Changes in physicochemical properties at different development stages of *Hexachlamys edulis* fruit, an underutilized South American species

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**A R T I C L E   I N F O**

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**A B S T R A C T**

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" W1, 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 \(\beta\)-cryptoxanthin and \(\beta\)-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 Km$^2$ 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.](image-url)
constant weight), fruit water content, maximum equatorial fruit diameter, minimum equatorial fruit diameter and polar fruit diameter (using a constant weight), fruit water content, maximum equatorial fruit diameter, and cumulative rainfall (R) at Moreno city, Buenos Aires, Argentina.

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   |

2.5. 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 2695 HPLC on a C-18 reverse-phase analytical column (waters spherosorb 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.

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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 ripe fruits of *Annona cherimola*, 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 solids contents between unripe and ripe stages, like as *Ejderoxa 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 Caez, 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 Caez, 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 FW), decreasing in Medium ripe, Ripe and Overripe fruits (426.1–130.4 mg tannin acid/100 g FW). Also, DPPH radical scavenging activity was significantly higher in Unripe fruits (94.7%) compared with Medium ripe, Ripe and Overripe fruits (62.0–22.9%). 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* (Belhadj et al., 2019), as well as for *Punus 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 ± S.D. (n = 3).

| Stages      | SS (% Brix) | pH     | TTA (%) | SS/TTA | TP (mg/100 g FFW) | DPPH (%) |
|-------------|-------------|--------|---------|---------|-------------------|----------|
| Unripe      | 7.6 ± 0.1c  | 3.4 ± 0.3a | 1.8 ± 0.1a | 4.3 ± 0.1b | 905.8 ± 176.2a    | 94.7 ± 6.7a |
| Medium ripe | 6.0 ± 0.0d  | 3.2 ± 0.0a | 1.6 ± 0.0ab | 3.7 ± 0.0b | 426.1 ± 46.3b     | 62.0 ± 24.9b |
| Ripe        | 8.2 ± 0.1b  | 2.3 ± 0.3b | 1.8 ± 0.2a | 4.5 ± 0.5b | 337.4 ± 23.6bc    | 80.1 ± 17.8ab |
| Overripe    | 10.1 ± 0.1a | 3.4 ± 0.1a | 1.4 ± 0.1b | 7.3 ± 0.4a | 130.4 ± 5.7c      | 22.3 ± 30.0c |
| F           | 2068.46     | 15.78   | 9.31    | 77.30   | 38.27             | 24.96    |
| p           | 0.000       | 0.001   | 0.005   | 0.00    | 0.00              | 0.00     |

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

corymbosum (Castrejón et al., 2008) and in *Rubus* hybrids (Sirivoharn 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 tanninc acid/100 g FFW and 1687 mg tanninc acid/100 g DFW) is higher than those found in the latest ripening stages in fruits of *Myrica esculenta*, *Pyracantha crenulata* 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 DWF) (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 *Byroniana 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 birea* (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 Caez, 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 Caez (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 b 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-Kurdziel, 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 ± standard error of the mean (n = 3).](image-url)
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 –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 Myr-
taceae family (Pereira et al., 2012; Silva et al., 2014). Changes during
fruit development are also reflected in changes in the amount of indi-
vidual 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 pre-
venting their degradation (Hornero-Méndez, 2019). This process leads to
a change and increases in color, making the fruit more attractive to an-
imals 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 carot-
enoid content (Figure 4 and Table 6). Thus, during fruit development,
synthesis and esterification of xanthophylls are stimulated, and β-crypt-
oxanthin and its esters, in particular, seem to be preferred. Lutein was
also the predominant carotenoid in Mangifera indica (31.7 μg/g FW),
Carica papaya (237 μg/g FW), Cucumis melo (172 μg/g FW) (Saini
et al., 2015), Euterpe edulis (297.7 μg/100 g FW) and in Psidium cat-
tleyanum (26.38 μg/g FW) (Pereira et al., 2012; Silva et al., 2014).

Besides, β-cryptoxanthin was the major carotenoid in Eugenia brasiliensis
(286.7 μg/100 g FW), Campomanesia xanthocarpa (121.08 μg/g FW)
and in Eugenia pyrifolia (521 μg/100 g FW or 159 μg/g FW, 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.

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

**Table 5.** Content of individual chlorophylls and pheophytins (μg/g DFW) determined in the four development stages of H. edulis fruits harvested during November and December 2018. Values represent mean ± S.D. (n = 3).

| Stages     | Chlorophyll a | Pheophytin a | Chlorophyll b | Pheophytin b |
|------------|---------------|--------------|---------------|--------------|
| Unripe     | 5.5 ± 1.4a    | 24.6 ± 7.2a  | 18.7 ± 5.7a   | 5.45 ± 1.3a  |
| Medium ripe| 3.7 ± 0.4     | 17.5 ± 4.9b  | 9.0 ± 1.4b    | 5.5 ± 0.8a   |
| Ripe       | -             | 7.9 ± 1.2c   | 4.6 ± 0.9c    | 5.9 ± 0.9a   |
| Overripe   | -             | 12.0 ± 1.7c  | 1.6 ± 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 ≤ 0.05). Only the values higher than 0 were statistically analyzed.
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 Overripe 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 stated 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 maximum at ~220 nm and

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).
Table 6. Content of individual carotenoids (μg/g DFW) detected in the four development stages of *H. edulis* fruits harvested during November and December 2018. Values represent means ± 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 ± 8.4a | 4.3 ± 1.3c      | 56.3 ± 13.9b| 24.8 ± 1.7c                          | 51.7 ± 7.9c                        |
| Medium ripe| 242.9 ± 6.4a | 45.5 ± 11.5b    | 59.5 ± 1.2b | 174.8 ± 20.3b                        | 119.5 ± 11.4b                      |
| Ripe       | 265.1 ± 40.5a| 50.1 ± 5.6b     | 44.7 ± 8.1b | 174.5 ± 12.9b                        | 172.1 ± 12.4a                      |
| Overripe   | 290.6 ± 50.9a| 92.7 ± 24.2a    | 91.2 ± 25.4a| 409.4 ± 52.1a                        | 194.3 ± 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 < 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).
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/Vis 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 region 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 flavanone decreases its concentration from 373 ± 40 μg/g DFW to 71 ± 9 μg/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 (peak 1) and syringic acid (peak 7) were identified, which decreases its area with fruit development. The chromato-}

| Peaknumber | Biophenol     | Retention time (min) | λ (nm) | Unripe | Medium ripe | Ripe | Overripe | F | p   |
|------------|--|--|--|--|--|--|--|--|--|--|
| 1          | Gallic acid   | 6.49                   | 215, 271 | 271±4a | 132±6b | 117±3c | 80±2d | 1078 | 0.000 |
| 2          | Galloy derivative | 10.4                  | 223, 257 | 123±17a | 524±5b | 350±6c | 42±7d | 599.6 | 0.000 |
| 3          | Catechol      | 12.5                  | 220, 285 | 129±5a | 166±1b | 163±5c | 360±5 | 224.2 | 0.000 |
| 4          | Galloy derivative | 13.2                 | 220, 260 | 119±2.2a | 591±8b | 173±1c | 47±6d | 186.8 | 0.000 |
| 5          | Galloy derivative | 16.5                 | 220, 275 | 498±2a | 517±7a | 175±9b | 94±2b | 22.6 | 0.006 |
| 6          | Galloy derivative | 17.6                 | 220, 271 | 951±1a | 1088±7a | 492±2b | 124±2c | 228.8 | 0.000 |
| 7          | Syringic acid  | 20.5                  | 218, 276 | 278±3a | 103±2b | 108±1b | 30±7c | 54.4 | 0.001 |
| 8          | Catechin      | 22.1                  | 215, 279 | 373±4a | 130±2b | 122±1b | 71±9c | 24.2 | 0.005 |
| 9          | Tannic acid   | 24.5                  | 280      | 541±4b | 178±5a | 218±7a | 181±1a | 11.9 | 0.018 |
| 10         | Tannic acid   | 25.8                  | 283      | 58±3c | 505±3b | 791±3a | 726±6a | 168.6 | 0.000 |
| 11         | Rutin         | 30.6                  | 253, 363 | 135±4a | 45±2b | 21±4b | 40±2b | 46.6 | 0.001 |

Total phenols
5877±6a 3981±9b 2735±182c 1488±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 ≤ 0.05).

### 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 to balanced effect of selected uses (e.g., for some beverages, for fresh market, for industrial processing). H. edulis fruit appears to possess good levels of polyphenols and pigment together with antioxidant activity, so it could be considered as a functional food. The obtained results are relevant for understanding 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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