

# Physicochemical characterisation of the mature-green Golden apple (*Spondias cytherea* Sonnerat)

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## Physicochemical characterisation of the mature-green Golden apple (*Spondias cytherea* Sonnerat).

**Abstract — Introduction.** Mature-green Golden apples (*Spondias cytherea* Sonnerat) are used in the French West Indies for the preparation of a nectar. With the aim of improving the fruit processing conditions for a better quality of this beverage, morphological and compositional characteristics of the mature-green fruits were determined. **Materials and methods.** Mature-green fruits were harvested in Martinique (French West Indies) and characterised: diameter, length, mass, and colour were determined; pH, titratable acidity, soluble solids, sugars, proteins, starch, fibre, vitamin C, phenols and green pigments were measured. **Results and discussion.** Average fruit length, diameter and weight were 71 mm, 54 mm and 116.4 g, respectively. With a low pH (2.6) and a high titratable acidity (1.3 g citric acid Eq·100 g<sup>-1</sup> fresh material), mature-green Golden apple is an acidic fruit comparable to the lemon. The fruit is rich in vitamin C (52.0 mg·100 g<sup>-1</sup>), phenols (349.5 mg gallic acid Eq·100 g<sup>-1</sup>) and starch (7.1 g·100 g<sup>-1</sup>). The pale green colour of the pulp is due to pheophytins a and b. **Conclusion.** Our study provided a detailed analysis of mature-green Golden apples, which will be taken into account for the improvement of nectar processing conditions.

France / Guadeloupe / *Spondias cytherea* / Anacardiaceae / fruits / chemical composition / ascorbic acid / starch / phenols / chlorophylls / pheophytins

## Caractérisation physico-chimique de la prune de Cythère mature verte (*Spondias cytherea* Sonnerat).

**Résumé — Introduction.** La prune de Cythère verte mature (*Spondias cytherea* Sonnerat) est utilisée aux Antilles françaises pour la préparation d'un nectar. Les caractéristiques morphologiques et la composition de ce fruit ont été déterminées avec l'objectif d'améliorer à terme ses conditions de transformation pour une meilleure qualité de la boisson obtenue. **Matériel et méthodes.** Des fruits verts matures ont été récoltés en Martinique (Antilles françaises) et caractérisés : le diamètre, la longueur, le poids et la couleur ont été déterminés ; le pH, l'acidité titrable, les solides solubles, les sucres, les protéines, l'amidon, les fibres, la vitamine C, les phénols et les pigments verts ont été mesurés. **Résultats et discussion.** Les longueur, diamètre et poids moyen des fruits sont respectivement de 71 mm, 54 mm et 116,4 g. Avec un pH faible (2,6) et une acidité titrable élevée (1,3 g Eq acide citrique·100 g<sup>-1</sup> matière fraîche), la prune de Cythère verte est un fruit acide comparable au citron. Le fruit est riche en vitamine C (52,0 mg·100 g<sup>-1</sup>), en phénols (349,5 mg Eq acide gallique·100 g<sup>-1</sup>) et en amidon (7,1 g·100 g<sup>-1</sup>). La couleur vert pâle de la pulpe industrielle est due aux phéophytines a et b. **Conclusion.** Notre étude a fourni une analyse détaillée de prunes de Cythère vertes matures qui sera prise en compte pour améliorer les conditions de leur transformation en nectar.

France / Guadeloupe / *Spondias cytherea* / Anacardiaceae / fruits / composition chimique / acide ascorbique / amidon / phénol / chlorophylle / phéophytine

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## 1. Introduction

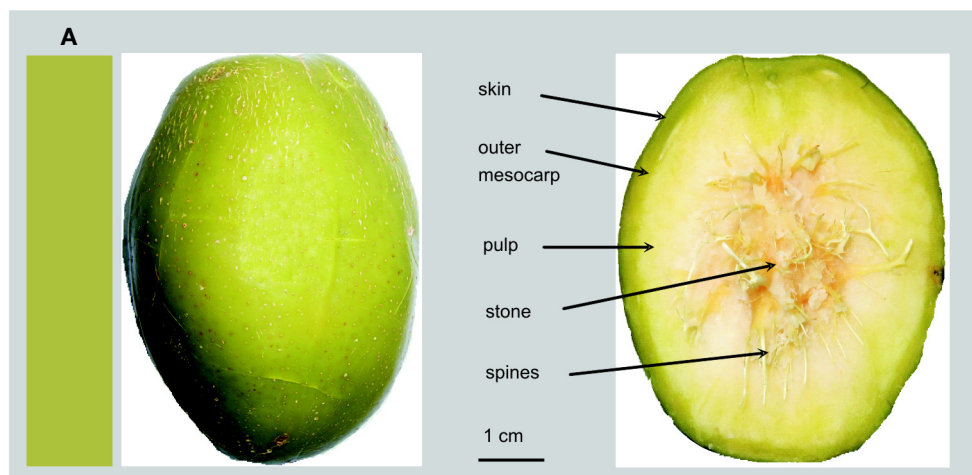
The Golden apple (*Spondias cytherea* Sonnerat or *Spondias dulcis* Forst.), also called Otaheite apple, a native of Polynesia, belongs to the Anacardiaceae family which includes the mango, *Mangifera indica* L., and the cashew, *Anacardium occidentale* L. [1]. It is mainly cultivated in the Caribbean region, Asia, Central America, South America and, in a lesser proportion, in Africa. This wide distribution of the fruit is reflected by a multitude of vernacular names according to the considered area: e.g., “pomme-Cythère” in Trinidad and Tobago, St. Lucia and Dominica, “prune de Cythère” in the French West Indies, “june plum” in Jamaica and Grenada, “ciruela dulce” in Cuba, “càja-manga” in Brazil, “ambarella” in Florida and “jobo” in India [2, 3].

The fruit is an ovoid drupe with a spiny stone (*figure 1*). Two forms of Golden apples are encountered: the large type (diameter 5–6 cm, length 9–10 cm, average weight 200 g), and the miniature type (diameter 4–5 cm, length 5–6 cm, average weight 65 g) [4]. The growth and development to maturity of the miniature Golden apple was studied by Graham *et al.* [4], showing that the harvest maturity or the mature-green stage of development is attained after 19 to 21 weeks after fruit-set. From this point to the fully ripe stage, the skin turns progressively from green to bright yellow to orange, and the flesh from whitish to yellow [2].

The ripe fruits are used to make jams, preserves, juices [5], and sparkling beverages [6]. The mature-green fruits are also used for pickling and relish dishes. Geographically more restricted is the use of mature-green fruits to elaborate a drink much appreciated by the consumers of the French West Indies (Martinique and Guadeloupe) and Grenada due to its slight acidity and astringency, and its olive green colour provided by the green pigments entrapped in the outer layers of the fruit (*figure 1*). Briefly, fruits are washed, crushed, pressed, and the crude pulpy juice is sieved prior to conditioning as a nectar (addition of sucrose and water), pasteurisation, and bottling (François-Haugrin, pers. commun., 2003). However, this drink elaborated with mature-green Golden apples contains starch which alters the visual appearance of the drink since, on one hand, a white sediment could form in storage on the container bottom, and on the other hand, the whiteness provided by starch makes the olive green colour paler; moreover, pasteurisation of the drink partially destroys the olive green colour of the nectar. Consequently, restoring of this characteristic colour is achieved by adding a chlorophyll extract from corn leaves (François-Haugrin, pers. commun., 2003). Moreover, as pasteurisation is not well controlled, this drink has a short shelf life that limits its export to non-ethnic markets.

In order to improve the quality of the drink, it has been suggested to use commercial

**Figure 1.** Structure of the mature-green Golden apple. (A) skin colour reconstituted from measured ( $L^*$ ,  $a^*$ ,  $b^*$ ) parameters (*table 1*) (see colour picture on *Fruits'* website).



enzymes which would degrade both cell-walls and starch to, on one hand, increase the juice extraction yield, and on the other hand, get rid of starch, a source of visual defect. A previous study showed that a commercial amyloglucosidase allowed total degradation of the mature-green Golden apple starch into glucose, thus increasing the total soluble solids [7].

Thus, the objective of our study was to measure the physical and physicochemical characteristics of a population of mature-green Golden apples representative of fruits used in the French West Indies for the nectar elaboration. Based on these results, enzymatic treatments could thereby be designed.

## 2. Materials and methods

### 2.1. Plant material

One hundred and forty-three (143) sound mature-green to turning-green Golden apples were harvested on several trees in domestic orchards in Martinique (French West Indies), cleaned, and washed. Fruit description is presented in *figure 1*. Amongst them, 79 mature-green Golden apples were sorted out by visual appreciation of the skin colour; then 15 fruits were randomly chosen and 5 batches of 3 fruit each were created ( $G_1$  to  $G_5$ ). Fruits were frozen ( $-20\text{ }^\circ\text{C}$ ) before analysis.

### 2.2. Physical methods of analysis

The physical analyses were performed on whole fresh fruits. Size (mm) was measured with a calliper (Mitutoyo Digimatic model CD-15B). Mass (g) was measured on each fruit with a Sartorius balance (precision 0.1 g). Colour of the skin ( $L^*$ ,  $a^*$ ,  $b^*$ ) was determined using a tristimulus Minolta CR-200 chromameter (Minolta Corp., Ramsey, N.J., USA); the true colour was reconstituted taking into account measured ( $L^*$ ,  $a^*$ ,  $b^*$ ) values using the software EasyRGB-PC Ver. 1.20 [8] (*figure 1*).

### 2.3. Physicochemical methods of analysis

Fruits were stoned, cut into pieces, freeze-dried, then pulverised in liquid nitrogen

with a Danguoumeau 300 ball mill (Prolabo, Paris, France) for 5 min (top impact frequency). Water content was thus deduced by the difference between fresh and freeze-dried matters. Total soluble solids, pH and titratable acidity were measured after reconstituting the fresh initial flesh by addition of an appropriate amount of distilled water. All data were expressed on a fresh weight basis.

A pH meter (pH-Vision 6071, Jenco Elec. Ltd, Taiwan) was used for measuring pH. Total soluble solids ( $^\circ\text{Brix}$ ) were measured at room temperature on centrifuged juice using an Abbe refractometer with a measuring range of  $0\text{--}30\text{ }^\circ\text{Brix}$  [9]. Titratable acidity ( $\text{g citric acid Eq}\cdot 100\text{ g}^{-1}$ ) was estimated by titration with a  $0.1\text{ M NaOH}$  up to pH 8.1 using phenolphthalein as indicator [9]. Proteins ( $\text{N} \times 6.25$ ) were determined by the Kjeldahl procedure [10]. Sugars and organic acids were simultaneously determined by HPLC [11] using a cation exchange ION-300 column ( $300\text{ mm} \times 7.8\text{ mm i.d.}$ , Mandel Scientific Co., Rockwood, Canada); sugars and organic acids were eluted at  $0.4\text{ mL}\cdot\text{min}^{-1}$  with  $0.0065\text{ N H}_2\text{SO}_4$  at  $25\text{ }^\circ\text{C}$ , and monitored with a differential refractive index detector (model R410; Waters Associates, Milford, USA). Starch was measured by the UV-method [12] with correction for endogenous glucose. The fibre constituents (cellulose, hemicelluloses and lignin) were determined by the method of Van Soest and Roberston [13]. Ashes were obtained by heating samples at  $100\text{ }^\circ\text{C}$  (12 h), then  $525\text{ }^\circ\text{C}$  (12 h) [9]. Vitamin C was measured by the 2,6-dichloroindophenol method [14].

### 2.4. Determination of phenols

Phenols were extracted from freeze-dried powder (100 mg) by stirring in 70% acetone (10 mL); after 10 min, the slurry was homogenised with an Ultra Turrax and left under stirring for an additional 3 min. The slurry was then filtered on a Whatman filter paper (No. 1), and the filtrate was tested for phenols and interfering substances, *e.g.*, ascorbic acid, by the Folin-Ciocalteu reagent [15]. The actual phenol content was expressed as  $\text{mg gallic acid Eq}\cdot 100\text{ g}^{-1}$  fresh material after correction for vitamin C.

**Table I.**  
Physical characterisation of mature-green Golden apples (*Spondias cytherea* Sonnerat).

Size (mm)		Weight (g)	Colour		
Length	Diameter		$a^*$	$b^*$	$L^*$
71.0 ( $\pm$ 5.3)	54.0 ( $\pm$ 4.8)	116.4 ( $\pm$ 25.6)	-12.2 ( $\pm$ 1.7)	+36.0 ( $\pm$ 1.8)	+51.7 ( $\pm$ 1.7)

## 2.5. Qualitative analysis of green pigments

Fresh fruit outer layers (*i.e.*, skin and outer mesocarp; 2–3 mm thickness) were obtained with a scalpel, pulverised in liquid nitrogen with a Danguomeau 300 ball mill for 2 min (top impact frequency), and the pigments were extracted by stirring a powder aliquot (1 g) in distilled water (25 mL) with diethyl ether (60 mL) containing 0.01% 2,6 di-*t*-butyl *p*-cresol for 10 min. The organic phase was recovered and the aqueous phase reextracted twice with ether. The extracts were pooled and, after filtration onto a porous glass crucible (porosity No. 4), brought to dryness under vacuum; the residue was dissolved in diethyl ether (5 mL). Pigments were separated by HPLC using an Agilent system (1100 series) including a quaternary pump, a manual Rheodyne 7525i injector, an automated gradient controller and a diode array detector; detection wavelengths were (410, 434, 642 and 663) nm. The column was a reverse phase Lichrospher RP-18 (Merck, Darmstadt, Germany) [(250  $\times$  4.6) mm *i.d.*, 5  $\mu$ m packing] protected with a guard column of the same material. Flow rate was 1 mL·min<sup>-1</sup> and injected volume was 20  $\mu$ L. Three solvents were used: acetonitrile (A), *n*-hexane/dichloromethane 1:1 (B), and methanol / distilled water 9:1 (C) [16]; the elution program was: from 100% (C) at 0 min linearly to 45% (C), 40% (A) and 15% (B) at 18 min; linearly to 80% (A), 15% (B) and 5% (C) at 28 min.

## 3. Results and discussion

### 3.1. Physical characterisation of the fruits

The fruit of a mature-green Golden apple is an ovoid green drupe with average length

of ~ 70 mm, diameter of ~ 55 mm, and weight of ~ 115 g (*table I*). These values agree with previously published morphological characteristics [2, 7], but are smaller than values given by Graham *et al.* [4] for large Golden apples. Its longitudinal section (*figure 1*) shows a green outer mesocarp (thickness 2–3 mm) clearly separated from whitish central and inner mesocarps (pulp) embedding a hard-to-remove spiny stone.

Objective colour measurement ( $L^*$ ,  $a^*$ ,  $b^*$ ) indicated that fruit skin was green (negative  $a^*$  value) and yellow (positive  $b^*$  value). Our ( $L^*$ ,  $a^*$ ,  $b^*$ ) values agree well with previously published data for miniature mature-green Golden apples (19–21 weeks after fruit-set) [4]. Reconstitution of the true fruit colour [9] (*figure 1*) agreed with its actual colour. Thus, from a technological point of view, nectars from mature-green Golden apples will exhibit a pale olive green colour, balance between whitish and green, and colour intensity depending, respectively, on the relative proportions and amounts of pulp and outer mesocarp passing the sieving step and found ultimately in the beverage.

### 3.2. Physicochemical characterisation of the fruits

The fruit contents (*table II*) in total soluble solids (10 g·100 g<sup>-1</sup>) were higher than those reported by Graham *et al.* [4] for mature-green Golden apples at 19–21 weeks after fruit-set (7.5 g·100 g<sup>-1</sup>). Sugars were mainly sucrose, fructose and glucose. Due to its mature-green status, the fruit flesh has a low pH comparable to a lime or a lemon juice [17]. The mature-green Golden apples show a high titratable acidity (1.3 g citric acid Eq·100 g<sup>-1</sup>), a value far higher than those given by Graham *et al.* [4] for miniature mature-green Golden apples at 19–21 weeks after fruit-set (~ 0.7 g citric acid Eq·100 g<sup>-1</sup>). Contrary to previously published data [2] which placed malic acid as the most represented organic acid (278 mg·100 g<sup>-1</sup>) [citric acid being found at 54 mg·100 g<sup>-1</sup>], in our study, citric acid is the major organic acid (900 mg·100 g<sup>-1</sup>), accounting for ~ 80% of the anions present. Malic acid is also present at a level (200 mg·100 g<sup>-1</sup>) resembling the value presented by Daulmerie [2].

**Table II.**Physicochemical characterisation of mature-green Golden apple, *Spondias cytherea* Sonnerat (fm = fresh material).

pH	Total solids (g·100 g <sup>-1</sup> fm)	Total soluble solids (°Brix)	Sucrose (g·100 g <sup>-1</sup> fm)	Fructose (g·100 g <sup>-1</sup> fm)	Glucose (g·100 g <sup>-1</sup> fm)	Titrateable acidity (g citric acid Eq·100 g <sup>-1</sup> fm)	Citric acid (g·100 g <sup>-1</sup> fm)	Malic acid (g·100 g <sup>-1</sup> fm)	Oxalic acid (g·100 g <sup>-1</sup> fm)
2.6 (± 0.04)	20.4 (± 1.8)	10.0 (± 0.1)	3.1 (± 0.3)	1.5 (± 0.2)	1.2 (± 0.2)	1.3 (± 0.03)	0.9 (± 0.1)	0.2 (± 0.02)	0.03 (± 0.01)
Proteins (g·100 g <sup>-1</sup> fm)	Starch (g·100 g <sup>-1</sup> fm)	Cellulose (g·100 g <sup>-1</sup> fm)	Hemicelluloses (g·100 g <sup>-1</sup> fm)	Lignin (g·100 g <sup>-1</sup> fm)	Vitamin C (mg·100 g <sup>-1</sup> fm)	Phenolic compounds (mg gallic acid Eq·100 g <sup>-1</sup> fm)		Ashes (g·100 g <sup>-1</sup> fm)	
0.8 (± 0.1)	7.1 (± 0.5)	1.1 (± 0.1)	0.8 (± 0.2)	0.5 (± 0.1)	52.0 (± 4.9)	349.5 (± 52.3)		0.5 (± 0.06)	

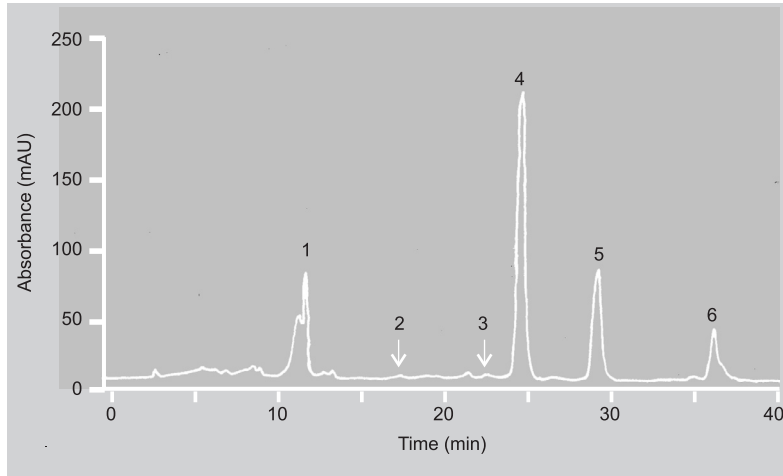
Starch was present at a significant level (7.1 g·100 g<sup>-1</sup>), a value higher than that reported for miniature mature-green Golden apples (19–21 weeks after fruit-set; ~ 4.5 g·100 g<sup>-1</sup>) [4]. Thus, in the industrial process, after heat treatment of fruits or juices to gelatinise starch, full enzymatic hydrolysis of starch into glucose by a commercial amyloglucosidase would theoretically increase the °Brix by 7 units. Proteins (0.8 g·100 g<sup>-1</sup>) and fibre constituents (sum of cellulose, hemicelluloses and lignin = 2.4·100 g<sup>-1</sup>) were found in the range of values reported by Persad [3].

The mature-green Golden apple is rich in vitamin C (52 mg·100 g<sup>-1</sup>) as the sweet orange *Citrus sinensis* L. (on average ~ 50 mg·100 g<sup>-1</sup>) [17]. Our average value is not in agreement with data reported by Graham *et al.* [4] for miniature mature-green Golden apples (19–21 weeks after fruit-set; 10–14 mg·100 g<sup>-1</sup>), and are closer to those reported by Daulmerie [2] and Persad [3] (36–42 mg·100 g<sup>-1</sup>). However, the vitamin C content varies with the harvest time and can decrease during the technological process applied to the fruit (*e.g.*, oxidation during the pulp defrosting, degradation due to the pasteurisation). Thus, it is imperative to define a precise harvest stage and to estimate the incidence of the technological treatments on vitamin C present in the Golden apple before using it as a selling argument for the marketable product (antioxidant).

These mature-green Golden apples show a high phenol content (~ 350 mg gallic acid Eq·100 g<sup>-1</sup>), a level higher than those known to be rich in these substances [18], *e.g.*, apples and red grapes. Our value is in full disagreement with previously published ones (green fruits; ~ 4 mg gallic acid Eq·100 g<sup>-1</sup> of pulp) [2]. It is worth mentioning that Corthout *et al.* [19] reported the presence of antiviral phenolic caffeoyl esters in a related species, *Spondias mombin* L.

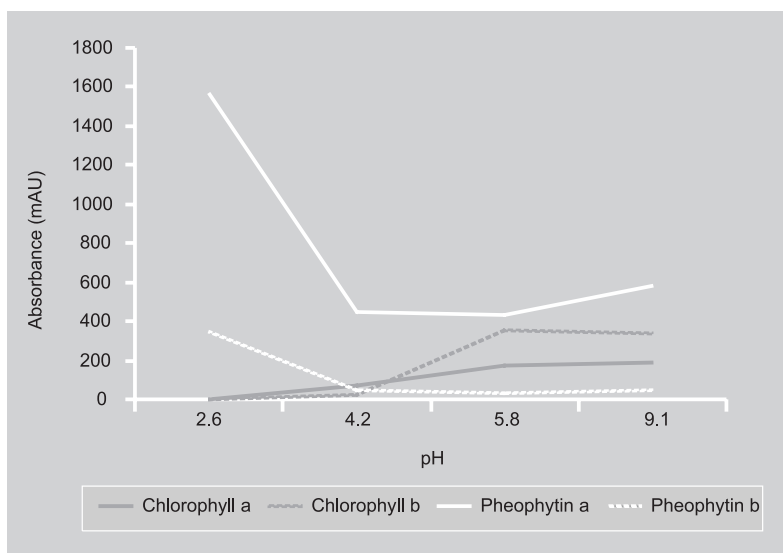
### 3.3. Green pigments of Golden apple

Since the pale olive green colour of Golden apple nectars is of prime importance to the consumers, the green pigments located in the outer layers of the fruit (*figure 1*) were extracted by diethyl ether and separated by HPLC, giving a typical chromatogram of a pigment extract (*figure 2*). Six peaks were consistently observed: two of them were carotenoids [peak No. 1, ( $\lambda_{\max}$  = 423, 446, 472 nm); peak No. 6 ( $\lambda_{\max}$  = 435, 452, 480 nm)]; four peaks were chlorophylls and chlorophyll-derived pigments (peaks No. 2 to 5). Analysis of spectra revealed that peaks No. 2 and 3 were chlorophyll b ( $\lambda_{\max}$  = 313, 343, 436, 464, 550, 600, 649 nm) and chlorophyll a ( $\lambda_{\max}$  = 336, 384, 414, 431, 580, 616, 664 nm), respectively [16, 20, 21]; they were present in minute amounts. The largely dominant peaks No. 4 and No. 5 were identified as pheophytin b ( $\lambda_{\max}$  = 330, 372, 416,



**Figure 2.** Reversed phase HPLC separation of the pigments isolated with diethyl ether from mature-green Golden apples. The analytical wavelength is 434 nm and the injection volume 20  $\mu$ L.

**Figure 3.** Influence of pH on the distribution of green pigments. Absorbance was measured at 654 nm for chlorophyll b and pheophytin b and at 664 nm for chlorophyll a and pheophytin a.



436, 528, 599, 654 nm) and pheophytin a ( $\lambda_{\max} = 324, 410, 506, 536, 606, 666$  nm), respectively [16, 20, 21]; these pigments are formed from chlorophylls by dissociating magnesium ion below pH 3 and its replacement by two hydrogen atoms [22].

The presence of pheophytins as the sole pigments in juices from mature-green Golden apples was already detected on the basis of their visible spectra [23]. Due to the very acid nature of the flesh of mature-green Golden apples (pH 2.6), and since, when crushing fruits, chloroplasts are brought into contact with the acid vacuolar juice, this observation

was expected. Confirmation of this result was obtained by spreading various amounts of powdered sodium carbonate onto aliquots of outer layer pieces prior to ball-milling to achieve instant pH adjustment (from pH 2.6 to 9.1) during ball-milling (figure 3). Clearly, while in the native pulp (pH 2.6), pheophytins a and b were almost exclusively present, their levels decreased drastically at pH 4.2, then remained constant up to pH 9.1; on the other hand, chlorophylls a and b were regenerated at pH 5.8, then remained stable. Due to the spectral characteristics of pheophytins compared with chlorophylls, this could explain why the acid pulpy juice obtained from mature-green Golden apple is olive green rather than bright green [24]. This technique will be used in the near future to estimate the capacity of enzymes in the industrial process to improve the pale olive green colour of the nectars.

## 4. Conclusion

A population of mature-green Golden apples (*Spondias cytherea* Sonnerat), representative of the fruits used in the French West Indies for the elaboration of a nectar, was analysed for its physical and physico-chemical characteristics. Fruits exhibited a low pH, a high titratable acidity with citric acid as the dominant anion, and a fairly high content in starch. Moreover, due to a high level in phenols, and a fair content in vitamin C, these fruits are a good source of antioxidants. The green pigments found in outer layers of mature-green Golden apples are pheophytins generated from chlorophylls by the high acidity of the fruits.

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### **Caracterización físico-química de la ciruela de Citera madura verde (*Spondias cytherea* Sonnerat).**

**Resumen — Introducción.** La ciruela de Citera verde madura (*Spondias cytherea* Sonnerat) se utiliza en las Antillas francesas para la preparación de un néctar. Las características morfológicas y la composición de esta fruta se determinaron con el fin de mejorar a largo plazo sus condiciones de transformación para una calidad mejor de la bebida obtenida. **Material y métodos.** Se recogieron en Martinica (las Antillas francesas) frutas verdes maduras y se caracterizaron: el diámetro, la longitud, el peso y el color; se midieron el pH, la acidez valorable, los sólidos solubles, los azúcares, las proteínas, el almidón, las fibras, la vitamina C, los fenoles y los pigmentos verdes. **Resultados y discusión.** La longitud, el diámetro y el peso medio de las frutas fueron respectivamente 71 mm, 54 mm y 116,4 g. Con un pH bajo (2,6) y una elevada acidez valorable (1,3 g Eq ácido cítrico·100 g<sup>-1</sup> materia fresca), la ciruela de Citera verde es una fruta ácida comparable al limón. El fruto es rico en vitamina C (52,0 mg·100 g<sup>-1</sup>), en fenoles (349,5 mg Eq ácido gálico·100 g<sup>-1</sup>) y en almidón (7,1 g·100 g<sup>-1</sup>). El color verde pálido de la pulpa industrial se debe a los feofitinas a y b. **Conclusión.** Nuestro estudio proporcionó un análisis detallado de ciruelas de Citera verdes maduras que se tendrá en cuenta para mejorar las condiciones de su transformación en néctar.

**Francia / Guadalupe / *Spondias cytherea* / Anacardiáceas / frutas / composición química / ácido ascórbico / almidón / fenol / clorofilas / feofitinas**

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