an interesting fact that on October 18th the fruit on tree No. 1 clung to the tree tenaciously, and the stems were apparently alive, whereas the fruit of tree No. 2 which had probably attained full maturity was readily separated from the tree. Another fact that is of striking interest is that the sum of marc and soluble tannin was almost uniform during the entire period of our observation of the fruit.

Both marc and tannin increased in weight very slightly from August 7th to September 21st. After September 21st in the fruit from the three trees of wild persimmons which have already been discussed, and in the two varieties of Japanese persimmons, the weight of marc increased while the weight of tannin showed a corresponding decrease.

We had intended the pickings of November 4th and 7th should be the last. Since the weight of sugar and other solids appeared to be increasing at that time, however, it seemed desirable to secure still another sample, if possible, for comparison with those previously examined. The fruit was still clinging to tree No. 1, though it had all fallen from tree No. 2. Accordingly a large sample (70 fruits) was secured on December 18th from tree No. 1. The results of the analysis show considerable desiccation, but no marked changes in the constituents. During the interval between November 4th and December 18th, temperatures below freezing were recorded twenty-eight times, and on December 1st a temperature of 13° F. was recorded.

The following theories to account for the disappearance of tannin seem worthy of consideration:

First, that it is decomposed with the formation of carbon dioxide and water, owing to the respiration of the fruit.

Second, that it is converted into sugar.

Third, that it is converted into undetermined solids.

Fourth, that it unites with some constituent of the undetermined solids.

Fifth, that it unites with the marc with the formation of an insoluble compound.

Sixth, that it is converted into an insoluble form.

The first theory was advanced by Gerber (see p. 692) as a result of investigations which appear to be very inadequate. We have satisfactorily demonstrated in our studies of the ripening of other fruits that the respiration of those fruits is accounted for by the diminution of sugar,[1] whereas their content of tannin was not sufficient to furnish the amount of carbon dioxide liberated during the respiration of the fruit. Here, again, we find the sugars decrease materially on storage of the fruit, and the loss of sugar sustained is evidently sufficient to account for the respiration during storage.

We are not aware that the second theory mentioned has been advanced as the result of any experimental work. It is the general impression, however, and was the impression of the writers when this study was begun, that the tannin was probably converted into sugar as the fruit ripened. If this were true, however, we should not look for the parallelism that exists between the total solids and sugar, but rather we would expect that the total solids should increase less rapidly than the sugars.

The same argument may be advanced with reference to the third theory. This possibility seems to be precluded by the parallelism of the curves representing the total solids and determined solids.

The same line of argument applies to the fourth theory mentioned above. If the loss of tannin were to be explained by this combination with undetermined solids, the weight of undetermined solids would increase relatively to the weight of total solids. If such a combination is formed, the relative amount of the combining body or complex is so small as to produce no apparent effect on the curves representing the total solids and the determined solids.

We are reduced therefore to the conclusion that the tannin either combines with some substance of very low molecular weight during the ripening of the fruit, or is converted into an insoluble form by a change in the nature of the tannin itself.

The fifth theory seems improbable. If the insolubility of the tannin were produced by a portion of the marc becoming soluble and combining with the tannin while the latter is distributed throughout the fruit, sufficient diffusion after the formation of

[1] U. S. Dept. Agric. Bur. Chem., Bull. 94, p. 42.

the compound to permit its passing to the specialized cells in which it finally collects is improbable. Moreover, the weight of marc would presumably decrease for a time before the tannin becomes insoluble.

It is a matter of great satisfaction to us that just at this point we were able to demonstrate definitely that the tannin itself became insoluble within certain specialized cells of the fruit. It is thus evident that there is no marc accessible with which it can go into combination with the exception of the walls of the particular cells in which it is located. Moreover, as the tannin solidifies it frequently shrinks away from the cell walls, leaving them apparently intact.

The first three theories mentioned above are especially negatived by the fact that the tannin does not disappear from the fruit at any stage, but is left in an insoluble form, and can be rendered soluble by warming with decinormal solutions of acids.

MICROSCOPICAL STUDY OF THE PERSIMMON.

The microscopical study shows that in the early stages of growth of the persimmon the tannin is diffused fairly uniformly throughout the fruit. During its ripening the persimmon flesh softens to an unusual extent. This change is attended as has been shown by the analytical results by a largely increased weight of marc, and a corresponding decrease in the weight of determinable tannin.

Shortly before the softening of the fruit begins, an examination of the fruit shows that the tannin is becoming condensed in certain specialized cells with a corresponding decrease in its occurrence in other parts of the tissue. These cells are often of a larger size than the other cells with which they are associated, but are difficult to differentiate from them by features other than that of size. This condensation of the tannin increases until the cell contents become so firm, owing to the solidification of the tannin, that they break into fragments upon the application of pressure. At this stage it is found that the tannin has all been deposited in these tannin cells.

The characteristic micro-chemical property of the tannin does not seem to have undergone any change, for throughout the whole ripening process, up to and including full ripeness, the tannin gives the characteristic reactions with tannin indicators.

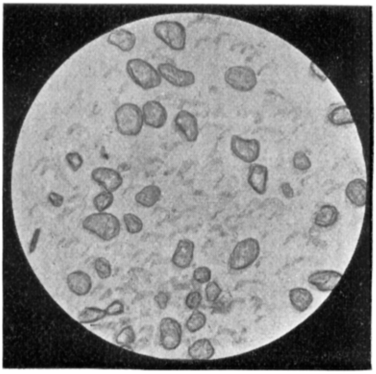

Fig. 1. Tannin cells from ripe American persimmon (*Diospyros Virginiana*). Magnified fourteen times.

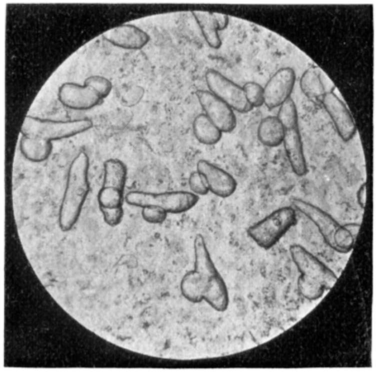

Fig. 2. Tannin cells from partially ripe Japanese persimmon (*Diospyros Kaki*) showing the bursting of cell wall and partial discharge of contents by absorption of water. Magnified fourteen times.

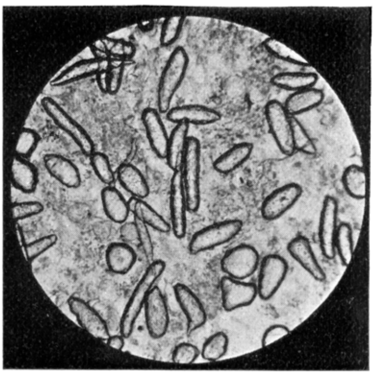

Fig. 3. Tannin cells from fully ripe Japanese persimmon, showing characteristic form. Magnified. fourteen times.

Fig. 4. Tannin cells like those of Fig. 3, but broken into fragments by pressure applied to cover glass. Magnified fourteen times.

The change of the tannin from the perfectly soluble mass diffused uniformly through the fruit to the solid insoluble body isolated in certain cells is very gradual in the growing persimmon, but takes place within a few days after picking, in the stored samples.

The soluble tannin is deposited within the specialized cells where it gradually assumes a jelly-like consistency, and shows an increase in refraction and a decrease in fluidity. If cells from a fruit that has shown softening, be mounted in water, a swelling occurs, and finally a bursting of the cell walls and a discharge of the cell contents into the surrounding media results (Fig. 1). Such swelling and rupturing does not occur after full ripeness has been attained; but instead there results on ripening a solidifying of the cell contents, so that when pressure is applied the cell mass ruptures into fragments. (Fig. 2.) The fact that when a nearly ripe persimmon is eaten the astringent taste is not developed for several seconds after the pulp has been placed in the mouth, finds an interesting explanation in the above experiment, because practically no astringency can be developed until sufficient time has elapsed for the saliva, which is a fluid of low osmotic power, to penetrate the cells, swell and burst them and thus bring the tannin in contact with the nerve terminals.

This phenomenon does not take place if the cells be mounted in the juice of the persimmon, or in any solution of similar or higher osmotic pressure. The size and shape of the tannin cells vary quite widely in the three species, the American, Japanese and Chinese varieties. The cells of two of these, the American and Japanese, are illustrated in Figs. 3 and 4. In the Chinese persimmon the cells are about the size and general shape of those of the American type, but broadly tapering at each end. These features could probably be utilized in determining the kind of pulp used in persimmon fruit products.

The analytical work connected with this article was done by Mr. Gore, and the microscopical work by Mr. Howard.