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High-Performance Liquid Chromatographic Analysis of D-*manno*-Heptulose, Perseitol, Glucose, and Fructose in Avocado Cultivars

Philip E. Shaw,* Charles W. Wilson, III, and Robert J. Knight, Jr.

The monosaccharides fructose, glucose, and *manno*-heptulose and the seven-carbon alcohol perseitol in freeze-dried pulp of ripe fruit from 21 avocado cultivars were quantitated by high-performance liquid chromatography (LC). For one cultivar (Lula), several samples of ripe and unripe pulp were analyzed. The results confirmed some earlier reports, but disagreed with others, concerning total sugars, total *manno*-heptulose plus perseitol content, and changes in sugar content with ripening. *manno*-Heptulose, which can cause "instant diabetes" in laboratory animals and in man, probably is not present in sufficient quantity in avocados to affect the blood sugar levels of normal humans, but its effect on those predisposed to diabetes is less certain. Correlation of *manno*-heptulose content with race of avocado cultivar showed that it might be possible to breed new cultivars with low *manno*-heptulose levels.

Avocados are the richest known natural source of the seven-carbon sugar D-*manno*-heptulose (Simon and Kracier, 1966), but a systematic study of the *manno*-heptulose content in avocados has only been carried out on a few cultivars (Simon and Kracier, 1966; Ogata et al., 1972). The related seven-carbon alcohol perseitol is also present in avocados (Richtmeyer, 1963). *manno*-Heptulose possesses the physiological ability to cause inhibition of insulin secretion in humans, thereby producing "instant diabetes" (Simon and Kracier, 1966; Johnson and Wolff, 1970). There is a wide variation in its reported content among cultivars and those grown in California apparently contain relatively low levels. Cochran and Poucher (1964) fed diets high in avocados to 30 diabetics and found no adverse effects on blood sugar levels, but the avocado cultivar was not specified. Since the study was carried out in California, it was likely one low in *manno*-heptulose content. Thus, a more thorough systematic study of the variation in *manno*-heptulose content among cultivars is needed if the possible physiological effects of avocados on both normal humans and on those predisposed to diabetes is to be assessed. In addition, the conflicting data on change in *manno*-heptulose content with ripening of the fruit and on total *manno*-heptulose plus perseitol content need to be clarified (Simon and Kracier, 1966; Davenport and Ellis, 1960).

Previous studies on *manno*-heptulose content in avocados have shown widely varying amounts depending on cultivar, on degree of ripeness, and (allegedly) on whether the fruit was attached to the tree during ripening (LaForge, 1917; Montgomery and Hudson, 1939; Davenport and Ellis, 1960; Richtmyer, 1962; Simon and Kracier, 1966; Ogata et al., 1972).

In two of those studies, the *manno*-heptulose contents in several cultivars were determined and compared. Thus, Simon and Kracier (1966) reported the *manno*-heptulose content in 12 cultivars, but provided no experimental details for their analyses. All samples except one were picked in the unripe state and allowed to ripen at room temperature. The one sample (of Lula) the authors stated had ripened on the tree contained slightly less than a sample picked in the unripe state and then allowed to ripen. The other systematic study of *manno*-heptulose content in avocados was reported by Ogata et al. (1972). They studied the *manno*-heptulose content in both pulp and peel of four avacado cultivars. The unripe pulp contained relatively high levels (0.64–2.5%), while the ripe pulp contained consistently lower levels (0.03–0.5%) of *manno*-heptulose. No data were reported in either study to indicate variability in *manno*-heptulose content from tree to tree.

Perseitol is found in some avocado cultivars in relatively high quantity, especially in the seed. Simon and Kracier (1966) concluded, without presenting experimental evidence, that the total perseitol plus *manno*-heptulose content of avocado flesh was about 5% of the fresh weight and that only the proportion of the two varied. However, Richtmyer (1962) had previously summarized the available data on perseitol content of avocado flesh and concluded that the yields of *manno*-heptulose and perseitol differ from one cultivar to another.

In the current study, the quantities of monosaccharides and perseitol were determined in 21 avocado cultivars grown in Florida. For one cultivar (Lula) these contents were measured at various stages of fruit maturity from a single tree during a season. Levels of monosaccharides and perseitol were correlated with variety, production area (California vs. Florida), and ripening procedures so that conflicting data previously reported might be clarified.

EXPERIMENTAL SECTION

All fruit were obtained from the Subtropical Horticulture Research Station, Miami, FL, and were either processed 1–2 days after picking (unripe samples) or were stored at 15–21 °C until ripe (7–10 days). Samples of pulp for freeze-drying were weighed, blended with an equal weight of water in a Waring Blendor, and dried in a Stokes Model 12-F freeze-drier. Freeze-dried samples were packed under nitrogen and stored at -21 °C.

Extraction Methods. To 15 g of freeze-dried avocado powder was added 150 mL of hexane. The mixture was allowed to stand 30 min and then filtered with a coarse sintered glass funnel. The filter cake was washed with an additional 50 mL of hexane, dried under gentle suction, and then mixed to a fine paste with 85 mL of distilled water. To the avocado paste was added 250 mL of distilled water, and the mixture was allowed to stand 1 h, then filtered under vacuum with a coarse sintered glass funnel. The filter cake was washed three times with 100-mL

U.S. Citrus & Subtropical Products Laboratory, U.S. Department of Agriculture, Science and Education Administration, Agricultural Research, Winter Haven, Florida 33880 (P.E.S., C.W.W.) and U.S. Subtropical Horticulture Research Station, U.S. Department of Agriculture, Science and Education Administration, Agricultural Research, Miami, Florida 33158 (R.J.K.).

Table I. Analyses of Monosaccharides and Flavor Evaluation of Avocado Cultivars

		monosaccharides, % fresh wt				flavor	
cultivar	race[a]	Fru	Glu	Manh	Pers	rating[b]	comments
Ile de France	GxWI[c]	0.57	0.84	3.82	0.42	G	nutty, sweet
Belize	WI	0.24	0.70	3.07	1.65	G	mild, nutty
Pollock	WI	0.33	0.55	3.03	1.04	E	nutty
General Bureau	GxWI	0.65	0.79	2.87	1.04	F	mild
Dade	WI	0.64	0.70	2.6	1.16	E	nutty, sweet
Simmonds[d]	WI	0.56	1.28	2.30	1.17	E	nutty, sweet
Ruehle	WI	Tr	0.25	2.16	2.06	G	mild
Booth 7 (immature)	GxWI	0.22	Tr	1.35	N[e]	E[f]	nutty, mild
Lula, ripe,[g] 2/20/79	GxWI	0.36	0.32	1.75	0.25	*h*	*h*
Lula, ripe, 12/18/78		Tr	Tr	1.72	Tr	E	nutty, mild
Lula, ripe, 11/27/78		Tr	Tr	1.80	Tr	*h*	*h*
Lula, ripe, 10/28/78		N	N	1.84	0.60	*h*	*h*
Lula, unripe,[g] 11/17/78		Tr	N	3.07	0.72	*h*	*h*
Lula, unripe, 10/18/78		N	N	3.14	0.75	*h*	*h*
Lula, unripe, 9/20/78		Tr	N	3.12	0.84	*h*	*h*
Vero Beach	MxWI	Tr	Tr	1.28	N	P	nutty, bitter
Trapp	WI	0.08	Tr	1.22	0.62	*h*	*h*
Duke	M	Tr	Tr	1.19	N	*h*	*h*
Collinson, ripe[g]	GxWI	0.16	0.14	1.75	N	E	nutty
Collinson, unripe[g]		N	N	3.38	Tr	*h*	*h*
Bacon	M	Tr	N	0.97	N	G	nutty
Suardia	GxWI[i]	0.23	Tr	0.46	N	G	mild
Waldin	WI	0.14	Tr	0.55	N	*h*	*h*
Cellons Hawaii (Green)	WI	Tr	Tr	0.52	Tr	G	mild, smooth
Booth 8	GxWI	0.29	0.31	0.51	0.33	G	nutty
Young No. 2	M	Tr	N	0.34	N	*h*	*h*
Utuado	GxWI	Tr	Tr	0.27	N	*h*	*h*
Fuerte	GxM	Tr	N	N	N	F	nutty
Antigua Market	GxM	N	N	N	N	F	nutty

[a] G = Guatemalan; WI = West Indian; M = Mexican race. [b] Using a hedonic scale of 1-9, E = 7-9, G = 6-7, F = 5-6, and P = <5. [c] Introduced from Morocco; apparent racial composition deduced from morphology of tree and fruit. [d] Average of three samples from one batch of fruit, SD 0.09 (Fru), 0.10 (Glu), 0.19 (Manh), 0.07 (Pers). [e] N, not detected. [f] Mature sample used for flavor evaluation. [g] Ripe indicates fruit kept 7-10 days at 15-21 °C after picking until soft; unripe indicates firm fruit processed 1-2 days after picking. [h] Not determined. [i] Introduced from Cuba; apparent racial composition deduced from morphology of tree and fruit.

portions of distilled water. The aqueous filtrate was concentrated to less than 200 mL on a rotary evaporator at 60 °C and 50 mmHg, preservative (25 mg of cycloheximide and 25 mg of chloramphenicol in 20 mL of distilled water) was added (0.1 mL/5 mL of extract) and the final volume was made up to 200 mL.

High-Performance Liquid Chromatography. Samples of the above extract were purified for LC analysis by use of C_{18} Sep-Pak cartridges (Waters Associates, Inc., Milford, MA). The Sep-Pak was washed with 2 mL of acetonitrile prior to sample preparation, then 10 mL of sample was taken up in a 10 mL syringe, the Sep-Pak was attached to the syringe, and 7 mL of sample was passed through the cartridge. The first 2 mL of eluate was discarded.

Samples were chromatographed on a Waters Model 202 LC equipped with a differential refractometer, and a Waters μ Bondapak/carbohydrate column. The instrument was modified with an Altex Model 905-42 injector that was fitted with a 20-μL injection loop and a Waters Model 6000A pump. The mobile phase was acetonitrile–water (87.5:12.5, v/v). The flow rate was 1.1 mL/min for 20 min and then increased to 2.1 mL/min until perseitol was eluted.

Peak areas were determined using height × width at half-height. The average for two consecutive runs was calculated and compared with a standard curve for each sugar and for perseitol determined under identical conditions. For glucose and *manno*-heptulose, standard mixtures of the two sugars were used. Lower limit of detection for the sugars (signal-to-noise ratio less than 2) was about 0.1%. The value for the Simmonds sample in Table I was obtained by extracting three separate samples of freeze-dried pulp from one batch of fruit and averaging the results from duplicate runs from the three samples. The standard deviation for each sugar determined for the Simmonds cultivar is shown in footnote *d*.

Flavor Tests. Samples of fresh, ripe avocado meat from a single cultivar were presented to an experienced 12-member taste panel. Panelists were asked to rank the sample from 1–9, where 1 is dislike extremely and 9 is like extremely. Each panelist was asked to describe the flavor from the following list of descriptive words: mild, nutty, waxy, rancid, bitter, sweet. The average hedonic score was calculated, and each sample ranked in Table I as: excellent (E), 7–9; good (G), 6–7; fair (F), 5–6; poor (P), <5.

RESULTS AND DISCUSSION

The monosaccharides *manno*-heptulose, glucose, and fructose, and the seven-carbon alcohol, perseitol, in 21 avocado cultivars were quantitated by LC. Freeze-dried samples of avocado flesh rather than fresh samples were analyzed because they were easier to extract and the results were more reproducible.

The carbohydrate column used for the LC analyses did not completely resolve glucose and *manno*-heptulose under any conditions tried (Wilson et al., 1979). However, the resolution was sufficient for quantitative measurements (Figure 1) since the standard curves for these two sugars were determined as mixtures in the approximate ratios found in the avocado samples. Davenport and Ellis (1960) using paper chromatography were also unable to resolve these two sugars.

Another problem encountered during these analyses was the gradual plugging of the carbohydrate column by the avocado extracts unless a precolumn was used. Sequential

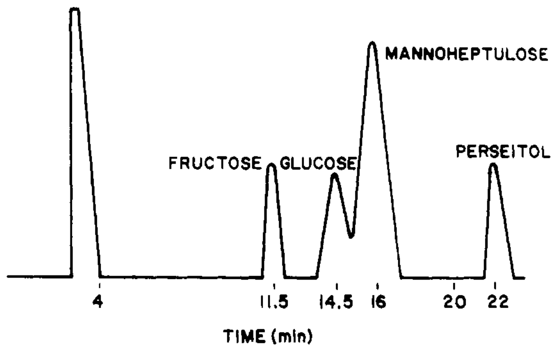

Figure 1. LC analysis of Simmonds avocado.

filtration with a C_{18} Sep-Pak cartridge and a 0.45 μm Millipore filter was inadequate to prevent column plugging, although Conrad and Palmer (1976) have found filtration with a Millipore filter adequate for many food samples. Samples, including standards, were treated with an antibacterial agent (see Experimental Section) to further protect the column from possible plugging. With these precautions, resolvability of the sugars was maintained through more than 100 sample injections.

Sugar content varied widely among the cultivars studied, as can be seen from Table I, which shows the cultivars listed in decreasing order of their *manno*-heptulose content. Those with high levels of fructose and glucose tended to contain relatively high levels of *manno*-heptulose and perseitol, as well. We found no consistent level of *manno*-heptulose plus perseitol in avocado cultivars, thus confirming the conclusion of Richtmyer (1962) rather than that of Simon and Kracier (1966).

Lula was the cultivar studied most extensively. Variation in sugar content of fruit from a single tree at different stages of maturity and degree of ripeness is shown in Table I. The *manno*-heptulose content of the green, unripe fruit was consistent at about 3.1% even though the samples were picked about a month apart. When fruit from two of these samples (10/18/78 and 11/17/78) were allowed to ripen prior to analysis, the *manno*-heptulose level had dropped to less than 2% in both cases. The *manno*-heptulose content of four samples of ripe fruit harvested a month apart was fairly consistent, with no decrease as the season progressed. There was a similar significant decrease in the perseitol content (from about 0.8% to about 0.2%) and a slight increase in the fructose and glucose contents associated with ripening, as shown by ripe and unripe Lula fruit from a single tree. Similarly, Davenport and Ellis (1960) noted the almost complete disappearance of *manno*-heptulose and perseitol, and a slight or no increase of C_6 sugars, in picked Fuerte avocados during ripening.

Simon and Kracier (1966) reported that Lula fruit allowed to ripen on the tree had a slightly lower *manno*-heptulose content that Lula fruit picked green and then allowed to ripen (3.9% vs. 4.9% *manno*-heptulose). We were not able to obtain a sample of fruit that had ripened on the tree. It is almost impossible to let avocados ripen on the tree—they tend to fall when unripe and then soften on the ground (Leopold, 1964; Lewis, 1978). The one composite sample we studied (11/27/78 in Table I) of several fruit that had fallen from the tree naturally prior to fully ripening showed no significant difference in its *manno*-heptulose content when compared with fruit that had been picked green from the same tree and allowed to ripen.

Comparison of our results on *manno*-heptulose content in those cultivars for which levels of this sugar were previously reported showed some variations. We found slightly less *manno*-heptulose (1.22%) in the Trapp cultivar than the 1.4–1.5% reported earlier (LaForge, 1917; Montgomery and Hudson, 1939), and less of this sugar in the Lula than have other workers [3.9–4.9% by Simon and Kracier (1966)]. We also found more *manno*-heptulose in the Collinson (1.75%) than previously reported [0.7% by Simon and Kracier (1966); 0.2% by Richtmyer (1962)]. Our Florida-grown sample of the Fuerte, a major cultivar produced in California, contáined a low level of *manno*-heptulose (<0.1%) that fell within the values reported earlier [0.07% by Ogata et al. (1972); 0.23% by Simon and Kracier (1966)]. Since *manno*-heptulose content decreases rapidly during ripening in many cultivars, and no standard definition of ripeness in avocados exists (Lewis, 1978), it is possible that variation in degree of ripeness for the samples tested accounts for much of the reported variation.

Our results differed from those of some previous workers in other respects. We found no measurable sucrose and no evidence in any sample for an "unknown disaccharide" described by Davenport and Ellis (1960). Unlike us, they did find fructose and glucose present in the Fuerte cultivar, but glucose was inseparable from *manno*-heptulose by paper chromatography and a difference in intensity between two spray reagents had to be used to indicate the presence of glucose. Of those that reported quantitative data for avocados, Simon and Kracier (1966) and Ogata et al. (1972) quantitated only *manno*-heptulose. The researchers in the latter study used a procedure that destroyed the six-carbon sugars to measure the seven-carbon sugars present in avocado and other subtropical fruit.

Of the 21 avocado cultivars we studied, eight are interracial hybrids of Guatemalan × West Indian origin, eight are purely West Indian, three are Mexican, and two, "Fuerte" and "Antigua Market", are mixtures of Guatemalan × Mexican race (Table I). No cultivars of purely Guatemalan racial origin were among the group tested. The only cultivars found to contain no measureable *manno*-heptulose were "Fuerte" and "Antigua Market"—the only two Guatemalan × Mexican hybrids investigated. The three Mexican avocados in the test group, "Young No. 2", "Bacon", and "Duke", ranged in content of this sugar from 0.27 to 1.19%, and the only Mexican × West Indian hybrid in the group, "Vero Beach" (M-20535 on the Miami Station inventory), contained 1.28% *manno*-heptulose. The seven pure West Indian cultivars in the group averaged 2.21% in their content of this sugar (if measurements on the most mature samples are averaged), but ranged from lows of 0.52 and 0.55 [Cellon's Hawaii (Green) and Waldin] to 3.03 and 3.07 (Pollock and Belize). The Guatemalan × West Indian hybrids ranged from a low of 0.27% (Utado) to Ile de France's high of 3.82, but averaged 1.52% *manno*-heptulose—0.69 percentage points lower than the average of the pure West Indians.

The lack of any pure Guatemalan-race cultivars in the group observed leaves unanswered the question of whether the interracial hybrids derive their elevated content of *manno*-heptulose from the West Indian parent alone. The fact, however, that individual West Indian cultivars such as Waldin and Cellon's Hawaii (Green) were quite low in this sugar, casts some doubt on such a hypothesis. None of the pure Guatemalan-race cultivars analyzed by previous workers contained a high level of *manno*-heptulose (Ogata et al., 1972; Simon and Kracier, 1966). The four samples studied contained a relatively low average level of 0.3% *manno*-heptulose for the Guatemala (0.01), Itzamma (0.2), Benik (0.37), and Nabal (0.68%) cultivars. Ogata et al. (1972) reported the Hass cultivar as pure Guatemalan, but it is now believed to be a hybrid (Guatemalan × Mexican)

(Knight, 1979). In that study, no Mexican or partly-Mexican cultivar contained more than 1.28% *manno*-heptulose.

The flavor of most cultivars used in this study was determined on fresh, ripe slices by an experienced 12-member taste panel, who rated the samples on a hedonic scale of 1–9. The three samples for which a majority of panelists noted a sweet flavor were all among those that had relatively high levels of the sugars measured. In none of the samples with relatively low measured levels of sugars did the panel note a sweet flavor. One cultivar that consistently received a relatively low score was Antigua Market, which had no detectable level of any of the sugars. The Dade consistently received the highest score of any cultivar tested. In addition to the nutty, sweet character, this fruit had a mildness with no one flavor character predominating. The sample of Vero Beach used in this study had a characteristic bitterness that lowered the flavor score. Apparently, not all fruit of this cultivar have this bitterness (Berry et al., 1977), so the rating in Table I may not be typical of this fruit.

Flavor, size, and durability during storage and handling all affect the commercial marketability of avocado cultivars, but *manno*-heptulose content may be an important consideration for food safety. Of the 10 most important commercial cultivars grown in Florida (Knight, 1980), six were analyzed in this study, and most contained relatively large quantities of *manno*-heptulose. Two major commercial cultivars produced in greatest quantity in Florida are Booth 8 and Lula; the latter contains a much greater quantity of *manno*-heptulose than the former. Booth 8 contained the lowest concentration of *manno*-heptulose in any Florida commercial cultivar studied. Our study suggests that selective breeding to lower the content of this sugar in all commercial cultivars is feasible.

It is unlikely that normal humans consume enough *manno*-heptulose from avocados to significantly affect their blood sugar levels. We calculated that a person would have to consume 524 g of Ile de France avocado meat (the cultivar with the highest *manno*-heptulose content in our study) in one sitting to ingest 20 g of *manno*-heptulose, which is the amount reported by Johnson and Wolff (1970) to cause a measurable increase in blood sugar level of humans. This is equivalent to about 2.5 medium-sized avocados. One source for error in this approximation is that *manno*-heptulose in avocado is probably absorbed differently than when ingested in the pure form (Johnson and Wolff, 1970). Since about 65% of the ingested *manno*heptulose is excreted within 24 h (Simon and Kracier, 1966), cumulative effects on blood sugar from daily consumption of avocado would likely not be significant. From our approximation, it appears unlikely that blood sugar levels in normal humans would be affected by normal avocado consumption. However, in persons predisposed to diabetes considerably less *manno*-heptulose might increase their blood sugar levels. Ogata et al. (1972) reached a similar conclusion, but reported no calculations.

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