Food Research International 105 (2018) 645–653

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

HPLC-DAD-APCI/ESI-MS^n analysis of carotenoids and α-tocopherol in Costa Rican *Acrocomia aculeata* fruits of varying maturity stages

Roland Schex[a], Veronika M. Lieb[a], Víctor M. Jiménez[b,c], Patricia Esquivel[d], Ralf M. Schweiggert[a], Reinhold Carle[a,e], Christof B. Steingass[a,*]

[a] Institute of Food Science and Biotechnology, Chair Plant Foodstuff Technology and Analysis, University of Hohenheim, Garbenstrasse 25, 70599 Stuttgart, Germany
[b] CIGRAS, University of Costa Rica, 2060 San Pedro, Costa Rica
[c] Food Security Center, University of Hohenheim, 70599 Stuttgart, Germany
[d] School of Food Technology, University of Costa Rica, 2060 San Pedro, Costa Rica
[e] Biological Science Department, King Abdulaziz University, P.O. Box 80257, Jeddah 21589, Saudi Arabia

ARTICLE INFO

Keywords:
Macauba
Coyol
Palm fruit
Underutilised crop
Carotenoid precursor
Xanthophyll cycle

ABSTRACT

Carotenoids and tocopherols were characterised in the meso- and exocarp of wild-growing Costa Rican *Acrocomia aculeata* fruits. Comprehensive profiling of these lipophilic micronutrients in fruits of three varying maturity stages was conducted for the first time. A method for the simultaneous extraction and quantitation of carotenoids and α-tocopherol was developed and validated. Detailed HPLC-DAD-APCI/ESI-MS^n analyses enabled the identification of α-tocopherol and 25 carotenoids. The latter comprised antheraxanthin, β-carotene, lutein, luteoxanthin, neoxanthin, phytoene, phytofluene, violaxanthin, zeaxanthin, and several (*Z*)-isomers of the aforementioned compounds. Quantitation by HPLC-DAD/FLD revealed total carotenoid concentrations of 872 ± 178 and 3075 ± 407 μg/100 g fresh weight in the meso- and exocarp of fully ripe fruits, respectively. In both fruit fractions, progressing maturation resulted in the accumulation of phytoene, phytofluene, (all-*E*)-zeaxanthin, (all-*E*)-antheraxanthin, and (all-*E*)-violaxanthin. Carotenoid profiling was supported by multivariate data analysis. Carotenoid precursors and xanthophyll cycle pigments characterised Macauba fruits of full maturity.

1. Introduction

The Macauba palm (*Acrocomia aculeata* (Jacq.) Lodd. ex Mart.), also known as Coyol in Costa Rica, represents a high yielding oil crop with wild-growing populations all over the Neotropics. Central America as well as Paraguay and Brazil were highlighted as major territories for its future cultivation (Plath et al., 2016). Fruits of various *Acrocomia* sp. were already used as food sources by the native Americans. Moreover, the sap from the palms is used to produce an alcoholic beverage termed 'Coyol-palm wine' (Brücher, 1989).

A. aculeata is adapted to wet and dry tropical to subtropical climates, withstanding short-period temperatures down to − 4 °C and dry years with rainfalls of only 500 mm (FAO, 2007). Its robustness enables a wider geographical distribution and cultivation compared to *Elaeis* oil palms (Falasca, Ulberich, & Pitta-Alvarez, 2016). Extensive cultivation of *Elaeis guineensis* Jacq. raised various environmental, social, and economic issues during the past decades. *Elaeis* monocultures intensified habitat degradation and pollution. Its high susceptibility to periodic climate phenomena such as "El Niño" influenced global prices of edible oil (Fitzherbert et al., 2008; USDA, 2016). Nevertheless, *Elaeis* oil palms still represent the leading sources of vegetable oil with a production volume of ~64 million t in 2014 (FAO, 2017). As an underutilised crop, current research highlighted the domestication and exploitation potential of *A. aculeata* to produce biodiesel and edible oil (César, Almeida, De Souza, Silva, & Atabani, 2015; Montoya, Motoike, Kuki, & Couto, 2016; Plath et al., 2016; Poetsch, Haupenthal, Lewandowski, Oberländer, & Hilger, 2012). Its annual oil yield was estimated to be 2.5 t/ha (Poetsch et al., 2012). This yield is slightly

Abbreviations: APCI, atmospheric pressure chemical ionisation; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; CID, collision induced dissociation; ESI, electrospray ionisation; HCA, hierarchical cluster analysis; HPLC-DAD, high performance liquid chromatography-diode array detection; LOD, limit of detection; LOQ, limit of quantitation; MS^n, multiple-stage mass spectrometry; PCA, principal component analysis; *t*BME, *tert*-butyl methyl ether

* Corresponding author.
E-mail addresses: roland.schex@dsm.com (R. Schex), veronika.lieb@uni-hohenheim.de (V.M. Lieb), victor.jimenez@ucr.ac.cr (V.M. Jiménez), patricia.esquivel@ucr.ac.cr (P. Esquivel), ralf.schweiggert@dsm.com (R.M. Schweiggert), reinhold.carle@uni-hohenheim.de (R. Carle), christof.steingass@uni-hohenheim.de (C.B. Steingass).

https://doi.org/10.1016/j.foodres.2017.11.041
Received 1 September 2017; Received in revised form 15 November 2017; Accepted 19 November 2017
Available online 21 November 2017
0963-9969/

lower than obtainable by *Elaeis* oil palms (3.4–4.1 t/ha), but significantly higher compared to other major oil crops such as rapeseed (0.7 t/ha), sunflower (0.5–0.6 t/ha), and soybean (0.4–0.5 t/ha) (Corley & Tinker, 2016; Lieberei & Reisdorff, 2012).

Crude *Elaeis* palm oils contain high carotene levels. During technical refinements carotenoids are commonly removed to decolourise and prevent the oils from excessive oxidation (Corley & Tinker, 2016; Prada, Ayala-Diaz, Delgado, Ruiz-Romero, & Romero, 2011). Accruing carotenes can be further processed to obtain highly pure carotene fractions that are legally approved as food additives in the EU (E160a (i)) (EFSA, 2012). Likewise, crude Macauba mesocarp oil containing carotenoid levels of 378 μg/g total lipids expressed as β-carotene may be used to recover such food additives (Nunes, Favaro, Galvani, & Miranda, 2015). Ramos, Ramos Filho, Hiane, Braga Neto, and Siqueira (2008) have reported 49 μg (all-*E*)-β-carotene per g fresh mesocarp, accounting for ~80% of the total carotenoids. HPLC-DAD analyses further revealed (all-*E*)-zeaxanthin and (all-*E*)-lycopene as well as its geometrical isomers as additional mesocarp constituents of *A. aculeata* fruits. Besides carotenoids, Coimbra and Jorge (2012) have reported 213 μg tocopherols per g total lipids, represented by the vitamers α-, β-, γ-, and δ-tocopherol. However, mass spectrometric studies of lipophilic micronutrients in Macauba fruits are lacking to date. Comprehensive reports on the lipid composition of Macauba fruits, in particular concerning carotenoid and tocopherol profiles, are scarce compared to *Elaeis* fruits (Irías-Mata et al., 2017; Lieb et al., 2017; Prada et al., 2011; Tranbarger et al., 2011). Moreover, existing data is limited to Macauba fruits from Brazilian provenances, thereby not reflecting the wide distribution of *A. aculeata*.

Thus, the presented study aimed at an in-depth characterisation of carotenoids and tocopherols in Costa Rican Macauba fruits of three progressing maturity stages. Carotenoids and α-tocopherol were identified by HPLC-DAD-APCI/ESI-MS^n and quantitated in the meso- and exocarp via HPLC-DAD/FLD. Multivariate statistics, i.e., principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used for an indicative carotenoid assignment to the maturity stages. In our study, we sought to amplify scientific data on lipophilic micronutrients in maturing *A. aculeata* fruits as promising nutritional source of carotenoids and tocochromanols.

2. Materials and methods

2.1. Reagents

Ammonium acetate, L-ascorbic acid, and potassium hydroxide were obtained from VWR International (Leuven, Belgium), *tert*-butyl methyl ether (*t*BME) for HPLC-DAD/FLD, methanol, and light petroleum (b.p. 40–60 °C) from VWR International (Fontenay-sous-Bois, France). *t*BME for HPLC-DAD-APCI/ESI-MS^n, butylated hydroxytoluene (BHT), calcium carbonate, sodium thiosulfate, iodine, acetone, ethyl acetate, ethanol, diethyl ether, and *n*-hexane were purchased from Merck (Darmstadt, Germany), butylated hydroxyanisole (BHA) from Sigma-Aldrich (Steinheim, Germany). All chemicals were at least of HPLC or analytical grade. Double distilled water was used throughout all experiments.

Authentic reference standards of (all-*E*)-antheraxanthin, (9*Z*)-β-carotene, (*E/Z*)-phytoene, (*E/Z*)-phytofluene, (all-*E*)-neoxanthin, and (all-*E*)-violaxanthin were obtained from CaroteNature (Ostermundingen, Switzerland). (all-*E*)-Zeaxanthin was purchased from BIOMOL (Hamburg, Germany), (all-*E*)-β-carotene, α-, β-, γ-, and δ-tocopherol from Sigma-Aldrich. (all-*E*)-Lutein was isolated from marigold extract beadlets (BioActives Europe, Frankfurt/Main, Germany) using *n*-hexane and a Sonopuls UW 3100 sonicator equipped with an MS72 microtip (Bandelin Electronic, Berlin, Germany). Iodine-catalysed isomerisation of (all-*E*)-lutein and (all-*E*)-zeaxanthin was performed according to Nguyen, Francis, and Schwartz (2001) with slight modifications. Briefly, reference standards were dissolved in 2 mL *n*-hexane, mixed with 10 μL iodine solution (1% w/v in *n*-hexane), and exposed to UV/vis light. Subsequently, iodine was washed out with sodium thiosulfate and water as previously described by Hempel et al. (2014).

2.2. Macauba samples

Samples were harvested in April 2016 from four wild Macauba palms ($n = 4$) grown approximately 5 km to the east of Bagaces (Guanacaste, Costa Rica). Each palm site was geographically determined by GPS data (N10 31.122 W85 12.418; N10 31.124 W85 12.408; N10 31.116 W85 12.362; N10 31.111 W85 12.349). Experts from Green Integrated Energies S.A. (San José, Costa Rica) supported the identification and harvest of Macauba samples. According to commercial harvesting techniques, samples comprised unripe (A) and ripe (B) fruits harvested from the bunch as well as fully ripe (C) fruits collected from the ground. Unripe fruits (A) were characterised by a green exocarp, a whitish mesocarp with green tonalities, and tough coherence to the bunch. Ripe fruits (B) featured a mottled green to brown exocarp, a cream white to light yellow mesocarp, and weak coherence to the bunch. Fully ripe fruits (C) showed mainly brown exocarp and yellow mesocarp. Their natural fall indicated full maturity (Crocomo & Melo, 1996; Montoya et al., 2016).

Exocarp and mesocarp of five intact, randomly sampled fruits of each palm and maturity stage were separated manually, frozen with liquid nitrogen, vacuum-sealed in laminated foil and stored at − 60 °C. Subsequently, samples were lyophilised for 72 h under light protection in a laboratory freeze-dryer (Alpha 1–2 LD, Christ, Osterode, Germany). Moisture loss was determined gravimetrically in duplicate. Dried samples were vacuum-sealed, transported from Costa Rica to Germany, and cryo-milled with the addition of liquid nitrogen using a laboratory blender (type 8011ES, Waring, Torrington, CT, USA). Ground samples were stored in airtight aluminium pouches at − 25 °C until further analyses.

2.3. Extraction of carotenoids and tocopherols

Sample workup was conducted under dimmed light to prevent carotenoids and tocopherols from isomerisation and degradation. An aliquot of 100 mg sample with the addition of 50 mg calcium carbonate was extracted with 2 mL ice-cooled acetone containing each 0.1 g/L BHA and BHT, using the aforementioned sonicator at 70% amplitude for 15 s. After centrifugation (4500 rpm, 3 min, Heraeus Labofuge 200, Thermo Fisher Scientific, Waltham, MA, USA), the supernatant was collected and the solid remainder was re-extracted twice with acetone (2 × 2 mL). The combined extract was evaporated to dryness under a gentle nitrogen steam and re-dissolved in 2 mL diethyl ether. 2 mL methanolic potassium hydroxide (20% w/v) and 200 μL aqueous L-ascorbic acid solution (20% w/w) were added. The mixture was flushed with nitrogen and saponified for 3 h under continuous stirring. The saponified extract was washed with 2 mL water and the organic phase was recovered. The residual aqueous phase was re-extracted twice with diethyl ether (2 × 1 mL). The combined organic phase was washed with another 2 mL water, evaporated to dryness under a gentle nitrogen steam, and stored at − 80 °C until HPLC analyses. Standard addition experiments were used to determine the recoveries of (all-*E*)-lutein (98%), (all-*E*)-β-carotene (95%), (all-*E*)-violaxanthin (90%), and α-tocopherol (91%) applying the extraction and saponification procedure detailed above.

2.4. HPLC-DAD-APCI/ESI-MS^n analysis

The extracts were re-dissolved in each 250 μL *t*BME and methanol, membrane-filtered (polytetrafluoroethylene, 0.45 μm, Pall Life Sciences, Ann Arbor, MI, USA), and filled into amber glass vials for HPLC analyses.

An Agilent series 1100 HPLC system was equipped with a