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Development and Ripening of Peaches as Correlated with Physical Characteristics, Chemical Composition, and Histological Structure of the Fruit Flesh: III. Macrochemistry

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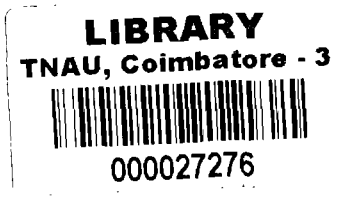

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Development and Ripening of Peaches as Correlated with Physical Characteristics, Chemical Composition, and Histological Structure of the Fruit Flesh: III. Macrochemistry

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This paper is one of a series of publications (1, 10) dealing with the growth and ripening of Elberta peaches from highly vegetative trees abundantly supplied with nitrogen, and from trees much less vegetative and from which nitrogen had been withheld. The highly vegetative trees produced luxuriant twig growth and many large dark green leaves. The less vegetative trees were shown by analysis to be relatively high in carbohydrates but very low in nitrogen; the twigs were short, and the leaves were few, small, and yellowish green.

Blake and Davidson (10) have contributed to our knowledge of external phases of the growth and ripening of peaches borne on such trees and in addition have suggested certain indexes of the degree of ripeness of peach fruits that may be of direct practical value in the commercial production, marketing, and storage of peaches.

Anatomical and microchemical studies of fruits from the same trees are reported by Addoms (1), and in the following pages are presented the results of macrochemical studies in their relationship to her investigations and to work of Blake and Davidson (10). Such a comparison and correlation of the results of each part (1, 10) of this investigation are entirely permissible and may be made with considerable assurance and accuracy, as each phase of this work was conducted on aliquots of the same lots of peaches.

Experimental Methods

Two Elberta peach trees each 7 years old and located about 20 feet apart in the New Jersey Agricultural Experimental Station orchard were selected for this work. One tree, the fruits of which will be designated by *N*, had received heavy applications of nitrogenous fertilizers for several years, whereas the other tree during the same period had received little nitrogen except that present in the rather poor loam soil in which it was growing. The fruits from the second tree will be indicated by the letter *C*. Both trees received an abundant supply of water throughout the period of this work (10). Further details of cultural treatments are given in another paper of this series (10). Fruits *N* and *C* were harvested for analysis as indicated under "Chemical Methods," on the several dates listed in table 3.

Each time fruits *C* and *N* were harvested from the Elberta trees, fruits were harvested also from a tree of the variety Shipper Cling, which bears

[1]The Kjeldahl and ash determinations were made by C. S. Cathcart, for whose cooperation the authors wish to express their appreciation.

peaches of the canning type, with non-melting flesh, whereas Elberta is a melting-fleshed variety. The Shipper Cling tree had received average commercial treatment with respect to fertilizer applications and other cultural practices (10) and was intermediate in vegetative vigor between the two Elberta trees. The Shipper Cling fruits received the same treatment as the Elberta fruits, with the exception that ripe Shipper Cling fruits picked on September 4 were stored at about 25° C. for 24 hours before analysis.

Chemical Methods

Harvesting and Sampling.—Seventy or more peaches of each series, as nearly uniform and comparable as possible, were picked at 7 a.m. on the several dates indicated in tables 3 and 4. Thirty-five of the fruits were used for macrochemical analysis and the remainder for microchemical and histological examination (1), hydrometer determinations of expressed juice, and pressure tests (10).

Both flesh and skin are included in all analytical samples, because of the impracticability of separating the two. In non-melting-fleshed varieties there is no zone of clear differentiation of flesh and skin tissue at any time, and in melting-fleshed varieties such differentiation does not occur until the fruits are completely ripe (1).

During the early stages of development and ripening, the flesh of the 35 fruits was removed from the stones, minced, and mixed, and the duplicate aliquots of 100 or 150 gm. were taken for preservation in alcohol. Samples of the fresh minced peach flesh were also used immediately for determinations of nitrogenous fractions, acidity, and dry matter. While the flesh was comparatively firm, mincing resulted in practically no loss of moisture, but at the time of the last three harvests of any series it was necessary to employ a different procedure in order to avoid considerable loss of juice from the ripe or nearly ripe fruits. At that time the fruits were cut in half with a sharp, thin-bladed knife. The stone was removed and the halves of the flesh were cut into small segments, in such a manner that there was little loss of juice. Aliquots of these segments were further minced directly into tared receptacles containing weighed amounts of alcohol. Other samples to be used for the determination of nitrogenous fractions, dry matter, and acidity were minced directly into tared vessels, and treated at once. Several workers assisted in mincing the 35 fruits of any lot, with the result that not more than 10 minutes elapsed from the start of this operation until the aliquots of peach flesh were in alcohol and other samples were undergoing other treatments as described under "Acidity," "Dry Matter," and "Soluble Nitrogen" (p. 7).

The minced peach tissue was put into sufficient warm 95 per cent alcohol to give a final concentration of about 80 per cent, was boiled for 5 minutes, and maintained at a pH of about 5.8 to 6.0 with tenth-normal NaOH, bromcresol green or bromcresol purple being used as an indicator. The final extraction was with 80 per cent alcohol.

Reducing Sugars.—Aliquots of the alcoholic extract were cleared with neutral lead acetate and deleaded with potassium-oxalate as recommended by Loomis (26). The reduction for all forms of carbohydrates was carried out under the conditions of Quisumbing and Thomas (38) and the amount of reduced copper was determined by the Shaeffer and Hartman (41) method. All results are calculated and expressed as dextrose.

Sucrose.—The increase in reducing power due to the action of invertase is reported as sucrose.

Total Sugars.—This fraction is reported as the sum of reducing sugars plus sucrose. Direct determination of total sugars by acid hydrolysis under Herzfeld's conditions (6) for 24 hours at 20° C. gave results which were equal to or slightly less than the sum of reducing sugars and sucrose.

Starch and Dextrin.—Microchemical tests (1) showed starch to be present in only very small quantities in the flesh of the green peach and there was apparently none at all in the ripe or nearly ripe fruits. Macroanalyses were made for starch and dextrin (33) but even at a period (1) when microchemical examination indicated that starch was present there was less than 0.10 per cent of starch and dextrin as computed on a green weight basis.

Tannin.—This fraction was determined on aliquots of the alcoholic extract. According to the method of Menual (28). Probably not all tannins are soluble in alcohol (29) yet the results may have some relative value. They are expressed as percentage of gallotannic acid.

Soluble Pectin.[2]—The alcohol-insoluble residue was dried at 80°, passed through a drug mill, and ground in a ball mill until all the tissue would pass through a sieve of 150 meshes to a square inch. A small quantity of water was added to aliquots of the ground alcohol-insoluble material and the mixture was allowed to stand for one hour at 20° C. with frequent stirring [slight destruction of pectin may result on prolonged standing in water], after which it was heated to boiling in 3 minutes, and filtered at once with slight suction through paper pulp in a Buchner funnel and washed with hot water. The residue was discarded, the filtrate cooled to 20° C., and pectin determined according to the Carre and Haynes method (15) which gives pectin in terms of calcium pectate[3].

The usual method has been to use fresh tissue for the determination of pectin in apples (13, 15) and in peach flesh (3). Table 1 gives pectin deter-

TABLE 1

Pectin Determinations Made on Fresh Tissue and on the Alcohol-Insoluble Residue of the Flesh of Ripe Cumberland Peaches

(Results computed as percentage of green matter and expressed as calcium pectate)

Material	Treatment	Pectin
Alcohol insoluble residue	Cold water 30 minutes	.51
Alcohol insoluble residue	Cold water 60 minutes	.55
Alcohol insoluble residue	Cold water 120 minutes	.52
Alcohol insoluble residue	Cold water 240 minutes	.50
Fresh tissue	After Appleman and Conrad (3)	.59
Fresh tissue	After Appleman and Conrad	.64
Fresh tissue	After Appleman and Conrad	.61

[2]The empirical terms used in this publication for the different pectic fractions are of a physiological significance but of course are not exact terms in a chemical sense. For the chemistry of pectin, the reader is referred to the appended list of references especially to a very recent paper by Ehrlich (21). Branfoot (11) gives a historical review of the pectic substances of plants. The several methods of nomenclature are presented in condensed form by Ahmann and Hooker (2).

[3]The calcium pectate precipitates were completely soluble in warm ammonium oxalate and did not contain sufficient nitrogen to be detected by the usual Kjeldahl determination.

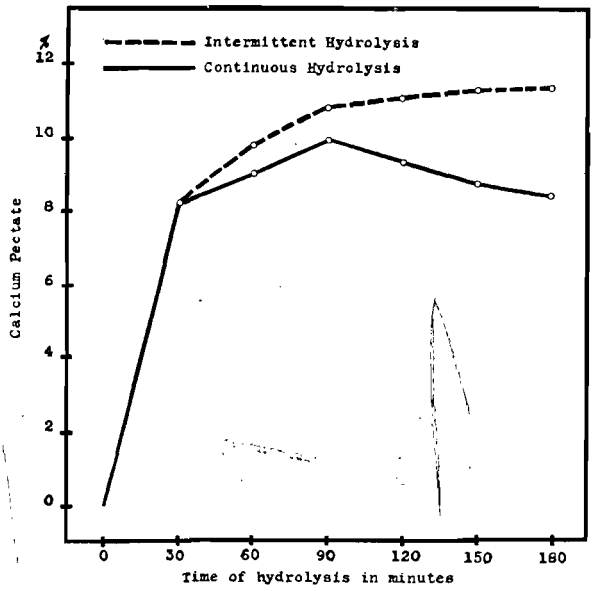

FIG. 1. CONTINUOUS AS COMPARED WITH INTERMITTENT HYDROLYSIS OF PROTOPECTIN
Aliquots of the alcohol-insoluble residue of the flesh of green Elberta peaches were subjected to *continuous hydrolysis* with boiling thirtieth-normal hydrochloric acid for different periods of time. In case of *intermittent hydrolysis* pectin was removed every half hour and fresh thirtieth-normal hydrochloric acid added.

minations for ripe Cumberland peaches by direct extraction of the fresh tissue with water according to the method of Appleman and Conrad (3), as compared with extraction of pectin from the alcohol-insoluble fraction of aliquots of the same lot of peaches. The results of the two methods are essentially the same, although in this trial of the two methods and in other trials, pectin as determined by the fresh tissue method was consistently comparatively high, possibly because of a slight enzymatic hydrolysis of protopectin during the period of about 20 minutes which is required for extraction of the fresh peach pulp.

Total Pectic Material.—Conrad (18) in his hydrolysis of insoluble protopectin of potato tissue employed continuous boiling with thirtieth-normal

TABLE 2

Yield of Calcium Pectate from the Alcohol-Insoluble Residue of the Flesh of Green Elberta Peaches by Continuous as Compared with Intermittent hydrolysis with Boiling Thirtieth-Normal Hydrochloric Acid

(Results expressed as percentage of the alcohol-insoluble residue)

Time	Continuous Hydrolysis	Intermittent Hydrolysis
minutes		
30	8.26	8.26
60	9.08	9.72
90	9.84	10.87
120	9.32	11.21
150	8.86	11.41
180	8.60	11.45

HCl. The same method was also used in other work on peaches (3). He obtained the maximum yield of pectin at the end of one hour and found that a longer period of heating at boiling temperature with thirtieth-normal acid destroyed some of the liberated pectin. Of course, some of the liberated pectin was also destroyed during the first hour, but the fact was not apparent until after one hour when pectin was destroyed faster than it was formed. There is, therefore, no assurance that all protopectin has been removed from any plant tissue simply because the maximum yield of pectin is obtained at the end of any period of hydrolysis.

Table 2 and figure 1 show that with tissue of green Elberta peaches the maximum yield of pectin was obtained by continuous hydrolysis for 90 minutes, yet tests of the cell wall fragments of the residue with ruthenium red (1) showed that much protopectin had not been hydrolized and was still present in the cell wall tissue. It required 2½ hours of hydrolysis with boiling thirtieth-normal acid to liberate all protopectin from the cell wall tissue; but continuous hydrolysis for this period greatly decreased the yield of pectin as compared with continuous treatment for 90 minutes (table 2, fig. 1), therefore, the method finally adopted for the determination of total pectic material was as follows:

Aliquots of the ground alcohol-insoluble fraction were boiled with thirtieth-normal HCl in a wide-mouthed Erlenmyer flask, under a reflux condenser for 30 minutes, after which the mixture was filtered and washed with hot thirtieth-normal HCl. The filtrate was cooled and neutralized at once with tenth-normal NaOH. The residue on the filter paper was washed back into the original Erlenmyer flask with hot thirtieth-normal HCl and hydrolyzed for another 30 minutes. This procedure was repeated after each 30-minute period of hydrolysis until the total time of hydrolysis was 2½ hours. All filtrates were combined and pectin was determined as calcium pectate[4] by the Carre and Haynes method (15).

As may be seen from table 2 and figure 1, this method of intermittent hydrolysis gives a much higher yield of pectin than is possible by continuous hydrolysis and, in addition, removes all protopectin from the tissue of the peach flesh.

Insoluble Protopectin.—The insoluble protopectin fraction is expressed as calcium pectate, and was calculated by the difference between total pectic material and soluble pectin.

Pectic Acid and Pectates.—All the usual macrochemical and microchemical (1) tests were made on the flesh of both green and ripe peaches for the fractions commonly called pectic acid and pectates. However, these terms are simply relative, not absolute (21). We find, as did Appleman and Conrad (3), that pectic acid and pectates, as such, must either be absent from peach flesh or be present in extremely small amounts.

Hemicellulose.—The residue remaining after the determination of total pectic material, was washed from the filter paper, with hot 2½ per cent H_2SO_4 and boiled with this reagent for 45 minutes under a reflux condenser. After hydrolysis, the mixture was filtered, the filtrate cooled, neutralized, cleared, and made to volume, and the reducing power determined as in the case of reducing sugars.

Cellulose.—The residue left after the determination of hemicellulose, was probably chiefly cellulose. It contained little ash and was almost completely soluble in Schweitzer's ammoniacal cupric oxide reagent.[5] Micro-

[4]See footnote 3.

[5]Schweitzer's reagent is, of course, not a specific solvent for cellulose. It will dissolve pentosans, some lignin, and even some protein.

chemical tests (1) have shown that the peach flesh contains very little lignin except for a trace in the vascular bundles and in the hairs of the skin, which are made up of both cellulose and lignin. The hairs, however, formed only a very small part of any analytical sample. Accordingly the residue from the hemicellulose determination was dried for 24 hours at 80° and weighed. A Kjeldahl determination was run on the weighed material, and the original weight was corrected for nitrogen after being multiplied by the conventional factor 6.25. Although such a method of nitrogen correction may be questionable, the amount of nitrogen in the hemicellulose residue was so low that the figure as presented for cellulose is practically the same as if nitrogen multiplied by 6.25 had not been deducted.

Acidity.—Aliquots of the expressed juice of fresh peach pulp were titrated with fiftieth-normal NaOH. The results are expressed as cubic centimeters of fiftieth-normal NaOH required to neutralize 10 cc. of juice. According to Nelson (31), "the non-volatile acids of the peach consist of malic and citric acid in almost equal proportions."

Dry Matter.—The percentage of dry matter in the peach flesh was estimated by drying aliquots of minced peach pulp for 48 hours at 80° in a current of air. The results, however, particularly as the fruit approached maturity, are undoubtedly low, because of the continuous loss of volatile material other than moisture. However, the determinations are strictly comparable with respect to temperature and time of drying.

Ash.—Aliquots of the dried ground peach flesh were used for total ash determinations (6), and the results are expressed as percentage of green matter.

Total Nitrogen.—Aliquots of the dried ground peach flesh were used for total nitrogen determinations (6, 39).

Soluble Nitrogen.—One hundred-gram aliquots of fresh peach flesh were macerated and extracted with water. After removal of the coagulable nitrogen as previously described (33), a Kjeldahl determination was run on aliquots of the filtrate. The results are designated by the term "soluble nitrogen." As far as could be detected, this fraction contained neither nitrate nor ammonia nitrogen.

Protein Nitrogen.—This fraction was calculated as the difference between total nitrogen and soluble nitrogen. For a further discussion of the significance of this fraction the reader is referred to Chibnall (17) and Nightingale and Robbins (33).

Experimental Results

The high-nitrogen (low-carbohydrate) Elberta tree which received liberal applications of nitrogen produced many large, dark green leaves, and a total twig growth for the current growing season of 33,233 inches; whereas the high-carbohydrate (low-nitrogen) Elberta tree which lacked a liberal external supply of nitrogen produced relatively few and small yellowish green leaves, and a total seasonal twig growth of only 5,828 inches (10).

At various periods during the summer and fall, the twigs and older wood of the trees were examined microchemically for starch by the usual iodine method (1). Invariably much more starch was found in the twigs and wood of the slow-growing tree than in comparable tissues of the vigorously vegetative tree. In addition, on September 5, aliquots of the current season's twigs were analyzed for total nitrogen. The twigs of the high-carbohydrate tree contained only 0.34 per cent of nitrogen, expressed on a green

Analysis of the Flesh of Elberta Peaches—C, *Fruits from the*

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Date of Picking	Reducing Sugars		Sucrose		Total Sugars		Soluble Pectin		Insoluble Proto-pectin		Total Pectic Material		Her Cell lo:
	N	*C*	*N*	*C*	*N*	*C*	*N*	*C*	*N*	*C*	*N*	*C*	*N*
July 22...	2.80	3.10	0.17	0.20	2.97	3.30	.36	.38	.47	.82	0.83	1.19	.62
30...	3.00	3.00	0.49	0.95	3.49	3.95	.39	.33	.76	.72	1.16	1.09	.41
Aug. 12...	1.97	2.78	1.63	2.22	3.60	5.00	.31	.26	.74	.64	1.04	.91	.41
16...	1.97	2.65	1.73	2.16	3.70	4.81	.44	.45	.73	.43	1.17	.87	.36
20...	2.00	2.50	1.96	2.83	3.96	5.38	.63	.51	.51	.37	1.14	.88	.37
24...	2.16	4.19	3.52	5.24	5.68	9.43	.46	.34	.36	.37	.82	.71	.33
27...	2.49	4.26	3.25	5.20	5.74	9.46	.45	.41	.39	.36	.83	.77	.17
31...	2.23	2.63	3.84	6.44	6.07	9.07	.45	.44	.23	.13	.67	.57	.25
Sept. 4...	2.15	....	3.87	....	6.02	....	.32	...	.26	...	.58	...	.19
9...	1.53	....	5.69	....	7.22	....	.34	...	.07	...	.41	...	.17

*Acidity as cubic centimeters of fiftieth-normal NaOH required to neutralize 10 cc. of

weight basis, whereas in the vigorously vegetative tree, low in carbohydrates, there was 0.58 per cent of nitrogen.

A more detailed description, and plates illustrating these trees and the fruits produced by them are given in another report (10).

The results of the macrochemical analysis of the flesh of the fruits produced on the two trees described in the foregoing are given in table 3. It is significant that practically all the fruits on the high-carbohydrate tree were *soft ripe*[6] on August 31 and had a very desirable golden yellow ground color that was almost completely overlaid with a deep red blush, whereas the first soft ripe peaches were picked from the high-nitrogen tree on September 9 and not all fruits on that tree were ripe then. Many unripe *green* peaches remained after the last harvest for analysis on September 9. The peaches on this tree were comparatively large but were conspicuously lacking in color even when soft ripe. Further exact details as to the appearance, percentage of stone and flesh, rate of growth, and dimensions of these two lots of peaches are given in another report (10).

In table 4 are presented the results of macrochemical analysis of the flesh of the Shipper Cling non-melting fruits.

Discussion

The Elberta peach trees employed for this work represent two extreme conditions of vegetative vigor for trees producing a commercial crop of fruit. Even greater extremes of growth of bearing trees, however, may be found

[6]The following arbitrary terms are employed in this series of publications to indicate the degree of ripeness of Elberta peaches as measured in resistance to pressure; the measurements were made with a Blake pressure tester (9) provided with a 3/16-inch plunger and are recorded in pounds: *Green*, 9 pounds and above; *Shipping ripe*, 7 to 8½ pounds; *Hard ripe*, 5 to 7 pounds; *Firm ripe*, 3½ to 5 pounds; *Soft ripe*, 3 pounds or less. Further details are given in another publication (10).

te Tree and N, *Fruits from the High-Nitrogen Tree*

ed as percentage of green matter)

Acidity*		Total Nitrogen		Protein Nitrogen		Soluble Nitrogen		Ash		Tannin		Per Cent Dry Matter	
N	*C*	*N*	*C*	*N*	*C*	*N*	*C*	*N*	*C*	*N*	*C*	*N*	*C*
37	37	.262	.123	.077	.061	.185	.062	1.39	1.26	.005	.082	12.40	13.86
41	42	.271	.122	.078	.042	.193	.080	1.27	1.29	.004	.067	13.10	13.00
62	66	.245	.087	.071	.042	.174	.045	1.17	.89	.006	.050	12.58	12.60
73	67	.242	.076	.072	.048	.170	.028	1.18	.66	.004	.040	11.87	11.80
62	64	.183	.066	.065	.038	.118	.028	.74	.54	.007	.041	11.89	11.93
67	63	.142	.061	.028	.035	.114	.026	.65	.61	.009	.033	11.42	12.73
68	75	.151	.051	.039	.025	.112	.026	.64	.54	.008	.007	11.13	12.80
57	48	.104	.045	.047	.032	.057	.013	.61	.51	.008	.003	11.86	13.60
63	...	.127	....	.047	....	.080	....	.66	....	.003	....	12.14	.....
45	...	.115	....	.032	....	.083	....	.69	....	.004	....	12.68	.....

commercial orchards. The nature of the growth (24) of these trees ıd specifically the analysis of twigs (page 7) and fruit (table 3) show :yond question that the vigorously vegetative tree with dark green leaves ıd luxuriant twig growth may be correctly spoken of as a high-nitrogen low-carbohydrate tree, and the other, with its short, slow-growing twigs and small yellowish green leaves, as a high-carbohydrate or low-nitrogen tree.

The purpose of this work was to compare, as to ripening process and quality of fruit, peaches from a high-carbohydrate tree with those from a high-nitrogen tree. The particular set of environmental conditions responsible for the two extremes of growth is not an important part of this investigation, although details are given elsewhere (10). An infinite number of sets of fertilizer treatments or cultural practices will produce a high-nitrogen type of Elberta tree practically identical with the one described in degree of vegetative vigor as well as in the size and quality of fruits. Likewise, a high-carbohydrate type of Elberta tree may be produced under many different conditions of environment and cultural practice, but always a high-carbohydrate tree will produce fruits essentially like those designated in this paper as fruits *C*.

It is not meant to imply that fertilizer trials and other studies of commercial practices may not be of great value. There is need of more field work and especially of work under controlled conditions. However, many combinations of treatments will give practically identical vegetative and reproductive responses, and the results of fertilizer treatments, pruning, cultivation, and cover crops will vary with the season and are often chiefly local in value. Accordingly, for this investigation it seemed more efficient and accurate to study the problem of quality, development, and ripening of peach fruits as associated with the character of growth of the tree itself rather than with the treatments given it.

Softening of Peaches

Addoms (1) finds that the cell walls of peach flesh apparently consists of an intimate combination of cellulose and protopectin. Also chemical investigations by others (16, 19, 20, 22) and, recently by Sucharipa (42), support this view. Addoms' (1) investigations further show that in any one cell wall, the cellulose is uniformly distributed, whereas protopectin is more abundant in areas that are exposed to intercellular spaces than in areas of contact with other walls. As the fruit ripens, the cell walls become thinner, because of a decrease in protopectin and cellulose, and in the flesh of soft ripe fruit some of the cell walls break and the cell contents fill the intercellular spaces, forming the watery areas commonly observed in melting-fleshed varieties of peaches. Carre (13), working with the apple, obtained similar results; in fact it has been generally recognized, since the time of Fremy (23),

TABLE 4

Analysis of the Flesh of the Non-Melting Shipper Cling Peach

(Results expressed as percentage of green matter)

Date	Soluble Pectin	Insoluble Protopectin	Total Pectic Material	Hemicellulose	Cellulose
July 22	.34	.66	1.00	.41	1.60
30	.39	.58	.96	.38	1.44
Aug. 12	.34	.65	1.00	.31	1.08
16	.38	.46	.85	.28	.92
20	.48	.34	.82	.29	.99
24	.43	.32	.76	.22	.84
27	.40	.36	.76	.24	.78
31	.29	.34	.63	.17	.70
Sept. 5	.27	.35	.62	.17	.66

that as fruit ripens, the insoluble protopectin of the cell walls is changed to soluble pectin. In the case of the Elberta peaches that were picked green and allowed to soften off the tree (10), pectin accumulated in an amount about equal to the decrease of protopectin. These results agree with those of others for peaches (3, 7), apples (8, 12, 13), and other fruit (4, 23), that were analyzed after a period of softening subsequent to picking.

Comparatively little work, however, has been done on the pectic changes occurring in fruits while on the tree. As may be seen from table 3, protopectin decreased markedly as the peaches approached the final soft ripe condition. This stage of complete ripeness occurred at the time of the last harvest of fruits *C* from the high-carbohydrate trees, on August 31, and of fruits *N* from the high-nitrogen tree, on September 9. There was, however, no corresponding increase in soluble pectin. It necessarily follows, therefore, that total pectic material decreased.

It does not seem probable that pectin is translocated from the fruit flesh. It may be that in fruits on the tree, pectin is changed in part to sugars about as fast as it is formed from protopectin. However, such a statement cannot be demonstrated, because any change of pectin to sugars might be masked by carbohydrates translocated to the ripening fruits from the vegetative organs.

Although in interpretation of the softening of fruits the chief emphasis has been placed, perhaps correctly, upon protopectin[7], it is clear from the data presented in table 3 that cellulose and hemicellulose decrease as the peach ripens, and that this decrease corresponds very closely to loss of protopectin. There is considerable practical significance to the fact that as the fruit ripens, pressure tests of the flesh with a 3/16-inch plunger (9, 10) show a decrease in resistance that closely parallels the decrease in protopectin and cellulose as expressed in table 3, and the decrease in thickness of cell walls as determined by Addoms (1).

Softening Process in Fruits C as Compared with Fruits N

On July 22, at the time of the first macrochemical analysis, the flesh of fruits *C* contained (table 3) nearly 100 per cent more protopectin and a higher percentage of cellulose than the flesh of fruits *N*. In addition, microchemical and histological investigations (1) show that the cell walls were thicker in the flesh of the fruit from the high-carbohydrate tree. Likewise, in complete accord with chemical and anatomical determinations, the resistance to pressure (10) of fruits *C* was higher than that of fruits *N*.

It does not seem strange that such a relative condition exists in the flesh of peach fruits during the early stages of growth, for it has been demonstrated repeatedly (24) that the vegetative organs of a slow-growing, high-carbohydrate type of plant are made up of tissues typically thick-walled as compared with those of a vigorously vegetative individual of the same variety that is lower in carbohydrates.

Regardless of the vegetative vigor of the tree, all peaches as they ripen become softer fleshed, and softening does not take place without a decrease in protopectin, in cellulose (table 3), and in thickness of cell walls (1). However, fruits *C* became soft ripe on August 31, nine days before fruits *N*. Accordingly it was found, as might be expected, that for a time after July 22 (table 3) the protopectin and cellulose content of the two lots of fruit was about the same; so also were pressure tests (10) and thickness of cell walls (1). But on August 20 (table 3), there was very much less protopectin and cellulose in the flesh of fruits *C* than in the flesh of fruits *N*, and at that time the cell walls of fruits *C* were very noticeably thinner (1).

When fruits *C* and *N* were in the best condition for eating, that is soft ripe, on August 31 and September 9, respectively, the cell walls of the flesh of both lots of fruit were so very thin that it was impossible to distinguish differences in cell wall thickness with accuracy (1).

Nevertheless the percentage of cellulose and protopectin (table 3) is a little higher in the flesh of fruits *C* than in that of fruits *N*. Although the differences in percentages of cellulose and protopectin are not large, they probably represent the actual existing conditions, for they are confirmed in the results of the pressure tests, namely, that fruits *C* showed greater resistance to pressure than did fruits *N*. The pressure tests were made at the time of harvesting for analysis, on August 31 and September 9; and, in addition, to insure the ripest possible condition, both lots of fruits were kept for a short period at 25° C. and pressure determinations were repeated. The relative results were the same. The peaches from the high-carbohydrate tree were a little firmer.

[7]It should be kept in mind that the figures presented are for protopectin in terms of calcium pectate. It would be very desirable if protopectin could be expressed in terms of protopectin, the molecular weight of which is presumably very much higher than that of the calcium pectate equivalent (11).

Partial Softening of the Non-melting or Canning Type of Peach

This phase of the work will be discussed more extensively in other publications of this series (1, 10), but the results (table 4) of analyses of the fruits of the non-melting-fleshed Shipper Cling for comparison with parallel determinations run on the melting-fleshed Elberta, are presented in table 3.

A comparison of tables 3 and 4 shows little difference between Elberta and Shipper Cling as to the trend of changes in protopectin and cellulose, until the last analysis of the non-melting-fleshed fruits, on September 5. The Shipper Cling peaches analyzed on that date were picked from the tree on September 4. The fruits were just ready to drop, but to insure the maximum possible degree of softness they were kept at about 25° C. for 24 hours before analysis. At that time (September 5) these completely ripe, non-melting Shipper Cling peaches were over 100 per cent higher in insoluble protopectin (table 4) than were the fruits of the soft ripe melting-fleshed Elberta (table 3). There was, however, no significant difference in cellulose content.

The results of these macrochemical analyses were confirmed in histological and microchemical studies by Addoms (1). It will be recalled that the cell walls of soft ripe Elberta fruits are very thin and contain little cellulose or protopectin, and that many of the walls break, filling the intercellular spaces with cell contents. The completely ripe Shipper Cling fruits examined on September 5 showed rather striking differences. Although the cellulose content was about the same as that of soft ripe Elberta fruits, the amount of protopectin was much greater, and the cell walls were noticeably thicker. Of the several other varieties of peaches examined, all of those having melting flesh were found to be similar to Elberta, all of those having non-melting flesh, similar to Shipper Cling. Her observations (1) combined with the macrochemical determinations given in tables 3 and 4 demonstrate that the non-melting character of the canning type of peach is directly associated with the fact that the cells of the flesh of the ripe fruits have walls that are intact and comparatively thick, with a large content of insoluble protopectin. This is undoubtedly a varietal or genetical character which may not be eliminated by cultural or nutritional treatments.

Carbohydrates.—It appears probable that the starch found in the flesh of green peaches is a rather temporary photosynthetic product and not in any sense an important storage constituent (1, 8, 43). Reducing sugars and sucrose appear, therefore, to be the chief carbohydrates of peach flesh. Of the reducing sugars, much is glucose, though there is some fructose (1).

The very young, green fruits are low in total sugars, most of which are reducing sugars (table 3). Sucrose increases as the fruits develop but there is also an increase in percentage of reducing sugars, particularly in the flesh of the fruits from the high-carbohydrate tree. It would seem quite apparent, however, that sucrose is the storage form, whereas reducing sugars are a comparatively temporary translocation product. This conclusion is indicated by the almost analogous results of Bigelow and Gore (7), and is further substantiated by the fact that reducing sugars are lower and sucrose is higher at the soft ripe stage (at the time of the last analysis of the two lots of fruits), than at any other period.

The sugar content (table 3) of the two lots of fruits, as might be expected, reflects the carbohydrate condition of the vegetative organs of the trees. Fruits *C* are very much higher in reducing sugars, but particularly

in sucrose, than fruits *N*. Moreover, the high sugar content was very apparent in the sweeter taste and better quality of the fruit from the high-carbohydrate tree[8].

The importance of sugar content in relation to time of picking and storage will be demonstrated in another report (10). It will be sufficient in this paper to call attention to the comparatively early accumulation of sugar in fruit *C*, which, in a large measure, made possible the maturation of high quality fruits even though they were picked from the tree before the soft ripe stage, whereas similar early picking of peaches from the high-nitrogen tree resulted in fruits of very poor quality.

Acidity.—There does not appear at any time to be a significant difference in the titratable acidity of the juice of the two lots of fruits (table 3), and the results are in general agreement with those of others (7) who show that the developing fruits increase in acidity until the soft ripe stage, when the percentage of acid suddenly decreases.

It may, however, be briefly mentioned that fruits *N* increased tremendously in acidity during the period of softening off the tree when picked before the hard ripe stage, whereas peaches from the high-carbohydrate tree showed little change in acidity even when harvested before they were hard ripe.

Nitrogen.—The percentage of total nitrogen (table 3) is highest in the young fruits, and as the sugars increase it necessarily follows that there must be a decrease in percentage of nitrogen unless there is a considerable intake of it after the early stages of fruit development. Such an intake is not indicated.

The sugar content of the peach fruits accurately reflects the carbohydrate condition of the vegetative organs of the tree upon which they were borne. The nitrogen content of the peach flesh also shows beyond any doubt the difference in nitrogen content of wood and leaves of the two trees.

The flesh of fruits *N* throughout the entire series of analyses is in every case over 100 per cent higher in total nitrogen than the flesh of fruits *C*. No inorganic nitrogen was detected, but a consistent and striking difference in quality of organic nitrogen is shown throughout the analyses. In every case a comparatively high percentage of the nitrogen of the flesh of fruits *C* is in a complex or protein-like form, whereas in the other series of fruits more nitrogen is present but most of it is in simpler amino acid form. This difference in nitrogen quality is not surprising in view of the fact that it has been frequently observed (32, 33, 34, 36) that the vegetative organs of high-carbohydrate plants often contain a comparatively high percentage of their organic nitrogen in a complex protein-like form, although exceptions to this general statement have been found, as when the plant is high in carbohydrates but a lack of phosphorus (25) or potassium (35) limits protein synthesis.

Tannin.—The results of the tannin determination, expressed as percentage of gallotannic acid in the flesh of the peach and presented in table 3, may be considered on a relative basis, but are not in any sense absolute. In the flesh of green fruits from the high-carbyhydrate tree there is very much more tannin than in the flesh of the green peaches borne on the high-nitrogen tree. Such a difference, however, is not surprising, as it is well known that fruits borne on a peach tree that is extremely high in carbohydrates as the result of girdling (24) are very astringent (10). Likewise it

[8]On the other hand, fruits from peach trees *extremely* high in carbohydrates are of very poor quality and often astringent (10).

is common knowledge that oak trees of a high-carbohydrate type grown in a poor, sandy soil, low in nitrogen, yield a bark higher in percentage of tannin than those grown on a lowland soil rich in nitrogen.

As the peaches from the high-carbohydrate tree ripened, there was a decrease in percentage of tannin. This decrease was so great that when peaches *C* and *N* were soft ripe, on August 31 and September 9, respectively, there was no significant difference in their tannin content (table 3). It is worthy of note, however, that if a commercial picking of fruits *N* had been made on August 31, at the time of the last picking of fruits *C*, the peaches from the high-nitrogen tree would have been twice as high in tannin as the fruits borne on the high-carbohydrate tree (table 3), and, in taste, much more astringent.

It is not clear why the peaches from the high-nitrogen tree increased in percentage of tannin from August 20 to August 31 (table 3) whereas the fruits from the high-carbohydrate tree during the same period decreased in tannin, though it may be associated with the comparatively late accumulation of sugars in the peaches from the high-nitrogen tree.

Ash.—The percentage of total ash is higher in young than in old fruits and apparently at all stages of growth higher in fruits *N* than in fruits *C*. As sugars increase, the percentage of ash decreases, apparently indicating, as in the case of nitrogen, that there is no considerable intake of the mineral elements after the early stages of fruit development.

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