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Flavonols and Anthocyanins of Bush Butter, *Dacryodes edulis* (G. Don) H.J. Lam, Fruit. Changes in Their Composition during Ripening

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Dacryodes edulis is a multipurpose tree presently undergoing domestication in central Africa and the countries bordering the Gulf of Guinea, the fruits of which are a good source of essential fatty acids. Polyphenols were characterized in the skin zone and in the pulp of bush butter [*Dacryodes edulis* (G. Don) H.J. Lam] fruits at different stages of ripeness, from unripe to soft fruits. Total polyphenols, assayed according to the Folin−Ciocalteu method, accounted for 3.0−4.2 mg/g of fresh skin, corresponding to 18.6−21.6 mg/g of defatted dry skin, higher concentrations than in the pulp, with 1.1−1.4 mg/g of fresh weight, corresponding to 5.4−12.3 mg/g of defatted dry weight. Reversed-phase high-performance liquid chromatography revealed the presence of flavonols and anthocyanins. Flavonols appeared to be the main class, with quercitrin as the main individual compound, and the highest concentrations occurred in the skin zone. Hyperin, isoquercitrin, isorhamnetin rhamnoside, and isorhamnetin hexoside were also present in relatively high amounts. Petunidin, cyanidin, and peonidin hexosides were identified by mass spectrometry. In the course of ripening, the total polyphenols as well as the major flavonols increased slightly between unripe and preripe stages and then declined gradually as ripening progressed. Anthocyanin profiles also showed a substantial change during ripening, concomitant with the color change from pink to purple.

KEYWORDS: Bush butter; *Dacryodes edulis*; polyphenols; quercitrin; ripening; ESI-MS

INTRODUCTION

The bush butter tree [*Dacryodes edulis* (G. Don) H.J. Lam] is an important multipurpose plant found particularly in central Africa and countries bordering the Gulf of Guinea. It belongs to the family Burseraceae. Fruits are ellipsoidal. A recent morphological study of fruits from different countries in central Africa showed the medium size of ∼6−7 cm long and 3−4 cm wide and weighing 40−50 g (*1*). The fruit is composed of a smooth epicarp, a pulpy endocarp, and a seed surrounded by a tegument, which corresponds to the mesocarp (*2*). The fruits are picked at the mature stage, indicated by the shift of the color of the epicarp from pink to purple. They are consumed after cooking in boiling water or after braising. The production of bush butter is ∼10−20 tonnes/ha a year (*3*, *4*), and most of the fruit is consumed locally. To determine the nutritional value of bush butter fruit, many authors have investigated the composition of the pulp, which corresponds to the edible part of the fruit (*5*−*8*). According to these studies, the pulp contains high amounts of oil (22.1% of fresh tissue) in addition to a substantial amount of protein (4%), carbohydrate (5%), and dietary fibers (8.7%). The major fatty acids of lipids are palmitic (45%), oleic (30%), and linoleic (20%) acids. Therefore, bush butter fruit is an important source of essential fatty acids. However, no information is available on the composition of polyphenols of bush butter fruit.

Polyphenols are important secondary metabolites of plants. They are widespread in nature, with >8000 phenolic structures currently known (*9*). They are ubiquitous in all plant organs and are, therefore, widely distributed in the human diet. In recent years, polyphenols have become an intense focus of research interest because of their potential health-beneficial effects with regard to their antioxidant capacity. Besides the health-protective effects, polyphenols are partially responsible for many quality criteria in plant-derived foods and beverages. The color, astringency, bitterness, and aroma of foods and beverages can depend on the content of polyphenolic compounds (*10*). In contrast to the positive aspects mentioned above, the oxidation of phenolic compounds is largely responsible for the browning of fruits during handling and storage. Polyphenols are also responsible for undesirable hazes and flavor during the preparation of fruit juices, wine, and beer (*11*−*13*).

Following a previous study on chemical composition, the present study reports an investigation on the flavonol and anthocyanin profile of bush butter fruit.

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MATERIALS AND METHODS

Solvents and Standard Phenolics. Methanol, acetonitrile, and acetone (Merck, Darmstadt, Germany) were of chromatographic grade. Hexane was of analytical grade (Merck). Deionized water was obtained with a Milli-Q water system (Millipore, Bedford, MA). Hyperin (quercetin 3-*O*-galactoside), quercitrin (quercetin 3-*O*-rhamnoside), and isoquercitrin (quercetin 3-*O*-glucoside) were provided by Extrasynthese S.A. (Lyon, France). (+)-Catechin, (−)-epicatechin, and (−)-epicatechin benzylthioether were kindly furnished by J. M. Souquet (Unité de Recherche des polymères et des Techniques Physico-Chimiques, INRA, Montpellier, France) and were previously characterized (*14*).

Materials. Bush butter fruits at different stages of ripeness were simultaneously picked from a single tree in April 2002 in Libreville (northwestern Gabon). Fruits at the same stage of ripeness were grouped together in the same perforated bag and immediately transported in optimal conditions to the Institut National de la Recherche Agronomique (Rennes, France). Four stages of ripeness were selected with regard to the color of the epicarp: unripe fruits (full-sized fruits, uniformly pink in epicarp color); preripe fruits (pink fruits with first appearance of purple coloration); midripe fruits (fruits showing ∼50−75% purple coloration); and ripe fruits (fruits uniformly purple in epicarp color). Some of the ripe fruits was stored at 25 °C to obtain three postharvest stages: over-ripe fruits (fruits showing evident sign of dehydration, after 1 day of storage); partly soft fruit (2 days of storage); and soft fruit (3 days of storage). For each stage, three replicates of 10−15 fruits were used. Fruits were peeled, and the seed was discarded. The skin tissues and the flesh (pulp tissue) were separated immediately, frozen in liquid N_2, and freeze-dried. About 1−2 g of freeze-dried samples was crushed in 15 mL of hexane and extensively washed (7 × 15 mL)with the same solvent. The defatted freeze-dried powders were finally air-dried overnight at 40 °C and kept in a desiccator until analysis. In this study, the dry weight corresponds to the freeze-dried material.

Thiolysis of Defatted Freeze-Dried Powders. Thiolysis was performed according to the procedure previously published for the polyphenol analysis of apple powders (*15*). Freeze-dried defatted powders (50 mg) were placed in Eppendorf vials, and 3.3% methanolic HCl (400 μL) and 5% toluene α-thiol in methanol (800 μL) were added. Vials were closed and incubated at 40 °C for 30 min with agitation on a vortex every 10 min. The vials were then cooled in an ice bath for at least 5 min, and 200 μL of the mixture was immediately filtered through a poly(tetrafluoroethylene) (PTFE) membrane (0.45 μm) into insert vials, which were closed with a butyl-Teflon cap and analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC).

Direct Solvent Extraction of Polyphenols. The defatted powders (400 mg) were dispersed in 5 mL of acidic methanol (0.2 N HCl), sonicated for 20 min at 0 °C, and centrifuged (20000*g*, 10 min). The extraction was repeated three times. The three extracts were pooled and stored at −18 °C before being used for Folin−Ciocalteu, RP-HPLC, and mass spectrometry (MS) analysis.

Total Polyphenols Assay. The total phenolic compounds in the methanol extracts were assayed according to the Folin−Ciocalteu method adapted from Singleton and Rossi (*16*). The extracts were diluted 5-fold with 2.5% (v/v) acetic acid. Folin−Ciocalteu reagent (0.25 mL) (Merck) was added to 0.5 mL of the diluted extract. 1 mL of 200 g/L Na_2CO_3 was added, and the volume was adjusted to 5 mL with distilled water. The mixture was then heated at 70 °C for 10 min. After cooling, the absorbance was measured at 700 nm with a Uvikon 860 spectrophotometer (Kontron, Milano, Italy) using blank samples composed of distilled water and reagents. Quercetin was used as standard for the quantification of polyphenols.

RP-HPLC Analysis. The filtered (PTFE, 0.45 μm) methanol extracts (10 μL) and the thiolysis reaction medium (10 μL) were injected onto a 250 × 4 mm i.d., 5 μm, Purospher RP18 end-capped column (Merck). The HPLC apparatus was a Waters (Milford, MA) system (Alliance 2690 separations module, 996 photodiode array detector, and the Millenium^{32} version 3.20 system manager). The autosampler was equipped with a cooling system set at 4 °C to increase the stability of the thiolysis derivatives, thus allowing a larger series of injections. The solvent system was a gradient of solvent A (2.5% aqueous acetic acid) and solvent B (acetonitrile), and the following gradient was applied: initial, 3% B; 0−5 min, 9% B linear; 5−15 min, 16% B linear; and 15−45 min, 50% B linear; the gradient was followed by washing and reconditioning of the column. HPLC peaks were identified on chromatograms according to their retention times and their UV−vis spectra by comparison with available standard compounds, as described by Guyot et al. (*17*). Quantification of the identified compounds was performed by correlating the measured peak area to the calibration curves obtained with reference compounds. Integration of HPLC peaks was performed at 350 nm for flavonols, using the extinction coefficient of quercitrin, and at 515 nm for anthocyanins, using the extinction coefficient of ideain.

HPLC Peak Collections for MS Analysis. The rest of the methanol extract of the fruits at the midripe stage was used for the purification of the major flavonols and anthocyanins. Pure water (30 mL) was added to 30 mL of the methanol extract, and the mixture was evaporated at 40 °C under reduced pressure to ∼30 mL. This operation was repeated once to largely remove methanol. This aqueous mixture was distributed onto 5 g C18 Sep-Pak cartridges (Waters) previously conditioned by 2.5% aqueous acetic acid. Each cartridge was washed with 30 mL of 2.5% aqueous acetic acid. These elution conditions permitted phenolic compounds to be retained on the stationary phase while removing sugars and other high-polarity compounds. Polyphenols were then recovered by elution with 30 mL of aqueous acetonitrile (1:1 v/v), the water being acidified with 2.5% aqueous acetic acid. The recovered extract was evaporated at 40 °C to remove organic solvent. Then, 20 mL of distilled water was added and the mixture evaporated again at 40 °C. This operation was repeated twice to eliminate acetic acid. Finally, the volume was reduced to ∼5 mL, and this concentrated extract was filtered (PTFE, 0.45 μm) and injected (10 μL) on the HPLC system RP-HPLC column using the same gradient and column as previously described for analytical purposes. Fractions corresponding to peaks on the chromatograms at 350 and 515 nm were collected and introduced into the electrospray ionization (ESI) MS apparatus.

ESI-MS Analysis. MS analyses were performed in the positive mode on an LCQ DECA ion trap mass spectrometer (Thermo-Finnigan, San Jose, CA) equipped with an ESI source and run by Xcalibur (version 1.2) software. The parameters were as follows: ion spray voltage, 4800 V; capillary voltage, 3.0 V; capillary temperature, 225 °C; sheath nitrogen gas flow rate, 50 (arbitrary units). Samples corresponding to collected HPLC peaks were directly introduced into the ESI source by a built-in syringe pump at the flow rate of 10 μL/min.

For the generation of MS^n data, the precursor ions were fragmented by helium gas collision in the ion trap by optimizing the collision energy in order to obtain the intensity of the precursor ion close to 10% of the relative scale of the spectrum.

RESULTS AND DISCUSSION

Identification of Phenolic Compounds. Flavonols and anthocyanins were identified in the methanolic extracts of the bush butter fruits at several ripening stages using RP-HPLC coupled with UV−visible diode array detection. Only chromatograms of the skin zone are considered in the present study because the pulp tissues did not reveal any evident peaks. Not only crude methanol extracts but also thiolysis reaction media of the extracts were considered for HPLC analysis. MS was also performed on some collected chromatographic fractions corresponding to well-resolved peaks in order to complete the structural characterization of some compounds.

Thiolysis was used to detect proanthocyanidins in the extracts. The reaction allows simultaneous characterization and quantification of proanthocyanidins. For direct and thiolysis HPLC, chromatograms were nearly identical, characterized by one major peak at 25.1 min in addition to numerous small peaks. Thiolysis chromatograms revealed no peak corresponding to our available reference compounds for thioether adducts; moreover, no improvement of the baseline or of the peak resolution was observed on thiolysis chromatograms. These observations were

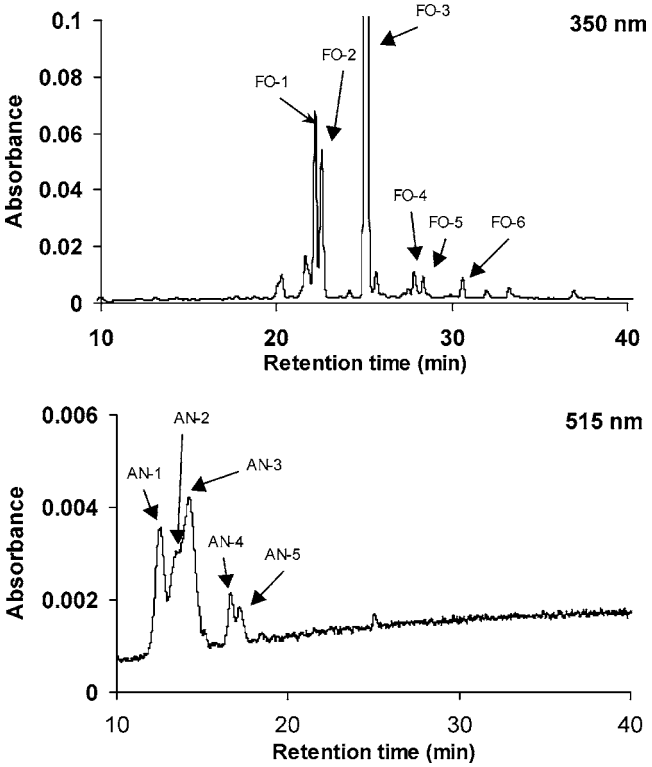

Figure 1. Reversed-phase HPLC trace of flavonol and anthocyanin profiles of a methanol extract from the skin zone tissue of bush butter fruit at the midripe stage.

in accordance with the absence of proanthocyanidins in bush butter fruits, contrasting with many other fruits including apple (*15*), *Litchi chinensis* (*18*), and grapes (*19*, *20*).

Two classes of phenolic compounds (i.e., flavonols and anthocyanins) were detected according to their typical UV−vis spectra. Flavonols are yellow pigments exhibiting a large absorption band with a shoulder, λ_{max} being in the range of 340−370 nm. Anthocyanins show specific UV−vis spectra with maximum absorbance in the 500−540 nm region. Their elution profiles are presented in **Figure 1**. Six flavonols (named FO-1 to FO-6) and five anthocyanins (named AN-1 to AN-5) indicated on the chromatograms were characterized in this study. From **Figure 1**, it was apparent that FO-3 is the main phenolic compound in bush butter fruit. Its retention time (25.1 min) and its UV−vis spectrum corresponded to that of quercitrin (quercetin 3-*O*-rhamnoside), which was available as a standard (**Table 1**). The other flavonols, FO-1 and FO-2, eluted at 22.1 and 22.6 min, respectively. These retention times corresponded to those of authentic hyperin (quercetin 3-*O*-galactoside) and isoquercitrin (quercetin 3-*O*-glucoside), respectively. For FO-4, FO-5, and FO-6 as well as for all anthocyanins, the retention times did not correspond to those of the available standards.

These selected peaks were therefore characterized by MS in the positive mode. For flavonols, mass spectra showed the pseudomolecular ion $[M + H]^+$ corresponding to the protonated molecule of the flavonoid glycosides. The fragmentation pattern of the major flavonol (FO-3) showed a pseudomolecular ion $[M + H]^+$ at *m*/*z* 449 (**Table 1**). The corresponding MS^2 spectrum exhibited a main ion product at *m*/*z* 303, which corresponded to the loss of 146 in accordance with the mass of a rhamnose moiety. The *m*/*z* 303 ion was indicative of a quercetin moiety. This was confirmed by the product ion spectrum of the aglycon ion (MS^3), which showed the characteristic fragments (*m*/*z* 285, 257, and 229) of authentic quercetin. Accordingly, flavonol FO-3 was identified as quercetin rhamnoside (quercitrin), confirming results on RP-HPLC. This flavonol has already been detected in numerous fruits including apple (*21*−*24*) and berries (*25*).

Mass spectra of FO-1 and FO-2 exhibited the pseudomolecular ion $[M + H]^+$ at *m*/*z* 465, as well as the characteristic ion at *m*/*z* 303 $[M + H - 162]^+$, which was identified as quercetin. The mass loss (i.e., 162) suggested hexose conjugates. Therefore, flavonols FO-1 and FO-2 corresponded to quercetin hexosides. According to their retention times mentioned above, we can confirm that FO-1 corresponded to hyperin and FO-2 to isoquercitrin as previously identified in several fruits such as apple (*21*, *23*, *24*), berries (*25*), and pears (*26*). For flavonols FO-5 and FO-6, the pseudomolecular ion $[M + H]^+$ was detected at *m*/*z* 463 and 479, respectively. For these two compounds, the MS^2 experiment on the pseudomolecular ions generated the characteristic ion at *m*/*z* 317, which was assigned to isorhamnetin according to a previous study (*27*). The mass difference between the pseudomolecular ions $[M + H]^+$ and the aglycon (146 for FO-5 and 162 for FO-6) suggested the loss of rhamnose and a hexose, respectively. Therefore, flavonol FO-5 was identified as isorhamnetin rhamnoside, and the tentative structure of FO-6 was proposed to be isorhamnetin hexoside. The presence of isorhamnetin glucoside and isorhamnetin galactoside has already been mentioned in some fruits including pears (*26*) and apples (*24*). For flavonol FO-4, the mass spectrum did not allow the determination of its tentative structure.

For the anthocyanins (**Table 1**), the fragmentation pattern on ESI-MS of AN-1 exhibited the molecular ion $[M]^+$ at *m*/*z* 449, which corresponded to the molecular mass of a cyanidin hexoside, such as cyanidin glucoside or cyanidin galactoside. Similar anthocyanins have already been detected in *Coriaria myrtifolia* fruit (*28*), strawberry (*29*), apple (*30*), and lychee (*31*). Anthocyanin AN-2, corresponding to a shoulder on the peak AN-3 in the extract used for the identification of flavonoids, was not identified. For AN-3, the mass spectrum showed the molecular ion $[M]^+$ at *m*/*z* 479. MS^2 analysis of *m*/*z* 479 generated a characteristic ion at *m*/*z* 317, assigned to petunidin. The mass loss (i.e., 162) suggested hexose conjugates. Therefore, AN-3 was suspected to be petunidin hexoside. The mass spectrum of AN-4 revealed an $[M]^+$ ion at *m*/*z* 463 and the characteristic fragment $[M - 162]^+$ at *m*/*z* 301 corresponding to the molecular mass of peonidin. Accordingly, we identified AN-4 as peonidin hexoside. Escribano-Bailon et al. (*28*) have already mentioned the presence of petunidin 3-*O*-glucoside and peonidin 3-*O*-glucoside in *C. myrtifolia* fruit. The mass spectrum of anthocyanin AN-5 also exhibited the molecular ion $[M]^+$ at *m*/*z* 463. The corresponding MS^2 spectrum showed a main ion product $[M - 162]^+$ at *m*/*z* 301. Therefore, we assumed that this compound might also correspond to a peonidin hexoside.

Variation of Phenolic Compounds during Ripening. In the fresh skin, the dry material accounted for 14.9% for unripe fruits. As ripening progressed, this yield markedly increased to reach 52.8% of fresh weight at the soft stage (**Table 2**). After removal of fats by hexane treatment, the yield of defatted dry weight remained almost constant (~14% of fresh weight) from unripe to ripe stages and then it increased to 22.3% for the soft fruits. A similar variation was also observed in the pulp: the dry weight increased from 12.4 to 48.2% of fresh weight between unripe and soft fruits, and the defatted dry weight increased from 11.6 to 20.0% between unripe and soft fruits. Accordingly, the marked increase in the yield of dry weight was due to the

Table 1. Retention Times, UV Spectra, and Characteristic Ions of Flavonoids from Bush Butter Skin Tissue

peak[a]	retention time (min)	HPLC-DAD-UV spectrum λ_{max} (nm)	$[M + H]^+$ or $[M]^+$ ion (*m/z*)[b]	HPLC-ESI-MS MS experiments *m/z* (relative abundance)	identity
FO-1	22.1	256, 266sh, 295sh, 355	465	MS^2 [465]: 465 (30), 303 (100) MS^3 [465 → 303]: 303 (100), 285 (38), 275 (10), 57 (54), 247 (22), 229 (25), 165 (24)	hyperin
FO-2	22.6	256, 266sh, 295sh, 355	465	MS^2 [465]: 465 (26), 303 (100)	isoquercitrin
FO-3	25.1	256, 264sh, 298sh, 349	449	MS^2 [449]: 449 (30), 303 (100) MS^3 [449 → 303]: 303 (100), 285 (41), 257 (52), 229 (26), 165 (14)	quercitrin
FO-4	27.7	264, 345			unidentified
FO-5	28.2	255, 264sh, 347	463	MS^2 [463]: 463 (25), 317 (100)	isorhamnetin rhamnoside
FO-6	30.4	256, 268sh, 300sh, 355	479	MS^2 [479]: 479 (39), 317 (100)	isorhamnetin hexoside
AN-1	12.3	279, 512	449	MS: 803 (13), 449 (100), 287 (51), 214 (45), 205 (24), 163 (14)	cyanidin hexoside
AN-2	13.3				unidentified
AN-3	14.3	279, 515	479	MS: 803 (18), 479 (100), 317 (63), 288 (20), 214 (26) MS^2 [479]: 479 (6), 317 (100)	petunidin hexoside
AN-4	16.5	279, 520	463	MS: 803 (12), 635 (20), 617 (12), 463 (52), 316 (32), 301(34), 288 (100), 214 (47), 163 (34), 158 (15)	peonidin hexoside
AN-5	17.2	279, 520	463	MS: 803 (6), 463 (14), 416 (6), 332 (7), 316 (34) 301 (12), 288 (100), 244 (7), 214 (26), 163 (14), 158 (7) MS^2 [463]: 463 (20), 301 (100)	peonidin hexoside

[a] See **Figure 1** for correspondence on chromatograms. [b] Flavonols were observed by their protonated form $[M + H]^+$ ions, whereas anthocyanins were observed under the flavylium form $[M]^+$.

Table 2. Total Polyphenols, Total Flavonols, and Total Anthocyanin Contents in the Skin and Pulp Tissues of Bush Butter Fruit at Different Stages of Ripeness

			total polyphenols		total flavonols		total anthocyanins	
ripeness stage	dry wt (%)	defatted dry wt (%)	mg/g of fresh wt	mg/g of defatted dry wt	mg/g of fresh wt	mg/g of defatted dry wt	mg/g of fresh wt	mg/g of defatted dry wt
				Skin Tissue				
unripe	14.9 (0.6)[a]	14.0 (0.2)[a]	3.0 (0.19)[a]	21.6 (0.95)[a]	1.5	11.2	0.035	0.25
preripe	18.1 (0.7)	14.6 (0.7)	3.3 (0.13)	22.6 (1.72)	1.8	13.1	0.046	0.32
midripe	27.7 (2.1)	13.9 (0.4)	3.0 (0.09)	21.6 (0.84)	1.7	12.6	0.051	0.37
ripe	36.4 (0.5)	14.5 (0.6)	3.0 (0.26)	20.9 (0.90)	1.6	11.7	0.068	0.47
over-ripe	38.1 (0.6)	16.7 (1.6)	3.4 (0.36)	20.5 (0.45)	1.6	10.3	0.079	0.47
partly soft	43.6 (2.7)	18.3 (1.3)	3.8 (0.18)	20.6 (1.42)	1.7	10.0	0.083	0.46
soft	52.8 (2.1)	22.3 (1.1)	4.2 (0.41)	18.6 (0.89)	1.9	9.0	0.075	0.34
				Pulp Tissue				
unripe	12.4	11.6	1.4	12.3	0.023	0.21	0.004	0.04
soft	48.2	20.0	1.1	5.4	nd[b]	nd	nd	nd

[a] Standard deviation in parentheses ($n = 3$). [b] Not detected.

gradual accumulation of fats in the tissue in the course of ripening. In soft fruits, fats contributed ~50% to the yield of dry weight. We believe that for over-ripe fruits and particularly for partly soft and soft fruits, hexane treatment did not lead to the complete removal of fats. This could explain the higher yields of defatted dry weight recorded for these stages of ripeness. For a better comparison between the different stages of ripeness, results in this study were therefore related to both fresh weight and defatted dry weight. Total polyphenols content, assayed according to the Folin−Ciocalteu method, accounted for 3.0−4.2 mg/g of fresh weight or 18.6−21.6 mg/g of defatted dry weight in the skin zone (**Table 2**). These values were ~2−4 times higher than those recorded in pulp tissue, namely, 1.1−1.4 mg/g of fresh weight, corresponding to 5.4−12.3 mg/g of defatted dry weight. These results are in agreement with those of many publications (*13*, *22*, *32*, *33*), which mentioned that the highest concentrations of phenolic compounds in fruits occurred in the skin tissue.

Flavonols appeared to be the main class of polyphenols. In the skin tissue, total flavonols, assayed by RP-HPLC, accounted for 1.5−1.9 mg/g of fresh weight or 9.0−13.2 mg/g of defatted dry weight, corresponding to 46−56% of total polyphenols. Anthocyanins (0.035−0.083 mg/g of fresh skin or 0.25−0.47 mg/g of defatted dry skin) were present in very low quantities compared to flavonols. They accounted for 1−2% of total polyphenols (**Table 2**). In the pulp, traces of flavonols and anthocyanins were detected in unripe fruits, but no phenolic compound was detected in soft fruits. When the two methods used for polyphenol quantification were compared, the Folin−Ciocalteu method gave about twice the sum of flavonols and anthocyanins assayed by RP-HPLC for the skin tissue and 50 times for the pulp. Two hypotheses can explain this great difference between the two methods. The first is underestimation of phenolic compounds on RP-HPLC analysis, as numerous unidentified peaks appeared on chromatograms at 280 nm and were not quantified. The second hypothesis concerns the probable presence of several interfering substances in the methanolic extracts. Investigations of Singleton et al. (*34*) have already mentioned that some nonphenolic compounds (i.e., ascorbic acids and amino acids) could interfere in the Folin−Ciocalteu assay, thus increasing the overall response.

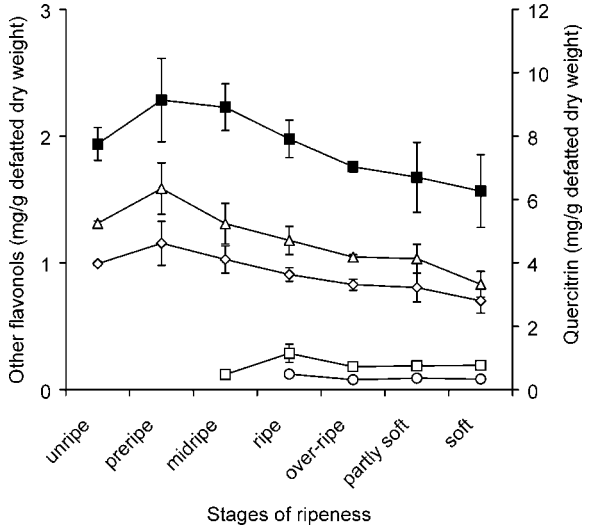

Figure 2. Evolution of the concentrations of flavonols in the skin zone tissue of bush butter fruit during ripening: (■) quercitrin; (◇) isoquercitrin; (□) isorhamnetin rhamnoside; (○) isorhamnetin hexoside; (△) hyperin. Bars represent ± standard deviation.

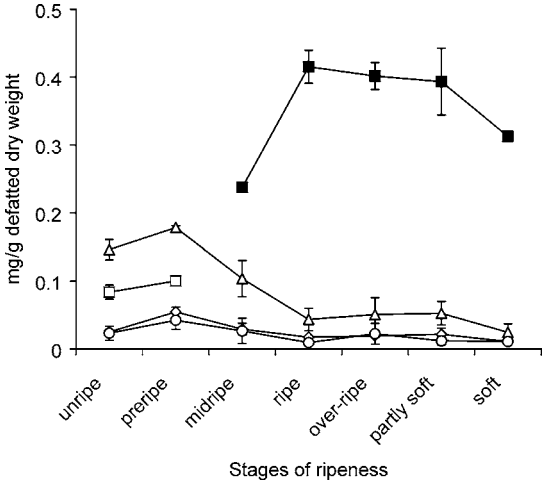

Figure 3. Evolution of the concentrations of anthocyanins in the skin zone tissue of bush butter fruit during ripening: (■) petunidin hexoside; (△) cyanidin hexoside; (□) unidentified (AN-2); (◇) peonidin hexoside (AN-4); (○) peonidin hexoside (AN-5). Bars represent ± standard deviation.

Comparing the different stages of ripeness, analysis of variance revealed significant differences for results based on fresh weight as well as for those based on defatted dry weight (**Table 2**). Total polyphenols in the skin tissue, based on fresh weight, remained nearly constant from unripe to ripe stages and then increased. Soft fruits showed the highest amount of polyphenols (4.2 mg/g). Regarding results based on defatted dry weight, total polyphenols showed a slight but nonsignificant increase from 21.6 to 22.7 mg/g between unripe and preripe stages and then significantly decreased to reach 18.6 mg/g at the last stage (soft fruits). These results may be due to the gradual degradation of phenolic compounds during ripening. In the pulp, the total polyphenol concentration decreased by about half between unripe and soft stages, suggesting an important degradation of polyphenols in soft fruits. As for total polyphenols, total flavonols based on defatted dry weight showed a slight increase between the two first stages of ripeness followed by a gradual decrease as ripening progressed. When each compound was considered, quercitrin (6.3−9.1 mg/g of defatted dry skin) was the main phenolic compound (**Figure 2**). As well as quercitrin, hyperin (0.8−1.6 mg/g of defatted dry skin) and isoquercitrin (1.0−1.2 mg/g of defatted dry skin) were also present in relatively high quantities; all of the other flavonols were present in minor quantities. In the course of ripening, the three important flavonols (quercitrin, hyperin, and isoquercitrin) increased slightly between the two first stages of ripeness and then declined gradually as ripening progressed. Anthocyanins showed a gradual accumulation in the course of ripening, but at the last stage (soft fruits), the total anthocyanin content significantly declined (**Table 2**). With regard to the evolution of each compound, cyanidin hexoside (AN-1) and peonidin hexosides (AN-4 and AN-5) increased slightly between unripe and preripe stages and then declined to reach a steady level from ripe to soft fruits (**Figure 3**). An unidentified anthocyanin, AN-2, occurred only in unripe and preripe fruits; its concentration also increased between these two first stages of ripeness. The disappearance of AN-2 and the marked decrease of cyanidin hexoside and peonidin hexosides coincided with the appearance at midripe stage of anthocyanin AN-3, assigned to petunidin hexoside. Its concentration was much higher than that of the other anthocyanins from midripe to soft stages. All of these results suggested that cyanidin hexoside, peonidin hexosides, and/or anthocyanin AN-2 were transformed into petunidin hexoside in the course of ripening. Many authors have already examined changes in phenolic compounds during fruit development and ripening. For apple (*30*, *35*), flavonols were highest in young fruit but decreased during fruit development to reach a steady level during maturation and ripening. Investigations of Reay (*36*) and Awad and de Jager (*37*) on apples also revealed that quercetin glycosides, the main flavonols of this fruit, remained relatively constant during storage. Concerning anthocyanins, strawberry, blackberry, and raspberry (*29*, *38*) showed significant accumulations of anthocyanin during ripening.

As in many fruits and vegetables, the main polyphenols in the skin of bush butter fruits were flavonols, more precisely, mostly quercetin derivatives. Ripening was accompanied by a marked color change from pink to purple, which was concomitant with an increase in concentration of a petunidin hexoside. An interesting feature of this color change is that it is not uniform in the skin but appears as spots which expand to cover the whole surface. It might be interesting for a detailed study of polyphenol evolution in bush butter (or of the biosynthetic mechanisms leading to color change) either to fractionate the individual fruits according to color zones or to relate the evolution of the percentage of purple surface to the evolution of the concentrations. The present study also revealed the presence of numerous unidentified peaks having a maximum absorbance at 280 nm, which may correspond to phenolic compounds. These investigations have to be completed by the identification of these unknown peaks and compounds for which structures were not formally elucidated. No proanthocyanidols could be detected. Overall, the polyphenol concentrations were low, and bush butter fruit is not likely to contribute significantly to antioxidant status, although there might be an effect on oil stability.

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LITERATURE CITED

(1) Silou, Th.; Rocquelin, G.; Mouaragadja, I.; Gallon, G. Chemical composition and nutritional characteristics of safou of Cameroon,

the Congo-Brazzaville, the Congo-Kinshasa and Gabon. *Riv. Ital. Sostanze Grasse* **2002**, *79*, 177−182.
(2) Silou, T. Le safoutier (*Dacryodes edulis*): Un arbre mal connu. *Fruits* **1996**, *51*, 47−60.
(3) Giacomo, R. *Etude de la biologie florale du safoutier, Dacryodes edulis au Gabon*; Internal Report, Project FAO/CIAM: Libreville, Gabon; 1982; 8 pp.
(4) Silou, T., Kiakouama, S.; Koucka-Gokana, B. Evaluation de la production et étude de la variabilité morphologique et résultats préliminaires. In *Séminaire sur la Valorisation du Safoutier*, Oct 4−6, 1994; J Nya Ngachou et J kengué: Yaoundé, Cameroon, 1994; pp 30−44.
(5) Omoti, U.; Okiy, P. A. Characterisation and composition of the pulp oil and the cake of the African pear, *Dacryodes edulis*. *J. Sci. Food Agric*. **1987**, *38*, 67−72.
(6) Bezard, J.; Silou, Th.; Semporé, G.; Kiakouama, S. Contribution à l'étude de *Dacryodes edulis*. Variation de la composition de la fraction glycéridique de l'huile de la pulpe de safou en fonction de la maturité. *Rev. Fr. Corps Gras* **1991**, *38*, 233−241.
(7) Kapseu, C.; Mapongmetsem, P. M.; Silou, Th.; Roques, M. Physico-chimie des fruits du safoutier (*Dacryodes edulis*) camerounais. *Tropicultura* **1998**, *16*, 38−42.
(8) Favier, J.-C.; Ireland-Ripert, J.; Laussucq C.; Feinberg M. *Répertoire Général des Aliments. 3. Table de Composition des Fruits Exotiques, Fruits de Cueillette d'Afrique*; INRA: Paris, France, 1993; p 185.
(9) Bravo, L. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. *Nutr. Rev.* **1998**, *56*, 317−333.
(10) Fleuriet, A.; Uhel, C.; Dédaldéchamp, F. Les composés phénoliques et la qualité des produits d'origine végétale consommés par l'homme. *Acta Bot. Gallica* **1996**, *143*, 493−500.
(11) Mathew, A. G.; Parpia, H. A. B. Food browning as a polyphenol reaction. *Adv. Food Res*. **1971**, *19*, 75−145.
(12) Ho, C.-T.; Lee, C. Y.; Huang, M.-T. *Phenolic Compounds in Food and Their Effects on Health. I: Analysis, Occurrence and Chemistry*; ACS Symposium Series 506; American Chemical Society: Washington, DC, 1992.
(13) Shahidi, F.; Naczk, M. *Food Phenolics: Sources, Chemistry, Effects, Applications*; Technomic Publishing: Lancaster, PA, 1995.
(14) Prieur, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry* **1994**, *36*, 781−784.
(15) Guyot, S.; Marnet, N.; Drilleau, J.-F. Thiolysis-HPLC characterization of apple procyanidins covering a large range of polymerisation states. *J. Agric. Food Chem*. **2001**, *49*, 14−20.
(16) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic−phosphotungstic acid reagents. *Am. J. Enol. Vitic*. **1965**, *16*, 144−158.
(17) Guyot, S.; Marnet, N.; Laraba, D.; Sanoner; P.; Drilleau, J.-F. Reversed-phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic compounds in the different tissue zones of a French cider apple variety (*Malus domestica* Var. Kermerrien). *J. Agric. Food Chem*. **1998**, *46*, 1698−1705.
(18) Le Roux, E.; Doco, T.; Sarni-Manchado, P.; Lozano, Y.; Cheynier, V. A-type proanthocyanidins from pericarp of *Litchi chinensis*. *Phytochemistry* **1998**, *48*, 1251−1258.
(19) Saucier, C.; Mirabel, M.; Daviaud, F.; Longieras, A.; Glories, Y. Rapid fractionation of grape seed proanthocyanidins. *J. Agric. Food Chem*. **2001**, *49*, 5732−5735.
(20) Yang, Y.; Chien, M. Characterization of grape procyanidins using high-performance liquid chromatography/mass spectrometry and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J. Agric. Food Chem*. **2000**, *48*, 3990−3996.
(21) Lu, Y.; Foo, L. Y. Identification and quantification of major polyphenols in apple pomace. *Food Chem.* **1997**, *59*, 187−194.
(22) Pérez-Ilzarbe, J.; Hernández, T.; Estrella, I. Phenolic compounds in apples: varietal differences. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 551−554.
(23) Burda, S.; Oleszek, W.; Lee, C. Y. Phenolic compounds and their changes in apples during maturation and cold storage. *J. Agric. Food Chem*. **1990**, *38*, 945−948.
(24) Schieber, A.; Keller, P.; Streker, P.; Klaiber, I.; Carle, R. Detection of isorhamnetin glycosides in extracts of apples (*Malus domestica* cv. "Brettacher") by HPLC-PDA and HPLC-APCI-MS/MS. *Phytochem. Anal*. **2002**, *13*, 87−94.
(25) Häkkinen, S.; Auriola, S. High-performance liquid chromatography with electrospray ionization mass spectrometry and diode array ultraviolet detection in the identification of flavonol aglycones and glycosides in berries. *J. Chromatogr. A* **1998**, *829*, 91−100.
(26) Duggan, M. B. Identity and occurrence of certain flavonol glycosides in four varieties of pears. *J. Assoc. Off. Anal. Chem*. **1969**, *17*, 1098−1101.
(27) Ma, Y. L.; Li, Q. M.; Van den Heuvel, H.; Claeys, M. Characterization of flavone and flavonol aglycones by collision-induced dissociation tandem mass spectrometry. *Rapid Commun. Mass Spectrum.* **1997**, *11*, 1357−1364.
(28) Escribano-Bailon, M. T.; Santos-Buelga, C.; Alonso, G. L.; Salinas, M. R. Anthocyanin composition of the fruit of *Coriaria myrtifolia* L. *Phytochem. Anal*. **2002**, *13*, 354−357.
(29) Yoshida, Y.; Koyoma, N.; Tamura, H. Color and anthocyanin composition of strawberry fruit: Changes during fruit development and differences among cultivars, with specific reference to the occurrence of pelargonidin 3-malonylglucoside. *J. Jpn. Soc. Hortic. Sci*. **2002**, *71*, 355−361.
(30) Awad, M. A.; de Jager, A.; van der Plas, L. H. W.; van der Krol, A. R. Flavonoid and chlorogenic acid changes in the skin of 'Elstar' and 'Jonagold' apples during development and ripening. *Sci. Hortic*. **2001**, *90*, 69−83.
(31) Rivera-Lopez, J.; Ordorica-Falomir, C.; Wesche-Ebeling, P. Changes in anthocyanin concentration in lychee (*Litchi chinensis* Sonn.) pericarp during maturation. *Food Chem.* **1999**, *65*, 195−200.
(32) Kühnau, J. The flavonoids: a class ofsemi-essential food compounds: their role in human nutrition. *World Rev. Nutr. Diet* **1976**, *24*, 117−191.
(33) Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. *J. Agric. Food Chem*. **1992**, *40*, 2379−2383.
(34) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin−Ciocalteu reagent. *Methods Enzymol*. **1999**, *299*, 152−178.
(35) Li, Z. H.; Sugaya, S.; Gemma, H.; Iwahori, S. Flavonoid biosynthesis and accumulation and related enzyme activities in the skin of 'Fuji' and 'Oorin' apples during their development. *J. Jpn. Soc. Hortic. Sci*. **2002**, *71*, 317−321.
(36) Reay, P. F. The effects of maturity on colour and concentration of pigments in the blush and shaded sides of 'Gala' apple fruit during cool storage. *J. Hortic. Sci. Biotechnol*. **1998**, *73*, 841−845.
(37) Awad, M. A.; de Jager, A. Flavonoid and chlorogenic acid concentrations in skin of "Jonagold" and "Elstar" apples during and after regular and ultra-low oxygen storage. *Postharvest Biol. Technol*. **2000**, *20*, 15−24.
(38) Wang, S. Y.; Lin, H. S. Antioxidant activity in fruits and leaves of blackberry, raspberry, strawberry. *J. Agric. Food Chem*. **2000**, *48*, 140−146.

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