

Article

# Nutraceutical Characteristics of the Extracts and Juice of Chayote (*Sechium edule* (Jacq.) Sw.) Fruits

María de la Luz Riviello-Flores <sup>1</sup>, Ma. de Lourdes Arévalo-Galarza <sup>2</sup>, Jorge Cadena-Iñiguez <sup>3</sup> , Ramón Marcos Soto-Hernández <sup>2,\*</sup>, Lucero del Mar Ruiz-Posadas <sup>2</sup> and Fernando C. Gómez-Merino <sup>4</sup> 

<sup>1</sup> Departamento de Procesos Alimentarios, Universidad Tecnológica de Tehuacán, 75859 Tehuacán, Puebla, Mexico; marluriviello@gmail.com

<sup>2</sup> Colegio de Postgraduados, Campus Montecillo, 56230 Texcoco, Mexico; larevalo@colpos.mx (M.d.L.A.-G.); lucpo@colpos.mx (L.d.M.R.-P.)

<sup>3</sup> Colegio de Postgraduados, Campus San Luis Potosí, 78622 Salinas de Hidalgo, San Luis Potosí, Mexico; jocadena@colpos.mx

<sup>4</sup> Colegio de Postgraduados, Campus Córdoba, 94500 Córdoba, Veracruz, Mexico; fernandg@colpos.mx

\* Correspondence: msoto@colpos.mx; Tel: + 52-(595)-952-0200 (ext. 1361)

Received: 8 March 2018; Accepted: 23 April 2018; Published: 1 May 2018



**Abstract:** Fruits of chayote [*Sechium edule* (Jacq.) Swartz] are a non-traditional vegetable widely consumed in Latin America, with the state of Veracruz, México being the world's main producer, but little is known about the nutraceutical potential. This study aimed to determine the chemical compositions and antioxidant activities from the juice fruits from two commercial varieties of chayote cultivated in Mexico, as well as a proposal for the elaboration of chayote juices with stevia leaves and pineapple juice. The physicochemical properties of juice from *virens levis* (VL) and *nigrum spinosum* (NS) varieties were determined using standard methods. The juice of the two varieties differ significantly regarding the concentrations of total soluble solids and total sugars, but not vitamin C. The total concentration of phenolics in NS extracts was slightly higher than in VL (1005 and 856 mg 100 g<sup>-1</sup> dry-weight, respectively), but the total flavonoid contents were similar (27 and 26 mg 100 g<sup>-1</sup> dry-weight, respectively). Cucurbitacin D was predominant in both varieties. The radical scavenging capacities of VL and NS extracts varied slightly (IC<sub>50</sub> = 0.45 to 0.65 mg mL<sup>-1</sup>), while the antioxidant activities were similar (~80%). The NS variety is particularly promising regarding nutraceutical application. The chayote juice combined with stevia and pineapple maintained the original nutraceutical characteristics of the fruit, but enhanced the organoleptic characteristics like density and sugar/acid balance.

**Keywords:** fruits; juice; flavonoids; cucurbitacins

## 1. Introduction

Recent epidemiological studies have demonstrated that oxidative stress is associated with the development of various human diseases including cancer, the global incidence of which was estimated to be in the region of 14 million new cases per year according to statistics from 2012, but is expected to rise to 22 million new cases per year over the next two decades [1]. Fruits and vegetables tend to be rich in natural antioxidants, and the increased consumption of these dietary components has been proposed as an alternative strategy for health improvement. Indeed, many of these food materials exhibit important pharmacological properties including cytotoxic and anticancer activities, and insufficient intake is believed to be the cause of up to 19% of gastrointestinal cancers [1]. Moreover, according to Gonzales and Valerio [2], 62% of new drugs approved by the United States Food and

Drug Administration (FDA) during the period 1981–2002 were of natural origin and possessed complex and diverse molecular structures with biological activities that were higher than their synthetic counterparts.

The family Cucurbitaceae encompasses a large number of species that are appropriate for human consumption, and many of these contain compounds with functional properties, for example, *Cucurbita pepo* L., *Cucumis sativus* L., *Trichosanthes dioica* Roxb., and various members of the genera *Momordica* and *Sechium* [3–5]. The tuberous rooted perennial *Sechium edule* (Jacq.) Swartz., commonly known as chayote, produces fleshy fruits that weigh between 250 and 400 g and are normally consumed in the same manner as vegetables. The species is native to Mesoamerica and the main producers are Mexico and Costa Rica. The Mexican states of Chiapas, Oaxaca, and Veracruz exhibit a particularly wide biological diversity with respect to *S. edule*. In the commercial scenario, the most appreciated varieties of chayote are *virens levis*, produced in subtropical and tropical regions, and *nigrum spinosum*, cultivated in temperate zones and high valleys with altitudes of 2000 to 2800 m [6].

Chayote is mainly used in cooked form and is valued for its nutritional content, which includes vitamins, minerals, fiber, water, and amino acids (lysine, histidine, arginine, aspartic acid, glutamic acid, cysteine, valine, isoleucine, serine, alanine, and tyrosine). Recent research has shown that chayote fruits possess diuretic, anti-inflammatory, and hypotensive activities owing to the presence of  $\beta$ -sitosterol  $\beta$ -D-glucopyranoside and stigmasterol  $\beta$ -D-glucopyranoside [5]. *S. edule* fruits also contains alkaloids, saponins, phenols, polyphenols, flavonoids, and cucurbitacins; however, information regarding which chayote variety was employed in the published investigations has rarely been provided [6].

As a non-traditional vegetable, there is not information about the way to consume this fruit, so the consumption of juice represents as a global tendency is recommended because they do not contain fat, and are rich in vitamins, minerals, and phytonutrients that help promote good health. Additionally, the flavor of the fruit is neutral and easily combined with other fruits.

The literature describes an abundance of information about the secondary metabolites of plants, particularly those with biological activity, which vary greatly depending on environmental and genetic factors. It is described, for example, that the agronomic strategies may significantly modify and/or improve the concentrations and profile of them. Factors such as light, temperature, stress, etc. can be related to these strategies and when managed properly, can result in a good quality of the medicinal plant [7,8]. Taking these facts into account, we postulate that the chemical profiles and pharmacological activities of the two commercial varieties of *S. edule* are distinct, even when they grow under the same agronomic conditions.

In order to test this hypothesis, we have examined the chemical compositions and antioxidant activities of the juice and methanol/ethanol extracts of fruit from *S. edule* var. *virens levis* and *S. edule* var. *nigrum spinosum*, as well as a proposal for the elaboration of chayote juices combined with stevia (*Stevia rebaudiana* Bert.) and pineapple (*Ananas comosus*) as a way to promote the consumption of chayote fruit.

## 2. Materials and Methods

### 2.1. Plant Materials and Chemical Reagents

Fruits (120) from each biological variant of *S. edule* var. *virens levis* (VL) and *S. edule* var. *nigrum spinosum* (NS) (Figure 1) were harvested between 18 days and 21 days after anthesis from a commercial farm located in Huatusco, Veracruz, Mexico (19°08'48' N, 97°57'00'' W; 1340 m altitude) during the summer season, and both variants experienced growth under the same agricultural conditions. The area has an annual mean temperature of 19–22 °C, relative humidity of 85–90%, and 2250 mm of annual mean precipitation. The soils are vitric luvisol, rich in nutrients, having moderate fertility, a thick texture, and fragments of volcanic glass (pH 4.3–6.5), rich in organic matter, low in calcium, and high in iron, manganese, and zinc.

Solvents, reagents, and reference standards were obtained from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated, and were used as received.



**Figure 1.** Varieties of chayote (*Sechium edule*) from Veracruz, México. (a) *virens levis* (VL) and (b) *nigrum spinosum* (NS).

## 2.2. Characterization of Fruit Juices

Fruits were washed with chlorinated water ( $100 \text{ mg L}^{-1}$ ), cut into small pieces, processed in a juice extractor (Model E-17, Turmix™, Mexico) industrial extractor, and subsequently filtered. The resulting juices were stored in the freezer at  $-70 \text{ }^{\circ}\text{C}$  until required for analysis. Total soluble solids were measured using a refractometer (Atago™, Tokyo, Japan) model PAL-1 digital refractometer according to the standard technique adopted by the Association of Official Analytical Chemists (AOAC) [9] and expressed as °Brix. Vitamin C was determined using the 2,6-dichlorophenolindophenol (DCPIP) method and concentrations ( $\text{mg } 100 \text{ mL}^{-1}$ ) were estimated using a calibration curve constructed using ascorbic acid as the reference standard. Chlorophylls a and b were determined by mixing 3 mL of juice and 5 mL of 80% acetone (*v/v*), transferring the liquid to flasks wrapped in aluminum foil, and storing in the refrigerator overnight in total darkness. Samples were subsequently filtered through filter paper and concentrations ( $\text{mg } 100 \text{ mL}^{-1}$ ) determined spectrophotometrically at 645 and 663 nm for chlorophylls a and b, respectively, using a Spectronic™ 20 spectrophotometer (Thermo Fisher, Waltham, MA, USA). Values of pH were measured using a Hanna Instruments (Carrollton, TX, USA) model H12211 benchtop pH meter. The anthrone/sulfuric acid method was used to determine total sugars with concentrations ( $\text{g } 100 \text{ g}^{-1}$ ) estimated by reference to a glucose standard curve calibrated at 600 nm. Color index (CI) was evaluated using a Hunter Lab D25-PC2 colorimeter (Hunter Associates Laboratory, Reston, VA, USA) and calculated according to Equation (1) (Commission Internationale de l’Eclairage  $L^*a^*b^*$  system) in which  $L^*$  represents lightness,  $a^*$  is the red/green coordinate, and  $b^*$  is the yellow/blue coordinate. All analyses were performed in triplicate.

$$\text{Color index} = (a^* \times 1000)/(L^* \times b^*) \quad (1)$$

## 2.3. Extraction and Quantitative Analysis of Functional Compounds

Twenty fruits from each variety were cut into small pieces, dried in a forced-air oven at  $45 \text{ }^{\circ}\text{C}$  for four days until reaching a constant weight, and subsequently reduced to a fine powder using a General Electric mill (Fairfield, CT, USA) model AC-160. A portion (200 g) of the powder was extracted exhaustively (15 times) with methanol for 48 h at room temperature ( $20 \pm 2 \text{ }^{\circ}\text{C}$ ). After each extraction, the liquid phase was separated from the solid material by decantation and filtration and a new solvent was added. The solid residue was subsequently extracted 12 times with ethanol in a similar manner. The bulked methanol and ethanol extracts were dried separately under reduced pressure using a Büchi (Flawil, Switzerland) Rotavapor™ R-114 at  $45 \text{ }^{\circ}\text{C}$  [10].

Total phenolics were quantified using the Folin-Ciocalteu method [11]. Briefly, samples (10 mg) of dried methanol and ethanol extracts were resuspended separately in 1 mL distilled water. Aliquots (30  $\mu\text{L}$ ) of these solutions were transferred to test tubes together with 470  $\mu\text{L}$  of distilled water, 25  $\mu\text{L}$  of 2M Folin-Ciocalteu reagent in distilled water (1:1;  $v/v$ ), and 975  $\mu\text{L}$  of 2.5% sodium carbonate solution. After homogenization, the samples were incubated in the dark for 1 h and the absorbances were measured at 740 nm. The concentrations of total phenols were estimated by means of a calibration curve constructed with gallic acid as the reference standard. For the juice, 1 mL of the juice was diluted with 1 mL distilled water, was mixed in a vortex by 5 s, and was then centrifuged to  $13,000\times g$  by 1 min. For the juice combined with pineapple juice, 500  $\mu\text{L}$  of the supernatant was taken, and 500  $\mu\text{L}$  de distilled water was added. Then, 30  $\mu\text{L}$  of the supernatant was taken along with 470  $\mu\text{L}$  of distilled water, and the above procedure was followed.

Quantitation of flavonoids: samples of 50 mg of dried methanol and ethanol extracts were dissolved in 1 mL of 80% methanol and transferred aliquots (20  $\mu\text{L}$ ) to test tubes containing 900  $\mu\text{L}$  of 80% methanol, 2 mL of 1M potassium acetate solution, and 2 mL of 10% aluminum chloride solution. After homogenization, the samples were incubated in the dark for 1 h and the absorbances measured at 415 nm. The concentrations of flavonoids were estimated by means of a calibration curve constructed with quercetin as the reference standard.

#### 2.4. Separation and Identification of Flavonoids and Cucurbitacins by High Performance Liquid Chromatography (HPLC)

Two different methods of HPLC were used to analyze the samples: one for flavonoids and one for cucurbitacins. Samples (20 mg) of dried methanol and ethanol extracts were dissolved separately in 2 mL of 80% methanol and the solutions were filtered through 0.45  $\mu\text{m}$  Acrodisc<sup>®</sup> syringe filters with a nylon membrane (Sigma-Aldrich, St. Louis, MS, USA) prior to analysis. Chromatography was performed using an Agilent Technologies (St. Clara, CA, USA) Infinity series 1220 instrument equipped with a Thermo Fisher Hypersyl<sup>™</sup> ODS C18 column (125  $\times$  4 mm; 5  $\mu\text{m}$  particle size). Flavonoids were analyzed at 30  $^{\circ}\text{C}$  under isocratic elution with a mobile phase comprising water: acetonitrile (65:35;  $v/v$ ), with pH adjusted to 2.5 with trifluoroacetic acid, supplied at a flow rate of 1 mL  $\text{min}^{-1}$  (179 bar pressure). The sample injection volume was 20  $\mu\text{L}$ , the detection wavelength was 235 nm, and the standard reference compounds employed were rutin, phloretin, phlorizidin, myricetin, quercetin, naringenin, and galangin. Cucurbitacins were analyzed at 25  $^{\circ}\text{C}$  using the instrument specified above equipped with a Waters (Milford, MA, USA) Symmetry Shield RP18 column (250  $\times$  4.4 mm i.d.; 5  $\mu\text{m}$  particle size). Isocratic elution was carried out with a mobile phase comprising water:methanol:acetonitrile (50:30:20;  $v/v/v$ ) supplied at a flow rate of 1 mL  $\text{min}^{-1}$  (179 bar pressure). The sample injection volume was 20  $\mu\text{L}$ , the detection wavelength was 235 nm, and the standard reference compounds employed were cucurbitacins B, D, E, and I.

#### 2.5. Evaluation of Antioxidant Properties

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was employed to evaluate antioxidant activities as previously described [12]. Aliquots (500  $\mu\text{L}$ ) of solutions containing crude methanol and ethanol extracts at concentrations of 2.5 mg  $\text{mL}^{-1}$ , 5 mg  $\text{mL}^{-1}$ , 10 mg  $\text{mL}^{-1}$ , 20 mg  $\text{mL}^{-1}$ , and 30 mg  $\text{mL}^{-1}$  were transferred to test tubes and mixed with 500  $\mu\text{L}$  of methanol and 2 mL of 0.1 mM DPPH solution in methanol. The reaction mixtures were incubated for 30 min at room temperature in the dark and absorbances were then measured at 517 nm. The control comprised 0.1 mM DPPH solution without extract and the blank was pure methanol. All measurements were performed in duplicate. Percentage DPPH inhibition was calculated according to Equation (2), in which  $A_0$  is the absorbance of 0.1 mM DPPH solution and  $A_1$  is the absorbance of 0.1 mM DPPH solution containing the sample.

$$\text{DPPH inhibition (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (2)$$

The concentration of extract required to scavenge 50% of DPPH radicals (IC<sub>50</sub> value) was established from dose response data by linear regression.

Lipid peroxidation was assessed using the  $\beta$ -carotene-linoleic acid assay as described previously [13,14], but with slight modifications. The reagent solution, containing 0.02 mL of linoleic acid, 0.2 mL of Tween-20, and 1 mL of  $\beta$ -carotene solution (0.2 g mL<sup>-1</sup>) in chloroform, was prepared by homogenizing the components in a 50 mL round-bottomed flask and removing the chloroform. Hydrogen peroxide (25 mL) was added to the flask, the whole sample was mixed thoroughly, and the absorbance was measured at 470 nm against a blank of reagent solution prepared in the same manner but without  $\beta$ -carotene. Subsequently, aliquots (4.8 mL) of the reagent solution were transferred to test tubes and samples (0.2 mL) of methanol and ethanol extracts at two different concentrations (10 and 50 mg mL<sup>-1</sup>) were added separately. Positive and negative controls were prepared in exactly the same manner, except that butylated hydroxytoluene (BHT; 0.1 mg g<sup>-1</sup>) and methanol, respectively, replaced the plant extracts. Assay mixtures and controls were stirred thoroughly for 2 min and then incubated at 50 °C for 140 min to induce thermal oxidation. Absorbances (470 nm) of the assay mixtures were monitored at 0, 20, 60, 100, and 140 min of reaction time. All measurements were performed in triplicate. Percentage antioxidant activity (% AA) was calculated according to Equation (3), in which  $A_0$  ( $A_{00}$ ) and  $A_t$  ( $A_{0t}$ ) are the absorbances of the test sample (control) at times 0 and  $t$ , respectively.

$$\text{Antioxidant activity (\%)} = [1 - (A_0 - A_t / A_{00} - A_{0t})] \times 100 \quad (3)$$

## 2.6. Juice Elaboration

Fruits from both varieties of chayote were ground in a food processor, to get juice that was mixed with dry and milled leaves of stevia (*Stevia rebaudiana*) at a ratio of 0.7% ( $p/v$ ) in *virens levis* and 0.8% ( $p/v$ ) in *nigrum spinosum*, as well as adding 50% pineapple juice (*Ananas comosus*) ( $v/v$ ). The chayote and pineapple fruits were selected and washed with 100 mg L<sup>-1</sup> hypochlorite solution, and rinsed thoroughly with distilled water. The pineapple was peeled with a stainless steel knife, cut into small pieces, and processed in a juice extractor (Model E-17, Turmix™, Mexico) with a filtration system.

The variables were defined after conducting a tasting panel, where the main characteristic was not detecting the bitter taste characteristic of stevia. In total, three treatments by variety of chayote were evaluated, with three replicates per treatment. Then, juices were pasteurized at 60 °C for 30 min, in an incubator (Model FE-372, Felisa®, Mexico), and immediately chilled at 6 °C, after the quality evaluation was performed at room temperature.

## 2.7. Statistical Analysis

Data were expressed as mean  $\pm$  standard deviation and compared using analysis of variance (ANOVA) and the Tukey test. The level of statistical significance was set at  $p < 0.05$ . All analyses were performed with the aid of SAS® version 9.0 software (SAS Institute, Cary, NC, USA).

# 3. Results and Discussion

## 3.1. Characteristics of Fruit Juices

The levels of total soluble solids, total sugars, and chlorophyll a were significantly higher ( $p < 0.05$ ) in fruit juice from the NS variety of *S. edule* in comparison with the VL juice (Table 1). Additionally, fruit juices from the two varieties differed significantly ( $p < 0.05$ ) with respect to color. Thus, while the juice from the NS variety was blue violet to deep green, that from VL was bright green to yellowish green. Interestingly, the juice of the VL variety was significantly more acidic than that of the neutral NS variety, while the concentration of vitamin C was lower in NS compared with VL, although the difference was not statistically significant.

Cadena-Iñiguez et al. [6] reported vitamin C contents of  $4.95 \pm 0.49$  and  $6.76 \pm 0.16$  mg 100 g<sup>-1</sup>, respectively, for the NS and VL varieties of *S. edule*. While these values appear to be appreciably

higher than those obtained in the present study, it should be noted that the earlier investigation was performed using fruit pulp rather than fruit juice, and it is known that vitamin C degrades rapidly in solution, especially on exposure to light and at increased temperature or pH. The difference in source material would also explain the higher values reported previously [6] in pulp for the content of chlorophyll a and b, i.e., 8.4 and 9.2 mg 100 g<sup>-1</sup>, respectively, for the NS variety and 6.0 and 7.1 mg 100 g<sup>-1</sup>, respectively, for the VL variety.

**Table 1.** Physicochemical characteristics of juices from *Sechium edule* var. *nigrum spinosum* (NS) and *S. edule* var. *virens levis* (VL).

Variable <sup>1</sup>	Variety	
	NS	VL
Total soluble solids (%)	5.1 ± 0.0 <sup>a</sup>	4.3 ± 0.0 <sup>b</sup>
Total sugars (g 100 g <sup>-1</sup> )	3.6 ± 0.3 <sup>a</sup>	2.03 ± 0.0 <sup>b</sup>
pH	6.8 ± 0.0 <sup>a</sup>	6.0 ± 0.0 <sup>b</sup>
Vitamin C (mg 100 mL <sup>-1</sup> )	2.7 ± 0.3 <sup>a</sup>	3.24 ± 0.0 <sup>a</sup>
Chlorophyll a (mg 100 mL <sup>-1</sup> )	4.0 ± 0.5 <sup>a</sup>	2.0 ± 0.6 <sup>b</sup>
Chlorophyll b (mg 100 mL <sup>-1</sup> )	5.0 ± 0.5 <sup>a</sup>	4.0 ± 0.5 <sup>a</sup>
Color index	-26.1 ± 1.2 <sup>b</sup>	-16.4 ± 0.12 <sup>a</sup>

<sup>1</sup> Data are expressed as the mean ± standard deviation of three replicates. In each row, values bearing dissimilar superscript lower-case letters (a, b) are significantly different ( $p \leq 0.05$ ; ANOVA and the Tukey test).

### 3.2. Identification of Phenolics and Flavonoids

Phenolic compounds protect plant cells against oxidative damage caused by reactive oxygen species (ROS) produced as a result of biotic or abiotic stress. The concentration of plant phenolics is determined by numerous factors including cultivar, agronomic management, climate, and developmental stage of the plant. For example, Nagarani et al. [3] reported that the content of gallic acid in the fruit of bitter squash *Momordica charantia* L. (Cucurbitaceae), a vine used in both culinary and traditional medicine, increases from 95.6 mg L<sup>-1</sup> in green fruit up to ~202 mg L<sup>-1</sup> as the fruit matures.

In the present study, there were no significant differences between the two varieties of *S. edule* regarding the concentration of phenolics in methanol extracts of the fruit (Table 2). However, the ethanol extract of the NS variety contained a significantly higher ( $p < 0.05$ ) level of phenolics compared with the VL variety and, for this reason, the overall phenolic content of NS fruit was somewhat higher (1005 mg 100 g<sup>-1</sup> dry weight) than that of VL fruit (856 mg 100 g<sup>-1</sup> dry weight). A previous report [15] stated that the phenolic contents of the leaves, stems, and seeds of an unidentified variety of *S. edule* were within the respective ranges of 0.15 to 2.06, 0.06 to 2.81, and 0.13 to 5 mg g<sup>-1</sup>, depending on the method of extraction employed.

**Table 2.** Concentration of phenolics and flavonoids in extracts of fruit from *Sechium edule* var. *virens levis* (VL) and *S. edule* var. *nigrum spinosum* (NS).

Variety	Extract	Phenolics <sup>1</sup>	Flavonoids <sup>1</sup>
		(mg 100 g <sup>-1</sup> Dry Weight)	(mg 100 g <sup>-1</sup> Dry Weight)
VL	Methanol	489 ± 50 <sup>a</sup>	10 ± 1 <sup>bc</sup>
	Ethanol	367 ± 24 <sup>b</sup>	16 ± 1 <sup>ab</sup>
NS	Methanol	525 ± 14 <sup>a</sup>	19 ± 4 <sup>a</sup>
	Ethanol	480 ± 17 <sup>a</sup>	8 ± 1 <sup>c</sup>

<sup>1</sup> Data are expressed as the mean ± standard deviation of three replicates. In each column, values bearing dissimilar superscript lower-case letters (a, b, c) are significantly different ( $p \leq 0.05$ ; ANOVA and the Tukey test).

There were significant differences ( $p < 0.05$ ) between the two varieties of *S. edule* with respect to the concentration profiles of flavonoids in the methanol and ethanol extracts of the fruits (Table 2).

However, the overall flavonoid contents of the two varieties were similar, with that of NS being slightly higher in comparison to VS (27 and 26 mg 100 g<sup>-1</sup> dry weight, respectively). Four flavonols (rutin, myricetin, quercetin, and galangin), two dihydrochalcones (phloretin and phlorizidin), and one flavanone (naringenin) were detected unambiguously in extracts of chayote fruit. Phenolic acids and corresponding esters, together with flavonoids and glycosylated flavonoids (including quercetin, myricetin, naringenin, and apigenin), have been detected previously in extracts of seeds from species of the genus *Cucurbita* (Cucurbitaceae) [4].

### 3.3. Identification of Cucurbitacins

Cucurbitacins are tetracyclic triterpenes that impart a bitter taste to plant tissues. Despite their toxicity, cucurbitacin-rich plants are used in traditional medicine and the pharmaceutical industry since they possess a wide range of therapeutic activities. In the present study, cucurbitacins B, D, E, and I were identified in the ethanol extracts of both varieties of *S. edule*, while the methanol extracts contained cucurbitacins D and E (VL) and B and E (NS) (Table 3). Cucurbitacin D was the most abundant member of this class of secondary compounds in all extracts in which it was detected. The overall cucurbitacin content of VL fruit was substantially higher than that of NS fruit (752.96 and 168.02 mg 100 g<sup>-1</sup> dry weight, respectively).

As verified in the present study, cucurbitacin E and its glycoside are found most commonly in edible plants. However, cucurbitacin D is the most toxic because it increases capillary permeability, produces irritation of the intestinal mucosa, and increases intestinal motility in experimental animals [16]. Fatope et al. [17] reported that leaves of wild *M. charantia* and *M. balsamina* L. are rich in cucurbitacins that stimulate intestinal secretions and favor food digestion (eupeptic activity). Melon (*Cucumis melo* L.), a fruit that is much appreciated in many parts of the world, contains significant amounts of cucurbitacins B and E and is used in Chinese traditional medicine as a liver protector agent [18]. Cucurbitacin B also exhibits cytotoxic activity against HeLa and KB cell lines and antitumor activity against sarcoma 280 and Ehrlich's ascites carcinoma, while cucurbitacins D and E have also been shown to inhibit the growth of carcinoma cells [13].

It is interesting to observe that although the cucurbitacins have been isolated and analyzed by HPLC by different co-workers [19,20], in different species of the *Cucurbitaceae* family, there is no information about their quantitation in the plant of our study and this supports the novelty of the research. In the case of flavonoids, as quite widespread metabolites in plants, there is no problem in their analysis using techniques such as spectrophotometry and chromatography, which are easily adapted from methods described in the literature.

**Table 3.** Concentration of cucurbitacins in extracts of fruit from *Sechium edule* var. *virens levis* (VL) and *S. edule* var. *nigrum spinosum* (NS).

Variety	Extract	Type of Cucurbitacin	Concentration mg 100 g <sup>-1</sup> Dry Weight <sup>1</sup>
VL	Methanol	D	353.41
		E	0.33
	Ethanol	B	0.16
		D	395.48
		E	3.25
NS	Methanol	I	0.33
		B	24.62
	Ethanol	E	5.85
		B	0.19
		D	134.51
	E	2.61	
	I	0.24	

<sup>1</sup> Data are expressed as the mean ± standard deviation of three replicates.

### 3.4. Antioxidant Properties of *S. edule* Extracts

The DPPH assay is a simple and sensitive method for the determination of the radical scavenging capacity of compounds and extracts. DPPH is a cell-permeable stable free radical with a strong absorption at 517 nm (purple), while reduced DPPH, which is formed by reaction with an antioxidant, is colorless or pale yellow.

The antioxidant activities exhibited in DPPH assays by fruit extracts of *S. edule* varieties (Table 4) can be attributed to the presence of phenolic acids and polyphenols, most especially flavonoids such as quercetin and its glycoside and, to a lesser extent, rutin [21,22]. The IC<sub>50</sub> values of the methanol and ethanol extracts of *S. edule* fruits were within the range 0.45 to 0.65 mg mL<sup>-1</sup>; however, while the difference between VL extracts was statistically significant, this was not the case for NS. Lim et al. [23] compared the antioxidant potential of some tropical fruits using the DPPH test and reported that the radical scavenging capacity of common guava (*Kampuchea* cultivar GU8; *Psidium guajava* L., Myrtaceae) with seeds was higher than that of sweet orange (Valencia cultivar; *Citrus x sinensis*), as demonstrated by the lower IC<sub>50</sub> value (1.71 ± 0.61 and 5.4 ± 1.3 mg mL<sup>-1</sup>, respectively). These results suggest that the radical scavenging activities of fruits from the two varieties of *S. edule* are high in comparison with those of other fruits. However, the IC<sub>50</sub> values of extracts from different plant species can vary significantly even within the same genus, as exemplified by ethanol extracts from leaves of the annonaceous plants, *Annona squamosa* L. (sugar-apple; 0.065 mg mL<sup>-1</sup>), *A. reticulata* L. (custard-apple; 0.080 mg mL<sup>-1</sup>), and *A. muricata* (soursop; 0.070 mg mL<sup>-1</sup>) [24]. Interestingly, the methanol extract of leaves from *Calia secundiflora* (Ortega) Yakovlev, a medicinal plant from Mexico that presents insect repellent activity, exhibited an IC<sub>50</sub> of 0.109 mg mL<sup>-1</sup> [25].

**Table 4.** Percentage inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) by extracts of fruit from *Sechium edule* var. *virens levis* (VL) and *S. edule* var. *nigrum spinosum* (NS).

Variety	Extract	Percentage Inhibition of DPPH					IC <sub>50</sub> (mg mL <sup>-1</sup> )
		Concentration of Extract (mg mL <sup>-1</sup> ) <sup>1</sup>					
		5	3.33	1.67	0.83	0.417	
VL	Methanol	89.13 ± 0.7 <sup>a</sup>	83.49 ± 0.8 <sup>a</sup>	68.42 ± 0.1 <sup>a</sup>	57.65 ± 0.3 <sup>a</sup>	49.11 ± 0.5 <sup>a</sup>	0.45
	Ethanol	82.42 ± 1.6 <sup>b</sup>	75.00 ± 2.2 <sup>b</sup>	62.13 ± 1.3 <sup>b</sup>	53.92 ± 1.2 <sup>b</sup>	45.48 ± 1.8 <sup>a</sup>	0.62
NS	Methanol	82.93 ± 0.3 <sup>b</sup>	75.28 ± 0.5 <sup>b</sup>	61.66 ± 0.7 <sup>b</sup>	53.92 ± 0.7 <sup>b</sup>	45.38 ± 0.5 <sup>a</sup>	0.63
	Ethanol	74.86 ± 1.1 <sup>c</sup>	69.40 ± 0.3 <sup>c</sup>	59.19 ± 1.4 <sup>b</sup>	50.98 ± 0.5 <sup>b</sup>	47.06 ± 1.0 <sup>a</sup>	0.65

<sup>1</sup> Data are expressed as the mean ± standard deviation of three replicates. In each column, values bearing dissimilar superscript lower-case letters (a, b, c) are significantly different ( $p \leq 0.05$ ; ANOVA and the Tukey test).

The β-carotene-linoleic acid test is a simple and rapid method for screening antioxidants and relies on the oxidation of unsaturated fatty acids by peroxide, such as linoleic and arachidonic acids, that are typically present in lipid bilayer membranes [26]. Free radicals formed in such reactions initiate the oxidation and, consequently, the discoloration of β-carotene. Antioxidants present in the test sample can inhibit the oxidation process and, thereby, reduce the extent of discoloration of the assay solution.

In the present study, the percentage antioxidant activities (% AAs) of the methanol and ethanol extracts (50 mg mL<sup>-1</sup>) from both *S. edule* varieties measured at different reaction times were comparable, but notably lower than those of the positive control BHT (Table 5), which is an efficient synthetic antioxidant used in food preservation [27]. However, after 60 min of reaction, the % AAs of the fruit extracts had diminished to 80%, lower than the 90% levels previously reported for ethanol and/or water extracts of leaves and seeds of *S. edule* [14]. On the other hand, seed samples from *Capsicum baccatum* L. (sweet pepper, green and red) and *Artocarpus altilis* (bread fruit) exhibited AA values of around 67% and 54% that were lower than those of the extracts of *S. edule* fruits employed in the present study [28].

### 3.5. Juice Quality

It was observed that the juice from chayote VL has a neutral pH and a low content of total soluble solids. These characteristics remained with the addition of stevia. Since pineapple juice is characterized by a high content of sugars (13–19%) and high acidity, it modifies the properties of the chayote juice and stevia. Monday et al. (2016) evaluated the characteristics of mixtures of pineapple and orange juice at a 1:1 ratio, with a pH of 3.64, acidity of 0.89, and 13.8% total soluble solids (TSS), with a good acceptance by consumers [29]. In the chayote juices, it was observed that the addition of pineapple juice reduces the pH of the juice significantly, with an increased value of the acidity and content of TSS (Table 6). There were no significant differences observed between chayote juice alone or with stevia, in these variables, but the higher content of total phenols in those added with stevia is notable. A high antioxidant activity has been reported in stevia (*Stevia rebaudiana* Bert.), with a content of phenols of 56.73 mg g<sup>-1</sup> [30], which explains the increased values in the mixtures with stevia leaves, and a decrease of these values when the mixtures are diluted with pineapple juice.

**Table 5.** Percentage antioxidant activity (AA) of extracts of fruit from *Sechium edule* var. *virens levis* (VL) and *S. edule* var. *nigrum spinosum* (NS) as determined by the  $\beta$ -carotene-linoleic acid test.

Variety/Control	Extract/Control (Concentration)	% AA <sup>1</sup> Determined After Reaction Time (min)			
		20	60	100	140
VL	Methanol (50 mg mL <sup>-1</sup> )	117.27 ± 0.004 <sup>ab</sup>	75.48 ± 0.02 <sup>b</sup>	41.92 ± 0.02 <sup>cd</sup>	17.50 ± 0.01 <sup>cd</sup>
	Ethanol (50 mg mL <sup>-1</sup> )	119.45 ± 0.01 <sup>ab</sup>	80.72 ± 0.02 <sup>b</sup>	49.62 ± 0.02 <sup>b</sup>	26.38 ± 0.02 <sup>b</sup>
NS	Methanol (50 mg mL <sup>-1</sup> )	118.18 ± 0.02 <sup>ab</sup>	79.19 ± 0.01 <sup>b</sup>	46.48 ± 0.03 <sup>bc</sup>	24.96 ± 0.02 <sup>bc</sup>
	Ethanol (50 mg mL <sup>-1</sup> )	118.83 ± 0.02 <sup>ab</sup>	80.28 ± 0.03 <sup>b</sup>	52.57 ± 0.03 <sup>bc</sup>	31.58 ± 0.04 <sup>bc</sup>
BHT <sup>2</sup>	(0.1 mg g <sup>-1</sup> )	120.76 ± 0.03 <sup>a</sup>	88.65 ± 0.02 <sup>a</sup>	64.35 ± 0.02 <sup>a</sup>	47.42 ± 0.02 <sup>a</sup>

<sup>1</sup> Data are expressed as the mean of three replicates. <sup>2</sup> Butylated hydroxytoluene (BHT) was employed as the positive control. In each column, values bearing dissimilar superscript lower-case letters are significantly different ( $p \leq 0.05$ ; ANOVA and the Tukey test).

Regarding the juices of NS, slightly higher values of pH were observed compared to those reported for the variety VL; however, similarly, in this case, there were no significant differences between chayote juice with and without stevia in the parameters of pH, titratable acidity, and total soluble solids. The addition of pineapple juice provides a higher density and flavor to both types of juices determined by the sugars/acidity relation, which favors the acceptance by consumers. The content of total phenols in the juice of VL had a significant increased value with the addition of stevia leaves (Table 6). The presence of cucurbitacins I, E, B, and D, and flavonoids such as galangin, naringin, and myricetin, was detected in all the juices.

**Table 6.** Quality variables of chayote juice of *virens levis* (VL) and *nigrum spinosum* (NS) and its combination with stevia and pineapple juice.

Juice	pH	Titratable Acidity (%)	TSS (%)	Phenols Content mg·mL <sup>-1</sup>
VL	6.9 ± 0.1 <sup>a</sup>	0.0853 ± 0.02 <sup>b,ε</sup>	4.7 ± 0.6 <sup>b</sup>	0.134 ± 0.02 <sup>b</sup>
VL + stevia (0.7%)	6.9 ± 0.0 <sup>a</sup>	0.1280 ± 0.03 <sup>b</sup>	5.0 ± 0.0 <sup>b</sup>	0.527 ± 0.004 <sup>a</sup>
VL + stevia + pineapple (50%)	3.8 ± 0.0 <sup>b</sup>	0.4352 ± 0.02 <sup>a</sup>	10.7 ± 0.6 <sup>a</sup>	0.180 ± 0.01 <sup>b</sup>
NL	7.4 ± 0.0 <sup>a</sup>	0.1024 ± 0.02 <sup>b</sup>	6.0 ± 0.0 <sup>b</sup>	0.177 ± 0.03 <sup>b</sup>
NL + stevia (0.8%)	7.4 ± 0.0 <sup>a</sup>	0.0469 ± 0.0 <sup>b</sup>	4.7 ± 0.6 <sup>b</sup>	0.437 ± 0.004 <sup>a</sup>
NL (50%) + stevia (0.8%) + pineapple (50%)	4.3 ± 0.0 <sup>b</sup>	0.4779 ± 0.04 <sup>a</sup>	9.0 ± 0.0 <sup>a</sup>	0.254 ± 0.01 <sup>b</sup>

<sup>ε</sup> Average values with the same letter in a column are not statistically different (Tukey  $p \leq 0.05$ ).

## 4. Conclusions

Fruits from two varieties of *S. edule* differed significantly with respect to their physicochemical characteristics (total soluble solids, total sugars, pH, chlorophyll a and color). Moreover, the phenolic and flavonoid contents of the VL variety were somewhat lower compared with those of the NS variety.

Cucurbitacin D was the most abundant in both varieties, while the concentration of cucurbitacin B was much higher in NS compared with VL. The radical scavenging capacities (DPPH test) of the methanol and ethanol extracts from VL and NS varieties were comparable (IC<sub>50</sub> values in the range 0.45 mg mL<sup>-1</sup> to 0.65 mg mL<sup>-1</sup>), while the antioxidant activity ( $\beta$ -carotene-linoleic test) was approximately 80%. These results support our hypothesis that the two commercial varieties of *S. edule* are chemically and pharmacological distinct. As far as the authors are aware, this report is the first to compare the bioactivities of two commercial varieties of chayote with potential application as nutraceuticals and, in this respect, the NS variety appears to be particularly promising. Additionally, the chayote juice combined with stevia and pineapple maintains the original nutraceutical characteristics of the fruit, and can be an option to increase the consumption of this vegetable.

**Author Contributions:** María de la Luz Riviello Flores, Ma. de Lourdes Arévalo-Galarza and Jorge Cadena-Iñiguez: Design and performed the experiments; Ramón Marcos Soto-Hernández, Lucero del Mar Ruíz-Posadas and Fernando C. Gómez-Merino, participated in the corrections of the manuscript and supported in the laboratory with the experiments.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. OMS. Estrategia Mundial Sobre Régimen Alimentario, Actividad Física y Salud Fomento al Consumo Mundial de Frutas y Verduras. Hoja Informativa. 2014. Available online: <http://www.who.int/dietphysicalactivity/fruit/es/index1.html> (accessed on 31 January 2018).
2. Gonzales, G.F.; Valerio, L.G. Medicinal plants from Peru: A review of plants as potential agents against cancer. *Anticancer Agents Med. Chem.* **2006**, *6*, 429–444. [[CrossRef](#)] [[PubMed](#)]
3. Nagarani, G.; Abirami, A.; Siddhuraju, P. Food prospects and nutraceutical attributes of Momordica species: A potential tropical bioresources—A review. *Food Sci. Hum. Wellness* **2014**, *3*, 117–126. [[CrossRef](#)]
4. Yasir, M.; Sultana, B.; Nigam, P.S.; Owusu-Apenten, R. Antioxidant and genoprotective activity of selected cucurbitaceae seed extracts and LC-ESIMS/MS identification of phenolic components. *Food Chem.* **2016**, *199*, 307–313. [[CrossRef](#)] [[PubMed](#)]
5. Cadena-Iñiguez, J.; Arévalo-Galarza, L.; Avendaño-Arrazate, C.H.; Soto-Hernández, M.; Ruiz-Posadas, L.M.; Santiago-Osorio, E.; Acosta-Ramos, M.; Cisneros-Solano, V.M.; Aguirre-Medina, J.F.; Ochoa-Martínez, D. Production, genetics, postharvest management and pharmacological characteristics of *Sechium edule* (Jacq.) Sw. *Fresh Prod.* **2007**, *1*, 41–53.
6. Cadena-Iñiguez, J.; Soto Hernández, M.; Arévalo Galarza, L.; Avendaño Arrazate, C.; Aguirre Medina, J.F.; Ruiz Posadas, L. Caracterización bioquímica de variedades domesticadas de chayote (*Sechium edule* (Jacq.) Sw.) comparadas con parientes silvestres. *Rev. Chapingo Ser. Horticu.* **2011**, *7*, 45–55.
7. Leskovar, D.I.; Crosby, K.; Jiffon, J.L. Impact of agronomic practices on phytochemicals and quality of vegetable crops. *Acta Hort.* **2009**, *841*, 317–322. [[CrossRef](#)]
8. Neube, B.; Finnie, J.B.; Van Staden, J. Quality from the field: The impact of environmental factors as quality determinants in medicinal plants. *S. Afr. J. Bot.* **2012**, *82*, 11–20.
9. Association of Official Analytical Chemist (AOAC). *Official Methods of Analysis*, 12th ed.; William, H., Ed.; AOAC: Washington, DC, USA, 1980; p. 1094.
10. Kuklinski, C. *Farmacognosia, Estudio de Las Drogas y Sustancias Medicinales de Origen Natural*; Ediciones Omega: Barcelona, Spain, 2000; pp. 32–45. ISBN 84-282-1191-4.
11. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178.
12. Liu, Y.; Sun, Y.; Laura, T.; Liang, X.; Ye, H.; Zeng, X. Determination of polyphenolic content and antioxidant activity of kudingcha made from *Ilex kudingcha* C. J. Tseng. *Food Chem.* **2009**, *112*, 35–41. [[CrossRef](#)]
13. Jayaprakasam, B.; Seeram, N.P.; Nair, N.G. Anticancer and anti-inflammatory activities of cucurbitacins from *Cucurbita andreana*. *Cancer Lett.* **2003**, *189*, 11–16. [[CrossRef](#)]
14. Ordoñez, A.; Gómez, J.; Vattuone, M.; Islas, M. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food Chem.* **2006**, *97*, 452–258. [[CrossRef](#)]

15. Ordoñez, A.A.L.; Gomez, J.D.; Cudmani, N.M.; Vattuone, M.I.; Isla, M.I. Antimicrobial activity of nine extracts of *Sechium edule* (Jacq.) Swartz. *Microb. Ecol. Health Dis.* **2003**, *15*, 33–39. [[CrossRef](#)]
16. Gry, J.; Soborg, I.; Andersson, H.C. *Cucurbitacins in Plant Food*; TemaNord; Nordic Council of Ministers: Copenhagen, Denmark, 2006; p. 556. ISBN 92-893-1381-1.
17. Fatope, M.O.; Takeda, Y.; Yamashita, H.; Okabe, H.; Yamauchi, T. New cucurbitane triterpenoids from *Momordica charantia*. *J. Nat. Prod.* **1990**, *53*, 1491–1497. [[CrossRef](#)]
18. Hu, R.; Peng, Y.; Chen, B.; Chen, Y.; Hou, X. Study on Tian Gua Di (*Cucumis melo* L.), an antihepatitis Chinese medicine. Preparation and assay of Gua Di Su Cucurbitacins B E. *Zhongcaoyao* **1982**, *13*, 445–447.
19. Sturm, S.; Stuppner, H. Analysis of cucurbitacins in medicinal plants by high-pressure liquid chromatography-mass spectrometry. *Phytochem. Anal.* **2000**, *11*, 121–127. [[CrossRef](#)]
20. Dinna, L.; Harmatha, J.; Lafont, R. Chromatographic procedures for the isolation of plant steroids. *J. Chromatog. A* **2001**, *935*, 105–123. [[CrossRef](#)]
21. Rice-Evans, C.A.; Miller, N.J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.* **1996**, *20*, 933–956. [[CrossRef](#)]
22. Lien, E.J.; Ren, S.; Bui, H.; Wang, R. Quantitative structure-activity relationship analysis of phenolic antioxidants. *Free Radic. Biol. Med.* **1999**, *26*, 285–294. [[CrossRef](#)]
23. Lim, Y.; Lim, T.; Tee, T. Antioxidant properties of several tropical fruits: A comparative study. *Food Chem.* **2007**, *103*, 1003–1008. [[CrossRef](#)]
24. Baskar, R.; Rajeswari, V.; Sathish-Kumar, T. In vitro antioxidant studies in leaves of *Annona* species. *Indian J. Exp. Biol.* **2007**, *45*, 480–485. [[PubMed](#)]
25. Barrón-Yáñez, R.M.; García-Mateos, R.; Soto-Hernández, R.M.; Colinas-León, T.; Kite, G. Flavonoides y actividad antioxidante de *Calia secundiflora* (Ort.) Yakovlev. *Rev Fitotec. Mex.* **2011**, *34*, 151–157.
26. Yu, L. Free radical scavenging properties of conjugated linoleic acids. *J. Agric. Food Chem.* **2001**, *49*, 3452–3456. [[CrossRef](#)] [[PubMed](#)]
27. Potterat, O. Antioxidants and free radical scavengers of natural origin. *Curr. Org. Chem.* **1997**, *1*, 1415–1419.
28. Rincón, A.; Pérez, M.; Bou, L.; Romero, A.; Bucarito, L.; Padilla, F. Métodos para la determinación de la actividad antioxidante de vegetales. *Rev. Fac. Farm.* **2011**, *74*, 24–28.
29. Monday, A.O.; Barine, K.D.; Onyedikachi, E.C. Quality Characteristics of Orange/Pineapple Fruit Juice Blends. *Am. J. Food Sci. Technol.* **2016**, *4*, 43–47. [[CrossRef](#)]
30. Shukla, S.; Mehta, A.; Mehta, P.; Bajpai, V.K. Antioxidant ability and total phenolic content of aqueous leaf extract of *Stevia rebaudiana* Bert. *Exp. Toxicol. Pathol.* **2012**, *64*, 807–811. [[CrossRef](#)] [[PubMed](#)]



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).