horticulturae

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Changes in Carbohydrates, Organic Acids, and Minerals at Different Development Stages of *Hexachlamys edulis* Fruit, a Wild South American Species with Horticultural Potential

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Abstract: The aim of this work was to study the patterns of the accumulation of carbohydrates, organic acids, and minerals at different development stages of *Hexachlamys edulis* fruit for its evaluation as a source of health-promoting compounds, which is necessary in order to be included in the Argentine Food Code. Additionally, the obtained results will allow for deciding the optimal time for consumption to receive a better flavour and a good contribution of the nutrients evaluated. The succinic acid concentration (the major organic acid) was high in unripe fruit (112.33 mg/g of the dry weight), then decreased to a minimum in medium ripe and ripe fruit (92.48 to 99.43 mg/g of the dry weight,), to increase again in overripe fruit (115.65 mg/g of the dry weight,). Sucrose increased significantly from 21.20 mg/g of the dry weight in unripe fruit to a maximum of 82.53 mg/g of the dry weight in ripe fruit. Glucose increased significantly from 95.59 mg/g of the dry weight in unripe fruit to a maximum of 163.13 mg/g of the dry weight in overripe fruit. Fructose followed the same behaviour, increasing significantly from 150.08 mg/g of the dry weight in unripe fruit to a maximum of 205.85 mg/g of the dry weight in overripe fruit. The starch concentration was at the maximum in unripe and medium ripe fruit (171.39 and 161.19 mg starch/g of the dry weight, respectively), to then decrease in ripe and overripe fruit (40.45 and 65.96 mg starch/mg of the dry weight, respectively). Maximum insoluble dietary fibre values were attained in unripe and medium ripe fruit (26.71 and 27.13 mg/100 g of the dry weight, respectively), to then decrease in ripe and overripe fruit (15.81 and 15.51 mg/100 g of the dry weight, respectively). Soluble dietary fibre oscillated between 9.03 and 11.26 mg/100 g of the dry weight during the development stages, although without significant differences. The mineral concentrations (Mg, K, Mn, and total cations) did not vary significantly during the different development stages. The obtained results allow us to consider *H. edulis* fruit as a promising natural source of sugars, organic acids, and minerals.

Keywords: underutilised fruit; fructose; glucose; dietary fibre; succinic acid

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Citation: Arena, M.E.; Povilonis, I.S.; Borroni, V.; Pérez, E.; Pellegrino, N.; Cacciatore, C.; Radice, S. Changes in Carbohydrates, Organic Acids, and Minerals at Different Development Stages of *Hexachlamys edulis* Fruit, a Wild South American Species with Horticultural Potential. *Horticulturae* **2023**, *9*, 314. https://doi.org/10.3390/horticulturae9030314

Academic Editors: Roberta Bulgari, Ada Baldi, Anna Lenzi and Antonios Chrysargyris

Received: 30 January 2023
Revised: 17 February 2023
Accepted: 22 February 2023
Published: 28 February 2023

1. Introduction

Fruits with market value but which are rarely available to consumers due to a lack of cultivation are known as underutilised fruits. Hence, most of them are yet wild or semi-domesticated. Likewise, they are related to the local culture and have been part of ancestral food and medicine. In general, they overlap or are located in areas close to traditional crops and are neglected by agricultural research organisations [1]. Many of them are resistant to biotic and abiotic factors and retain desirable genes which could be useful in crop improvement through genetic engineering [2]. Given their composition in bioactive compounds, fatty acid profile, organic acids, and carbohydrates, underutilised fruits are not only considered as a food source but also for their therapeutic potential [3]. So, in some underutilised fruits such as *Euterpe oleracea*, Andean berries, and *Myrciaria dubia*, the positive effect of the bioactive substances on the antioxidant status and oxidative stress in humans was verified [4]. Nowadays, consumers are aware of the nutritional value of the new products they incorporate into their diet. Many of the tropical and temperate climate indigenous fruits are still underexploited due to a lack of knowledge, market conditions, and crop yield. While some 3,000 species of tropical fruits make up the diversity, only a few are cultivated on a large scale [5,6].

Hexachlamys edulis (O. Berg) Kausel and D. Legrand, “ubajay”, is an underutilised species native from South America belonging to the Myrtaceae family. From the Atlantic Forest, it extends along the Paraná and Uruguay rivers. There are even references in the Paraná Jungle and the Paraguay river. In Argentina, it is found in the provinces of Entre Ríos, Corrientes, Misiones, Santa Fe, Formosa, and Chaco. *H. edulis* is a fruit tree with yellow globose drupes which are sweet–sour to very acidic, pleasant, and which quickly overripen. Fruit set and ripening occur during spring to early summer in South America. This species is undoubtedly prominent due to all its potential uses based on its nutraceutical properties, particularly in the leaves and in the yellow fruits rich in carotenoids [7,8]. The positive effects on the health of its leaves are well known. Bronchitis, cough, and whooping cough are all treated in alternative medicine with infusions of ubajay leaves, such as tea or mate. Many pharmacologic properties of leaf extracts are due to its powerful antioxidant action due to its flavonoids and tannins-rich content. Their effects on the balance of blood glucose levels for the treatment of diabetes and the reduction in hyperuricemia have been observed [7]. The consumption of carotenoid compounds has positive health impacts, including defence against cancer, cardiovascular disease, age-related disorders, and oxidative stress, as well as protection against macular degeneration [9]. The ripe fruit stands out for its antioxidant activity (near 100% DPPH radical scavenging activity with 5 mg methanolic fruit extract/mL) and nutraceutical profile, with a high content of carotenoids (706 µg of total carotenoids/g of the dry weight), making lutein the most abundant carotenoid [8]. Additionally, several efforts have been made to prospect and study the variability of this species in Entre Ríos for its subsequent selection and improvement [10] in its behaviour in other exotic environments and its reproductive phenological development regime [11], as well as in its post-harvest behaviour for its conservation [12].

Organoleptic characteristics are important determinants of consumer acceptability and, therefore, to the market possibilities of a fruit. In addition to colour and texture, flavour changes during fruit ripening due to variations in the ratio between organic acids (sourness) and sugar (sweetness) levels [8,13,14]. These two types of molecules are interconnected through the central carbon metabolism since both provides substrates for the respiratory processes. In addition, sugars and organic acids are intermediates in the biosynthesis of amino acids, vitamins, and terpenic aroma volatiles [13].

Various metabolite processes occur during fruit development and ripening, and products such as sugars and organic acids play an important role in developing fruit quality [15]. Internal and external factors determine the content of sugars, as well as their transport, metabolism, accumulation, and the relationship between them [16]. Glucose and fructose are the major simple sugars of fruits, and the relative amounts between them vary among fruits and in the same fruit in relation to maturity [17]. The health benefits of consuming dietary fibre can include things such as laxation, lowering blood sugar and cholesterol levels, and reducing the risk of developing colorectal cancer [18]. El-Zoghbi [19] has reported the variation in the dietary fibre content together with the fruit firmness during ripening.

Fruits have a variety of different organic acids in their fleshy parts, but the amount of each can vary a lot depending on the species and cultivars. Fruit flavour, to be consumed raw or used in fruit products, is influenced by the presence of organic acids in their fleshy parts. Malic, citric, isocitric, galacturonic, quinic, oxalic, and tartaric organic acids are very abundant in some fruits, while phenolic and ascorbic acids are ubiquitous in fruits [20].

The mineral nutrient fruit composition is important from a nutritional point of view, but it also can influence fruit ripening, quality, physiological disorders, storage behaviour, and other post-harvest physiological aspects (cell wall texture, non-enzymatic and enzymatic components of antioxidative system, plant/tissue defence, etc.) [21]. Different mineral elements such as sodium, potassium, iron, calcium, and many other trace elements are very essential for the human body [1]. There are several pre-harvest (genetics, plant density, temperature, soil composition, soil pH, and salt content) and post-harvest factors (harvesting time and gamma irradiation) that influence the contents of minerals in horticultural crops [21–24].

At this time, knowledge about the quality and potential use of wild fruits throughout the world is still scarce. Their biochemical analysis could provide information to breeders, producers, and consumers. The aim of this work was to study the patterns of the accumulation of carbohydrates, organic acids, and minerals during different stages of *H. edulis* fruit development for its evaluation as a source of health-promoting compounds, which is necessary in order to be included in the Argentine Food Code. Additionally, the obtained results will allow for deciding the optimal time for its consumption to achieve a better flavour and receive a good contribution of the nutrients evaluated.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

Seventeen *Hexachlamys edulis* plants were obtained from the seeds of fruits collected in Federación, Entre Ríos (Argentina) (30°59′ SL, 57°55′ WL, 50 m.a.s.l.). They were grown in the nursery and then were transplanted to 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.). The mean air daily temperatures in October, November, and December 2018, months when the flowering and fruit growth and ripening took place, were 17.3, 20.9, and 22.0 °C, respectively. Rainfall was 200, 81, and 157 mm along the mentioned months, respectively. Fruits (3 samples of 15 fruits each one) were harvested during November and December 2018 in 4 development stages according to Arena et al. [8]. 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).

Figure 1. *H. edulis* fruit at different development stages. Bar = 1 cm.

2.2. Analysis of Carbohydrates and Organic Acids

Immediately after harvest, the fruits were lyophilised and stored at −20 °C until analysis. The carbohydrate and organic acid extraction was achieved according to Colaric et al. [25]. An aliquot of the extract was filtered through a 0.45 µm MILLIPORE filter. A Waters e2695 HPLC system (Waters Associates, Milford, MA, USA) equipped with a refraction index detector (Waters 2414) was used. Carbohydrates were separated on Rezex RCM—Monosaccharide Ca^{2+} (8%) (300 mm length, 7.8 mm i.d., Phenomenex, Torrance, CA, USA). The operating conditions recommended by the supplier were used: the mobile phase included ultrapure water; a flow rate of 0.6 mL/min; and a column temperature of 65 ± 5 °C, and the injection volume loop was 10 µL. They were quantified by the external standard method using the reference standards of glucose, fructose, and sucrose (Anedra, Buenos Aires, Argentina). Then, the sum of the three quantified sugars was calculated (total sugars). Carbohydrate contents were expressed as mg per g of the dried weight. Additionally, the glucose/fructose ratio was calculated.

The separation of organic acids were carried out by Rezex ROA—Organic Acid H+ (8%) column (300 × 7.8 mm) (Bio-Rad, Hercules, CA, USA), associated with a photodiode array detector at 210 nm (Waters 2998). The HPLC equipment was the same as mentioned above. The column operated at 65 ± 5 °C. The sulfuric solution (0.005N) was a mobile phase at a flow rate of 0.6 mL/min. Tartaric, malic, quinic, and succinic acids were quantified by an external standard method. Then, the sum of the four quantified organic acids was calculated (total organic acids). Concentrations of the quantified organic acids were expressed as mg per g of the dry weight. In all cases, determinations were performed in duplicate. Additionally, the total sugars/total organic acids ratio was calculated.

Starch was quantified according to the methodology described by Lage-Yuste et al. [26]. Samples (0.2 g for unripe and medium ripe fruits, and 1.0 g for ripe and overripe fruits) were taken and homogenised with 5 mL of 52% perchloric acid and left to stand for 10 min. Then, the volume was completed to 100 mL, and 10 mL of this solution was taken out and mixed with 0.5 mL of 0.1:1 iodine:potassium iodide solution. The solutions were kept for 10 min in light–dark and then 1.5 mL of this solution were taken and centrifugated (10,000 rpm for 1 min). The absorbances were quantified at 600 nm in a Spectrum SP-2000 spectrophotometer. A calibration curve was prepared with starch (Sigma, Burlington, MA, USA) at 2, 3, 4, and 5 mg/100 mL, and the concentrations were reported as mg starch/g of the dry weight.

The determination of total, soluble, and insoluble dietary fibre was performed using the Dietary Fibre Assay Kit according to AOAC (991.43) and AACC (32.05.01) approved methods from Megazyme [27]. Fibre determination was performed in duplicate on samples of dry material. The gelatinisation, hydrolysis, and depolymerisation of starch were achieved by mixing the samples with heat-stable α-amylase at 100 °C. Then, the samples were incubated at 60 °C with protease (to solubilise and depolymerise proteins) and amyloglucosidase (to hydrolyse starch fragments to glucose) and they were treated with ethanol (rate 1:4, v/v) to precipitate the soluble fibre and remove the depolymerised protein and glucose (from starch). The residue was filtered, washed with 78% ethanol, 95% ethanol, and acetone, dried, and weighed. One duplicate was analysed for the protein content and the other was incubated at 525 °C to determine the ash. The total dietary fibre was determined by gravimetry based on the weight of the filtered residue less the weight of the protein and ash. For the soluble and insoluble fibre determination, before precipitation with 78% ethanol, the sample was filtered to obtain insoluble dietary fibre and then the residue was washed with warm distilled water. Filtered solution and rinse waters were collected and treated with 95% ethanol (rate 1:4 v/v) to precipitate the soluble dietary fibre. Precipitate was separated by being filtered and dried. Both the soluble dietary fibre and insoluble dietary fibre calculated values were corrected for the protein, ash, and blank content.

2.3. Analysis of Minerals

Approximately 1 g of each sample was calcined in a muffle at 500 °C for 5 h. The ash was raised with 5 mL of 20% HCl and completed to 50 mL with distilled water. Then, it was filtered and quantified by atomic absorption spectrometry in a Perkin Elmer AAnalyst 200 equipment. The minerals Mg, K, and Mn were quantified. Then, the sum of the three quantified minerals was calculated (total cations). Mg and K were selected because they are important minerals in the electrolytic balance, which present highlighted properties in populations of children, adults, and athletes, while was selected Mn for its antioxidant activity.

2.4. Statistical Analysis

Data were analysed through general linear models (GLM; mixed models) when corresponded. The assumption of normality was checked by the Shapiro–Wilk test and QQplots. Additionally, the homogeneity of variance was checked by the Levene test and scatter plots of the residuals versus the predicted values of each model. Data were analysed through an ANOVA and the means were separated by Tukey's test at $p \leq 0.05$ using RStudio. Pearson's correlation analysis was used to examine the relationship between the total titratable acidity [8] with each organic acid and with the total acids to understand how these variables were related.

3. Results and Discussion

The concentration of organic acids in the fleshy portions of the fruits can vary significantly depending on the species, environmental factors, the kind of the tissue, and the development stage of the fruit [20]. Usually, a decrease in the organic acid concentration and acidity is observed during fruit ripening. Examples of this behaviour are pears, peaches, and apples [28]. However, some fruit species show an increase in organic acids during ripening, as was demonstrated for "mangaba" fruit (*Hancornia speciosa*) and "soursop" (*Annona muricata*), which are considered bittersweet fruits [28,29].

Interestingly, the organic acid concentration varied significantly over the different development stages of *H. edulis* fruit (Table 1). The succinic acid concentration (the main organic acid) was high in unripe fruit (112.33 mg/g of the dry weight), then decreased to a minimum in medium ripe and ripe fruit (92.48 to 99.43 mg/g of the dry weight), to increase again in overripe fruit (115.65 mg/g of the dry weight). The malic acid concentration (the second main organic acid) was 44.90 mg/g of the dry weight in unripe fruit, to increase to a maximum of 105.14 mg/g of the dry weight in ripe fruit, after which it decreased again to 84.79 mg/g of the dry weight in overripe fruit. The quinic acid concentration was at the minimum in unripe fruit (0.37 mg/g of the dry weight), to then increase during fruit development (25.25 to 22.85 mg/g of the dry weight). Tartaric acid was detected only in overripe fruit (0.94 mg/g of the dry weight). Finally, the unripe fruits showed the minimum value for the total organic acids (157.67 mg/g of the dry weight) to increase during the fruit development between 207.98 and 227.27 mg/g of the dry weight (Figure 2).

Table 1. ANOVA for tartaric acid (TAR), malic acid (MAL), quinic acid (QUI), and succinic acid (SUC) expressed in dry weight and considering the four development stages of *H. edulis* "ubajay" harvested from the plants growing in Moreno (Buenos Aires). Values represent means ± standard error.

Factor	TAR (mg/g)	MAL (mg/g)	QUI (mg/g)	SUC (mg/g)
Stages				
Unripe	0.00 ± 0.10 b	44.90 ± 8.33 c	0.37 ± 0.24 b	112.33 ± 3.31 a
Medium Ripe	0.00 ± 0.10 b	90.19 ± 2.85 b	25.25 ± 1.80 a	92.48 ± 3.31 b
Ripe	0.00 ± 0.10 b	105.14 ± 1.95 a	22.62 ± 1.09 a	99.43 ± 3.31 b
Overripe	0.94 ± 0.10 a	84.79 ± 2.34 b	22.85 ± 1.78 a	115.65 ± 3.31 a
F	26.039	77.887	262.200	12.398
p	<0.001	<0.001	<0.001	<0.001

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

As in *H. edulis* fruits, succinic acid was the major organic acid present throughout most of the development stages of *Litchi chinensis* and *Amelanchier alnifolia* (saskatoon), although a higher malic acid concentration was found at maturity [30,31]. Similar to *H. edulis* fruits, in *Ziziphus jujuba*, the major components of organic acid were malic acid, quinic acid, and succinic acid, although malic acid was predominant [32]. In citrus fruits, quinic acid was the major organic acid at the beginning of the development; later, citric acid was predominant in acidic varieties, while in less acidic types, malic acid overtook it [33]. In apricot, plum, plumcot, and peach, quinic acid was the third predominant organic acid too, not showing a marked decrease in their concentrations [34], as was shown in *H. edulis* fruits.

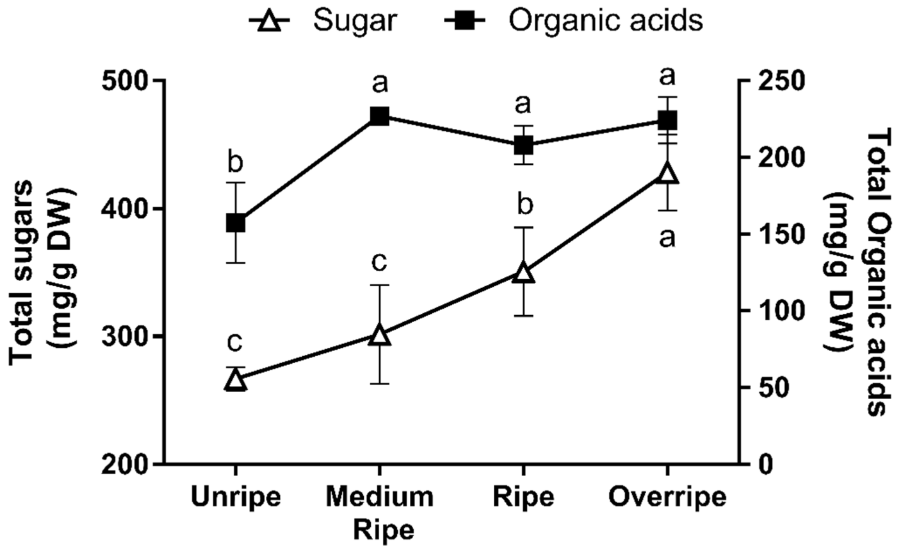

Figure 2. Total sugars and total organic acids (mg/g dry weight) (DW) during the development stages of *H. edulis* fruit: unripe, medium ripe, ripe, and overripe. Values represent means ± S.E. Different letters indicate significant differences according to the Tukey test ($p \leq 0.05$).

In *H. edulis* fruits, the total titratable acidity stayed high during unripe, medium ripe, and ripe stages, and decreased in the overripe stage (1.8, 1.6, 1.8, and 1.4%, respectively) [8] following different behaviour with respect to the total organic acids, a fact that explained the non-significant correlation found between both of these variables (r = −0.439, $p = 0.1537$). The only acid that decreased in the overripe stage compared with the ripe stage was malic acid, which showed a positive and significant correlation with the total acids (r = 0.930, $p < 0.001$), while quinic acid also presented a positive and significant correlation with the total acids (r = 0.855, $p < 0.001$). In both cases, these acids explained the increase in the total acid concentration throughout the fruit stages. In subtropical fruit *Annona cherimola*, a significant increase in the organic acid levels during ripening occurred as in *H. edulis*, and the increase in acidity was also related to the accumulation of malic acid [28,29]. The acid concentration in fruits affects the fruit's taste, as was cited for strawberries, blackberries, and mandarins [35].

The concentration of soluble sugars varies during fruit growth and development among species, usually peaking at maturity. Glucose and fructose predominate in the majority of fruits, whereas in others such as mandarin, peaches, and litchi, sucrose is the most important sugar [36]. The simple sugars varied significantly during the different development stages of *H. edulis* fruit (Table 2). Sucrose increased significantly from 21.20 mg/g of the dry weight in unripe fruit to a maximum of 82.53 mg/g of the dry weight in ripe fruit, to then it decreased to 59.38 mg/g of the dry weight in overripe fruit. Glucose increased significantly from 95.59 mg/g of the dry weight in unripe fruit to a maximum of 163.13 mg/g of the dry weight in overripe fruit. Fructose followed the same behaviour, increasing significantly from 150.08 mg/g of the dry weight in unripe fruit to a maximum of 205.85 mg/g of the dry weight in overripe fruit. The glucose concentration was lower than fructose. So, the glucose/fructose ratio increased significantly from 0.63 to 0.79 during fruit development. Finally, the total sugars in unripe fruits showed the minimum value (266.88 mg/g of the dry weight) to increase significantly during fruit development (301.68 to 428.36 mg/g of the dry weight) (Figure 2). The total sugars/total organic acids ratio was at the maximum in overripe fruit (1.90). The starch concentration was at the maximum in unripe and medium ripe fruit (171.39 and 161.19 mg starch/g of the dry weight, respectively), to then decrease in ripe and overripe fruit (40.45 and 65.96 mg starch/mg of the dry weight, respectively).

Table 2. ANOVA for sucrose (SUC), glucose (GLU), fructose (FRU), glucose/fructose ratio (GLU/FRU), total sugars/total organic acids (TS/TA), and starch (STR) expressed in dry weight and considering the four development stages of *H. edulis* "ubajay" harvested in Moreno (Buenos Aires). Values represent means ± standard error.

Factor	SUC (mg/g)	GLU (mg/g)	FRU (mg/g)	GLU/ FRU	TS/TA	STR (mg/g)
Stages						
Unripe	21.20 ± 4.80 b	95.59 ± 2.42 b	150.08 ± 6.50 bc	0.63 ± 0.01 b	1.72 ± 0.09 ab	171.39 ± 1.93 a
Medium Ripe	77.79 ± 4.80 a	89.49 ± 5.90 b	134.40 ± 6.50 c	0.66 ± 0.00 b	1.44 ± 0.09 b	161.19 ± 1.93 a
Ripe	82.53 ± 4.80 a	105.00 ± 77.59 b	163.08 ± 6.50 b	0.64 ± 0.00 b	1.54 ± 0.09 ab	40.45 ± 1.93 b
Overripe	59.38 ± 4.80 a	163.13 ± 6.57 a	205.85 ± 6.50 a	0.79 ± 0.00 a	1.90 ± 0.09 a	65.96 ± 1.93 b
F	33.672	42.000	33.660	118.100	4.972	11.705
p	<0.001	<0.001	<0.001	<0.001	0.045	0.003

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

As in the overripe fruit of *H. edulis*, the sucrose decrease was concomitant to an increase in the glucose and fructose levels during peach (*Prunus persica*) ripening, suggesting the degradation of this disaccharide [36]. In addition, starch degradation could contribute to the increase in the glucose content, as it was demonstrated for *Malus domestica Borkh.* cv. Gala (apples) and *Actinidia deliciosa* (kiwifruit) [37,38].

In *H. edulis* fruit, the main sugars present are fructose and glucose, as occurs in grapes, making the fructose concentration higher than the glucose concentration. In tomato, melon, grape berry, cherry, and peach, fructose and glucose are found in identical quantities. However, in apple, fructose is the major sugar [39]. The sweetness of fructose is higher than that of glucose, so changes in these sugar concentrations affect the sweet taste of the fruits as it occurs in grapes [40]. In addition, sugars are the main precursor of aroma in fruits [41]. The amount of carbohydrates in *H. edulis* ripe fruit (39.2 mg/100 g of the fresh weight) were higher than those reported for several underutilised species from India (0.50 to 34.40 mg/100 g of the fresh weight for *Carissa carandas* and *Terminalia belirica*, respectively) [6].

The decrease in the starch content in *H. edulis* fruit with the development stages has also been observed in several climacteric fruits, such as in tomato (10–20 dry weight to 0.1 mg/g of the dry weight), banana (100–300 to <150 mg/g of the dry weight), apple (20–25 to 0.5 mg/g of the dry weight), and mango (60 to 5 mg/g of the dry weight). This behaviour was not observed in non-climacteric fruits, where the starch content decreases sharply after anthesis and therefore the fruits accumulate simple sugars during development. Although many reports show the differences in the starch metabolism between climacteric and non-climacteric fruits, it is unclear if this contrast could be a key to differentiate both classes of fruits [42,43].

Interestingly, Colaric et al. [25] found that the sweetness of nectarine and peach was positively correlated with a higher sugars/organic acid ratio more than to the total amount of sugars alone. Additionally, in strawberries, a high sugar/organic acid ratio was associated with a strong sweetness and weak sourness [44]. In addition, acidity was related to the concentrations and type of the different organic acids, which are ordered according to its sourness relative to citric acid, as follows: citric (1.0) > malic (0.9) > tartaric (0.8) [45]. It is reported that malic acid in particular provides a smooth, tart taste. Taste was related to the malic/citric acid ratio, total sugars, sucrose, sorbitol, and malic acid concentrations in nectarine and peaches [25,46]. Therefore, the increase in the total sugar, together with the lack of changes in the total organic acids at the ripe and overripe stages, suggest a more sweet and less sour fruit. These results suggest that in order to obtain an appealing product, ubajay fruits should be consumed in a ripe or overripe state, although the overripe fruits presented lower antioxidant activity with respect to ripe fruits [8]. Additionally, considering that the post-harvest life of the overripe fruit could be short, the ripe stage is desirable to extend the time for consumption.

Maximum insoluble dietary fibre values were attained in unripe and medium ripe fruit (26.71 and 27.13 mg/100 g of the dry weight, each), to then decrease in ripe and overripe fruits to 15.81 and 15.51 mg/100 g of the dry weight, respectively (Table 3). Soluble dietary fibre oscillated between 9.03 and 11.26 mg/100 g of the dry weight during the development stages, although without significant differences. So, the total dietary fibre decreased from 36.80 to 25.94 mg/100 g of the dry weight between medium ripe and overripe fruit. The pattern of change in the amount of dietary fibre with ripening found in *H. edulis* is similar to other tropical fruits, where the decrease in the total fibre content was associated with softening of the flesh [19]. Hydrolysis of the cell wall by indigenous cellulolytic and pectinolytic enzymes was responsible for this change [19]. The amounts of total fibre in *H. edulis* ripe fruit (3.03 mg/100 g of the fresh weight) were higher than those reported for *M. indica* and *P. guajava* (1.46 to 1.80 and 1.81 mg/100 g of the fresh weight, respectively) [19], and higher than those cited for several underutilised species from India (0.10 to 3.00 mg/100 g of the fresh weight for *Morus indica* and *Aegle marmelos*, respectively) [6]. Therefore, *H. edulis* may constitute a new source of fibre for the consumers.

Table 3. ANOVA for insoluble dietary fibre (IDF), soluble dietary fibre (SDF), and total dietary fibre (TDF) expressed in dry weight and considering the four development stages of *H. edulis* "ubajay" harvested in Moreno (Buenos Aires). Values represent means ± standard error.

Factor	IDF (g/100 g)	SDF (g/100 g)	TDF (g/100 g)
Stages			
Unripe	26.71 ± 1.68 a	9.03 ± 1.35	35.74 ± 2.22 ab
Medium Ripe	27.13 ± 1.93 a	9.67 ± 1.35	36.80 ± 2.22 a
Ripe	15.81 ± 1.93 b	11.26 ± 1.35	27.07 ± 2.22 ab
Overripe	15.51 ± 1.93 b	10.44 ± 1.35	25.94 ± 2.22 b
F	14.895	0.505	6.511
P	0.001	0.689	0.015

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

The mineral concentrations (Mg, K, Mn, and total cations) did not vary significantly across the development of *H. edulis* fruit (Table 4). The Mg and K concentrations in ripe fruit were 0.40 and 22.85 mg/g of the dry weight, respectively, while the total cations concentration was 23.27 mg/g of the dry weight. Mn was absent in unripe, ripe and overripe fruits. In some underutilised fruits from India, Barua et al. [6] mentioned the absence of K in *Carissa carandas* and *Morus alba* fruits, while higher contents in *Terminalia chebula* fruit pulp (1270 mg/100 g of pulp) were cited. In different varieties of dates (*Phoenix dactylifera L.*), the K content was between 533.9 ± 0.95 and 1013 ± 0.86 mg/100 g and the Mg content was between 30.46 ± 0.40 and 76.74 ± 0.52 mg/100 g [47]. Czech et al. [48] studied different citrus fruits and reported that the fresh pulp contained 104–145 mg/100 g of K and 7.99–19.40 mg/100 g of Mg. Therefore, the mineral content of *H. edulis* is comparable with other fruits. K acquired through diet reduces the arterial pressure and the risk of stroke and coronary heart disease in adults [49]. On the other hand, Mg is a crucial mineral that works as a cofactor of different enzymes involved in antioxidant defences, the glucose metabolism, and blood pressure regulation. Therefore, increasing the K and Mg intake improves the cardiovascular function in adults [50]. Due to the importance for human health, the WHO recommends a minimum K level of 3510 mg/day and a Mg intake of 400 mg/day from food, depending on age and sex [51,52]. Considering that each fruit of ripe *H. edulis* is expected to contain ~140 mg of K and ~3.4 mg of Mg, the consumption of a portion of 5 fruits would incorporate ~16.0% and ~2.5% of the recommended intake of K and Mg, respectively.

Table 4. ANOVA for magnesium (Mg), potassium (K), manganese (Mn), and total cations (TC) expressed in dry weight and considering the four development stages of *H. edulis* "ubajay" harvested from the plants growing in Moreno (Buenos Aires). Values represent means ± standard error.

Factor	Mg (mg/g)	K (mg/g)	Mn (mg/g)	TC (mg/g)
Stages				
Unripe	0.33 ± 0.10	20.63 ± 7.63	0.00 ± 0.00	20.97 ± 7.74
Medium Ripe	0.34 ± 0.03	20.69 ± 2.55	0.01 ± 0.01	21.05 ± 2.57
Ripe	0.40 ± 0.02	22.85 ± 2.73	0.00 ± 0.00	23.27 ± 2.76
Overripe	0.24 ± 0.04	17.57 ± 3.14	0.00 ± 0.00	17.82 ± 3.17
F	3.559	0.761	2.815	0.787
p	0.087	0.556	0.130	0.543

F(p) = F statistic and probability of Fisher test.

4. Conclusions

Variations in carbohydrates, organic acids, and minerals through the development stages for *Hexachlamys edulis* fruit were analysed for the first time. The increase in the total sugar, together with the lack of changes in the total organic acids with the ripening process, suggest a more sweet and less sour fruit, i.e., an appealing product to be consumed at the ripe or overripe stage. Additionally, *H. edulis* may constitute a new source of fibre for consumers; additionally, the consumption of a portion of 5 ripe fruits would incorporate ~16% and ~2.5% of the recommended intake of K and Mg, respectively. These results enable us to propose *H. edulis* fruit as a promising natural source of sugars, organic acids, and minerals, information that is relevant for the introduction of *H. edulis* fruits into the Argentine Food Code.

Author Contributions: Conceptualization, M.E.A., I.S.P., V.B., E.P. and S.R.; Methodology, M.E.A., I.S.P., V.B., E.P., N.P., C.C. and S.R.; Software, I.S.P.; Validation, M.E.A. and I.S.P.; Formal analysis, M.E.A., I.S.P., E.P., N.P. and C.C.; Investigation, M.E.A., I.S.P., V.B., E.P., N.P., C.C. and S.R.; Resources, M.E.A., I.S.P., V.B., E.P., N.P., C.C. and S.R.; Data curation, M.E.A. and I.S.P.; Writing—original draft, M.E.A., I.S.P., V.B., E.P., N.P., C.C. and S.R.; Writing—review & editing, M.E.A., I.S.P., V.B., E.P., N.P., C.C. and S.R.; Visualization, M.E.A., I.S.P., V.B., E.P. and S.R.; Supervision, M.E.A. and S.R.; Project administration, M.E.A.; Funding acquisition, M.E.A. and S.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by MINCYT-UM PICTO 2019-00003, and CONICET PIP 11220200102292CO. The APC did not receive external funding.

Data Availability Statement: Data is contained within the article.

Acknowledgments: The authors thank Marta Alonso for his technical assistance.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hossain, M.M.; Rahim, M.A.; Haque, M.R. Biochemical properties of some important underutilized minor fruits. *J. Agric. Food Res.* **2021**, *5*, 100148. [CrossRef]
2. Murthy, H.N.; Bapat, V.A. Importance of Underutilized Fruits and Nuts. In *Bioactive Compounds in Underutilized Fruits and Nuts*; Murthy, H., Bapat, V., Eds.; Reference Series in Phytochemistry; Springer: Cham, Switzerland, 2020. [CrossRef]
3. Lamani, S.; Anu-Appaiah, K.A.; Murthy, H.N.; Dewir, Y.H.; Rikisahedew, J.J. Analysis of Free Sugars, Organic Acids, and Fatty Acids of Wood Apple (*Limonia acidissima* L.) Fruit Pulp. *Horticulturae* **2022**, *8*, 67. [CrossRef]
4. Gordon, A. Bioactive Compounds in Underutilized Tropical Fruits from Latin America. Ph.D. Dissertation, Universitäts-und Landesbibliothek, Bonn, Germany, 2012. Available online: https://nbn-resolving.org/urn:nbn:de:hbz:5n-29864 (accessed on 2 December 2022).
5. Nandal, U.; Bhardwaj, R.L. The role of underutilized fruits in nutritional and economic security of tribals: A review. *Crit. Rev. Food Sci. Nutr.* **2014**, *54*, 880–890. [CrossRef] [PubMed]
6. Barua, U.; Das, R.P.; Gogoi, B.; Baruah, S.R. Underutilized fruits of Assam for livelihood and nutritional security. *Agric. Rev.* **2019**, *40*, 175–184. [CrossRef]

7. Povilonis, I.S.; Arena, M.E.; Radice, S. *Hexachlamys edulis* (Berg) Kausel & Legrand, "ubajay", a native fruit species from South America. *AHS* **2021**, *35*, 389–397. [CrossRef]
8. Arena, M.E.; Povilonis, I.S.; Borroni, V.; Constenla, D.; Radice, S. Changes in physicochemical properties at different development stages of *Hexachlamys edulis* fruit, an underutilized South American species. *Heliyon* **2021**, *7*, e08323. [CrossRef]
9. Britton, G.; Khachik, F. Carotenoids in Food. In *Carotenoids: Nutrition and Health*; Britton, G., Pfander, H., Liaaen-Jensen, S., Eds.; Birkhäuser: Basel, Switzerland, 2009; Volume 5, pp. 45–66, ISBN 978-3-7643-7501-0. [CrossRef]
10. Povilonis, I.S.; Arena, M.E.; Radice, S. Caracterización de la variabilidad fenotípica de los frutos de ubajay (*Hexachlamys edulis*). In Proceedings of the 41° Congreso Argentino de Horticultura, La Plata, Argentina, 5–8 October 2021; Available online: http://sedici.unlp.edu.ar/bitstream/handle/10915/128494/Documento_completo.pdf-PDFA.pdf?sequence=1&isAllowed=y (accessed on 15 November 2022).
11. Radice, S.; Povilonis, I.S.; Arena, M.E. Flower and fruit formation of *Hexachlamys edulis* in Buenos Aires, Argentina. *JAEID* **2023**; *in press*.
12. Povilonis, I.S.; Arena, M.E.; Radice, S. Efecto de la temperatura de conservación sobre propiedades químicas de los frutos de *Hexachlamys edulis* en Concordia, Entre Ríos. In Proceedings of the VIII Congreso Internacional de Ciencia y Tecnología de Alimentos (CICYTAC), Cordoba, Argentina, 4–6 October 2022.
13. Batista-Silva, W.; Nascimento, V.L.; Medeiros, D.B.; Nunes-Nesi, A.; Ribeiro, D.M.; Zsögön, A.; Araújo, W.L. Modifications in Organic Acid Profiles during Fruit Development and Ripening: Correlation or Causation? *Front. Plant Sci.* **2018**, *9*, 1689. [CrossRef]
14. Shi, Y.; Li, B.J.; Su, G.; Zhang, M.; Grierson, D.; Chen, K.S. Transcriptional regulation of fleshy fruit texture. *J. Integr. Plant Biol.* **2022**, *64*, 1649–1672. [CrossRef]
15. Xie, F.; Chen, C.; Chen, J.; Yuan, Y.; Hua, Q.; Zhang, Z.; Zhao, J.; Hu, G.; Chen, J.; Qin, Y. Metabolic Profiling of Sugars and Organic Acids, and Expression Analyses of Metabolism-Associated Genes in Two Yellow-Peel Pitaya Species. *Plants* **2022**, *11*, 694. [CrossRef]
16. Zhi, W.S.; Li, Y.F.; Zi, S.W.; Xue, X.B. Sugar transport, metabolism, accumulation and their regulation in fruits. *J. Plant Physiol. Mol. Biol.* **2004**, *30*, 1–10. [PubMed]
17. Vicente, A.R.; Manganaris, G.A.; Sozzi, G.O.; Crisosto, C.H. Nutritional quality of fruits and vegetables. In *Postharvest Handling: A Systems Approach*, 2nd ed.; Florkowski, W.J., Shewfelt, R.L., Brueckner, B., Prussia, S.E., Eds.; 2009; pp. 57–106, ISBN 978-0-12-374112-7.
18. Ahmad, A.; Khalid, N. Chapter 5—Dietary Fibers in Modern Food Production: A Special Perspective with β-Glucans. In *Biopolymers for Food Design*; Grumezescu, A.M., Holban, A.M., Eds.; Academic Press: Philadelphia, PA, USA, 2018; pp. 125–156, ISBN 978-0-12-811449-0. [CrossRef]
19. EI-Zoghbi, M. Biochemical changes in some tropical fruits during ripening. *Food Chem.* **1994**, *49*, 33–37. [CrossRef]
20. Walker, R.P.; Famiani, F. Organic Acids in Fruits: Metabolism, Functions and Contents. In *Horticultural Review*, 1st ed.; Warrington, I., Ed.; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2018; pp. 371–430, ISBN 978-1-119-43107-7.
21. Paul, V.; Pandey, R.; Ramesh, K.V.; Singh, A. Role of mineral nutrients in physiology, ripening and storability of fruits. In *Advances in Plant Physiology*; Hemantaranjan, A., Ed.; An International Treatise Series: Nutriophysiological and Molecular Interventions for crop improvement under changing climate; Scientific Publishers: Jodhpur, India, 2012; Volume 13, pp. 56–96.
22. Rahman, M.M.; Roy, M.; Sajib, M.A.M.; Sarkar, A.; Hussain, M.S. Radiation effects on essential minerals content of cucumber (*Cucumis sativus*). *Amer. J. Food Nutr.* **2015**, *3*, 69–74.
23. Tyagi, S.; Sahay, S.; Imran, M.; Rashmi, K.; Mahesh, S.S. Pre-harvest factors influencing the postharvest quality of fruits: A review. *Curr. J. Appl. Sci. Technol.* **2017**, *23*, 12. [CrossRef]
24. Taghavi, T.; Siddiqui, R.; Rutto, L.K. The effect of preharvest factors on fruit and nutritional quality in strawberry. In *Strawberry Pre-and Post-harvest Management Techniques for Higher Fruit Quality*; IntechOpen: London, UK, 2019.
25. Colaric, M.; Stampar, F.; Solar, A.; Hudina, M. Influence of branch bending on sugar, organic acid and phenolic content in fruits of 'Williams' pears (*Pyrus communis* L.). *J. Sci. Food Agric.* **2006**, *86*, 2463–2467. [CrossRef]
26. Lage-Yusty, M.; Simal-Lozano, J.; Gómez, G.S. Determinación de almidón en alimentos. *Trab. Compostel. Biol.* **1983**, *10*, 15–25.
27. Megazyme. Total Dietary Fiber. Assay Procedure. 2017. Available online: https://www.megazyme.com/documents/Assay_Protocol/K-TDFR-200A_DATA.pdf (accessed on 10 June 2022).
28. González-Agüero, M.; Tejerina Pardo, L.; Zamudio, M.S.; Contreras, C.; Undurraga, P.; Defilippi, B.G. The Unusual Acid-Accumulating Behavior during Ripening of Cherimoya (*Annona cherimola* Mill.) is Linked to Changes in Transcription and Enzyme Activity Related to Citric and Malic Acid Metabolism. *Molecules* **2016**, *21*, 398. [CrossRef]
29. Carnelossi, M.A.G.; Costa de Sena, H.; Narain, N.; Yaguiu, P.; da Silva, G.F. Physico-Chemical Quality Changes in Mangaba (*Hancornia speciosa gomes*) Fruit Stored at Different Temperatures. *Braz. Arch. Biol. Technol.* **2009**, *52*, 985–990. [CrossRef]
30. Rogiers, S.Y.; Knowles, N.R. Physical and chemical changes during growth, maturation, and ripening of Saskatoon (*Amelanchier alnifolia*) fruit. *Can. J. Bot.* **1997**, *75*, 1215–1225. [CrossRef]
31. Pareek, S. Chapter 17—Nutritional and Biochemical Composition of Lychee (*Litchi chinensis* Sonn.) Cultivars. In *Nutritional Composition of Fruit Cultivars*; Simmonds, M.S.J., Preedy, V.R., Eds.; Academic Press: Cambridge, MA, USA, 2016; pp. 395–418, ISBN 9780124081178. [CrossRef]
32. Zhao, A.-L.; Xue, X.-F.; Wang, Y.-K.; Sui, C.-L.; Ren, H.-Y.; Li, D.-K. The Sugars and Organic Acids Composition in Fruits of Different Chinese Jujube Cultivars of Different Development Stages. *Acta Hortic. Sin.* **2016**, *43*, 1175–1185. [CrossRef]

33. Albertini, M.-V.; Carcouet, E.; Pailly, O.; Gambotti, C.; Luro, F.; Berti, L. Changes in organic acids and sugars during early stages of development of acidic and acidless citrus fruit. *J. Agric. Food Chem.* **2006**, *54*, 8335–8339. [CrossRef]
34. Bae, H.; Yun, S.K.; Jun, J.H.; Yoon, I.K.; Nam, E.Y.; Kwon, J.H. Assessment of organic acid and sugar composition in apricot, plumcot, plum, and peach during fruit development. *J. Appl. Bot. Food Qual.* **2014**, *87*, 24–29. [CrossRef]
35. Mao, Y.; Tian, S.; Qin, Y.; Cheng, S. An optimized organic acid human sensory sourness analysis method. *J. Sci. Food Agric.* **2021**, *101*, 5880–5887. [CrossRef]
36. Aslam, M.M.; Deng, L.; Wang, X.; Wang, Y.; Pan, L.; Liu, H.; Niu, L.; Lu, Z.; Cui, G.; Zeng, W.; et al. Expression patterns of genes involved in sugar metabolism and accumulation during peach fruit development and ripening. *Sci. Hortic.* **2019**, *257*, 108633. [CrossRef]
37. Zhang, W.; Lunn, J.E.; Feil, R.; Wang, Y.; Zhao, J.; Tao, H.; Guo, Y.; Zhao, Z. Trehalose 6-phosphate signal is closely related to sorbitol in apple (*Malus domestica Borkh.* cv. Gala). *Biol. Open* **2017**, *6*, 260–268. [CrossRef] [PubMed]
38. Wang, H.; Wang, J.; Mujumdar, A.S.; Jin, X.; Liu, Z.L.; Zhang, Y.; Xiao, H.W. Effects of postharvest ripening on physicochemical properties, microstructure, cell wall polysaccharides contents (pectin, hemicellulose, cellulose) and nanostructure of kiwifruit (*Actinidia deliciosa*). *Food Hydrocoll.* **2021**, *118*, 106808. [CrossRef]
39. Desnoues, E.; Gibon, Y.; Baldazzi, V.; Signoret, V.; Génard, M.; Quilot-Turion, B. Profiling sugar metabolism during fruit development in a peach progeny with different fructose-to-glucose ratios. *BMC Plant Biol.* **2014**, *14*, 336. [CrossRef] [PubMed]
40. Trad, M.; Boge, M.; Hamda, H.B.; Renard, C.M.G.C.; Harbi, M. The Glucose-Fructose ratio of wild Tunisian grapes. *Cogent Food Agric.* **2017**, *3*, 1374156. [CrossRef]
41. Song, J.; Bi, J.; Chen, Q.; Wu, X.; Lyu, Y.; Meng, X. Assessment of sugar content, fatty acids, free amino acids, and volatile profiles in jujube fruits at different ripening stages. *Food Chem.* **2019**, *270*, 344–352. [CrossRef]
42. Osorio, S.; Scossa, F.; Fernie, A.R. Molecular regulation of fruit ripening. *Front. Plant Sci.* **2013**, *14*, 198. [CrossRef] [PubMed]
43. Chervin, C. Should Starch Metabolism Be a Key Point of the Climacteric vs. Non-climacteric Fruit Definition? *Front. Plant Sci.* **2020**, *11*, 609189. [CrossRef]
44. Ikegaya, A.; Toyoizumi, T.; Ohba, S.; Nakajima, T.; Kawata, T.; Ito, S.; Arai, E. Effects of distribution of sugars and organic acids on the taste of strawberries. *Food Sci. Nutr.* **2019**, *7*, 2419–2426. [CrossRef]
45. Kader, A.A. Flavor quality of fruits and vegetables. *J. Sci. Food Agric.* **2008**, *88*, 1863–1868. [CrossRef]
46. Baccichet, I.; Chiozzotto, R.; Bassi, D.; Gardana, C.; Cirilli, M.; Spinardi, A. Characterization of fruit quality traits for organic acids content and profile in a large peach germplasm collection. *Sci. Hortic.* **2021**, *278*, 109865. [CrossRef]
47. Nadeem, M.; Qureshi, T.M.; Ugulu, I.; Riaz, M.N.; An, Q.U.; Khan, Z.I.; Dogan, Y. Mineral, vitamin and phenolic contents and sugar profiles of some prominent date palm (*Phoenix dactylifera*) varieties of Pakistan. *Pak. J. Bot.* **2019**, *51*, 171–178. [CrossRef] [PubMed]
48. Czech, A.; Zarycka, E.; Yanovych, D.; Zasadna, Z.; Grzegorczyk, I.; Kłys, S. Mineral content of the pulp and peel of various citrus fruit cultivars. *Biol. Trace Elem. Res.* **2020**, *193*, 555–563. [CrossRef]
49. Aburto, N.J.; Hanson, S.; Gutierrez, H.; Hooper, L.; Elliott, P.; Cappuccio, F.P. Effect of increased potassium intake on cardiovascular risk factors and disease: Systematic review and meta-analyses. *BMJ* **2013**, *346*, f1378. [CrossRef]
50. DiNicolantonio, J.J.; Liu, J.; O'Keefe, J.H. Magnesium for the prevention and treatment of cardiovascular disease. *Open Heart* **2018**, *5*, e000775. [CrossRef]
51. WHO. *Potassium Intake for Adults and Children*; WHO: Geneva, Switzerland, 2012; pp. 1–52, ISBN 978-92-4-150482-9. Available online: https://www.who.int/publications/i/item/9789241504829 (accessed on 20 January 2023).
52. World Health Organization. *Vitamin and Mineral Requirements in Human Nutrition*; World Health Organization: Geneva, Switzerland, 2004. Available online: https://www.fao.org/3/y2809e/y2809e00.htm (accessed on 20 January 2023).