



Research article

Changes in physicochemical properties at different development stages of *Hexachlamys edulis* fruit, an underutilized South American speciesMiriam E. Arena^{a,*}, Ignacio S. Povilonis^a, Virginia Borroni^b, Diana Constenla^c, Silvia Radice^a^a CONICET - Universidad de Morón, Laboratorio de Fisiología Vegetal, Machado 914 Lab. 501, Morón, B1708EOH, Buenos Aires, Argentina^b Instituto de Tecnología en Polímeros y Nanotecnología - Universidad de Buenos Aires-CONICET, Facultad de Arquitectura, Diseño y Urbanismo, Ciudad Autónoma de Buenos Aires, Argentina^c Planta Piloto de Ingeniería Química (PLAPIQUID), Universidad Nacional del Sur - Consejo Nacional de Investigaciones Científicas y Técnicas (UNS-CONICET), Bahía Blanca, Argentina

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ABSTRACT

The aim of this work was to study the evolution of fruit size and weight together with the soluble solid and total titratable acidity contents during development of *Hexachlamys edulis* fruit. Also, the patterns of accumulation of chlorophylls, carotenoids, phenols and antioxidant activity were analysed to define the optimal time for harvesting to obtain maximum nutraceutical characteristics. Fruits were harvested from *H. edulis* plants growing at the experimental field of the University of Morón (Moreno, Buenos Aires, 34°35'4.98" SL, 58°48'52.09" WL, 14 m.a.s.l.). Fresh fruit weight was significantly higher in Medium ripe, Ripe and Overripe fruits (40.1, 39.6 and 38.5 g, respectively) than in Unripe fruits (19.5 g). Soluble solids/total titratable acidity was significantly higher in Overripe fruits (7.3) than in Unripe, Medium ripe and Ripe fruits (3.7–4.5). Total polyphenols were maximum in Unripe fruits (905.8 mg tannic acid/100 g fresh fruit weight) decreasing during the fruit development (426.2–130.4 mg tannic acid/100 g fresh fruit weight). Also, DPPH radical scavenging activity was significantly higher in Unripe fruits (75.7%) compared with Medium ripe, Ripe and Overripe fruits (64.1–17.0%). Positive and significant correlations were observed between total polyphenol content and DPPH radical scavenging activity at each extract concentration ($r = 0.74, 0.87, 0.74$ and 0.60 for 1.25; 2.50; 5.00 and 12.50 mg/mL, respectively). Total carotenoid content increased during fruit development while at the same time decreased chlorophyll content. Chlorophyll b is the main chlorophyll found. Chromatographic analysis showed that lutein is the main carotenoid found in *H. edulis* fruits, followed by β -cryptoxanthin and β -carotene. As shown by the chromatograms at 280 nm, the concentration of biophenols and the complexity of the biophenol profile decreases during fruit development. Levels of polyphenols and pigments together with the antioxidant activity allow us to consider *H. edulis* fruit as a functional food.

1. Introduction

The lack of knowledge of several wild fruit species about their complete botanical information, food and nutrition value and consequently of their potential use, as well as the changes in their ecosystem, make them remain as underutilized status (Dandin and Krishna Kumar, 2016). However, these species are valuable since they usually contain nutritionally rich compounds that make them functional foods and a source of natural pigments (Brauch, 2016). They also play an important role considering increased food and nutritional insecurity, for their ability to recover from rigorous weather, to resist biotic and abiotic stress, and finally for being important gene donors for crop breeding. Based on the

foregoing, it is considered that non-traditional and underutilized fruits are important in mitigating the problems of world food in the presence of sustainable population growth and malnutrition (Nandal and Bhardwaj, 2014; S Ajay Vino and Sinija, 2016). Adaptation to social, economic and environmental changes can be favored by the diversity of the food system. In developing countries, the existence of diverse rural and agricultural landscapes can help in choosing healthy diets (Powell et al., 2015). Argentina has an important biological diversity, where its great variety of climates allows to obtain a very well-diversified flora with around 10,000 species of plants, a number that is in line with almost all of Europe's flora. However, little is known about it (Alonso and Desmarchelier, 2014), and several hundred species remain unused or underutilized.

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Hexachlamys edulis (O. Berg) Kausel & D. Legrand, “ubajay”, is certainly an underutilized and prominent species, distributed naturally in an important area of South America (Brazil, Paraguay, Bolivia, Uruguay and Argentina), and it grows spontaneously near water courses. According to several studies and mentions, *H. edulis* is distributed at least in one million Km² in South America (Povilonis et al., in press). It is a productive alternative framed within the underutilized species with nutraceutical properties, since it is a giver of health compounds, and considered as a non-timber forest product, facts that justify the growing demand for its products. In addition, it is a pioneering, rustic plant, a provider of abundant food for fauna and indispensable in mixed plantations destined to recompose degraded areas for permanent preservation. *H. edulis* is a fruit tree species with yellow globose drupes and can reach up to 15 m (Dematte, 1997). Fruit set and ripening occur in a few weeks from mid October to the end of November when the harvest can be done in Uruguay according to Vignale and Bisio (2005), or from September to January in Brazil (Dematte, 1997). Also, fruits have been described as sweet-sour to very acidic, pleasant, and with a quickly overripening (González, 2003; Chebez and Masariche, 2010).

Fruit development, the synthesis of secondary metabolites and the antioxidant activity are influenced by the genotype, the environmental conditions, and the cultural practices applied. Also, the knowledge of the properties of unripe fruits is important in the case of those species whose fruits are used unripe in the juice industry, for example, cranberries (Kähkönen et al., 2001; Ferreyra et al., 2007; Celik et al., 2008; Roussos et al., 2009; Arena et al., 2012). Thus, the aim of this work was to study the evolution of fruit size and weight together with the soluble solid and total acidity contents with the patterns of accumulation of chlorophylls, carotenoids, total phenols and antioxidant activity during different stages of *H. edulis* fruit development, to define the optimal time for harvesting to obtain maximum nutraceutical characteristics. The information obtained through this study could also be of value due to at present, this species is being considered for its introduction into the Argentinean food code.

2. Material and methods

2.1. Plant material and growing conditions

Seventeen *Hexachlamys edulis* plants were growing at the experimental field of the Faculty of Agriculture and Agrifood Sciences of the University of Morón (Moreno, Buenos Aires, 34°35'4.98" SL, 58°48'52.09" WL, 14 m.a.s.l.). Three samples of fruits (15 fruits each one) were manually harvested during November and December 2018 in four development stages; Stage 1: Unripe, fruits with all green skin (fruits with 21 days after full bloom: dafb); Stage 2: Medium ripe, fruits with green and yellow skin (fruits with 35 dafb); Stage 3: Ripe, fruits with all yellow skin (fruits with 42 dafb); and Stage 4: Overripe, fruits with yellow and brown skin (fruits with 49 dafb) (Figure 1).

Moreno's climate is classified as warm and temperate. According to Köppen (1936), this climate is classified as Cfa i.e., temperate rainy climate, or according to Peel et al. (2007), based on Köppen, determines the dominance of a humid subtropical climate. Maximal air daily temperature, minimal air daily temperature, mean air daily temperature and cumulative rainfall along 2018 at Moreno city, Buenos Aires (Argentina) are presented in Table 1. Maximal air daily temperature was observed in January (29.3 °C), while minimal air daily temperature was in June (7.5 °C). Mean air daily temperatures in October, November and December, months when the flowering and fruit growth and ripening took place, were 17.3, 20.9 and 22.0 °C, respectively. June and July were the months with the lowest rainfall (9 and 11 mm, respectively), while August and October were those with the highest rainfall (209 and 200 mm, respectively). Cumulative rainfall along 2018 was 1080 mm.

2.2. Determination of physicochemical parameters

The following characteristics were measured in each fruit (n = 12): fresh fruit weight (using an Ohaus Pioneer PX 0.001 g precision balance), dry fruit weight (fruits were dried in an oven at 50 °C for 7–10 days until

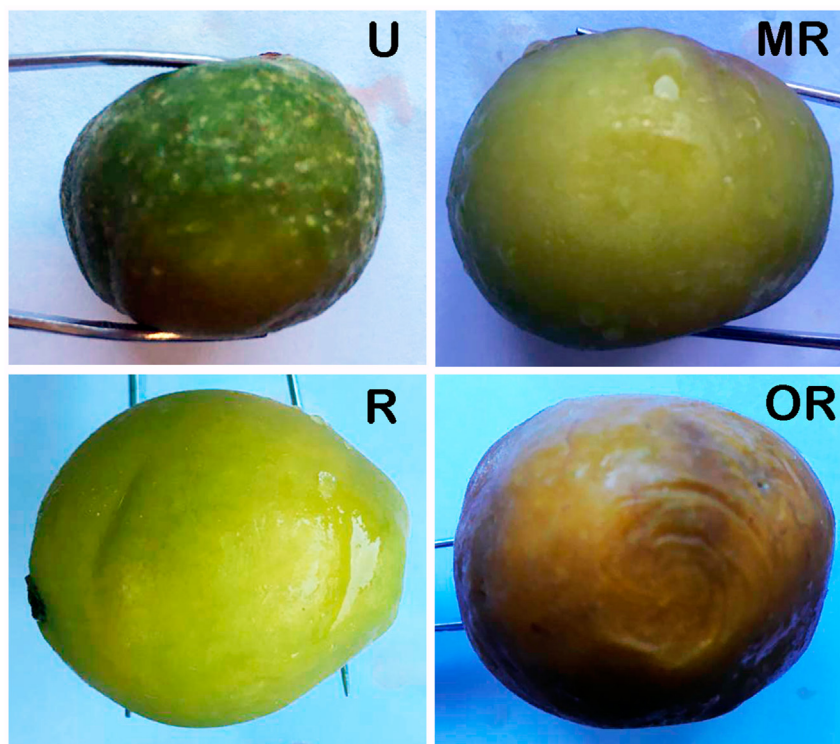


Figure 1. Development stages of *H. edulis* fruit. U: Unripe; MR: Medium ripe; R: Ripe, and OR: Overripe.

Table 1. Climatic data along the months of 2018. Maximal air daily temperature (MAX), minimal air daily temperature (MIN), mean air daily temperature (MEA) and cumulative rainfall (R) at Moreno city, Buenos Aires, Argentina.

Month	MAX °C	MIN °C	MEA °C	R mm
January	29.35	21.23	25.29	49
February	27.93	20.93	24.43	115
March	26.10	17.87	21.98	89
April	24.13	18.90	21.52	34
May	19.39	14.03	16.71	73
June	14.57	7.50	11.03	9
July	13.10	8.35	10.73	11
August	15.48	8.48	11.98	209
September	19.93	14.07	17.00	53
October	20.68	14.00	17.34	200
November	24.63	17.07	20.85	81
December	25.81	18.26	22.03	157

constant weight), fruit water content, maximum equatorial fruit diameter, minimum equatorial fruit diameter and polar fruit diameter (using a Mitutoyo caliper 0 to 6 "/0 to 150 mm measuring range, 0.0005 "/0.01 mm resolution), seed number, dry seed weight and dry seed weight/dry fruit weight ratio. Color was measured by Chincan NH300 colorimeter according to CIE L*a*b*. Differences in fruit color were obtained by applying Eq. (1).

$$\Delta E^* = [\Delta L^*2 + \Delta a^*2 + \Delta b^*2]^{1/2} \quad (1)$$

Soluble solids were quantified in fruit juice (n = 3, with 4–5 fruits each one) through an ATAGO N1-a refractometer with 0–32 °Brix measurement. Initial pH was also determined using a pH-meter. Total titratable acidity was determined by manual titration equipment and a pH-meter, using a 0.1 N NaOH solution. Total titratable acidity was expressed as citric acid (%), the most abundant organic acid in *H. edulis* fruits (Branco et al., 2016). Soluble solids/total titratable acidity ratio was also registered.

2.3. Total polyphenols

Total polyphenols were determined according to Makkar et al. (1993). Samples (n = 3, 5 g fresh fruit weight each one) (FFW) were extracted for 24 h in 50 mL 80% MeOH–H₂O at 4 °C. Aliquots (25, 50 and 100 µL) were set to 500 µL with water, and then 250 µL of 50% of the Folin-Ciocalteu reagent (Sigma-Aldrich) and 1.25 mL of 20% (w/v) aqueous sodium carbonate solution were put. The reaction mixture were kept at 24 °C for 40 min, and then the absorbance was read at 725 nm. The calibration curve was made with tannic acid (Sigma) and expressed as mg tannic acid equivalents/100 g FFW.

2.4. DPPH radical scavenging activity

A methanolic extract (n = 3, 5 g FFW in 50 mL of methanol each one) was prepared and kept under stirring for 1 h at room temperature. Aliquots (0.00; 0.025; 0.05; 0.10 and 0.25 mL) were then taken which in methanol (2 mL) were mixed with 0.25 mL of a methanolic solution containing 1,1-diphenyl-2-picrylhydrazyl (DPPH), resulting in a final concentration of 0.1 mM DPPH. The mixture was vigorously stirred and allowed to stand for 30 min in the dark. The absorbance at 517 nm was then measured against a blank using a Merck Spectroquant Pharo 300 UV-Vis spectrophotometer according to Shimada et al. (1992) method, briefly modified. A low absorbance of the reaction mixture indicates a high activity of free radical sequestration. Scavenging activity on DPPH radicals was calculated by applying Eq. (2).

$$\text{DPPH radical scavenging activity \%} = [(A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100](2)$$

Ablank is the absorbance of all reagents without the fruit extract), while A_{sample} is the absorbance of all reagents with the inclusion of the fruit extract.

2.5. Analysis of chlorophylls and carotenoids

Fruits were freeze-dried and carotenoids and chlorophylls were extracted with acetone (~25 mg/500 µL acetone) in a custom-designed ball-mill (n = 3). Briefly, 2 cycles of 1 min each at 25 cpm were used to break the cell wall, and 1 cycle of 1 min at 25 cpm was used to extract the carotenoids. After resting 20 min at room temperature, the extract was centrifuged at 15000 xg and 4 °C and immediately subjected to chromatographic analysis. Light was avoided during the process in order to prevent photodamage of the compounds under study. Separation of carotenoids and chlorophylls was achieved by a Waters Alliance e2695 HPLC on a C-18 reverse-phase analytical column (Waters Spherisorb ODS2 5 µm, 4.6 mm × 250 mm) kept at 25 °C, using an injection volume of 10 µL and a flow rate of 1 mL/min. The mobile phase consisted of a binary gradient of acetone and water. The initial composition was 75% acetone, which was linearly increased to 95% acetone in 10 min. It was maintained at this composition for the next 7 min, then raised to 100% acetone in 3 min and held for 10 min. The initial composition was reached in 5 min (Fernandez-Orozco et al., 2013). Peaks were monitored at 450 nm and online spectra were recorded between 320–700 nm with a Waters 2998 Photodiode Array Detector. A calibration curve was prepared with β-carotene and the concentration of each carotenoid was expressed as β-carotene equivalents/g dry fruit weight (DFW). A standard of chlorophylls was prepared from spinach by isolating it by TLC as in Minguez-Mosquera and Hornero-Mendez (1993), and was expressed as µg/g DFW. Identification was performed by comparing the retention time and UV-Vis spectra with standards prepared in the laboratory and literature data.

2.6. Analysis of polyphenols

Fruits were freeze-dried and polyphenols were extracted with methanol-water 80:20 (~20 mg/4 mL solvent) during 1 h at room temperature with agitation. At the end of the extraction, samples were centrifuged at 1500 g and filtered with a PVDF Millipore filter (0.45 µm). Separation was achieved by a Waters Alliance e2695 HPLC on a C-18 reverse-phase analytical column (Waters Spherisorb ODS2 5 µm, 4.6 mm × 250 mm) kept at 30 °C, using an injection volume of 20 µL and a flow rate of 1 mL/min (COI, 2009). The mobile phase consisted of a ternary gradient A: 0.2% (v/v) H₃PO₄ in water; B: Methanol; C: Acetonitrile. Elution was monitored at 280 nm and 335 nm and online spectra were recorded between 200–400 nm with a Waters 2998 Photodiode Array Detector. The individual biophenols were tentatively identified by their retention times, UV-Vis spectra, literature data and by comparison with available commercial standards. Syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid, ≥95%) and tyrosol [2-(4-hydroxyphenyl) ethanol, 98%] were provided by Sigma-Aldrich (Gillingham, England). The following standards were used for peak identification: caffeic acid (≥99%), vanillin (99%), p-coumaric acid (≥98%), trans-ferulic acid (99%), trans-cinnamic acid (97%), gallic acid (≥95%), tannic acid (≥95%), luteolin (≥98%), and apigenin (≥95%) from Sigma-Aldrich, and vanillic acid (≥97%) from Fluka. All the results are expressed as micrograms of tyrosol equivalents per gram of DFW and are averages of three replicates.

2.7. Statistical analysis

Data were analysed through the ANOVA and the means were separated with the Tukey test at p ≤ 0.05. Correlations between total polyphenol content and DPPH radical scavenging activity at different extract concentrations were made.

Table 2. Fresh fruit weight (FFW), dry fruit weight (DFW), fruit water content (FWC), minimum equatorial fruit diameter (MIEFD), maximum equatorial fruit diameter (MAEFD), polar fruit diameter (PFD), seed number (SN), dry seed weight (DSW) and dry seed weight/dry fruit weight ratio (DSW/DFW) determined in the four development stages of *H. edulis* fruits harvested during November and December 2018. Values represent means \pm S.D. (n = 12).

Stages	FFW (g)	DFW (g)	FWC (%)	MIEFD (mm)	MAEFD (mm)	PFD (mm)	SN	DSW (g)	DSW/DFW
Unripe	19.5 \pm 8.4b	4.1 \pm 2.2a	79.4 \pm 2.3c	31.4 \pm 4.7b	34.3 \pm 5.1b	36.3 \pm 6.1a	2.8 \pm 1.1a	2.2 \pm 1.2a	0.55 \pm 0.2a
Medium ripe	40.1 \pm 7.6a	6.6 \pm 2.3a	83.6 \pm 3.8b	41.4 \pm 3.8a	47.6 \pm 4.4a	43.8 \pm 7.6a	2.2 \pm 1.2a	3.5 \pm 1.6a	0.53 \pm 0.1a
Ripe	39.6 \pm 17.7a	5.0 \pm 2.8a	87.5 \pm 2.6a	42.4 \pm 8.8a	48.5 \pm 8.2a	38.6 \pm 5.6a	2.6 \pm 1.4a	3.0 \pm 1.6a	0.63 \pm 0.2a
Overripe	38.5 \pm 10.5a	6.5 \pm 2.4a	83.3 \pm 2.4b	43.6 \pm 4.0a	50.0 \pm 5.1a	39.8 \pm 7.9a	2.4 \pm 1.1a	3.5 \pm 1.6a	0.53 \pm 0.1a
F	12.713	2.564	13.234	8.816	14.142	2.148	0.414	1.689	0.701
p	0.000	0.071	0.000	0.000	0.000	0.113	0.744	0.188	0.558

F(p) = F statistic and probability of Fisher test. Different letters in each column indicate significant differences according to the Tukey test ($p \leq 0.05$).

3. Results and discussion

Morphological attributes such as fruit shape, size and biomass changed during the different development stages of *H. edulis* fruit. Fresh fruit weight, fruit water content, minimum and maximum equatorial fruit diameter varied significantly during the development (Table 2). Fresh fruit weight was significantly higher in Medium ripe, Ripe and Overripe stages (40.1, 39.6 and 38.5 g, respectively) than in Unripe stage (19.5 g). Fruit water content was significantly higher in Ripe stage (87.5%) than in Unripe, Medium ripe and Overripe stages (79.4–83.6%). The minimum equatorial fruit diameter was significantly higher in Medium ripe, Ripe and Overripe stages (41.4–43.6 mm) than in Unripe stage (31.4 mm). Maximum equatorial fruit diameter was also significantly higher in Medium ripe, Ripe and Overripe stages (47.6–50.0 mm) than in Unripe stage (34.3 mm). The fruit weight and size of other tropical species also increased during the development period, like in *Mangifera indica*, although the fruit length did not change (Wongmetha et al., 2015), following the same pattern that *H. edulis* fruit. Fresh fruit weight doubled between Unripe and Medium ripe stages, while minimum and maximum equatorial fruit diameters augmented 25 and 28% respectively, in the same period. The magnitude of these increments could also be related to the climatic conditions of the growing season (Arena and Curvetto, 2008). Dry seed weight/dry fruit weight ratio varied between 0.53 and 0.63, without significant differences, although it was maximum in Ripe stage, where half or more of the fruit weight corresponded to the seeds. The described behavior was not observed in other fruits such as barberries where this ratio decreased or at least stayed constant along the development stages (Arena and Curvetto, 2008), although also, depending on the climatic conditions in the growing season.

Fruit luminosity and "a" coordinate significantly varied during the stages of fruit development (Table 3). Fruit luminosity was significantly higher in Ripe and Overripe fruits (72.3 and 71.5, respectively) than in Unripe and Medium ripe fruits (68.7 and 67.7, respectively). The "a" coordinate (red/green) was significantly higher in Ripe fruits (5.7) than in Unripe fruits (1.3), which indicates that red pigments are synthesized

Table 3. Colorimetric parameters determined in the four development stages of *H. edulis* fruits harvested during November and December 2018. L: luminosity; a: red/green coordinate (+a indicates red. -a indicates green); b: yellow/blue coordinate (+b indicates yellow. -b indicates blue). Values represent means \pm S.D. (n = 12).

Stages	L	a	B
Unripe	68.7 \pm 0.9b	1.3 \pm 0.3b	6.8 \pm 1.6a
Medium ripe	67.7 \pm 2.1b	4.2 \pm 1.5ab	5.4 \pm 4.3a
Ripe	72.3 \pm 0.4a	5.7 \pm 2.9a	12.3 \pm 1.5a
Overripe	71.5 \pm 0.6a	4.3 \pm 1.2ab	6.8 \pm 4.7a
F	11.4	4.8	3.3
P	0.000	0.016	0.051

F(p) = F statistic and probability of Fisher test. Different letters in each column indicate significant differences according to the Tukey test ($p \leq 0.05$).

with the stages of fruit development. Also, the "b" coordinate (blue/yellow) was maximum in Ripe fruits, meaning that fruits have higher yellow pigments, although without significant differences among the rest of the values, probably due to the high variability. *H. edulis* fruits presented a similar luminosity than ripen fruits of *Annona chirimola*, although with higher content of red pigments and lower yellow color according to "a" and "b" coordinate values, respectively (Puccio et al., 2019).

Soluble solids, pH, total titratable acidity and soluble solids/total titratable acidity significantly varied during the development fruit period (Table 4). Soluble solids were significantly higher in Overripe fruits (10.1 °Brix) compared with Unripe, Medium ripe and Ripe fruits (7.6–8.2 °Brix). pH was significantly higher in Unripe, Medium ripe and Overripe fruits (3.2–3.4) than Ripe fruits (2.3). Total titratable acidity was significantly lower in Overripe fruits (1.4%) compared with Unripe and Ripe fruits (1.8%). Soluble solids/total titratable acidity was significantly higher in Overripe fruits (7.3) than in Unripe, Medium ripe and Ripe fruits (3.7–4.5). Some tropical and subtropical fruits showed a great variation in soluble solid contents between unripe and ripe stages, like as *Eleiodoxa conferta* and *Mangifera indica* fruits (20–25 and 60–65%, respectively), while other species presented a scarce variation, as was found in fruits of *Bouea oppositifolia* (5–10%), where soluble solid contents in ripe fruits of the mentioned species attained maxima values close to 4 °Brix (Mokhtar and Abd Aziz, 2015). However, fruits of other tropical species like as *Physalis peruviana* showed a solid soluble content close to 17 °Brix at ripe stage (Mier and Cález, 2011). If the type of carbohydrate accumulation during fruit development has yet not been determined in *H. edulis*, the increase in soluble solids content could be attributed to converting starch to sugar as was cited for different fruits (Mokhtar and Abd Aziz, 2015). The increase in the soluble solids/total titratable acidity ratio during fruit development was also shown in most tropical and subtropical fruits like in *Physalis peruviana* fruits (Mier and Cález, 2011), and several species (Mokhtar and Abd Aziz, 2015; Wongmetha et al., 2015). Decrease in acid content may be due to change of acids into sugars by some physiological and biochemical changes in the fruits (Mokhtar and Abd Aziz, 2015).

Total polyphenols and DPPH radical scavenging activity varied significantly during the fruit development period (Table 4). Total polyphenols were maximum in Unripe fruits (905.8 mg tannin acid/100 g FFW), decreasing in Medium ripe, Ripe and Overripe fruits (426.1–130.4 mg tannin acid/100 g FFW). Also, DPPH radical scavenging activity was significantly higher in Unripe fruits (94.7%) compared with Medium ripe, Ripe and Overripe fruits (62.0–22.3%). However, differences in DPPH radical scavenging activity depended on the extract concentration; indeed, the highest differences among fruit development stages were observed on 1.25 and 2.5 mg/mL extract concentration (Figure 2). Positive and significant correlations were observed between total polyphenol content and each extract concentration ($r = 0.74, 0.87, 0.74$ and 0.60 for 1.25; 2.50; 5.00 and 12.50 mg/mL, respectively). The highest total polyphenol concentration in Unripe fruits could be due to high flavonoid and tannin contents, as was found for some wild fruit species as *Myrica esculenta*, *Pyracantha crenulate* and *Rubus ellipticus* (Belwal et al., 2019), as well as for *Prunus persica* (Belhadj et al., 2016), *Vaccinium*

Table 4. Soluble solids (SS), pH (pH), total titratable acidity (TTA), soluble solids/total titratable acidity ratio (SS/TTA), total polyphenols (TP) and DPPH radical scavenging activity (DPPH) determined in the four development stages of *H. edulis* fruits harvested during November and December 2018. Values represent means \pm S.D. (n = 3).

Stages	SS ($^{\circ}$ Brix)	pH	TTA (%)	SS/TTA	TP (mg/100 g FFW)	DPPH (%)
Unripe	7.6 \pm 0.1c	3.4 \pm 0.3a	1.8 \pm 0.1a	4.3 \pm 0.1b	905.8 \pm 176.2a	94.7 \pm 6.7a
Medium ripe	6.0 \pm 0.0d	3.2 \pm 0.0a	1.6 \pm 0.0ab	3.7 \pm 0.0b	426.1 \pm 46.3b	62.0 \pm 24.9b
Ripe	8.2 \pm 0.1b	2.3 \pm 0.3b	1.8 \pm 0.2a	4.5 \pm 0.5b	337.4 \pm 23.6bc	80.1 \pm 17.8ab
Overripe	10.1 \pm 0.1a	3.4 \pm 0.1a	1.4 \pm 0.1b	7.3 \pm 0.4a	130.4 \pm 5.7c	22.3 \pm 30.0c
F	2068.46	15.78	9.31	77.30	38.27	24.96
p	0.000	0.001	0.005	0.000	0.000	0.000

F(p) = F statistic and probability of Fisher test. Different letters in each column indicate significant differences according to the Tukey test ($p \leq 0.05$).

corymbosum (Castrejón et al., 2008) and in *Rubus* hybrids (Siriwoharn et al., 2004). High total polyphenol concentration in green fruits could act as protection against several fruit diseases during pre-maturation stage (Prusky and Keen, 1993; Lattanzio et al., 2008). Total polyphenol concentration in Ripe *H. edulis* fruits (yellow fruits) (337.4 mg tannic acid/100 g FFW and 1687 mg tannic acid/100 g DFW) is higher than those found in the latest ripening stages in fruits of *Myrica esculenta*, *Pyracantha crenulate* and *Rubus ellipticus* (Belwal et al., 2019). Also, *H. edulis* fruit total polyphenol content was higher than in *Musa* spp. (24–72 mg gallic acid/100 g DFW) and *Psidium guajava* (109–191 mg gallic acid/100 g DFW) (Nitcheu et al., 2017), *Annona chirimola* (64.6–80.4 mg gallic acid/100 g FFW) (Puccio et al., 2019) and *Syzygium cumini* (787 mg gallic acid/100 g DFW), *Psidium guineense* (754 mg gallic acid/100 g DFW) and *Byrsonima crassifolia* (254 mg gallic acid/100 g DFW) (Gordon et al., 2011). The total polyphenol content in Ripe *H. edulis* fruits (yellow fruits) was comparable to that found in *Sclerocarya birrea* (700–2500 mg gallic acid/100 g DFW) (Nitcheu et al., 2017), and lower than *Pouteria macrophylla* (2915 mg gallic acid/100 g DFW) (Gordon et al., 2011). The highest DPPH radical scavenging activity in Unripe fruits could be related with the highest total polyphenol concentration. In fact, the positive and significant correlation between both variables explained this relationship, as was observed for the four underutilized fruits from the Amazon region (Gordon et al., 2011) and in *Physalis peruviana* (Mier and Cáez, 2011). DPPH radical scavenging

activity found in *H. edulis* fruits was comparable to the obtained in *Physalis peruviana* (78%) (Nitcheu et al., 2017), and lower than the obtained for this species by Mier and Cáez (2011).

Total carotenoid content increased significantly during development fruit period while at the same time decreased chlorophyll content (Figure 3A-B), which could be reflecting the changes in the color parameters. Chlorophyll b is the main chlorophyll found as it, although the high levels of pheophytin a suggest the conversion of chlorophyll a to this derivative during sample processing (Table 5). Some minor and constant amounts of pheophytin b are also observed that became undetectable in Overripe fruits. The conversion of chlorophylls to pheophytins, which can be a result of heat or acid treatment, could be favored in *H. edulis* fruit tissues due to its low pH (Table 4). During fruit development, chlorophyll b levels decreased, although in Ripe and Overripe stages some small quantity remained in the fruits. Pheophytin a, in contrast, seems to be more stable, and it is the most abundant chlorophyll present in Overripe fruits. Chlorophylls have been widely studied due to their relevance in plant physiology and their applications as food additives. In the food industry, chlorophylls are mainly used as colorants but, they also have health-promoting effects. Indeed, chlorophylls and their derivatives were suggested to have antioxidant and anti-inflammatory activities (Solymosi and Mysliwa-Kurziel, 2017; Pareek et al., 2018). Total chlorophyll content in *H. edulis* fruits was lower than the obtained for different varieties of *Psidium guajava* although in both cases chlorophyll contents

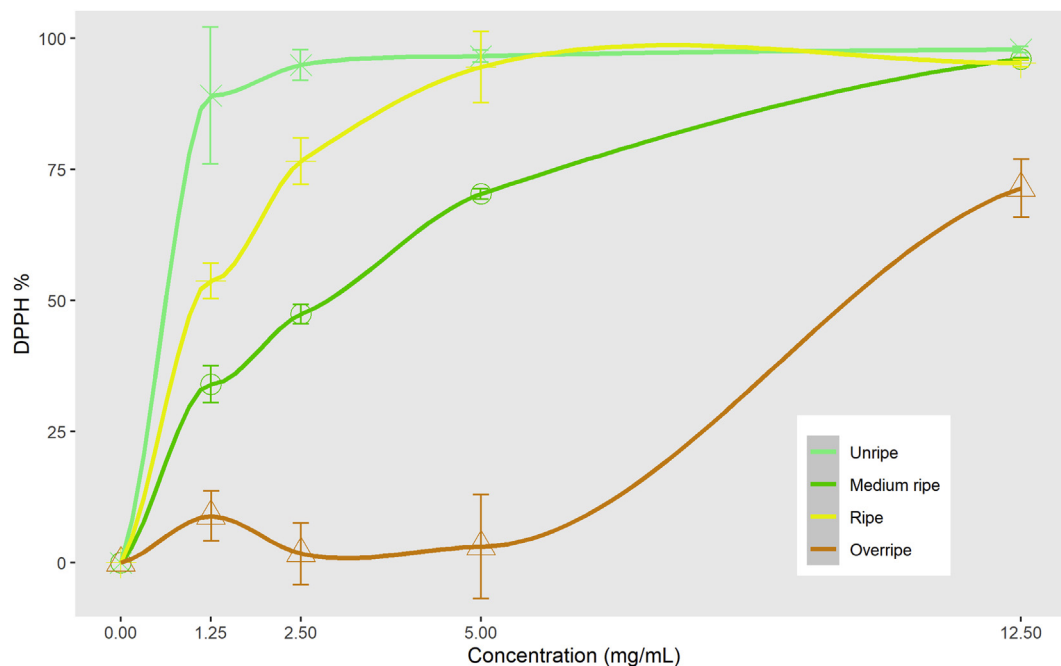


Figure 2. DPPH radical scavenging activity (DPPH%) of *H. edulis* fruits in different development stages and at different concentrations of the methanolic fruit extracts. Bars represent \pm standard error of the mean (n = 3).

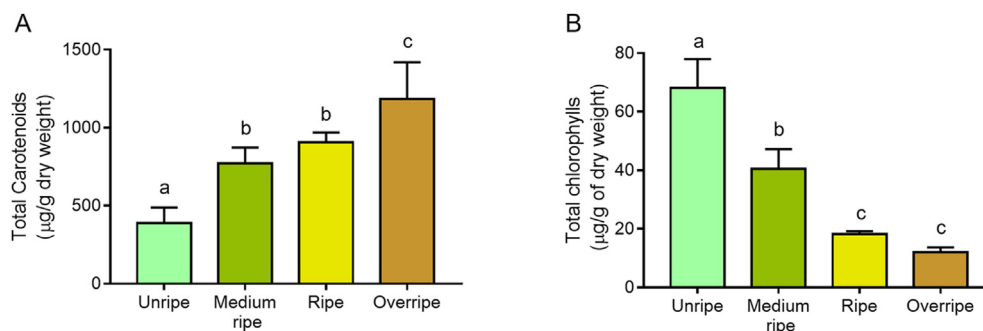


Figure 3. Total carotenoid (A) and total chlorophyll (B) content of *H. edulis* fruits in different development stages. Bars represent \pm standard error of the mean. ($n = 3$). Different letters indicate significant differences according to the Tukey test ($p \leq 0.05$).

Table 5. Content of individual chlorophylls and pheophytins ($\mu\text{g/g}$ DFW) determined in the four development stages of *H. edulis* fruits harvested during November and December 2018. Values represent means \pm S.D. ($n = 3$).

Stages	Chlorophyll a	Pheophytin a	Chlorophyll b	Pheophytin b
Unripe	5.5 \pm 1.4a	24.6 \pm 7.2a	18.7 \pm 5.7a	5.45 \pm 1.3a
Medium ripe	3.7 \pm 0.4	17.5 \pm 4.4b	9.0 \pm 1.4b	5.5 \pm 0.8a
Ripe	-	7.9 \pm 1.2c	4.6 \pm 0.9c	5.9 \pm 0.9a
Overripe	-	12.0 \pm 1.7c	1.6 \pm 0.9c	-
F	12.26	8.115	13.28	0.1643
P	0.151	0.008	0.002	0.852

F(p) = F statistic and probability of Fisher test. Different letters in each column indicate significant differences according to the Tukey test ($p \leq 0.05$). Only the values higher than 0 were statistically analyzed.

decrease during fruit development (Guavita-Vargas et al., 2018). The loss in chlorophyll is probably due to increased activities of chlorophyll degrading enzymes (Kuai et al., 2018). The significantly increase in total carotenoid contents during development in *H. edulis* fruits, was also found in *Psidium guajava* var Red (7–39 mg/100 g DFW) (Guavita-Vargas et al., 2018); however, in *Psidium guajava* var White, carotenoid content decreased from 10 to 5 mg/100 g DFW (Guavita-Vargas et al., 2018). In Ripe fruits, total carotenoid content was greater than the obtained by Silva et al. (2014) in the very related *Eugenia pyriformis* pulp (1306.6 $\mu\text{g}/100$ g FFW), but similar than the obtained by Pereira et al. (2012) in the same fruit at the same development stage (909.8 \pm 270.9 $\mu\text{g}/\text{g}$ DFW). Other fruits of the Myrtaceae family, like *Psidium cattleianum*, *Campomanesia xanthocarpa* and *Eugenia brasiliensis* have lower levels of total carotenoids at maturity (Pereira et al., 2012; Silva et al., 2014). Besides, compare with other tropical fruits, total carotenoid contents in Ripe and Overripe *H. edulis* fruits (706 and 1078 $\mu\text{g}/\text{g}$ DFW, respectively) were higher than the observed for *Annona cherimola* (2.7–5.5 μg β -carotene/100 g FFW) (Puccio et al., 2019) and for *Mangifera indica*, *Carica papaya* and *Syzygium cumini* fruits (Saini et al., 2015).

Chromatographic analysis showed that lutein (peak 1) is the main carotenoid found in *H. edulis* fruits, followed by β -cryptoxanthin (peak 2) and β -carotene (peak 3) (Figure 4A-B and Table 6). In its free form, lutein comprises \sim 60% of total carotenoid content in Unripe fruits, while this proportion decreases to 25% in Overripe fruits, although the absolute amount of free lutein ($\mu\text{g}/\text{g}$ DFW) does not change. β -cryptoxanthin, however, significantly increases its content along the development, with its maximum in Overripe fruits, while β -carotene only increases in Overripe fruits (Table 6). As shown in Figure 4A, during development also increases the complexity of the carotenoid profile of the fruits. The chromatograms showed the presence of five peaks with retention times longer than β -carotene (peaks 4–8), which increase their area during fruit development and correspond to esterified xanthophylls (Hornero-Méndez, 2019). The conjugation with a fatty acid (or two in the case of lutein) renders the xanthophylls less polar and thus explains the longer

retention time observed. However, since the chromophore is not affected by esterification, the UV-Vis spectrum remains the same. Therefore, it is possible to tentatively identify peak 4 and 5 as β -cryptoxanthin esters and peaks 6–8 as lutein esters. In the case of lutein, which contains two $-\text{OH}$ groups, mono- or di-esters could be formed. Besides, diesters can have the same or different fatty acid, leading to the possibility of diverse esterified molecules. However, it is reported previously that lutein monoesters elute before β -carotene, and lutein diesters after it (Hornero-Méndez and Minguez-Mosquera, 2000; Mattera et al., 2020). Therefore, the retention time and the UV-Vis spectrum suggest that peaks 6–8 correspond to lutein diesters and no partially esterified lutein is observed (Table 6). Lutein, β -cryptoxanthin and β -carotene are the main carotenoids found in *H. edulis* fruits, and this profile is similar to other members of the Myrtaceae family (Pereira et al., 2012; Silva et al., 2014). Changes during fruit development are also reflected in changes in the amount of individual carotenoids as well as in their esterification. In Unripe, Medium ripe and Ripe fruits the most abundant carotenoid was lutein, while in Overripe fruits the most abundant carotenoid was β -cryptoxanthin in its esterified form. It is suggested that esterification facilitates xanthophylls accumulation by increasing their insertion into membranes and/or preventing their degradation (Hornero-Méndez, 2019). This process leads to a change and increases in color, making the fruit more attractive to animals that are a vehicle for seed dissemination. Indeed, the esterification of carotenoids dramatically increases during development of *H. edulis* fruits and is the main factor responsible for the increase in total carotenoid content (Figure 4 and Table 6). Thus, during fruit development, synthesis and esterification of xanthophylls are stimulated, and β -cryptoxanthin and its esters, in particular, seem to be preferred. Lutein was also the predominant carotenoid in *Mangifera indica* (31.7 $\mu\text{g}/\text{g}$ FFW), *Carica papaya* (237 $\mu\text{g}/\text{g}$ FFW), *Cucumis melo* (172 $\mu\text{g}/\text{g}$ DFW) (Saini et al., 2015), *Euterpe edulis* (297.7 $\mu\text{g}/100$ g FFW) and in *Psidium cattleianum* (26.38 $\mu\text{g}/\text{g}$ DFW) (Pereira et al., 2012; Silva et al., 2014). Besides, β -cryptoxanthin was the major carotenoid in *Eugenia brasiliensis* (286.7 $\mu\text{g}/100$ g FFW), *Campomanesia xanthocarpa* (121.08 $\mu\text{g}/\text{g}$ DFW) and in *Eugenia pyriformis* (521 $\mu\text{g}/100$ g FFW or 159 $\mu\text{g}/\text{g}$ DFW, depending on the study) (Pereira et al., 2012; Silva et al., 2014). β -carotene was also present at significant levels, although its amount did not change along of fruit development. Carotenoids are important health-promoting molecules that humans must obtain from the diet. All of them act as antioxidants, and it is proposed that they may be important in the reduction of the risk of contracting chronic degenerative diseases (Britton and Khachik, 2009). Also, some individual carotenoids have more specific functions like for example, β -carotene and β -cryptoxanthin have provitamin A activity, while lutein and zeaxanthin constitute macular pigment in the eye. Moreover, dietary lutein was shown to be beneficial to age-related macula degeneration patients (Feng et al., 2019). The levels of individual carotenoids found in this study, allow us to consider *H. edulis* as a very good source of lutein and β -cryptoxanthin and a good source of β -carotene, according to Britton and Khachik (2009) classification.

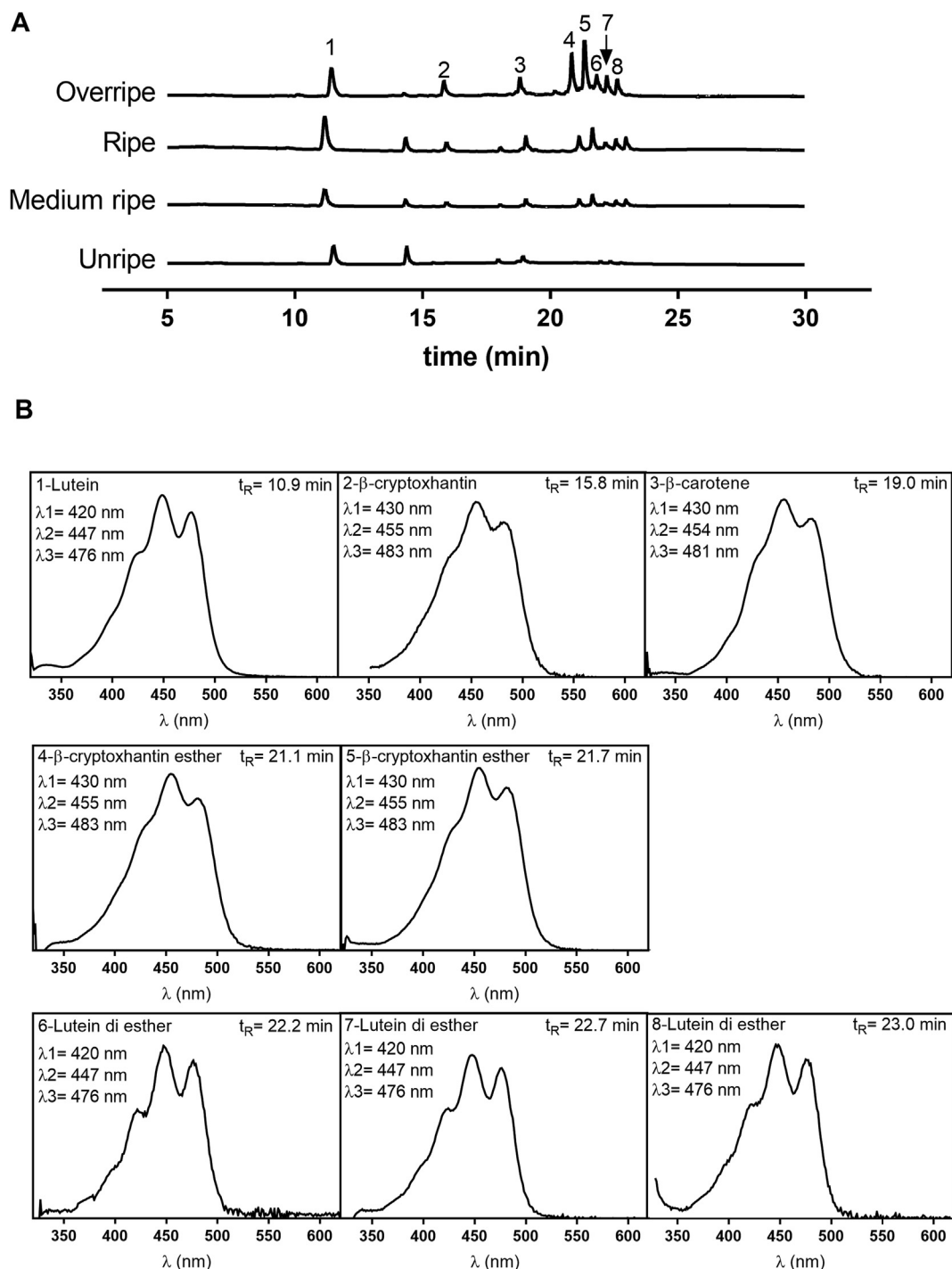


Figure 4. HPLC-DAD carotenoid chromatograms (A) of *H. edulis* fruits in different development stages recorded at 450 nm and UV/Vis spectrums of the different peaks present in the chromatograms (B).

As shown by the chromatograms at 280 nm (Figure 5A-D), the concentration of biophenols and the complexity of the biophenol profile decreases during fruit development. In Unripe fruits, many peaks are present, most of them at shorter retention times, i.e. below 25 min. A shift to larger retention times occurs during fruit development, and in Over-ripe fruits, fewer peaks remain. All the main peaks decrease their area during fruit development except for the one at 25.8 min, which increases it (Table 7).

According to their polarity, the elution order of biophenols in this chromatographic system is hydroxybenzoic acids, hydroxycinnamic acids, and lastly flavonoids, although some superposition is probable due

to substituents that may change the polarity of each compound (Robards, 2003). Among a specific flavonoid, flavanone-glycoside elutes before the flavonol, followed by flavone glycosides and lastly the free aglycone (Robards, 2003). These groups of compounds have some characteristics in their UV/Vis spectrum which can be used to tentatively assign each peak. As state below, hydroxybenzoic acids elute first and they have an absorption band between 260-280 nm. This group comprises gallic, p-hydroxybenzoic, protocatechuic, vanillic and syringic acids, which elutes between 6-22 min. As shown in the chromatograms, most of the peaks are in this time window in Unripe, Medium ripe and Ripe fruits and the UV/Vis spectrum of these peaks all have maximums at ~ 220 nm and

Table 6. Content of individual carotenoids ($\mu\text{g/g}$ DFW) detected in the four development stages of *H. edulis* fruits harvested during November and December 2018. Values represent means \pm S.D. ($n = 3$).

Stages	Lutein	β -cryptoxanthin	β -carotene	β -cryptoxanthin esters (peaks 4 + 5)	Lutein di-esters (peaks 6 + 7+8)
Unripe	297.7 \pm 8.4a	4.3 \pm 1.3c	56.3 \pm 13.9b	24.8 \pm 1.7c	51.7 \pm 7.9c
Medium ripe	242.9 \pm 6.4a	45.5 \pm 11.5b	59.5 \pm 1.2b	174.8 \pm 20.3b	119.5 \pm 11.4b
Ripe	265.1 \pm 40.5a	50.1 \pm 5.0b	44.7 \pm 8.1b	174.5 \pm 12.9b	172.1 \pm 12.4a
Overripe	290.6 \pm 50.9a	92.7 \pm 24.2a	91.2 \pm 25.4a	409.4 \pm 52.1a	194.3 \pm 52.8a
F	0.1112	22.56	14.86	92.08	35.09
p	0.951	0.000	0.000	0.000	0.000

F(p) = F statistic and probability of Fisher test. Different letters in each column indicate significant differences according to the Tukey test ($p \leq 0.05$).

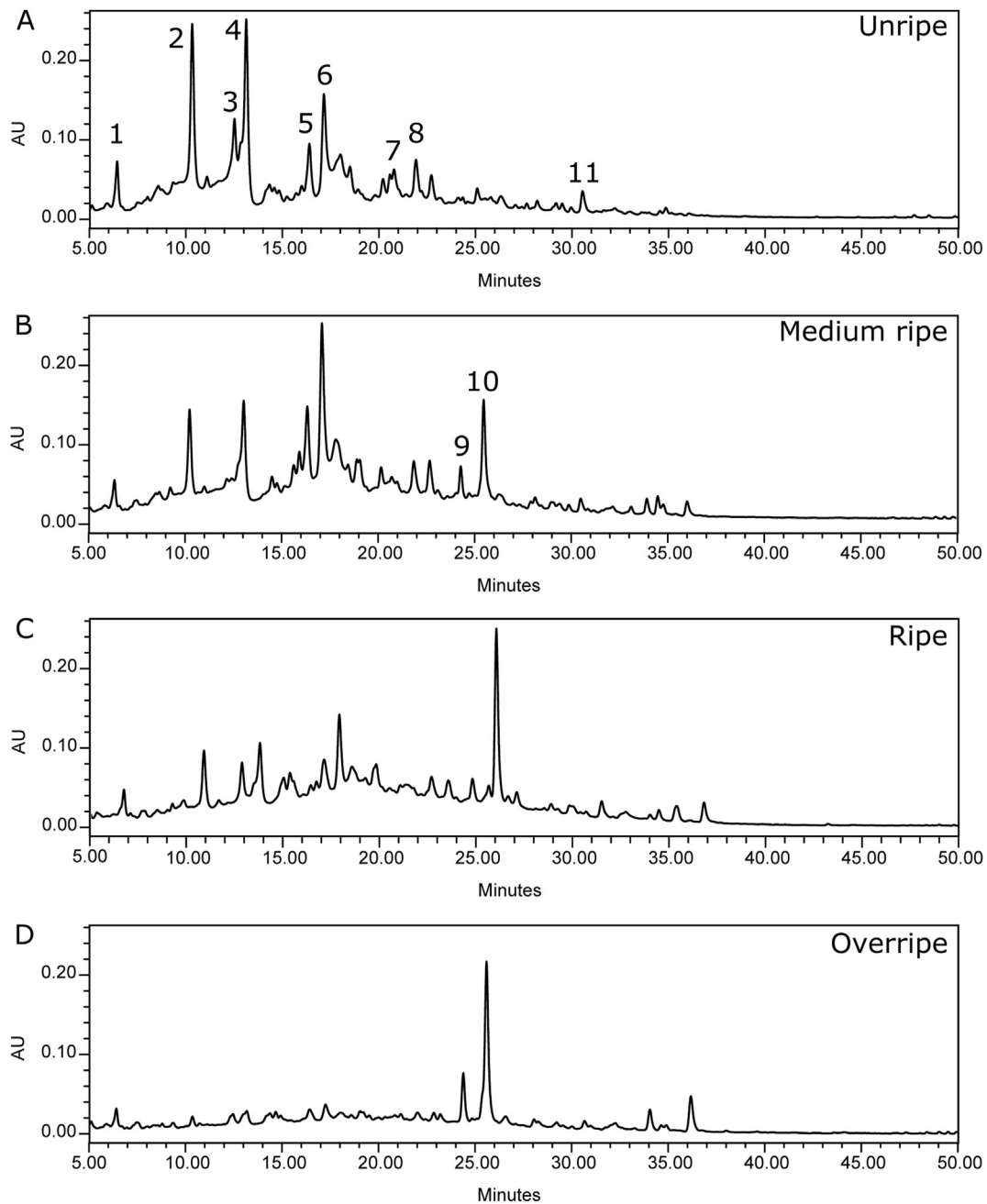


Figure 5. HPLC-DAD polyphenol chromatograms of *H. edulis* fruits in different development stages (A-Unripe, B-Medium ripe, C-Ripe and D-Overripe) recorded at 280 nm (Peak number refers to Table 7).

Table 7. Retention time and concentration of biophenols ($\mu\text{g/g}$ DFW) detected in the four development stages of *H. edulis* fruits harvested during November and December 2018. Values represent means \pm S.D. ($n = 3$).

Peaknumber	Biophe nol	Retention time (min)	λ (nm)	Unripe	Medium ripe	Ripe	Overripe	F	p
1	Gallic acid	6.49	215, 271	271 \pm 4a	132 \pm 5b	117 \pm 3c	80 \pm 2d	1078	0.000
2	Galloyl derivative	10.4	223, 257	1238 \pm 7a	524 \pm 5b	350 \pm 6c	42 \pm 7d	599.6	0.000
3	Catechol	12.5	220, 285	829 \pm 5a	166 \pm 1b	360 \pm 5c	53 \pm 8d	224.2	0.000
4	Galloyl derivative	13.2	220, 260	1192 \pm 2a	591 \pm 8b	173 \pm 1c	47 \pm 6d	186.8	0.000
5	Galloyl derivative	16.5	220, 275	498 \pm 2a	517 \pm 7a	175 \pm 9b	94 \pm 2b	22.6	0.006
6	Galloyl derivative	17.6	220, 271	951 \pm 1a	1088 \pm 7a	492 \pm 2b	124 \pm 2c	228.8	0.000
7	Syringic acid	20.5	218, 276	278 \pm 3a	103 \pm 2b	108 \pm 1b	30 \pm 7c	54.4	0.001
8	Catechin	22.1	215, 279	373 \pm 4a	130 \pm 2b	122 \pm 1b	71 \pm 9c	24.2	0.005
9	Tannic acid	24.5	280	54 \pm 4b	178 \pm 5a	218 \pm 7a	181 \pm 1a	11.9	0.018
10	Tannic acid	25.8	283	58 \pm 3c	505 \pm 3b	791 \pm 3a	726 \pm 6a	168.6	0.000
11	Rutin	30.6	253, 363	135 \pm 4a	45 \pm 2b	21 \pm 4b	40 \pm 2b	46.6	0.001
	Total phenols			5877 \pm 6a	3981 \pm 9b	2735 \pm 182c	1488 \pm 67d	459.3	0.000

F(p) = F statistic and probability of Fisher test. Different letters in each row indicate significant differences according to the Tukey test ($p \leq 0.05$).

between 270–280 nm, indicating that these group of compounds or their derivatives are the predominant biophenols present (Table 7). Gallic acid (peak 1) and syringic acid (peak 7) were identified at 6.5 min and 20.9 min respectively, and their concentration decreases during fruit development. At 12.6 min a peak with the UV/Vis spectrum compatible with catechol is present (peak 3). Peaks at 10.4 and 13.2 min (peaks 2 and 4) most probably correspond to low molecular weight hydrolyzable tannins with ellagic moiety, according to their UV/Vis spectrum and polarity inferred from the retention time (Salminen et al., 1999). The next peaks have a compatible UV/Vis spectrum with the different galloyl derivatives (peaks 5 and 6) containing a different degree of substitution. Peaks 9 and 10 correspond to tannic acid according to retention time and UV/Vis spectrum. Hydrolyzable tannins represent a group of polyphenolic compounds whose structure is formed by esters of β -D-glucose with either gallic (gallotannins) or hexahydroxydiphenic (ellagitannins) acids. According to the degree of glucose esterification, they can be simple or very complex compounds (i.e. tannic acid) and elute at different times (Salminen et al., 1999). Among cinnamic acids, the most common are p-coumaric, caffeic, ferulic, synapic and chlorogenic (Natella et al., 1999). They usually appear between 20–30 min and they have an absorption band between 310–330 nm. None of the peaks in this region have this characteristic, therefore cinnamic derivatives may not be important biophenols in *H. edulis* fruits. Flavonoids which usually appear at retention times larger than 25 min, exhibit a characteristic UV spectrum with two major absorption bands, one between 330–380 nm, and the other between 240–280 nm (Mabry et al., 1970). The chromatograms and UV/Vis spectrum of the peaks at this area show that there are no important peaks with these characteristics. However, a peak with the retention time and UV/Vis spectrum similar to catechin at 22 min is present (peak 8). This flavanol decreases its concentration from $373 \pm 40 \mu\text{g/g}$ DFW to $71 \pm 9 \mu\text{g/g}$ DFW (Table 7). Also, a peak at 30.6 min (peak 11) with a similar spectrum and retention time as a true standard of rutin was detected, which decreases its area with fruit development. The amount of rutin in Unripe fruits was $135 \pm 4 \mu\text{g/g}$ DFW and decrease to $40 \pm 2 \mu\text{g/g}$ DFW in Overripe fruits. No peaks are detected beyond 37 min. Total phenolics determined by HPLC (Table 7) agree with total phenolics determined spectrophotometrically by Folin-Ciocalteu (Table 4), which decreases ~ 4 to 8 times from Unripe to Overripe fruits.

The results presented here regarding the biophenol profile of *H. edulis* fruits are in agreement with the ones reported by Silva et al. (2014), who determine by HPLC-DAD-MS that biophenols present in ripe fruits comprise mostly gallic acid and its derivatives. Also, Stafussa et al. (2018), determine the content of biophenols and found that hydroxybenzoic acid > flavonoids > hydroxycinnamic acids. Among the flavonoids, catechin was the main one in Medium ripe to Overripe stages of *H. edulis* fruits. Changes in the polyphenol profile from more complex to more simple phenolic derivatives also explain the decrease in antioxidant

power of the *H. edulis* extract. For instance, Li et al. (2019) show that a tannic acid derivative, trigalloyl glucose has less antioxidant power than gallic acid itself and Pulido et al. (2000) report that gallic acid is more potent antiradical than tannic acid. Polyphenols are supposed to be accumulated in green fruits as a mechanism of fruit protection from various fruit-borne diseases during pre-maturation stage. As maturation proceeds phenols get oxidized, thus the phenol concentration gets reduced in ripened fruits. This trend was clearly observed in our samples (Murata et al., 1995; Ivanova et al., 2011).

4. Conclusions

Variations in physicochemical properties at different development stages of *H. edulis* fruits were analysed. The definition of fruit traits at each development stage contributes in assigning the best alternative uses (e.g. for some beverages, for fresh market, for industrial processing). *H. edulis* fruit appeared to possess good levels of polyphenols and pigments together with antioxidant activity, so, it could be considered as a functional food. The obtained results are relevant for introducing *H. edulis* fruits in the Argentinean food code, and therefore to tend its commercialization.

Declarations

Author contribution statement

Miriam E. Arena: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ignacio S. Povilonis, Virginia Borroni: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Diana Constenla: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Silvia Radice: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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