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Development of a Rapid Method for the Sequential Extraction and Subsequent Quantification of Fatty Acids and Sugars from Avocado Mesocarp Tissue

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Methods devised for oil extraction from avocado (*Persea americana* Mill.) mesocarp (e.g., Soxhlet) are usually lengthy and require operation at high temperature. Moreover, methods for extracting sugars from avocado tissue (e.g., 80% ethanol, v/v) do not allow for lipids to be easily measured from the same sample. This study describes a new simple method that enabled sequential extraction and subsequent quantification of both fatty acids and sugars from the same avocado mesocarp tissue sample. Freeze-dried mesocarp samples of avocado cv. Hass fruit of different ripening stages were extracted by homogenization with hexane and the oil extracts quantified for fatty acid composition by GC. The resulting filter residues were readily usable for sugar extraction with methanol (62.5%, v/v). For comparison, oil was also extracted using the standard Soxhlet technique and the resulting thimble residue extracted for sugars as before. An additional experiment was carried out whereby filter residues were also extracted using ethanol. Average oil yield using the Soxhlet technique was significantly ($P < 0.05$) higher than that obtained by homogenization with hexane, although the difference remained very slight, and fatty acid profiles of the oil extracts following both methods were very similar. Oil recovery improved with increasing ripeness of the fruit with minor differences observed in the fatty acid composition during postharvest ripening. After lipid removal, methanolic extraction was superior in recovering sucrose and perseitol as compared to 80% ethanol (v/v), whereas mannoheptulose recovery was not affected by solvent used. The method presented has the benefits of shorter extraction time, lower extraction temperature, and reduced amount of solvent and can be used for sequential extraction of fatty acids and sugars from the same sample.

KEYWORDS: ***Persea americana*; lipids; Soxhlet**

INTRODUCTION

Avocado fruit is valued for its high lipid content in mesocarp tissue, which can vary between 15 and 30% (on a fresh weight basis) depending on cultivar (*1, 2*) and seasonality. Avocado fruit is considered to be one of the most important natural sources of monounsaturated food-derived lipids and essential fatty acids such as linoleic and linolenic acid (*3*). Recent research has shown that diets enriched with avocado pulp have a cholesterol-lowering effect (*4–6*). Oil accumulates during fruit growth and maturity on the tree, with a large increase in the oleic acid fraction. Once fruit has been harvested, oil no longer accumulates, and it has been shown that there is little change in fatty acid profile during postharvest ripening (*7*).

Soluble sugars content in avocado mesocarp tissue is dominated by the seven-carbon (C_7) sugar D-mannoheptulose and its reduced form polyol, perseitol (*8–10*), whereas sucrose, glucose, and fructose are present in lower concentrations. Sugars play an essential role in avocado fruit growth and development but are also considered to be important respiratory substrates during fruit ripening (*9*). It has also been suggested that the C_7 sugars could be involved in the ripening inhibition of the fruit while still attached on the tree and shortly after harvest (*8, 9*). Mannoheptulose has long been linked to improved health (*11*), and recent research has reported that it may inhibit insulin secretion and have anticancer activity (*12–14*).

The standard method for determining lipid content in various foodstuffs is the Soxhlet technique, which commonly uses conventional solvents such as hexane with a boiling point (bp) of 66−69 °C or petroleum ether (bp 40−60 °C). Accordingly, the Soxhlet method has been used extensively to extract oil from avocado mesocarp (*2, 7, 15–18*). However, this technique has the disadvantage of being time-consuming and requires operation at relatively high temperature. Alternative lipid extraction techniques such as homogenization with a solvent (e.g., petroleum ether) or supercritical carbon dioxide ($SC\text{-}CO_2$) have been compared to Soxhlet extractions in avocado fruit (*15, 18*). In contrast to fatty acid analysis in avocado, there is a paucity of published methods describing the extraction and quantification of soluble sugars from avocado mesocarp tissue. Most protocols

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rely on the use of ethanol 80% (v/v) (*8–10*) as the extracting solvent. Crucially, lipids are not recovered during this process, and to the best of our knowledge, no method has yet been reported whereby both lipids and sugars have been extracted from the same mesocarp sample.

The aim of this work was, therefore, to develop a simple and rapid procedure to sequentially extract and quantify both neutral lipids and sugars from the same mesocarp sample of avocado fruit at three different ripening stages, and compare this to previously published methods that have measured fatty acids or sugars separately.

MATERIALS AND METHODS

Reagents, Plant Material, and Sample Preparation. All chemicals used were of analytical grade. Hexane, methanol, and ethanol were purchased from Fisher Scientific Chemicals (Leics., U.K.). Methyl palmitate, methyl palmitoleate, methyl oleate, methyl linoleate, methyl linolenate, sucrose, D-glucose, D-fructose, and D-mannoheptulose standards were purchased from Sigma (Dorset, U.K.). Perseitol (D-glycero-D-galacto-heptitol) was obtained from Industrial Research Ltd. (IRL - Fine Chemicals, New Zealand).

Early season avocado (*Persea americana* Mill.) cv. Hass fruit ($n = 72$), originating from Malaga (Spain), were harvested on January 25, 2007, and supplied by M. W. Mack Ltd. (Kent, U.K.). Fruits were stored overnight at 12 °C. Fruits were not pretreated with 1-methylcyclopropene. On arrival at the laboratory, fruits were 4 days old after harvest and were considered to be preclimacteric. Fruits were held in 3 L jars at 12 °C for 3 days and then removed to avoid CO_2 poisoning (*19*). On days 3, 5, and 9, a fruit subsample ($n = 18$) was removed, and lightness (L^*), chroma (color saturation; C^*), and hue angle ($H°$) were measured (*19*). Firmness was measured as described elsewhere (*19*) with slight modification in that force was recorded at the bioyield point. Three levels of ripeness, defined by firmness range [viz. underripe (>50 N), medium-ripe (50−15 N), and eating-ripe (<5N)], were selected comprising three fruits per maturity level and used for further lipids and soluble sugars analysis. Each fruit was a replicate and extracted for lipids and soluble sugars in triplicate.

Fruits were cut in half vertically into two equal sections. The stone and peel of one-half were removed manually and discarded. The remaining mesocarp was then immediately chopped into small chunks and randomly mixed. Approximately 30 g of sample was immediately snap-frozen in liquid nitrogen and held at −40 °C before being freeze-dried in a Christ ALPHA-RVC freeze-dryer with cooling-trap ALPHA 1-4 (Christ, Osterode, Germany) for 7 days. Dry weight (DW) was determined, and samples were returned to −40 °C prior to analysis.

Lipid Extraction. Lyophilized mesocarp tissue [1 g, ca. 3.8 g of fresh weight (FW)] was ground to a powder using a pestle and mortar and homogenized with hexane (30 mL) for 30 s using an Ultra-Turrax T25 homogenizer (Janken & Kunkel Ika-Labortechnik, Staufen, Germany). The mixture was allowed to stand at room temperature for 1 min before filtering under vacuum, using a Büchner flask and funnel, through a 5.5 cm diameter Fisherbrand QL 100 filter paper (Fisher Scientific, Leics., U.K.). The powdered residue was recovered from the filter paper and washed again with 20 mL of fresh hexane. The mixture was allowed to stand at room temperature for another 1 min before being filtered as before. Additional hexane (10 mL) was used to rinse the beaker and funnel. All lipid-containing filtrates were combined (60 mL), and the solvent was removed using a rotary evaporator (Buchi Rotovapor, Büchi Labortechnik AG, Flawil, Switzerland) under vacuum at 40 °C. The recovered oil was weighed and stored under nitrogen in capped amber glass vials at −40 °C until fatty acid analysis. The filter residue was allowed to stand for approximately 2 h at room temperature until no more hexane was present. The residue was weighed and stored in vials at −40 °C for subsequent extraction and analysis of nonstructural carbohydrates.

The Soxhlet technique was used for validation of the method described above and carried out according to AOAC 963.15 (*20*) with modifications. The thimble containing ground freeze-dried mesocarp from the same sample (1 g, ca. 3.8 g FW) was placed in the Soxhlet device, and 150 mL of hexane was placed in the round flask with a few defatted antibumping granules (Fisher Scientific). The sample was refluxed for approximately 1 h, with the heat adjusted so that the extractor siphoned eight times (approximately 70 °C). The flask was removed and the solvent evaporated on a rotary evaporator as previously described. The recovered oil was weighed and stored as before. The thimble residue was allowed to stand at room temperature until no more hexane was present, and the residue was then stored as described earlier.

Sugars Extraction. Extracts for soluble sugars determination were prepared from the residue obtained following either hexane homogenization or Soxhlet extractions, using either methanol or ethanol (following homogenization only) as solvents. Powdered residue (150 mg) was combined with 3 mL of 62.5% aqueous methanol (v/v) (*21*) or 3 mL of ethanol 80% (v/v) (*8*) and mixed well. Vials (7 mL polystyrene bijou vials; Sterilin, Staffs., U.K.) containing the slurry were placed in a shaking water bath at 55 °C for 15 min, removed briefly, and vortexed (Vortex Genie 2, Scientific Industries, New York) for 20 s every 5 min. The samples were then filtered through syringe filters (0.2 μm pore diameter; Millipore Corp., Bedford, MA) and stored at −40 °C until needed. Extracts were diluted 1:10 with water (HPLC grade) immediately before analysis.

Fatty Acid Identification and Quantification. Fatty acid methyl esters (FAMEs) were produced according to the method prescribed by the International Olive Oil Council (IOOC) (*22*) with modifications. Briefly, 0.2 mL of methanolic KOH (2 N) was added to 0.1 g of avocado oil extract in 2 mL of hexane. Hexane was chosen as the preferred solvent due to improved peak resolution. The mixture was shaken vigorously for 30 s and left to stratify until the upper layer became clear. The hexane layer containing the methyl esters was decanted and kept for no more than 12 h at 5 °C until needed. This solution was diluted 1:100 (v/v) with fresh hexane immediately before injection into an Agilent 6890N GC (Agilent Technologies, Cheshire, U.K.) equipped with a G1540N flame ionization detector (FID) and a 7683B autosampler. The identification and quantification of selected compounds were performed on a CP-Sil 88 fused silica capillary column (30 m × 0.25 mm i.d., 0.2 μm film thickness; Varian, Palo Alto, CA). Column temperature was programmed at 55 °C for 3 min, and then raised to 175 at 13 °C min^{-1} intervals followed by an isothermal period of 1 min, and increased again to a final temperature of 220 at 8 °C min^{-1}. The carrier gas was He at a constant flow rate of 1.6 mL min^{-1}. The injector and detector temperatures were set at 220 and 250 °C, respectively. The presence and abundance of fatty acids were calculated by comparison of peak area with standards (methyl palmitate, methyl palmitoleate, methyl oleate, methyl linoleate, and methyl linolenate).

Sugar Identification and Quantification. Concentrations of fructose, glucose, sucrose, mannoheptulose, and perseitol were determined using a HPLC system comprising a P580 pump, a Dionex STH column thermostat, and a GINA 50 autosampler (Dionex, Sunnyvale, CA) based on that described previously (*21*). The diluted avocado extract (20 μL) or standard sugar solution was injected into a Rezex RCM monosaccharide Ca^+ (8%) size exclusion column of 300 mm × 7.8 mm diameter, 8 μm particle size (Phenomenex, Torrance, CA; part 00H-0130-K0) with a Carbo-Ca^+ security guard cartridge of 4 mm × 3 mm diameter (Phenomenex). The mobile phase was HPLC grade water at a flow rate of 0.6 mL min^{-1}. Column temperature was held at 75 °C using a Dionex STH column thermostat. Eluted soluble sugars were monitored using an evaporative light scattering detector (ELSD 2420, Waters, Milford, MA) (*21*) connected to the Dionex system using a UCI-50 universal chromatography interface. The presence and abundance of the selected sugars were automatically calculated by comparison of peak area with peak area of known standards using Chromeleon version 4.6 software (Dionex).

Statistical Analysis. All statistical analyses were carried out using Genstat for Windows vers. 10 (VSN International Ltd., Herts., U.K.). Data were subjected to analysis of variance (ANOVA). Least significant difference values (LSD; $P = 0.05$) were calculated for mean separation using critical values of t for two-tailed tests. Tests for correlations between mean values for sugars concentrations were made using Pearson's product moment correlation. Correlations are presented with the Pearson's correlation coefficient (r) and P value based on a two-

Table 1. Effect of Ripening Stage [Under-ripe (UR), Medium-Ripe (MR), and Eating-Ripe (ER)] and Storage Time on Fresh Weight, Dry Matter Content, Firmness, Lightness (L^*), Chroma (C^*), and Hue Angle ($H°$) of Avocado cv. Hass Fruit Stored for 9 Days at 12 °C[a]

ripening stage	storage days	fresh wt (g)	dry matter (g 100 g^{-1} FW)	firmness (N)	L^*	C^*	$H°$
UR	2	181.74a	25.63a	74.67a	33.86a	17.33a	123.58a
MR	5	179.50a	26.71a	25.00b	30.46ab	12.90a	114.80a
ER	9	166.33b	27.09a	3.83b	26.76b	6.45b	48.11b

[a] Values with different letters are significantly different ($P < 0.05$).

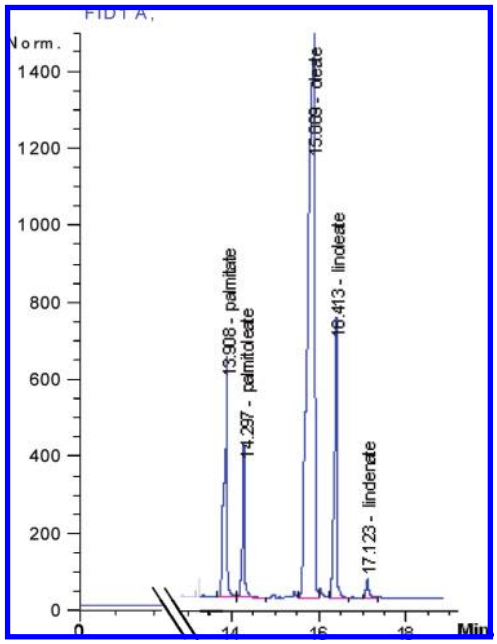

Figure 1. Typical GC-FID chromatogram of the main fatty acids present in oil extracted from avocado cv. Hass fruit mesocarp tissue.

tailed test. Unless otherwise stated significant differences were $P < 0.05$. Means with different letters in tables are significantly different from one another ($P < 0.05$) unless otherwise stated.

RESULTS

Physical Measurements. During 9 days of storage at 12 °C, there was a decrease in fresh weight, firmness, L^* and C^* values and $H°$ and an increase in dry matter content (**Table 1**). Fresh weight, L^*, C^*, and $H°$ were all significantly lower at eating-ripe stage as compared with medium- and under-ripe stages. Eating-ripe and medium-ripe fruits were significantly less firm as compared with under-ripe fruits. Concomitant to changes in color, weight, and firmness, dry matter content increased (but not significantly) as ripening advanced (**Table 1**).

Oil Yield and Fatty Acid Identification and Quantification. The Soxhlet extraction technique resulted in a significantly higher quantity of oil extracted from avocado mesocarp tissue (0.61 g g^{-1} total mesocarp tissue DW, 0.16 g g^{-1} FW) as compared with the homogenization extraction technique (0.54 g g^{-1} DW, 0.14 g g^{-1} FW), respectively. The gas chromatography method developed and presented in this study successfully identified and quantified fatty acids in avocado oil extracts. A final run time of <20 min was required to elute all fatty acids present (**Figure 1**). In all oil samples, oleic acid was predominant, constituting 56.93% of total fatty acids. In descending order of abundance, other fatty acids quantified were palmitic (20.92%), linoleic (12.16%), palmitoleic (8.88%), and linolenic acids (1.12%). The fatty acid profiles (percent total fatty acids) of the oils extracted by homogenization and by Soxhlet were very similar, with no significant differences found between extraction methods for palmitic, palmitoleic, oleic, and linoleic acids (**Table 2**). However, the proportion of the polyunsaturated fatty acid linolenic acid was significantly, yet slightly, higher following homogenization with hexane (1.14%) in comparison to the Soxhlet technique (1.10%).

Concentrations of each fatty acid were also determined in all oil samples and expressed as milligrams per gram of oil extracted and milligrams per gram of DW (**Table 2**). Greater amount of all the fatty acids measured were extracted via homogenization, resulting in a significantly higher value of total fatty acids (153.0 mg g^{-1} oil) as compared with the Soxhlet technique (141.86 mg g^{-1} oil). Crucially, the concentration of polyunsaturated fatty acids (linoleic and linolenic) was significantly higher in samples extracted by homogenization (20.47 mg g^{-1} oil) than by Soxhlet (18.70 mg g^{-1} oil). There was an interaction between extraction method and ripening stage for palmitic, oleic, and linoleic acids at medium- and eating-ripe stages, with the highest concentrations (per oil weight) found in the homogenization extracts versus Soxhlet extracts (**Table 2**). Conversely, significant differences for palmitoleic acid were found between the methods at the medium-ripe stage only, with greater concentrations obtained in homogenization extracts. On DW and FW basis, there was no significant interaction between extraction method and ripening stage for concentration of all fatty acids (**Table 2** and data not shown, respectively).

The effect of ripening stage on oil yield, oil fatty acid composition, and concentrations was investigated for samples extracted by homogenization. Oil recovery was improved as fruit ripened, with values significantly higher at the eating-ripe stage (0.61 g g^{-1} DW) as compared with the medium- and under-ripe stages (0.53 and 0.48 g g^{-1} DW, respectively). Additionally, ripening stage had a main effect on the fatty acid profile, whereby the dominant fatty acid, oleic acid, was significantly lower at the medium-ripe stage (54.90%) as compared with the under-ripe and eating-ripe stages (57.72 and 58.25%, respectively). On the other hand, the second most abundant fatty acid, palmitic acid, was significantly higher at the medium-ripe (21.83%) and eating-ripe stages (20.89%) versus the under-ripe stage (19.84%). Per DW, concentrations of all fatty acids increased concomitantly to degree of ripeness. Specifically, amounts of palmitic, palmitoleic, and linoleic acid were significantly greater at the medium- and eating-ripe stages as compared with the under-ripe stage, whereas significantly greater concentrations of oleic acid were found at the eating-ripe stage versus the under- and medium-ripe stages (**Table 2**).

Soluble Sugars. Soluble sugars were extracted from the filter residue recovered after lipid extraction. For the extraction and quantification of sugars, the method described in this work was successfully adapted and slightly modified from previously reported methods applied to other fruits (*21*). Mannoheptulose, perseitol, and sucrose were the main sugars identified in all samples (**Figure 2**). Fructose and glucose were detected, but their presence was at or near the detection limit, and thus they were not considered (**Figure 2**). Sugars were differentially affected by the extraction method (**Table 3**). Specifically, sucrose and perseitol concentrations (residue and DW basis) were both significantly lower in ethanol extracts than in methanol extracts following either homogenization or Soxhlet extraction (**Table 3**). In contrast, the use of either methanol or ethanol as extraction solvent had no effect on mannoheptulose for residues obtained from homogenization with hexane. This

Table 2. Effect of Extraction Method on the Concentration of Fatty Acids in Avocado cv. Hass Fruit Mesocarp at Under-ripe (UR), Medium-Ripe (MR), and Eating-Ripe (ER) Stages, Expressed per Gram of Oil, per Dry Weight, and as Percentage of Total Fatty Acids (FA)[a]

		palmitic acid		palmitoleic acid		oleic acid		linoleic acid		linolenic acid	
	ripening stage	homog[b]	Soxhlet[c]	homog	Soxhlet	homog	Soxhlet	homog	Soxhlet	homog	Soxhlet
% total FA	UR	19.84a	19.87a	8.82a	8.82a	57.72a	57.76a	12.44a	12.43a	1.19a	1.11a
	MR	21.83b	22.06b	9.50b	9.44b	54.90b	54.70b	12.71b	12.64b	1.16b	1.16b
	ER	20.89c	21.01c	8.34c	8.38c	58.25c	58.26c	11.45c	11.31c	1.07c	1.03c
	mean	20.85x	20.98x	8.89x	8.88x	56.95x	56.91x	12.20x	12.13x	1.14y	1.10x
mg g^{-1} DW	UR	13.74a	16.43a	6.17a	7.29a	40.62a	47.95a	8.77a	10.30a	0.84a	0.92a
	MR	18.35b	18.04b	8.09b	7.65b	46.14b	44.87b	10.70b	10.35b	0.98b	0.95b
	ER	19.40c	20.09c	7.78c	8.03c	54.07c	55.45c	10.64c	10.78c	0.99c	0.98c
	mean	17.16x	18.19x	7.35x	7.65x	46.95x	49.43x	10.03x	10.47x	0.94x	0.95x
mg g^{-1} oil	UR	29.05a	29.19a	12.93a	12.95a	84.53a	84.93a	18.35a	18.17a	1.75a	1.62a
	MR	34.70c	30.04b	15.37c	12.82b	87.34c	74.48b	20.32c	17.13b	1.86b	1.58b
	ER	31.93d	29.98d	12.76d	11.96d	88.95e	83.14d	17.50e	16.13d	1.63c	1.47c
	mean	31.89y	29.73x	13.69y	12.58x	86.94y	80.85x	18.72y	17.14x	1.75y	1.56x

[a] LSD ($P < 0.05$) used for comparing means within the same levels of ripeness. Values with different letters within the same ripening stage between the methods are significantly different ($P < 0.05$). [b] Homogenization with hexane. [c] Soxhlet extraction with hexane.

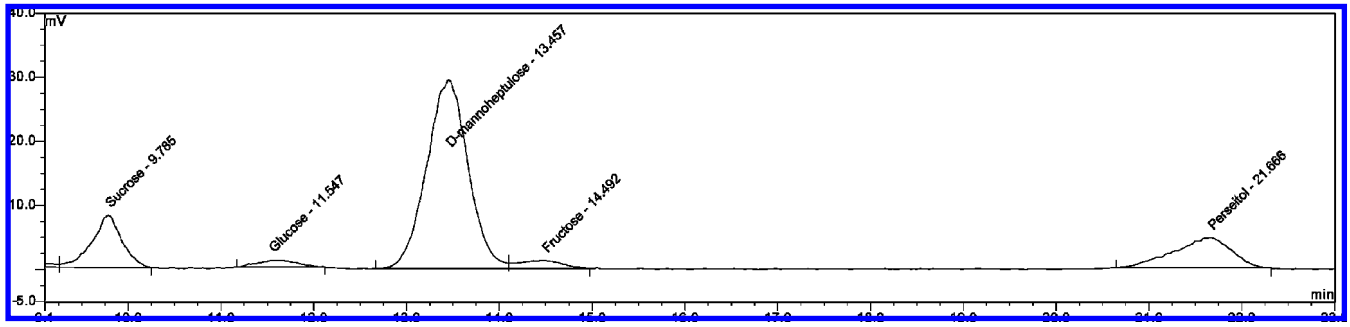

Figure 2. Typical HPLC-ELSD chromatogram of main sugars extracted from avocado cv. Hass fruit mesocarp tissue after lipid removal.

said, sugar extractions from Soxhlet residue consistently resulted in the highest concentrations of sucrose, mannoheptulose, and perseitol (**Table 3**). Concentrations of mannoheptulose and perseitol (residue weight, DW, and FW) were generally lowest at the eating-ripe stage. In particular, greater amounts of mannoheptulose, on a residue and DW basis, were extracted from under-ripe and medium-ripe fruits versus eating-ripe fruit, whereas perseitol concentration was significantly higher at under-ripe versus medium- and eating-ripe stages. In contrast, little difference between ripeness stages was seen for sucrose concentrations on a residue basis (**Table 3**). Again, there was a significant interaction between extraction method and ripening stage for all sugars, whereby significantly lower amounts of sucrose, mannoheptulose, and perseitol (residue basis) were obtained in ethanol extracts versus methanol extracts at the under-ripe stage. At the medium-ripe stage, sucrose concentration was also significantly lower following ethanol extraction as compared with methanol, whereas no differences at medium- and eating-ripe stages were found between methods for mannoheptulose and perseitol. Per DW, significant differences between the methods for sucrose and perseitol were found at the under-ripe stage, with greater concentrations in methanol extracts as compared with ethanol extracts, whereas no differences were found between methods for mannoheptulose (**Table 3**).

Pearson's product moment correlations were drawn between the three extraction method for sugars, namely, methanol extraction following either Soxhlet or homogenization and ethanol extraction following homogenization. There was generally a good correlation ($r = 0.8-0.9$) between the methanol and ethanol extractions following homogenization, for all sugars and all ripening stages. However, the correlation between the methanol extractions following homogenization or Soxhlet was much poorer ($P > 0.05$) for sucrose (0.48) and for perseitol (0.47) at the eating-ripe stage.

DISCUSSION

Oil content and dry matter of avocado fruit are associated with avocado fruit horticultural maturity and therefore have often been used as a basis for determining harvesting time. Fatty acid composition defines oil quality. Therefore, it is important to have an appropriate method that adequately recovers lipids from avocado fruits. Oil yield from avocado mesocarp is variable and differs according to genotype, harvesting time, and post-harvest ripening (*1, 7*). Nevertheless, the results of the present study (0.54−0.61 g g^{-1} DW; 0.14−0.16 g g^{-1} FW) are in general agreement with those reported in earlier works, where values of 56−58% DW were found for avocado cv. Hass fruit (*7*) and ca. 54% DW was found for an unknown cultivar extracted with hexane (*17*). Higher values of 74−75% DW (ca. 23.5% FW) (*15*) and ca. 70% have been reported for avocado cv. Fuerte (*18*).

The fatty acid profile from all tested samples was as expected for avocado and consistent with that reported in the literature for avocado fruit in general (*7, 16, 17, 23*). The predominance of the monounsaturated fatty acid oleic acid (from 55 to 58%) has been reported previously (*7, 16, 17, 24*). Palmitic acid, the major saturated fatty acid, was the second most abundant fatty acid (20−22%). Linolenic acid was very scarce in the neutral fraction of the mesocarp lipids (<1.2%), but in agreement with others (*24*), who found <1.1% linolenic acid.

Table 3. Effect of Extraction Method on the Concentrations of Sugars in Avocado cv. Hass Fruit Mesocarp at Under-ripe (UR), Medium-Ripe (MR), and Eating-Ripe (ER) Stages, Expressed per Residue Weight and per Dry Weight (DW)[a]

	ripening stage	sucrose			mannoheptulose			perseitol		
		homog + MeOH[b]	Soxhlet + MeOH[c]	homog + EtOH[d]	homog + MeOH	Soxhlet + MeOH	homog + EtOH	homog + MeOH	Soxhlet + MeOH	homog + EtOH
mg g^{-1} residue	UR	41.20c	38.99b	30.94a	57.49b	59.62b	53.75a	58.98b	66.95c	53.13a
	MR	29.63e	32.69f	26.59d	80.52c	94.12d	81.86c	44.77d	51.83e	43.73d
	ER	36.41g	44.03h	34.64g	32.12e	35.83f	32.57e	40.66f	43.59g	39.33f
	mean	35.75y	38.57z	30.72x	56.71x	63.19y	56.06x	48.14y	54.13z	45.40x
mg g^{-1} DW	UR	19.74c	17.72b	14.56a	27.82a	27.51a	25.85a	28.14b	30.62c	25.16a
	MR	14.01de	14.95e	12.37d	37.96b	42.64b	38.32b	21.03d	23.47e	20.44d
	ER	13.70f	15.91g	13.17f	12.14c	12.83c	12.26c	15.54f	15.66f	14.93f
	mean	15.81y	16.19y	13.37x	25.97x	27.66y	25.48x	21.57y	23.25z	20.18x

[a] LSD ($P < 0.05$) used for comparing means within same levels of ripeness. Values with different letters within the same ripening stage between the methods are significantly different ($P < 0.05$). [b] Homogenization with hexane followed by methanolic extraction. [c] Soxhlet extraction with hexane followed by methanolic extraction. [d] Homogenization with hexane followed by ethanolic extraction.

Effect of Method on Oil Yield. Oil extraction from avocado mesocarp with petroleum using the Soxhlet technique has previously been compared to sample homogenization with petroleum ether (*15*), and similarities in oil recovery were found for both methods (74−75% DW; ca. 23.5% FW). More recently, the efficacy of extracting avocado oil using hexane or supercritical carbon dioxide (SC-CO_2) was investigated (*18*); better results were found with the former, possibly because hexane is less selective during extraction and better permeates whole plant material, leading to more exhaustive extraction. In the present study, the differences in the oil yielded by hexane extraction using either the conventional Soxhlet technique or homogenization, although statistically different, remained very slight. Any differences are probably due to a longer extraction time (i.e., eight times siphoning; approximately 1.5 h) at higher operating temperature (approximately 70 °C) when using the Soxhlet system. Yet, the new method described here requires only homogenization of the sample with hexane at ambient temperature and, once samples have been freeze-dried, the method takes ca. 10 min per sample as opposed to 1−6 h for Soxhlet extraction. Considerably less solvent (60 mL per sample) is required compared to Soxhlet extraction (150 mL). It must be noted, however, that a neutral solvent, such as hexane, will only tend to recover nonpolar lipids (triglycerides) and that a more polar solvent is usually required to extract more polar lipids, namely, glycolipids and phospholipids.

It has been reported that oil content in avocado fruit does not increase after harvest (*2, 25*). Greater recovery in oil yield during ripening of avocado fruit observed herein has been reported elsewhere (*7, 18*). Changes in the mesocarp at the ultrastructural level typically occur during fruit ripening and have previously been associated with the activity of the cell wall degrading enzymes, cellulase and polygalacturonase (*26–28*). It has been hypothesized that this structural degradation possibly causes oil to be liberated from cellular bodies, specifically triacylglycerols from parenchyma cells, making it more available for extraction (*18, 29*). This said, the idioblastic oil cells, which have a different composition and function from parenchyma cells, are less sensitive to the activity of these enzymes and remain relatively intact during ripening (*29*).

Effect of Extraction Method on Fatty Acid Composition. There was little change in the fatty acid profile during postharvest ripening, as already reported for avocado cv. Hass fruit (*7*). Crucially, this study showed that the fatty acid profile of oil obtained following hexane extraction with either Soxhlet or homogenization was not different, suggesting that high-temperature (approximately 70 °C) lipid extraction may not be detrimental to fatty acids. Most studies that have analyzed avocado oil have not quantified fatty acids on either a fresh or dry weight basis or per gram of oil recovered, but have rather stated the relative proportion of each fatty acid (*7, 16, 17, 23, 24*). This study presents concentrations of fatty acids, and results showed that the oil extracted by homogenization generally contained higher concentrations of fatty acids than the oil extracted by the Soxhlet method (**Table 2**). A possible explanation may be that Soxhlet extracted in a more exhaustive manner than homogenization with hexane, therefore recovering more nontarget compounds other than triglycerides such as gums, waxes, and nonsaponifiable material (viz. sterols, pigments, and hydrocarbons), resulting in a higher overall oil value. Although the fatty acid profile (percent total fatty acid) did not change as fruit ripened, there was an overall increase in fatty acid concentrations (DW and FW basis) as fruit ripened, and this

was most probably caused by the apparent increase in oil content associated with ripening.

Effect of Extraction Method on Soluble Sugars. The principal sugars present in mesocarp tissue were mannoheptulose, perseitol, and sucrose, as previously reported (*8–10, 30*). Mannoheptulose and sucrose concentrations found in the present study were higher than previously reported (*9*), whereas perseitol concentrations were in agreement with others (*8, 9*). Differences in the harvest season and fruit origin could have accounted for these discrepancies as it has been shown that nonstructural carbohydrates, especially the seven-carbon (C_7) sugars, tend to decline throughout the season (*8*). Other work (*31*) that used a crude method to quantify total sugars and therefore did not discriminate between individual sugars also found a seasonal decline in the total sugar content of avocado cvs. Fuerte, Ettinger, and Hass fruit. Nevertheless, and in accordance with that previously found (*8, 9*), all sugars studied herein exhibited a decreasing trend during fruit ripening. Comparison of the efficacy of methanol (62.5%, v/v) and ethanol (80%, v/v) as extraction solvents has been reported for sucrose in onion (*32*), but not for sugars in avocado fruit. In the present study, the efficacy of these solvents was compared on residues obtained from homogenization with hexane. Results showed that methanolic extraction was ca. 1.2-fold more efficacious (on a residue basis) in extracting sucrose and ca. 1.1-fold better for perseitol than 80% ethanol (v/v) (*8, 9*), whereas mannoheptulose concentration was not affected by the solvent used. It is known that sucrose is nearly 3 times more soluble in a water/methanol mixture than in a water/ethanol mixture (*33, 34*), and this could explain the higher concentrations of sucrose in methanol-based extraction (*32*). Furthermore, methanol (62.5%, v/v), being a more polar solvent mixture than ethanol (80%, v/v), could simply have wetted the powdered sample more efficaciously (*32*). Additionally, it was noted that lyophilized under-ripe samples, when ground with a mortar, resulted in coarser particles than eating-ripe or medium-ripe samples. The difference in the physical nature of these powders may have accounted for discrepancies in solvent efficiency at the under-ripe stage. Moreover, sugar analysis of residue samples derived from Soxhlet extraction showed that some chromatograms had poorer peak separation (especially for sucrose; data not shown) as compared with the excellent peak separation obtained from residue following homogenization with hexane (**Figure 2**). This suggests that an alteration of some sugars might have occurred when samples were subjected to longer extraction at higher temperature when using the Soxhlet technique. Additionally, the nature of the solvent used for sugar extraction (viz. ethanol vs methanol) could have affected the recovery of sugars. For example, the use of proton-donating alcohols will tend to cause inversion of sucrose to a limited extent. In this context, spiking of the samples by adding external sugar standards to the freeze-dried sample prior to lipid extraction or to the filter residues prior to sugar extraction would provide additional information on the recovery of target analytes, hence facilitating the discrimination between the methods investigated.

Sequential extraction of lipids and sugars from the same mesocarp sample can be achieved by recovering and extracting filter residues with methanol following homogenization of freeze-dried avocado mesocarp tissue with hexane. The brevity of this method and its relative simplicity make it especially suitable for the extraction of large numbers of samples, without altering the fatty acid profile of the avocado oil. Removal of lipids before sugar analysis also has the advantage of extending HPLC column and guard column working life. The present study also demonstrated that the extraction efficiency for sucrose and perseitol is affected by the solvent used, with 62.5% methanol (v/v) being more efficacious than 80% (v/v) ethanol.

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