Article

Nutraceutic Characteristics of The Extracts and Juice of Chayote (*Sechium edule* (Jacq.) Sw.) Fruits

María de la Luz Riviello-Flores[1], Lourdes Arévalo-Galarza[2], Jorge Cadena-Iñiguez[3], Ramón Marcos Soto-Hernández[2], Lucero del Mar Ruiz-Posadas[2], Fernando C. Gómez-Merino[4]

[1] Universidad Tecnológica de Tehuacán, 75859, Tehuacán, Puebla, México. marluriviello@gmail.com

[2]Colegio de Postgraduados, Campus Montecillo, 56230, Texcoco, México. larevalo@colpos.mx; msoto@colpos.mx; lucpo@colpos.mx; fernandg@colpos.mx

[3] Colegio de Postgraduados, Campus San Luis Potosí, 78622, Salinas de Hidalgo, San Luis Potosí, México.

[4] Colegio de Postgraduados, Campus Córdoba, 94500, Córdoba, Veracruz, México. jocadena@colpos.mx

Abstract: Fruits of chayote [*Sechium edule* (Jacq.) Swartz] are widely consumed in Mesoamerica, but little is known about the nutraceutical potential. This study aimed to determine the chemical compositions, 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, 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^{-1} dry-weight, respectively) but the total flavonoid contents were similar (27 and 26 mg 100 g^{-1} dry-weight, respectively). Cucurbitacin D was predominant in both varieties. The radical scavenging capacities of VL and NS extracts varied slightly (IC50 = 0.45 to 0.65 mg mL^{-1}), while the antioxidant activities were similar (~80%). The NS variety is particularly promising regarding nutraceutical application. The chayote juice combined with stevia and pineapple maintain the original nutraceutical characteristics from the fruit, but enhanced the organoleptic characteristics like density and sugar/acidity balance.

Keywords: Cucurbitaceae; gourd family; nutraceutical; antioxidant

 doi:10.20944/preprints201803.0061.v1

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 as, 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 variety 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 used mainly 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 β-sitosterol β-D-glucopyranoside and stigmasterol β-D-glucopyranoside [5]. Unfortunately, information regarding which chayote variety was employed in the published investigations has rarely been provided.

As a no traditional vegetable, there is not information about the way to consume this fruit, so the consumption in juice represents as a global tendency is recommended because they do not contain fat, and rich in vitamins, minerals and with phytonutrients that affect good health. Also the flavor of the fruit is neutral and easily to combined with other fruits.

Considering that the chemical constituents, particularly those with biological activity, vary greatly depending on environmental and genetic factors, we postulate that the chemical profiles and pharmacological activities of the two commercial varieties of *S. edule* are distinct.

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.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 March 2018 doi:10.20944/preprints201803.0061.v1

2. Materials and methods

2.1. Plant materials and chemical reagents

Fruits (120) from each biological variant *S. edule* var. *virens levis* (VL) and *S. edule* var. *nigrum spinosum* (NS) (Fig. 1) were harvest between 18 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. Solvents, reagents and reference standards were obtained from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated, and were used as received.

Fig. 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 mg L-1), cut into small pieces, processed in a Turmix™ (Mexico) industrial extractor and subsequently filtered. The resulting juices were stored in the freezer at -70°C until required for analysis. Total soluble solids were measured using an Atago™ (Tokyo, Japan) model PAL-1 digital refractometer according to the standard technique adopted by the Association of Official Analytical Chemists (AOAC) [7] and expressed as °Brix. Vitamin C was determined using the 2,6-dichlorophenolindophenol (DCPIP) method and concentrations (mg 100 mL^{-1}) were estimated using a calibration curve constructed using ascorbic acid as 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 (mg 100 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 (g 100 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.

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

 doi:10.20944/preprints201803.0061.v1

106 2.3. Extraction and quantitative analysis of functional compounds

107 Twenty fruits from each variety were cut into small pieces, dried in a forced-air oven at 45°C for 4
108 days until constant weight and subsequently reduced to a fine powder using a General Electric mill
109 (Fairfield, CT, USA) model AC-160. A portion (200 g) of the powder was extracted exhaustively (15
110 times) with methanol for 48 h at room temperature (20 ± 2°C). After each extraction, the liquid phase
111 was separated from the solid material by decantation and filtration and new solvent was added. The
112 solid residue was subsequently extracted 12 times with ethanol in a similar manner. The bulked
113 methanol and ethanol extracts were dried separately under reduced pressure using a Büchi (Flawil,
114 Switzerland) Rotavapor™ R-114 at 45°C [8].

115 Total phenolics were quantified using the Folin-Ciocalteu method [9]. Briefly, samples (10 mg) of
116 dried methanol and ethanol extracts were resuspended separately in 1 mL distilled water. Aliquots
117 (30 µL) of these solutions were transferred to test tubes together with 470 µL of distilled water, 25 µL
118 of 2M Folin-Ciocalteu reagent in distilled water (1:1; v/v) and 975 µL of 2.5% sodium carbonate
119 solution. After homogenization, the samples were incubated in the dark for 1 h and the absorbances
120 measured at 740 nm. The concentrations of total phenols were estimated by means of a calibration
121 curve constructed with gallic acid as reference standard. For the juice, 1 mL of the juice was diluted
122 with 1 mL distilled water, was mixed in a vortex by 5 seconds and then was centrifuged to 13000 xg
123 by 1 min. For the juice combine with pineapple juice, 500 µL of the supernatant was taken, and 500
124 µL de distilled water was added. Then 30 µL of the supernatant was taken and 470 µL of destilled
125 water, and the above procedure was follow.

126 Quantitation of flavonoids: samples of 50 mg of dried methanol and ethanol extracts were disolved
127 in 1 mL of 80 % methanol and transferring aliquots (20 µL) to test tubes containing 900 µL of 80 %
128 methanol, 2 mL of 1M potassium acetate solution and 2 mL of 10 % aluminum chloride solution.
129 After homogenization, the samples were incubated in the dark for 1 h and the absorbances measured
130 at 415 nm. The concentrations of flavonoids were estimated by means of a calibration curve
131 constructed with quercetin as reference standard.

132

133 2.4. Separation and identification of flavonoids and cucurbitacins by high performance liquid
134 chromatography (HPLC)

135 Samples (20 mg) of dried methanol and ethanol extracts were disolved separately in 2 mL of 80%
136 methanol and the solutions filtered through 0.45 µm Acrodisc® syringe filters with nylon membrane
137 (Sigma-Aldrich) prior to analysis. Chromatography was performed using an Agilent Technologies
138 (St. Clara, CA, USA) Infinity series 1220 instrument equipped with a Thermo Fisher Hypersyl™ ODS
139 C18 column (125 x 4 mm; 5 µm particle size). Flavonoids were analyzed at 30°C under isocratic
140 elution with a mobile phase comprising water: acetonitrile (65:35; v/v), with pH adjusted to 2.5 with
141 trifluoroacetic acid, supplied at a flow rate of 1 mL min^{-1} (179 bar pressure). The sample injection
142 volume was 20 µL, the detection wavelength was 235 nm, and the standard reference compounds
143 employed were rutin, phloretin, phlorizidin, myricetin, quercetin, naringenin and galangin.
144 Cucurbitacins were analyzed at 25°C using the instrument specified above equipped with a Waters
145 (Milford, MA, USA) Symmetry Shield RP18 column (250 x 4.4 mm i.d.; 5 µm particle size). Isocratic
146 elution was carried out with a mobile phase comprising water: methanol: acetonitrile (50:30:20;
147 v/v/v) supplied at a flow rate of 1 mL min-1 (179 bar pressure). The sample injection volume was 20
148 µL, the detection wavelength was 235 nm, and the standard reference compounds employed were
149 cucurbitacins B, D, E and I.

150

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 March 2018 doi:10.20944/preprints201803.0061.v1

151 2.5. Evaluation of antioxidant properties

152 The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was employed to evaluate
153 antioxidant activities as previously described [10]. Aliquots (500 µL) of solutions containing crude
154 methanol and ethanol extracts at concentrations 2.5, 5, 10, 20 and 30 mg mL^{-1} were transferred to test
155 tubes and mixed with 500 µL of methanol and 2 mL of 0.1 mM DPPH solution in methanol. The
156 reaction mixtures were incubated for 30 min at room temperature in the dark and absorbances were
157 the measured at 517 nm. The control comprised 0.1 mM DPPH solution without extract and the
158 blank was pure methanol. All measurements were performed in duplicate. Percentage DPPH
159 inhibition was calculated according to equation 2 in which A0 is the absorbance of 0.1 mM DPPH
160 solution and A1 is the absorbance of 0.1 mM DPPH solution containing the sample.

161 DPPH inhibition (%) = [(A_0-A_1)/A_0] × 100 Eq. 2

162 The concentration of extract required to scavenge 50% of DPPH radicals (IC50 value) was
163 established from dose response data by linear regression.

164 Lipid peroxidation was assessed using the β-carotene-linoleic acid assay as described previously
165 [11,12] but with slight modifications. The reagent solution, containing 0.02 mL of linoleic acid, 0.2
166 mL of Tween-20 and 1 mL of β-carotene solution (0.2 g mL^{-1}) in chloroform, was prepared by
167 homogenizing the components in a 50 mL round-bottomed flask and removing the chloroform.
168 Hydrogen peroxide (25 mL) was added to the flask, the whole mixed thoroughly and the absorbance
169 measured at 470 nm against a blank of reagent solution prepared in the same manner but without
170 β-carotene. Subsequently, aliquots (4.8 mL) of the reagent solution were transferred to test tubes and
171 samples (0.2 mL) of methanol and ethanol extracts at two different concentrations (10 and 50 mg
172 mL^{-1}) were added separately. Positive and negative controls were prepared in exactly the same
173 manner except that butylated hydroxytoluene (BHT; 0.1 mg g^{-1}) and methanol, respectively, replaced
174 the plant extracts. Assay mixtures and controls were stirred thoroughly for 2 min and then incubated
175 at 50°C for 140 min to induce thermal oxidation. Absorbances (470 nm) of the assay mixtures were
176 monitored at 0, 20, 60,100 and 140 min of reaction time. All measurements were performed in
177 triplicate. Percentage antioxidant activity (% AA) was calculated according to equation 3 in which
178 A0 (A00) and At (A0t) are the absorbances of the test sample (control) at times 0 and t, respectively.

179 Antioxidant activity (%) = [1-(A_0-A_t/A_00 -A_0t)]× 100 Eq. 3

180

181 Juice elaboration

182 Fruits of both varieties of chayote were ground in a food processor, to get the juice that was mixtures
183 with dry and milled leaves of stevia (*Stevia rebaudiana*) in a ratio of 0.7 % (p/v) in *virens levis* and 0.8 %
184 (p/v) in *nigrum spinosum*, and well the addition of 50 % pineapple juice (*Ananas comosus*) (v/v). The
185 fruits of chayote and pineapple were selected and were washed with 100 mg L^{-1} hypochlorite
186 solution and rinsed thoroughly with distilled water. On the pineapple was peeled with stainless
187 knife, cut into small pieces and processed in a juice extractor (Turmix™) with filtration system.

188 The variables were defined after conduct a tasting panel, where the main characteristic was the not
189 detection of bitter taste characteristic of stevia. In total were evaluated three treatments by variety of
190 chayote with three replicates by treatment. Later on the juices were pasteurized at 60 °C by 30
191 minutes, in an incubator (Felisa®), and immediately chilled at 6 °C, after the quality evaluation was
192 perform at room temperature.

193

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 March 2018 doi:10.20944/preprints201803.0061.v1

194 2.7 Statistical analysis

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

198

199 **3. Results and discussion**

200 3.1. Characteristics of fruit juices

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

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

210

Variable[1]	Variety	
	NS	VL
Total soluble solids (%)	5.1 ± 0 [a]	4.3 ± 0 [b]
Total sugars (g 100 g^{-1})	3.6 ± 0.3 [a]	2.03 ± 0 [b]
pH	6.8 ± 0 [a]	6.0 ± 0 [b]
Vitamin C (mg 100 mL^{-1})	2.72 ± 0.26 [a]	3.24 ± 0 [a]
Chlorophyll *a* (mg 100 mL^{-1})	4.0 ± 0.5 [a]	2.0 ± 0.6 [b]
Chlorophyll *b* (mg 100 mL^{-1})	5.0 ± 0.5 [a]	4.0 ± 0.5 [a]
Color index	-26.08 ± 1.2 [b]	-16.4 ± 0.12 [a]

211 [1] Data are expressed as the mean ± standard deviation of three replicates. In each row, values
212 bearing dissimilar superscript lower-case letters are significantly different ($P \leq 0.05$; ANOVA and the
213 Tukey test).

214

215 Cadena-Iñiguez et al. [6] reported vitamin C contents of 4.95 ± 0.49 and 6.76 ± 0.16 mg 100 g^{-1},
216 respectively, for the NS and VL varieties of *S. edule*. While these values appear to be appreciably
217 higher than those obtained in the present study, it should be noted that the earlier investigation was
218 performed using fruit pulp rather than fruit juice, and it is known that vitamin C degrades rapidly in
219 solution, especially on exposure to light and at increased temperature or pH. The difference in
220 source material would also explain the higher values reported previously [6] in pulp for the content
221 of chorophyll a and b, i.e. 8.4 and 9.2 mg 100 g^{-1}, respectively, for the NS variety and 6.0 and 7.1 mg
222 100 g^{-1}, respectively, for the VL variety.

223

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 March 2018 doi:10.20944/preprints201803.0061.v1

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^{-1} in green fruit up to ~ 202 mg L^{-1} 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 II). 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^{-1} dry weight) than that of VL fruit (856 mg 100 g^{-1} dry weight). A previous report [14] stated that the phenolic contents of the leaves, stems and seeds of an unidentified variety of *S. edule* were within the respective ranges 0.15 to 2.06, 0.06 to 2.81, and 0.13 to 5 mg g^{-1} depending on the method of extraction employed.

Table II. 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[1] (mg 100 g^{-1} dry weight)	Flavonoids[1] (mg 100 g^{-1} dry weight)
VL	Methanol	489 ^{a}	10 ^{bc}
	Ethanol	367 ^{b}	16 ^{ab}
NS	Methanol	525 ^{a}	19 ^{a}
	Ethanol	480 ^{a}	8 ^{c}

[1] Data are expressed as the mean of three replicates.

In each column, values bearing dissimilar superscript lower-case letters 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 II). However, the overall flavonoid contents of the two varieties were similar, with that of NS being slightly higher in comparison with VS (27 and 26 mg 100g^{-1} 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].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 March 2018 doi:10.20944/preprints201803.0061.v1

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 therapeutical 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 III). Cucurbitacin D was the most abundant member of this class of secondary compound 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 $100g^{-1}$ 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 [15]. Fatope et al. [16] 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 an liver protector agent [17]. 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 [11].

Table III. 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^{-1} dry weight[1]
VL	Methanol	D	353.41
		E	0.33
	Ethanol	B	0.16
		D	395.48
		E	3.25
		I	0.33
NS	Methanol	B	24.62
		E	5.85
	Ethanol	B	0.19
		D	134.51
		E	2.61
		I	0.24

[1] Data are expressed as the mean of three replicates.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 March 2018 doi:10.20944/preprints201803.0061.v1

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 IV) 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 [18,19]. The IC50 values of the methanol and ethanol extracts of *S. edule* fruits were within the range 0.45 to 0.65 mg mL^{-1}; however, while the difference between VL extracts was statistically significant, this was not the case for NS. Lim et al. [20] 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 IC50 value (1.71± 0.61 and 5.4 ± 1.3 mg mL^{-1}, 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_{50} 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^{-1}), *A. reticulata* L. (custard-apple; 0.080 mg mL^{-1}) and *A. muricata* (soursop; 0.070 mg mL^{-1}) [21]. Interestingly, the methanol extract of leaves from *Calia secundiflora* (Ortega) Yakovlev, a medicinal plant from Mexico that presents insect repellent activity exhibited an IC50 of 0.109 mg mL^{-1} [22].

The β-carotene-linoleic acid test is a simple and rapid method for screening antioxidants and relies on oxidation by peroxide of unsaturated fatty acids, such as linoleic and arachidonic acids, that are typically present in lipid bilayer membranes [23]. 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.

Table IV. 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_{50}
		Concentration of extract (mg mL^{-1})[1]					(mg mL^{-1})
		5	3.33	1.67	0.83	0.417	
VL	Methanol	89.13 [a]	83.49 [a]	68.42 [a]	57.65 [a]	49.11 [a]	0.45 [b]
	Ethanol	82.42 [b]	75.00 [b]	62.13 [b]	53.92 [b]	45.48 [a]	0.62 [a]
NS	Methanol	82.93 [b]	75.28 [b]	61.66 [b]	53.92 [b]	45.38 [a]	0.63 [a]
	Ethanol	74.86 [c]	69.40 [c]	59.19 [b]	50.98 [b]	47.06 [a]	

[1] Data are expressed as the mean of three replicates.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 March 2018 doi:10.20944/preprints201803.0061.v1

In the present study, the percentage antioxidant activities (% AAs) of the methanol and ethanol extracts (50 mg mL^{-1}) from both *S. edule* varieties measured at different reaction times were comparable, but notably lower than those of the positive control BHT (Table V), which is an efficient synthetic antioxidant used in food preservation [24]. 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* [12]. 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 [25].

Table V. 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 β-carotene-linoleic acid test.

Variety / control	Extract / control (concentration)	% AA[1] determined after reaction time (min)			
		20	60	100	140
VL	Methanol (50 mg mL^{-1})	117.27 ^{ab}	75.48 ^{b}	41.92 ^{cd}	17.50 ^{cd}
	Ethanol (50 mg mL^{-1})	119.45 ^{ab}	80.72 ^{b}	49.62 ^{b}	26.38 ^{b}
NS	Methanol (50 mg mL^{-1})	118.18 ^{ab}	79.19 ^{b}	46.48 ^{bc}	24.96 ^{bc}
	Ethanol (50 mg mL^{-1})	118.83 ^{ab}	80.28 ^{b}	52.57 ^{bc}	31.58 ^{bc}
BHT[2]	(0.1 mg g^{-1})	120.76 ^{a}	88.65 ^{a}	64.35 ^{a}	47.42 ^{a}

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

3.5 Juice quality

It was observed that the juice of chayote *virens levis* has a neutral pH and a low content of total soluble solids. These characteristics maintained with the addition of stevia. By the way the pineapple juice, is characterized by a high content of sugars (13-19 %) and high acidity, modify the properties of the juice of chayote and stevia. Monday et al., (2006) evaluated the characteristics of mixtures of juice of pineapple and orange in a ratio 1:1, with a pH de 3.64, and an acidity of 0.89 and 13.8 % total soluble solids (TSS), with a good acceptation by the consumers [31]. In the juices of chayote, it was observed that the addition of pineapple juice reduces significantly the pH of the juice with an increased value of the acidity and content of TSS (Table VI). There were not observed significant differences between the juice of chayote alone or with stevia, in these variables, but it is notable the higher content of total phenols in those added with stevia. It has been reported a high antioxidant activity in stevia (*Stevia rebaudiana* Bert.) and a content of phenols of 56.73 mg g-1, was reported [32], and this explain the increased values in the mixtures with leaves of stevia, and a diminution of these values when are diluted with pineapple juice.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 March 2018 doi:10.20944/preprints201803.0061.v1

345 **Table VI.** Quality variables of chayote juice of *virens levis* and its combination with stevia and
346 pineapple juice.

Juice	pH	Titratable acidity (%)	TSS (%)	Phenols content mg·mL^{-1}
virens levis	6.9 a	0.0853 b	4.7 b	0.134 b
virens levis + stevia (0.7 %)	6.9 a	0.1280 b	5.0 b	0.527 a
virens levis + stevia + pineapple (50%)	3.8 b	0.4352 a	10.7a	0.180 b

347 £Average values with the same letter in a column are not statistically different (Tukey p≤0.05).
348

349 In connection with the juices of *nigrum spinosum*, it was observed values slightly higher of pH to
350 those reported for the variety *virens levis*, however in the same way in this case there were not
351 significant differences between the juice of chayote with and without stevia in those parameter of
352 pH, titratable acidity and total soluble solids. The addition of pineapple juice provides to both type
353 of a juice, higher density and flavor, determined by the relation sugars/acidity, this favors the
354 acceptation of the consumers. The content of total phenols in the juices of virens levis had in
355 significant increased value with the addition of stevia leaves (Table VII).

356 In all the juices it was detected the presence of cucurbitacins I, E, B y D and flavonoids as galangin,
357 naringin y myricetin.

358

359 **Table VII.** Quality variables of the juice of chayote *nigrum spinosum* and its combination with stevia
360 and pineapple**.**

Juice	pH	Titratable acidity	TSS (%)	Phenols content mg·mL^{-1}
nigrum spinosum	7.4 a	0.1024 b	6.0 b	0.177 b
nigrum spinosum + stevia (0.8 %)	7.4 a	0.0469 b	4.7 b	0.437 a
nigrum spinosum (50 %) + stevia (0.8 %) + pineapple (50 %)	4.3 b	0.4779 a	9.0 a	0.254 b

361 £ Average values with the same letter in a column are not statistically different (Tukey p≤0.05).
362
363

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 capacity (DPPH test) of the methanol and ethanol extracts from VL and NS varieties were comparable (IC50 values in the range 0.45 to 0.65 mg mL-1), while the antioxidant activity (β-carotene-linoleic test) was approximately 80%. These results support our hypothesis that the varieties of 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.

References

[1] OMS. Estrategia mundial sobre régimen alimentario, actividad física y salud Fomento al consumo mundial de frutas y verduras. Hoja Informativa. (2014). http://www.who.int/dietphysicalactivity/fruit/es/index1.html

[2] G.F. Gonzales, L.G. Valerio, Medicinal plants from Peru: a review of plants as potential agents against cancer. Anticancer Agents Med. Chem. 6 (2006) 429-444.

[3] G. Nagarani, A. Abirami, P. Siddhuraju, Food prospects and nutraceutical attributes of Momordica species: A potential tropical bioresources – A review. Food Science and Human Wellness 3 (2014) 117–126.

[4] M. Yasir, B. Sultana, P.S. Nigam, R. Owusu-Apenten, Antioxidant and genoprotective activity of selected cucurbitaceae seed extracts and LC–ESIMS/MS identification of phenolic components. Food Chemistry 199 (2016) 307–313

[5] J. Cadena-Iñiguez, L. Arévalo-Galarza, C. H. Avendaño-Arrazate, M. Soto-Hernández, L. M. Ruiz-Posadas, E. Santiago-Osorio, M. Acosta-Ramos, V. M. Cisneros-Solano, J. F. Aguirre-Medina, D. Ochoa-Martínez, Production, genetics, postharvest management and pharmacological characteristics of *Sechium edule* (Jacq.) Sw. Fresh produce 1 (2007) 41-53.

[6] J. Cadena-Iñiguez, M. Soto Hernández, L. Arévalo Galarza, C. Avendaño Arrazate, J.F. Aguirre Medina, L. Ruiz Posadas, Caracterización bioquímica de variedades domesticadas de chayote (*Sechium edule* (Jacq.) Sw.) comparadas con parientes silvestres. Revista Chapingo Serie Horticultura 7 (2011) 45-55.

[7] AOAC. Association of Official Analytical Chemist. In: Official Methods of Analysis. William Horwitz (ed). 12th edition. Washington, D. C., 1980, 1094 p.

[8] C. Kuklinski, Farmacognosia, estudio de las drogas y sustancias medicinales de origen natural. Edición Omega. Barcelona, 2000, p. 32-45.

[9] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventos, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: PACKER, L. (ed.) Oxidants and Antioxidants, Pt A. 1999. p.

[10] L. Liu, Y. Sun, T. Laura, X. Liang, H. Ye , X. Zeng, Determination of polyphenolic content and antioxidant activity of kudingcha made from *Ilex kudingcha* C. J. Tseng. Food Chem. 112 (2009) 35-41.

[11] B. Jayaprakasam, N.P. Seeram, M.G. Nair, Anticancer and antiflamatory activities of cucurbitacins from *Cucurbita andreana*. Cancer letters. 189 (2003) 11 16.

[12] A. Ordoñez, J. Gómez, M. Vattuone M. Islas, Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chemistry. 97 (2006) 452 – 258

[13] W.E. Kueng, E. Silver, V. Eppnberg, Quantification of cell cultured on 96-wels plates. Analytical Biochem. 186 (1989) 16-19.

[14] A. A. L. Ordoñez, J.D. Gomez, N.M. Cudmani, M.A. Vattuone, M.I. Isla, M.I.. Antimicrobial activity of nine extracts of *Sechium edule* (Jacq.) Swartz. Microb. Ecol. Health Dis. 15 (2003) 33-39.

[15] J. Gry, I. Soborg, H.C. Andersson, Cucurbitacins in plant food. TemaNord 2006:556. ISBN 92-893-1381-1.

[16] M.O. Fatope, Y. Takeda, H. Yamashita, H. Okabe, T. Yamauchi, New cucurbitacine triterpenoids from *Momordica charantia*. J. Natural Products. 53 (1990) 1491-1497.

[17] R. Hu, Y. Peng, B. Chen, Y. Chen, X. Hou, Study on Tian Gua Di (*Cucumis melo* L.), an antihepatitis chinese medicine. Preparation and assay of Gua Di Su and cucurbitacins B and E. Zhongcaoyao. 13 (1982) 445-447.

[18] C.A. Rice-Evans, N.J. Miller, G. Paganga, Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20 (1996) 933-956.

[19] E.J. Lien, S. Ren, H. Bui, R. Wang, Quantitative structure-activity relationship analysis of phenolic antioxidants. Free Radic. Biol. Med. 26 (1999) 285-294.

[20] Y. Lim, T. Lim, T. Tee. Antioxidant properties of several tropical fruits: a comparative study. Food Chem. 103 (2007) 1003-1008.

[21] R. Baskar, V. Rajeswari, T. Sathish-Kumar, In vitro antioxidant studies in leaves of Annona species. Indian J. Exp. Biol. 45 (2007) 480-485.

[22] R.M. Barrón-Yánez, R. García-Mateos, M. Soto-Hernández, T. Colinas-León, G. Kite, Flavonoides y actividad antioxidante de *Calia secundiflora* (Ort.) Yakovlev. Revista Fitotecnia Mexicana. 34 (2011) 151-157.

[23] L. Yu, Free radical scavenging properties of conjugated linoleic acids. Journal of Agricultural and Food Chemistry. 49 (2001) 3452-3456.

[24] O. Potterat, Antioxidants and free radical scavengers of natural origin. Current Organic Chemistry. 1997 p. 1415–1419.

[25] A. Rincón, M. Pérez, L. Bou, A. Romero, L. Bucarito, F. Padilla, Métodos para la determinación de la actividad antioxidante de vegetales. Revista Facultad de Farmacia 74(2011) 24-28.

[26] M. J. Simirgiotis, S. Adachi, S. To, H. Yang, K. A. Reynertson, M. J. Basile, R. R. Gil, I. B. Weinstein, E. J. Kennelly, Cytotoxic chalcones and antioxidants from the fruits of *Syzygium samarangense* (Wax Jambu). Food Chem. 107 (2008) 813-819.

[27] M. Verma, S. K. Singh, S. Bhushan, V. K. Sharma, P. Datt, B. K. Kapahi, A. K. Saxena, In vitro cytotoxic potential of *Polyalthia longifolia* on human cancer cell lines and induction of apoptosis through mitochondrial-dependent pathway in HL-60 cells. Chemico-Biological Interactions 171 (2008) 45-56.

[28] S. C. Chu, S.F. Yang, S.J. Liu, W.H. Kuo, Y.Z. Chang, Y.S. Hsieh, In vitro and in vivo antimetastatic effects of *Terminalia catappa* L. leaves on lung cancer cells. Food Chem. Toxicol. 45 (2007) 1194-1201.

[29] L. Yu, M. Zhao, J.S. Wang, C. Cui, B. Yang, Y. Jiang, Q. Zhao, Antioxidant, immunomodulatory and anti-breast cancer activities of phenolic extract from pine (*Pinus massoniana* Lamb) bark. Innov. Food Sci. Emerg. Technol. 9 (2008) 1122–1128.

[30] T.H. L. Wu, L. P. Chow, J. Y. Lin, Sechiumin, a ribosome inactivating protein from the edible gourd, *Sechium edule* Swartz purification, characterization, molecular cloning and expression. Chem. Pharma. Bull. 255 (1998) 400-408.

[31] A. O., Monday, K.D. Barine, E.C. Onyedikachi. 2016. Quality Characteristics of Orange/Pineapple Fruit Juice Blends. American Journal of Food Science and Technology 4 (2): 43-47.

[32] S. Shukla, A. Mehta, P. Mehta, V. K. Bajpai. 2012. Antioxidant ability and total phenolic content of aqueous leaf extract of *Stevia rebaudiana* Bert. Exp Toxicol Pathol. 64 (7-8): 807-811. doi: 10.1016/j.etp.2011.02.002.