

ORIGINAL ARTICLE

## Evolution of the nutritional composition of *Hovenia dulcis* Thunb. pseudofruit during the maturation process

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**Abstract – Introduction.** Native to Asia, *Hovenia dulcis* Thunb. (Rhamnaceae), known as Japanese grape, is also found in central and southern America, southern Europe and North Africa. The fruit is a small capsule, attached to peduncle or pseudofruit, which, when mature, is the edible part. In order to increase knowledge about the potential of these pseudofruits as an alternative to other fruits available in the market, or as a new ingredient for the food industry, this study aimed to characterize the nutritional value of *H. dulcis* peduncles harvested at different stages of maturation. **Materials and methods.** Samples of *H. dulcis* pseudofruit were collected for five months, from an immature stage to a senescent one (Hd01, Hd02, Hd03, Hd04 and Hd05), in Curitiba, Parana, Brazil. Analysis of moisture, ash, minerals (Ca, Mg, Na, K, Fe, Cu, Mn and Zn), proteins, lipids, vitamin C, available carbohydrates, soluble sugars and dietary fiber (soluble and insoluble) were performed. Analysis of variance (ANOVA) followed by Duncan's test were applied to the analytical results. **Results and discussion.** Soluble sugar content increased during maturation, probably due to starch hydrolysis and water loss. This product (Hd02 to Hd05) can be considered an interesting source of dietary fiber, according to Brazilian and European regulations. Total ash content increased during the development of these pseudofruits, due to the concentration of minerals, by reducing moisture in the mature product. **Conclusion.** Together with fibers, Cu, Ca and Mn were the most relevant dietary contributions of mature *H. dulcis* peduncles, being a good means to improve the nutritional quality of modern diets, requiring further investigation of their sensory and nutritional characteristics.

**Keywords:** *Hovenia dulcis* / maturation / nutrients / minerals / nutritional value

**Résumé – Évolution de la composition nutritionnelle des pseudofruits de *Hovenia dulcis* Thunb. au cours de leur maturation. Résumé – Introduction.** Originaire d'Asie et connu sous le nom de raisin japonais, *Hovenia dulcis* Thunb. (Rhamnaceae) est également présent en Amérique centrale et du Sud, en Europe du Sud et en Afrique du Nord. Le fruit est une petite capsule, attachée par un pédoncule ou pseudofruit qui, à maturité, est comestible. Afin d'accroître les connaissances sur le potentiel de ces pseudofruits comme alternative à d'autres fruits disponibles sur le marché, ou en tant que nouvel ingrédient pour l'industrie alimentaire, cette étude vise à caractériser la valeur nutritionnelle des pédoncules de *H. dulcis* récoltés à différents stades de maturité. **Matériel et méthodes.** Des échantillons de pseudofruits de *H. dulcis* ont été collectés durant cinq mois, du stade immature à la sénescence (Hd01, Hd02, Hd03, Hd04, Hd05), à Curitiba, Etat du Parana au Brésil. L'analyse de l'humidité, des cendres, des minéraux (Ca, Mg, Na, K, Fe, Cu, Mn, Zn), des protéines, des lipides, de la vitamine C, les glucides assimilables, des sucres solubles et des fibres alimentaires (solubles et insolubles) a été effectuée. Une analyse de variance suivie d'un test de Duncan ont été appliqués sur les résultats. **Résultats et discussion.** La teneur en sucres solubles a augmenté au cours de la maturation, probablement due à l'hydrolyse de l'amidon et aux pertes en eau. Ce pseudofruit aux stades Hd02 à Hd05 peut être considéré comme une source intéressante de fibres alimentaires, conformément aux réglementations brésiliennes et européennes. La teneur en cendres totales augmente au cours de l'élaboration de ces pseudofruits, la concentration minérale étant vraisemblablement liée aux pertes en eau dans le produit mature. Les teneurs élevées en fibres, Cu, Ca et Mn sont les contributions alimentaires les plus intéressantes des pédoncules de *H. dulcis* arrivés à maturité (Hd04).

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**Conclusion.** Ce produit constitue un bon moyen d'améliorer la qualité nutritionnelle des régimes alimentaires modernes, et nécessite maintenant des recherches complémentaires sur ses caractéristiques sensorielles et nutritionnelles.

**Mots clés :** *Hovenia dulcis* / maturation / nutriments / composition minérale / valeur nutritionnelle

## 1 Introduction

A large variety of fruits are grown in Brazil, due to its large area and climatic and soil diversity. However, there are numerous native and exotic fruits that are little exploited economically [1]. Fruits are essential components of the human diet; they play an important nutritional role in the daily diet, providing mostly vitamins, minerals, fiber and energy. In addition, they are products that appeal to vision, taste, smell and touch to meet consumers' demands. The diversification of food in the diet is considered as a strategy that positively contributes to the health status of the human being [2].

*Hovenia dulcis* Thunb. (Rhamnaceae), commonly named Japanese grape, is a tree, originally from Asia (China, Japan and South Korea), and introduced into other areas, where it may be intentionally cultivated as an ornamental, and then become an invasive species for the surrounding vegetation (for example, in East Africa). Nowadays it is widely used as an ornamental tree in many areas of Europe and America (Brazil, Argentina, Paraguay, Uruguay, United States, and Cuba). Its introduction into Brazil in the seventies was apparently due to the Chinese Academy of Forestry, who gave seeds of *H. dulcis* from two localities of the People's Republic of China to the Brazilian Agricultural Research Corporation (EMBRAPA) as a gift for ornamental purposes and reforestation. Then it was disseminated throughout the southern region of Brazil, and adapted well to the climate and soil of this country [3,4].

As for its phenology, *H. dulcis* in Brazil can bloom from August to February and its peduncles ripen from March to October, according to Cozzo [4]; these wide variations in the flower and fruit development are due to the influence of different climates. The fruit is a small, dry, globose capsule 6–7 mm in diameter containing 2–4 seeds, attached to a tanned stalked peduncle, which becomes thickened and fleshy when mature (figure 1), and is sweet and pleasant-tasting. The seeds are orange- or reddish- coloured when freshly harvested, becoming brown and black over time, and having a more or less circular shape, with 4–8 mm diameter [3].

Pseudofruits (peduncles) are from a single flower that, as a result of fertilization, has the development of this accessory organ in addition to the ovary [5]. This phenomenon is relatively infrequent in angiosperms, occurring commonly in several families such as Podocarpaceae, Anacardiaceae, Icacinaceae and Rhamnaceae, in which a dry drupe develops at the end of a fleshy peduncled fruit stalk [6]. Some authors have hypothesized that it obeys an adaptation to facilitate endozoochory: as fleshy-fruited plants are easily eaten by animals and seeds are disseminated in feces, dry-fruited plants may develop fleshy appendages to attract animals to swallow their fruit. The study of Zhou *et al.* [6] demonstrated that particularly *H. dulcis* peduncles attracted different species of mammals to consume the entire infructescence, and more new plants germinate from egested seeds than from unconsumed seeds.

In the case of *H. dulcis*, the mature peduncle can be considered as the edible part of the Japanese grape, either as fresh fruit or included as a functional ingredient in other food products. The meaty, juicy and tasty pulp of the more mature stage presents characteristics very similar to pear aroma, with good acceptance for human consumption [3].

Mature peduncles can also be used for preparation of juice, wine, vinegar and sweets such as jelly, or for nutritional fortification of bakery products, as a source of dietary fiber [3,7]. Although these uses are common in some countries such as China, in Brazil it has still not reached a high level of human consumption.

During the life cycle of Japanese grape, plant parts are supplied with the necessary nutrients for their growth, maturation and ripening. This step develops numerous biosynthesis and degradation processes of concomitant or sequential forms, resulting in sensitive modifications in their features such as flavor or nutritional value [5].

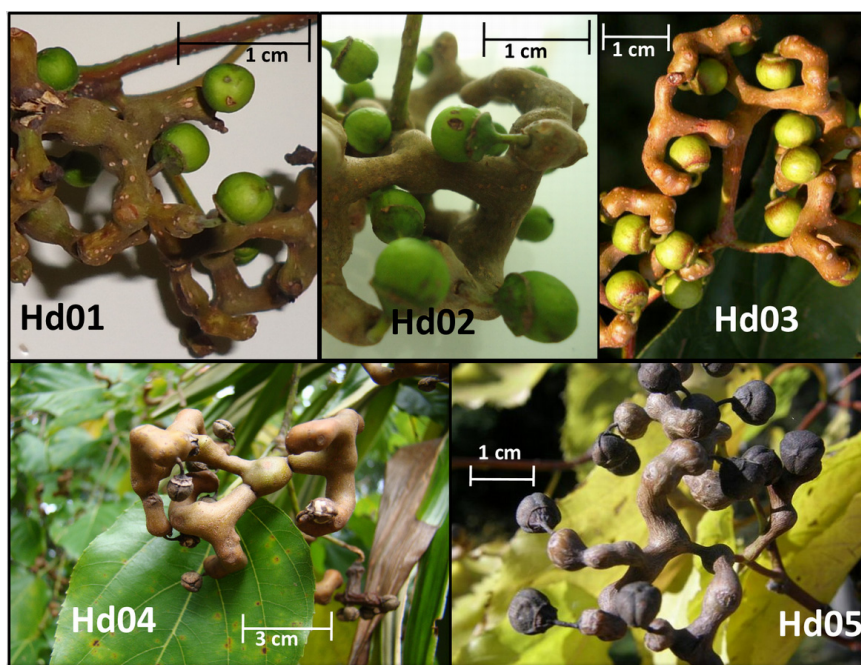
Despite the great interest in both agronomic characterization and sustainable harvest, few research groups have investigated the nutritional potential of Japanese grape, and very few have studied the different stages of maturation, or the influence of geographical and seasonal variation on the nutritional parameters, as has been studied in other fruit. Therefore, in order to increase knowledge about the nutritional potential of these pseudofruits, as an alternative to other fruit available in the market or as a new ingredient for the food industry, this study aimed to characterize the nutritional value of *H. dulcis* peduncles harvested at different stages of maturation.

## 2 Materials and methods

### 2.1 Sampling and moisture determination

The pseudofruits of *H. dulcis* (figure 1) were collected for five consecutive months, in the suitable season in this area, which was February (immature peduncles, named Hd01) to July (too-mature peduncles, named Hd05), in at least two different trees situated in the neighborhood of Jardim das Américas, Curitiba, Parana, Brazil, with the coordinates latitude 25° 20' 56 South and longitude 49° 13' 57 West.

Each sample constituted about 800–1,000 g of peduncles, collected from two different trees, and a composite sample for each stage was prepared by mixing all the pseudofruits harvested according to the recommendations of Greenfield and Southgate [8]. They were washed in water and left for 10 min under a concentration of 200 ppm sodium hypochlorite, then rinsed and freeze-dried (L101-Liotop, Brazil-São Carlos- São Polo). Dry matter was determined by desiccation to constant weight at 100 ± 2 °C following AOAC procedures [9]. Finally the samples were stored at –20 °C until analyzed. Determinations were performed on freeze-dried samples. Triplicate subsamples were taken for each analytical procedure.



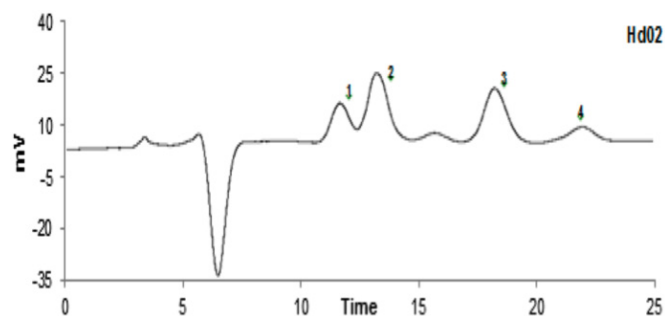
**Figure 1.** Images of *Hovenia dulcis* peduncles at different maturation stages (Hd04 shows the peduncles at the optimum stage for food consumption).

## 2.2 Total available carbohydrate (TAC) determination

The analysis of TAC was carried out by a colorimetric method using anthrone reagent, as described by Osborne and Voogt [10], on 0.5-g freeze-dried samples. Samples were pretreated with 15 mL of 52% (v/v)  $\text{HClO}_4$  and 10 mL distilled water and kept for 18 h in the dark. After this period, samples were filtered and the volume of filtrate was adjusted to 250 mL. Finally, the solution was further diluted to 8% (v/v), and 5 mL of 0.1% (w/v) anthrone solution in 70% (v/v)  $\text{H}_2\text{SO}_4$  was added to 1 mL extract. Samples were kept in a boiling water bath for 12 min where anthrone reacted with sugars, yielding a green-colored compound, and absorbance was measured at 630 nm on a UV/Vis Spectrometer EZ210 (Perkin Elmer, Waltham, MA, USA) equipped with Lambda software PESSW v. 1.2. The absorbance of the sample solution was compared with a 10–100 mg  $\text{mL}^{-1}$  concentration range standard glucose calibration curve.

## 2.3 Soluble sugars determination

Soluble sugars were determined by High-Performance Liquid Chromatography (HPLC) according to Sánchez-Mata *et al.* [11]. Triplicate subsamples of 0.5 g freeze-dried berries were extracted with 80% (v/v) ethanol in water at 55–60 °C for 45 min with constant stirring. The ethanol was evaporated by using a rotary vacuum evaporator (Büchi R-114) set to 40 °C and the concentrate was made up to 25 mL with distilled water. Then, the samples were passed through a previously washed (5 mL methanol followed by 5 mL water) Sep-Pak C18 cartridge (Waters, Milford, MA, USA). Two mL of filtrate were mixed with 8 mL of acetonitrile and the mixture was filtered



**Figure 2.** HPLC profile of soluble sugars in pseudofruit of *Hovenia dulcis* Thunb. at stage Hd02. Chromatographic conditions: Luna  $\text{NH}_2$  100 R (250 mm  $\times$  4.60 mm, 5  $\mu\text{m}$ ) column; mobile phase: acetonitrile/water (80/20); detection: refraction index; flow rate = 0.9  $\text{mL min}^{-1}$ . Fructose (1), glucose (2), sucrose (3) and maltose (4).

through a 0.45- $\mu\text{m}$  Millipore PVDF membrane (Millipore, Bedford, MA, USA) before injection (100- $\mu\text{L}$  aliquots) into the HPLC, equipped with a PU II isocratic pumping system (Micron Analytical, SA, Spain), a Rheodyne valve, and a differential refractometer R401 detector (Jasco, Madrid, Spain). The chromatographic column was a Luna 5  $\mu\text{m}$   $\text{NH}_2$  100 R (250 mm  $\times$  4.60 mm) (Phenomenex, Torrance, CA, USA). The mobile phase was acetonitrile:water (80:20), at a flow rate of 0.9  $\text{mL min}^{-1}$ . All chromatograms (*figure 2*) were processed using Cromane XP software (Micronec, Spain). The resultant peak areas in the chromatograms were plotted against calibration curves obtained from multiple standard solutions (external standard method), in a concentration range of 0.1 to 1.0 mg  $\text{mL}^{-1}$  for each compound.

## 2.4 Soluble and insoluble dietary fiber assay determination

AOAC enzymatic-gravimetric methods (993.19 and 991.42) were used for soluble dietary fiber and insoluble dietary fiber analysis [9]. In brief, freeze-dried samples were treated with alpha-amylase, protease and amyloglucosidase. The soluble and insoluble fractions were separated by vacuum filtration. Waste from the digests was dried at 100 °C, and ash and protein contents were determined in the residue. Total fiber was the sum of soluble and insoluble fibers.

## 2.5 Total proteins determination

Total proteins were determined as nitrogen content by the Kjeldahl method. An amount of 0.5 g of freeze-dried sample was digested in sulfuric acid; after alkalization, NH<sub>3</sub> was distilled over 0.1 N H<sub>2</sub>SO<sub>4</sub> and the excess of sulfuric acid was titrated against 0.1 N NaOH. Total nitrogen content was converted to protein content by using the conversion factor 6.25 [9].

## 2.6 Lipid determination

The crude fat was determined by extracting 0.5 g freeze-dried sample with petroleum ether. Containers were removed and dried at 105 °C, cooled and weighed [9].

## 2.7 Vitamin C determination

The content of ascorbic acid was quantified by High-Performance Liquid Chromatography (HPLC), based on the methods proposed by Vazquez-Odériz *et al.* [12] and Arella *et al.* [13]. An amount of 5 g of homogenized fresh fruits was extracted in 25 mL of 4.5% (w/v) *m*-phosphoric acid, with magnetic shaking (P-Selecta, Asincro) for 15 min in darkness. Extracts were filtered through Albert no.1242 paper; a portion was filtered through a 0.45- $\mu$ m PVDF membrane, for injection into the HPLC system, while another aliquot of 3 mL of filtrate was added to 2.5 mL of 4% (w/v) L-Cisteine, adjusted to pH 7 with 20% (w/v) K<sub>2</sub>HPO<sub>4</sub>, and left to stand for 5 min to allow the reduction of dehydroascorbic acid to ascorbic acid. Then, they were adjusted to pH 3 with 20% (w/v) metaphosphoric acid and completed with distilled water to a final volume of 10 mL, prior to filtration through a 0.45- $\mu$ m PVDF membrane and injection into the chromatographic system. The instrumental equipment was a liquid chromatographer (Micron Analytical, Madrid, Spain) equipped with an isocratic pump (model Pu II), an AS-1555 automatic injector (Jasco, Japan), a Spherclone ODS(2) (250  $\times$  4.60 mm, 5  $\mu$ m) Phenomenex column, a UV-visible detector (Thermo Separation Spectra Series UV100); and using Cromanec XP software (Micronec, Spain). The mobile phase was 1.8 mN H<sub>2</sub>SO<sub>4</sub> (pH 2.6). For AA analysis a flow rate of 0.9 mL min<sup>-1</sup> and UV detection at 245 nm were used. The identification of each compound was made by comparison of the retention times of each chromatographic

peak with those standard products prepared from ascorbic acid diluted in 4.5% (w/v) *m*-phosphoric acid. Quantification was performed by construction of calibration lines for each compound, after verification of the recovery rates of the analytical method.

## 2.8 Ash content and mineral composition

AOAC method 930.05 was used [9]. A sample of 500 mg was incinerated with high pressure in a microwave oven (muffle furnace MLS 1200) for 24 h at 550 °C, and ash was gravimetrically quantified. The residue of incineration was extracted with HCl (50% v/v) and HNO<sub>3</sub> (50%, v/v) and made up to an appropriate volume with distilled water, where Fe, Cu, Mn and Zn were directly measured at the suitable wavelength for each element, using standard solutions for calibration purposes. An additional 1/10 (v/v) dilution was performed in LaCl<sub>2</sub> (18 g L<sup>-1</sup>) for Ca and Mg determination, and CsCl<sub>2</sub> (2 g L<sup>-1</sup>) for Na and K analysis. All measurements were performed with atomic absorption spectroscopy using the Analyst 200 Perkin Elmer equipment.

## 2.9 Comparison with the Recommended Dietary Allowance – RDA

In food composition studies, the focus is not only on the amount of nutrients present, but also on the contribution that these levels represent to the human daily needs of nutrients. For that reason, the data obtained from the analysis of nutrient composition of the samples were compared with the Recommended Dietary Allowance (RDA) given by the Food and Nutrition Board (FNB) of the American Institute of Medicine of the National Academies (formerly National Academy of Sciences) [14]. This RDA was selected from among others for being one of the most widely accepted ones all over the world, and was used to calculate the potential contribution of a portion of 100 g of *H. dulcis* pseudofruit to the human daily needs for nutrients.

## 2.10 Statistical analysis

All the analyses were carried out in triplicate. Analysis of variance (ANOVA), followed by Duncan's test, was conducted using Statgraphics Plus 5.1 software, to analyze data at the 95% confidence level.

## 3 Results and discussion

The results of nutrient composition and energy values (expressed in fresh weight) in the samples of *H. dulcis* pseudofruit analyzed at five stages are given in *tables I–II*.

Significant differences were found for the moisture content of pseudofruit throughout the process of formation and maturation. Immature peduncles (Hd01) presented moisture values similar to other fruits of 93%. During maturation a decrease

**Table I.** Major components, vitamin C and energy values (per 100 g fresh weight) in pseudofruit of *Hovenia dulcis* Thunb. at different maturation stages (Hd01 to Hd05) (mean  $\pm$  SD,  $n = 3$ ). In each row, different letters indicate significant differences ( $P < 0.05$ ). n.d. = not detected.

Constituents	Hd01	Hd02	Hd03	Hd04	Hd05
Moisture (g)	93.21 $\pm$ 0.75e	79.39 $\pm$ 0.73d	75.45 $\pm$ 1.74c	67.64 $\pm$ 1.17b	51.51 $\pm$ 2.23a
Proteins (g)	0.06 $\pm$ 0.00a	0.20 $\pm$ 0.02b	0.32 $\pm$ 0.01c	0.41 $\pm$ 0.02d	0.69 $\pm$ 0.05e
Lipids (g)	0.12 $\pm$ 0.01a	0.32 $\pm$ 0.01c	0.36 $\pm$ 0.02c	0.23 $\pm$ 0.01b	0.46 $\pm$ 0.03d
Total available carbohydrate (g)	2.48 $\pm$ 0.11a	14.16 $\pm$ 0.49b	14.76 $\pm$ 1.34b	18.05 $\pm$ 1.41c	26.42 $\pm$ 2.10d
Total fiber (g)	1.51 $\pm$ 0.11a	9.22 $\pm$ 0.36b	9.92 $\pm$ 0.49c	15.17 $\pm$ 0.43d	25.63 $\pm$ 0.23e
Soluble fiber (g)	0.72 $\pm$ 0.02a	5.68 $\pm$ 0.20b	6.21 $\pm$ 0.51b	9.66 $\pm$ 0.44c	15.45 $\pm$ 0.19d
Insoluble fiber (g)	0.83 $\pm$ 0.04a	3.39 $\pm$ 0.13b	3.84 $\pm$ 0.17c	5.47 $\pm$ 0.05d	10.22 $\pm$ 0.06e
Vitamin C (mg)	4.07 $\pm$ 0.11a	16.98 $\pm$ 0.19c	18.41 $\pm$ 0.89d	4.25 $\pm$ 0.22a	6.83 $\pm$ 1.17b
Ascorbic acid (mg)	4.07 $\pm$ 0.11a	16.98 $\pm$ 0.19c	18.41 $\pm$ 0.89d	4.25 $\pm$ 0.22a	6.83 $\pm$ 1.17b
Dehydroascorbic acid (mg)	n.d.	n.d.	n.d.	n.d.	n.d.
Energy (kcal)	14.72	76.57	83.43	102.67	155.67

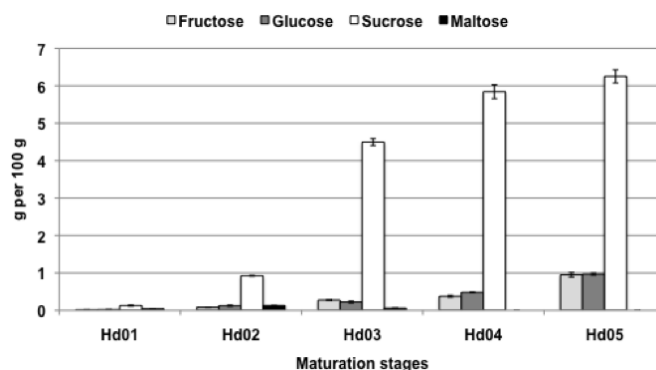
**Table II.** Mineral content (per 100 g fresh weight) in pseudofruit of *Hovenia dulcis* Thunb. at different maturation stages (Hd01 to Hd05) (mean  $\pm$  SD,  $n = 3$ ). In each row, different letters indicate significant differences ( $P < 0.05$ ).

Constituents	Hd01	Hd02	Hd03	Hd04	Hd05
Ash (g)	0.32 $\pm$ 0.00a	0.84 $\pm$ 0.03c	0.79 $\pm$ 0.03b	1.22 $\pm$ 0.02d	1.47 $\pm$ 0.02e
Na (mg)	14.3 $\pm$ 0.01a	45.3 $\pm$ 0.59b	97.1 $\pm$ 0.56c	109.2 $\pm$ 0.90d	165.9 $\pm$ 2.51e
K (mg)	60.2 $\pm$ 0.01a	185.9 $\pm$ 1.68b	195.1 $\pm$ 0.77c	344.4 $\pm$ 0.47d	431.8 $\pm$ 0.89e
Ca (mg)	43.4 $\pm$ 0.28a	112.2 $\pm$ 1.36d	84.2 $\pm$ 1.03b	110.0 $\pm$ 2.01c	187.8 $\pm$ 2.72e
Mg (mg)	9.4 $\pm$ 0.11a	21.2 $\pm$ 0.26b	21.0 $\pm$ 0.17b	25.5 $\pm$ 0.25c	44.3 $\pm$ 0.96d
Fe (mg)	0.14 $\pm$ 0.01a	0.21 $\pm$ 0.02b	0.31 $\pm$ 0.12c	1.16 $\pm$ 0.11e	0.58 $\pm$ 0.05d
Mn (mg)	0.08 $\pm$ 0.00c	0.06 $\pm$ 0.00b	0.03 $\pm$ 0.00a	0.06 $\pm$ 0.01b	0.37 $\pm$ 0.32d
Zn (mg)	0.07 $\pm$ 0.00a	0.21 $\pm$ 0.05b	0.25 $\pm$ 0.07b	0.33 $\pm$ 0.07b	0.80 $\pm$ 0.22c
Cu ( $\mu$ g)	55.5 $\pm$ 1.81a	118.7 $\pm$ 1.73b	284.7 $\pm$ 3.64c	576.3 $\pm$ 7.68e	452.0 $\pm$ 2.96d

occurred, reaching 51% (almost half of the initial value) in peduncles at the most advanced stage of senescence (Hd05).

The content of proteins usually represents only a small percentage of the wet mass of a fruit, being present in plants primarily as enzymes that catalyze metabolic processes, and training or activation of them may be physiologically important in various physiological processes such as ripening and senescence. Low values of lipids were found in all the stages of development of these pseudofruits.

The available carbohydrates contents are in agreement with values reported by Almeida and Valsechi [15] for the stages considered fit for consumption (Hd04 and Hd05), with values of 15.17 and 25.63 g 100 g<sup>-1</sup>, respectively. This apparent increase may be partially attributed to the decrease in moisture in the mature product, since data expressed as dry weight (data not shown) exhibit a maximum carbohydrate content at the Hd02 stage, with a progressive decrease after this stage. Also, starch hydrolysis and utilization of sugars for the metabolism of the plant tissues could explain this behavior. Available carbohydrates include starch and soluble sugars; fructose, glucose, sucrose and maltose were identified and quantified in all the analyzed samples (figure 2). Total amounts of sugars in fresh peduncles ranged from 0.17 g 100 g<sup>-1</sup> in Hd01 (which means that starch constituted almost all the available carbohydrates) to 8.2 g 100 g<sup>-1</sup> in Hd05 (starch representing two-thirds of available carbohydrates). These results show that the proportion of soluble sugars/starch in *H. dulcis*

**Figure 3.** Average soluble sugar content (in g 100 g<sup>-1</sup> fresh weight) in pseudofruit of *Hovenia dulcis* Thunb. at different maturation stages (Hd01 to Hd05) (mean  $\pm$  SD).

pseudofruit increases with maturation (figure 3), leading to the characteristic sweet taste of this product. Sucrose was the major sugar in *H. dulcis* pseudofruit, as also occurs in stalks of *Saccharum officinarum* L. [16].

An aspect of paramount importance is the study of fiber present in fruits, due to their association with bioactive compounds and their role in the quality of food and the health of the consumer. There are few studies that assess fibers and their fractions during stages of maturation of fruit; these kinds of studies are generally focused on the analysis of color, tex-

**Table III.** Contribution to the Recommended Dietary Allowance (RDA for adult males ♂ and females ♀) referred to by Trumbo *et al.* [16] of *Hovenia dulcis* Thunb. pseudofruit at different maturation stages (Hd01 to Hd05).

Constituents	Total fiber		Total vitamin C		Ca	Mg		Fe		Zn		Mn		Cu
	♂	♀	♂	♀	♂♀	♂	♀	♂	♀	♂	♀	♂	♀	♂♀
RDA (minimum)	38	25	90	75	1000	420	310	8	18	11	8	2.3	1.8	900
	g day <sup>-1</sup>		mg day <sup>-1</sup>		mg day <sup>-1</sup>	mg day <sup>-1</sup>		mg day <sup>-1</sup>		mg day <sup>-1</sup>		mg day <sup>-1</sup>		μg day <sup>-1</sup>
Hd01														
Content 100 g <sup>-1</sup>	1.51		4.07		43.36	9.43		0.14		0.07		0.08		55.48
% RDA	3.97	6.04	4.52	5.43	4.34	2.25	3.04	1.75	0.78	0.64	0.88	3.48	4.44	6.16
Hd02														
Content 100 g <sup>-1</sup>	9.22		6.98		112.24	21.19		0.21		0.21		0.06		118.70
% RDA	24.26	36.88	18.87	22.64	11.22	5.05	6.84	2.63	1.17	1.91	2.63	2.61	3.33	13.19
Hd03														
Content 100 g <sup>-1</sup>	9.92		18.41		84.23	21.01		0.31		0.25		0.03		284.69
% RDA	26.11	39.68	20.46	24.55	8.42	5.00	6.78	3.88	1.72	2.27	3.13	1.30	1.67	31.63
Hd04														
Content 100 g <sup>-1</sup>	15.17		4.25		110.03	25.47		1.16		0.33		0.06		576.30
% RDA	39.92	60.68	4.72	5.67	11.00	6.06	8.22	14.50	6.44	3.00	4.13	2.61	3.33	64.03
Hd05														
Content 100 g <sup>-1</sup>	25.63		6.83		187.78	44.32		0.58		0.80		0.37		452.03
% RDA	67.45	102.52	7.59	9.11	18.78	10.55	14.30	7.25	3.22	7.27	10.00	16.09	20.56	50.23

ture, pH, soluble solids and titratable acidity. Pseudofruit of *H. dulcis*, in Hd02 and subsequent maturation stages, can be considered as an interesting source of dietary fiber, according to European and Brazilian regulations [17, 18], as they have more than 3 g 100 g<sup>-1</sup> fiber (9–26 g 100 g<sup>-1</sup>). This is of great interest since modern diets are frequently deficient in fiber, and an increase in the intake of fiber-rich foods is highly recommended in many countries [19]. *Hovenia dulcis* pseudofruit would represent a very good contribution for this purpose, as 100 g edible Hd04 stalks (optimum for consumption) would provide about 60% of the dietary Recommended Dietary Allowances (RDA) for women, and up to 100% in Hd05 (table III).

Most of this fiber content is in the form of soluble fiber, which includes pectin, being higher than that found in most fruits considered rich in pectin such as apples, citrus, apricots, peaches and plums [20]. Soluble fiber (0.72 to 15.45 g 100 g<sup>-1</sup>) increased more than the fraction of insoluble fiber (0.83 to 10.22 g 100 g<sup>-1</sup>) throughout maturation, being predominant in practically every stage. Contrary to what happens to available carbohydrates, soluble and insoluble fiber contents increase during maturation of peduncles, with no dependence on moisture content (15 to 31 g 100 g<sup>-1</sup> dw, and 17 to 21 g 100 g<sup>-1</sup> dw, respectively).

Vitamin C in the fresh product increases during the first stages of maturation (from 4 mg 100 g<sup>-1</sup> in Hd01, to 18 mg 100 g<sup>-1</sup> in Hd03); then drastically declines in Hd04 and Hd05. In fact, when considering dw values (data not shown), a progressive decrease in ascorbic acid content was found in the pseudofruit (from 85 mg 100 g<sup>-1</sup> dw to 14 mg 100 g<sup>-1</sup> dw). This can be justified by the stage of maturation of the fruit, combined with the climatic conditions, since the formation of ascorbic acid is related to the intensity of solar radiation. Ito

*et al.* [21] also found that vitamin C content declines with maturation of acerola fruit. From the values obtained, it can be seen that the nutritional contribution of this food product to vitamin C intake is not of high relevance in edible Hd04 and Hd05 peduncles. However, it is important to note that all vitamin C is in the form of ascorbic acid, which is a potent antioxidant in food and in the human body by destroying free radicals of oxygen [22, 23].

Total ash content increased during the development of these pseudofruits, due to concentration of minerals by reduction of moisture in the mature product. According to Peñuelas *et al.* [24], these variations may be related to environmental conditions, such as rain, humidity and soil composition, which can influence levels of micronutrients; they can induce plant responses to physiological stress situations, in which the minerals could act as co-factors, regulating the metabolic pathways of plants, since the material in formation requires the presence of the metabolic process regulator metals. As for minerals, the results showed variation among the different stages of maturation of pseudofruit of *H. dulcis*.

Among the macroelements, K was the main mineral (table II), showing the highest contents in the mature pseudofruit (60.2–431.8 mg 100 g<sup>-1</sup>), followed by Ca (43.4–187.8 mg 100 g<sup>-1</sup>) and Na (14.3–165.9 mg 100 g<sup>-1</sup>). Mg content presented lower values, ranging between 9.4 and 44.3 mg 100 g<sup>-1</sup>. With regard to microelements, the highest values were achieved by Fe (0.14–1.16 mg 100 g<sup>-1</sup>) and Zn (0.07–0.80 mg 100 g<sup>-1</sup>). Mn had the smallest values, varying between 0.03–0.37 mg 100 g<sup>-1</sup>, since Cu exhibited a remarkable increase during maturation, from 55 μg 100 g<sup>-1</sup> in Hd01, to 576 μg 100 g<sup>-1</sup> in the optimum stage for consumption (Hd04). None of these minerals reach the Tolerable Upper Intake Levels established by Trumbo *et al.* [16].

Microelements, also called trace elements, include a large number of compounds with physiological activity, and some of them can play decisive roles in maintaining human health. The biological activities of the minerals Cu, Fe, Zn and Mn are strongly associated with the presence of electrons that enable their participation in redox reactions. It is assumed that these trace metals play a critical role in the mechanisms of protection for cleaning up free radicals [25]. From *table III*, it can be seen that the edible fresh stalks in the Hd04 stage can be considered as good sources of Cu (64% of RDA for adults), while Hd05 pseudofruits provide more than 15% of the Ca, Mn and Cu RDAs established for both men and women .

## 4 Conclusion

*Hovenia dulcis* pseudofruits are a good alternative to improve the nutritional quality of modern diets demanding diversification from either sensorial or nutritional points of view. In this respect, this neglected product can provide a very high fiber intake, as well as relevant Cu, Ca and Mn contributions in the habitually eaten mature stages. Less mature peduncles could also be good alternatives to be used as ingredients for foods or dietary supplements.

## References

- [1] Lago E.S., Gomes E., Silva R. Produção de geléia de jambolão (*Syzygium cumini* Lamarck): processamento, parâmetros físico – químicos e avaliação sensorial, *Cien. Tec. Alim.* 26 (2006) 847–852.
- [2] Martinez-Valverde I., Periago M.J., Ros G. Significado nutricional de los compuestos fenolicos de la dieta, *Arch. Latinoam. Nutr.* 50 (2000) 5–18.
- [3] Carvalho P.E.R. Ecologia, silvicultura e usos da uva-do-japão (*Hovenia dulcis* Thunberg), Circular Técnica EMBRAPA, Colombo: EMBRAPA Florestas, 1994.
- [4] Cozzo D. Resultados de las plantaciones florestais com *Hovenia dulcis* en la region Argentina subtropical y húmeda de Misiones, *Revista Florestal Argentina* 4 (1960) 107–117.
- [5] Chitarra M.I.F., Chitarra A. B. Pós-colheita de frutas e hortaliças: fisiologia e manuseio. Lavras: ESAL/FAEPE, 2005.
- [6] Zhou Y., Newman C., Xie Z. Macdonald D.W. Peduncles elicit large-mammal endozoochory in a dry-fruited plant, *Ann. bot.* 112 (2013) 85–93.
- [7] Bampi M., Bicudo M.O.P., Fontoura P.S.G., Ribani R.H. Composição centesimal do fruto, extrato concentrado e da farinha da uva-do-japão, *Cien. Rural* 40 (2010) 2361–2367.
- [8] Greenfield, H. Southgate, D.A.T. Food composition data: Production, management, and use, FAO, Rome, 2003.
- [9] Horwitz W., Latimer G. W. Official methods of analysis of AOAC international (18th ed.), Gaithersburg: EUA, 2005.
- [10] Osborne D.R., Voogt P. Análisis de los nutrientes de los alimentos, Zaragoza: Acribia, 1986.
- [11] Sánchez-Mata M. C., Peñuela-Teruel M. J., Cámara-Hurtado M., Díez-Marqués C., Torija-Isasa M. E. Determination of mono-, di- and oligosaccharides in legumes, by HPLC, using an amino bonded silica column, *J. Agric. Food Chem.* 46 (1998) 3648–3652.
- [12] Vazquez-Odériz M., Vázquez Blanco M.E., López Hernández J., Simal Lozano J., Romero-Rodríguez M.A. Simultaneous determination of organic acids and vitamin C in green beans by liquid chromatography, *J. AOAC Int.* 77 (1994) 1056–1059.
- [13] Arella F., Deborde J.B., Lahély S., Bourguignon J.B., Hasselmann C. Liquid chromatographic determination of vitamins B1 and B2 in foods. A collaborative study, *Food Chem.* 56 (1996) 81–86.
- [14] Trumbo P., Schlicker S., Yates A. A., Poos M. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids, *J. Am. Diet. Assoc.* 102 (2002) 1621–1630.
- [15] Almeida J.R., Valsechi O. Guia de composição de frutas. Piracicaba: ESALQ, Instituto Zimotécnico, 1966.
- [16] Ayaz F.A., Kucukislamlolu M., Reunanen M. Sugar, non-volatile and phenolic acids composition of strawberry tree (*Arbutus unedo* L. var. ellipsoidea) fruits, *J. Food Compos. Anal.* 13 (2000) 171–177.
- [17] Brasil. Portaria n.27 SVS/MS, de 13 de janeiro de 1998. A secretaria de vigilância sanitária do MS aprova o regulamento técnico referente à informação nutricional complementar, *Diário Oficial da União*, 1998.
- [18] European Parliament and Council (2011), Regulation (EU) No 1169/2011 of the European parliament and of the Council of 25 October 2011 on the provision of food information to consumers, *Official Journal of the European Union*, 22.11.2011: L304/18-L304/63 .
- [19] World Health Organization (WHO) Study Group. Nutrition and the prevention of chronic diseases, WHO, 1990, pp. 30–39.
- [20] Souci S. W., Fachmann W., Kraut H. Food composition and nutrition tables, MedPharm Scientific Publishers, 2008.
- [21] Ito S., Aiba M., Ishihata K. Comparison of ascorbic acid content in acerola fruit from different production region depend on degree of maturity, and it's stability by processing. *Nippon Shokuhin Kogyo Gakkaishi* 37 (1990) 726–729.
- [22] Sun J., Chu Y.F., Wu X., Liu R.H. Antioxidant and antiproliferative activities of common fruits, *J. Agric. Food Chem.* 50 (2002) 7449–7454.
- [23] Ruiz-Rodríguez B.M., Sánchez-Moreno C., De Ancos B., Sánchez-Mata M.C., Fernández-Ruiz V., Camara M., Tardío J. Wild *Arbutus unedo* L. and *Rubus ulmifolius* Schott fruits are underutilized sources of valuable bioactive compounds with antioxidant capacity, *Fruits* 69 (2014) 435–448.
- [24] Peñuelas J., Sardans J., Ogaya R., Estiarte M. Nutrient stoichiometric relations and biogeochemical niche in coexisting plant species: Effect of simulated climate change, *Polish Journal of Ecology* 56 (2008) 613–622.
- [25] Ferguson L.R. Micronutrients, dietary questionnaires and cancer, *Biomed. Pharmacother.* 8 (1997) 337–344.