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An integrative analytical study of the functional and antioxidant properties of selected varieties of pink guava *Psidium guajava* L.

[*]Odile Marcelin[1], Chloé Mazaloubeaud[2], Fabienne Priam[1], Roselyne Marcus[1], Fabien Belloir[3], Valériu Vrabié[3], Emilie Juliette Smith-Ravin[1]

[1]*EA929 Groupe BIOSPHERES, Université des Antilles, BP 7207, 97275 Schœlcher Cedex, Martinique.*
[2]*Dénel SAS, Usine Dénel, Gros-Morne, Martinique.*
[3]*Centre de Recherche en STIC (CReSTIC), EA 3804, Université de Reims Champagne-Ardenne (URCA), Campus du Moulin de la Housse, BP 1039, 51687 Reims, France*
Corresponding Author: Odile Marcelin

Abstract: *(Guava (Psidium guajava L.) is a tropical fruit widely consumed around the world. The study was carried out on four selected guava varieties grown in Martinique. Two fruit harvests were gathered at two-year intervals, at the green/yellow color change maturity stage. First, we established the homogeneity of all fruit batches by bootstrapping, ANOVA, box plot and multiple comparison statistical analysis on physical parameters. Red Supreme Ruby clearly differs from the other three varieties, whereas the results for Centeno Prolific are between Beauséjour and Enana Cuba. We determined on puree obtained from a homogeneous batch of each variety the physicochemical and functional properties as well as the antioxidant properties. Our results show that the combination of lycopene and vitamin C, and their synergistic mechanisms in the fruit extracts may be responsible for the high antioxidant activity of guava puree, and we demonstrate a statistically significant positive correlation between EC_{50} and lycopene.*

Keywords: *Psidium guajava L., Vitamin C, Lycopene, Total polyphenols, Antioxidant activity*

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I. Introduction

Despite the fact that the Caribbean island of Martinique is one of the hot spots for global biodiversity, this has not been the subject of many studies. Yet, knowledge and exploitation of this immeasurable wealth would represent a vector for significant economic benefits in the agrifoods, environmental and health sectors. These priorities have been defined in the regional development plan, in line with the needs and innovation capacity of industry. Between 2010 and 2013, although the total utilized agricultural area (UAA) dedicated to guava cultivation remained steady, a doubling of the dedicated agricultural area by farmers was observed, in parallel with the increasing organization of the sector. Growers operating within a cooperative structure carry out fruit production and their market is guaranteed by food processing companies, which carry out primary and secondary processing operations. The sector has made innovation one of the major strategies for its development and sustainability. Consequently, for the past 25 years, supported and encouraged by the sector, fundamental and applied research [1], [2], [3], [4] have been carried out, with the aim of meeting consumers expectations .

Tropical fruits, including guava, are a source of natural antioxidants, such as polyphenols, carotenoids and vitamins, which are known to have beneficial effects on human health [5]. The literature reveals that guava contains less vitamin C than acerola fruit but more than orange [6], that it can have a similar polyphenol content as pomegranate [7] and that it contains as much lycopene as watermelon and tomato [8]. The presence in the same fruit of all three of these beneficial micronutrients is rare. This partly explains our interest in guava as the subject of our research study. The nutritional and functional benefits of the guava varieties currently grown in Martinique have not yet been extensively investigated. Our research study was therefore undertaken in order to overcome this drawback and to promote higher consumption of fresh and processed guava, so as to increase the intake of natural antioxidants.

Psidium guajava L. is an important crop belonging to the genus Psidium and the family Myrtaceae. It is native to, and is widely distributed in, Mexico and Central America. The plant is cultivated today from the west coast of Africa to the Pacific region and has spread widely throughout the tropics [9]. It is a climacteric fruit, and its consumption in the raw state is therefore limited to a short period lasting approximately two weeks [10]. As a result, a dynamic processing industry is required in which guava puree for example is used as a raw material to make nectar, juice, jelly, fruit pastille, sorbet, jam and concentrates. In addition, since the market trend is towards an increasing focus on the health benefits of foods, the agrifoods sector is turning towards innovative secondary processed products in order to offer consumers high added-value foods such as pure guava juice [11].

For our research study, four guava varieties cultivated by local farmers to supply the primary and secondary processing industries were selected. We gathered two fruit harvests and carried out statistical analysis of the physical characteristics of the fruits (intra- and inter-varietal, inter-harvest) in order to assess the homogeneity of the batches constituted. This makes it possible to confirm, compare and interpret the results subsequently obtained with puree derived from processing of the fruit. We measured the content of three major micronutrients of interest: vitamin C, lycopene and polyphenol. The antioxidant activity of each of the four varieties was assessed and correlated with the three micronutrients of interest.

II. Materials and Methods

2.1 Plant materials

Four guava varieties - Centeno Prolific (CP), Red Supreme Ruby (RSR), Beauséjour (BS) and Enana Cuba (EC) - were chosen for the study. These varieties are widely consumed because of their physicochemical and sensory qualities when consumed raw or when subjected to primary or secondary processing.

2.2 Sampling

The four guava varieties were harvested from orchards in Martinique. For each of the varieties selected, two harvests were gathered at two-year intervals, in which 150 fruits were picked for Harvest 1 and 30 fruits for Harvest 2. The fruits were picked in October (Harvest 1) and November (Harvest 2) for the CP, EC and RSR varieties and in January (Harvest 1) and March (Harvest 2) for the BS variety. Both BS harvests were from the same orchard in southern Martinique. The harvests for the other three varieties were from different orchards in central and northern Martinique.

The varieties selected are green when mature and unripe. The ripening stage is characterized by a gradual change of skin color from green (mature) to yellow (fully ripe). The fruits were selected at the green/yellow transition stage on the basis of their homogeneity with respect to criteria which include weight, size, color and firmness. After weighing, measurement of the diameter and assessment of the color and firmness of individual fruits, the fruits were processed into puree.

The 150 fruits from the first harvest were grouped into five batches of 30 fruits. The 30 fruits from the second harvest formed a sixth batch. Each batch was then processed to produce a puree on which physicochemical and functional characterizations were performed in triplicate. The data were subjected to statistical analysis.

2.3 Physical characterization of the fruit

The physical characteristics measured on individual fruits were weight, diameter, firmness and color. The weight (g) was determined using a laboratory weighing scale BALCO - Mettler PJ3600 Delta Range. The diameter (mm) was measured using an OTMT digital caliper. The firmness (g) was measured by a texture analyzer Stable Micro Systems TA-XTplus using a 4-mm diameter module. Hunter color values were measured using a Minolta Color Measuring system Model CR-300. The color values were expressed as L, a, b, where L = lightness, a (+) = redness, a (-) = greenness and b (+) = yellowness.

2.4 Preparation of puree

After removing the stalks, the fruits were cut into quarters and crushed in a Moulinex 5000 blender for 2 minutes. The puree obtained was sieved on a sieve with a mesh size of 2 mm and the sieve residues consisting of seeds and skin fragments were discarded. The puree obtained was stored frozen at -80°C in 100-g portions for subsequent analysis of the micronutrients and determination of antioxidant activity.

2.5 Physicochemical characterization of puree

The physicochemical characteristics, including viscosity, pH, titratable acidity, Total Soluble Solids (TSS, Brix), Total Dry Extract (TDE) and color, were measured on freshly prepared puree. The viscosity was measured using a rotary viscometer VT500 HAAKE at 20°C equipped with the SVDIN measuring system. The pH was determined using the Hanna Instruments HI9321 pH-meter. The titratable acidity was determined using an electronic titrator Schott Easy. The Brix value was determined using an electronic refractometer Bellingham Stanley Ltd Model RFM330+. Hunter color values were measured as described in section 2.3. The determination of the TDE was based on the measurement of the water content using a moisture analyzer Sartorius MA45 operating at 105°C.

2.6 Statistical analysis

The statistical analysis was designed to assess the homogeneity of the batches, as well as similarities or differences between the varieties and harvests used in this study. The batch homogeneity was assessed based on the measurement of the physical characteristics. Once the homogeneity has been established for a given variety, it is then possible to make comparisons between varieties and between harvests based on the physicochemical characteristics.

2.6.1. Analysis of homogeneity

The tool conventionally used to analyze differences between groups is ANOVA [12], [13]. Since this is based on a linear model, assumptions regarding probability distribution need to be verified. Due to the construction of batches, the independence of the samples is obvious, as is the equality of data variances in each batch. However, the normality hypothesis should be verified. The simplest way to verify normality is to analyze the data histograms in each batch, which are estimators of the probability distribution of a quantitative variable [14]. Since the number of samples was small in our study, we employed bootstrapping to generate a large number of samples. Bootstrapping makes it possible to assign measurements of accuracy of estimates (defined in terms of bias, variance, confidence intervals, prediction error and other measurements of this type). This technique makes it possible to estimate the sample distribution of almost any statistics using random sampling methods [15]. Next, kernel density estimation is used to assess sample distribution. Kernel density estimation is a data smoothing algorithm in which inferences about the population are made, based on a finite data sample [16]. The normality of a batch can be simply quantified by estimating the skewness and kurtosis, which are measurements of the symmetry and tailedness of a distribution [17].

2.6.2. Homogeneity

As ANOVA does not provide information on which batch(es) is/are the most deviated, a multiple comparison test is used [18]. Multiple comparison analysis uses the one-way ANOVA results to determine which estimates (such as mean, slope or intercept) are significantly different. Contrary to box plot, which is a compact way of describing the distribution of a set of data by presenting the quartiles [19], the results of a multiple comparison test make it possible to visualize - and therefore discriminate between - batches which are statistically different.

2.6.3. Correlation between micronutrients and total antioxidant activity

To determine whether there is a linear correlation between micronutrients and total antioxidant activity, we used Pearson's statistic [20], implemented in the SPSS Statistics software. The Sig (2-tailed) value indicates whether there is a statistically significant correlation between the tested variables. If this value is less than or equal to a fixed value – usually 0.05 as in our study, then we can conclude that there is a statistically significant correlation between the two variables. The sign of the correlation coefficient indicates whether the correlation is positive or negative, while the magnitude indicates the strength of the correlation.

2.7 Assay of micronutrients of interest

The micronutrients of interest in guava in our study are vitamin C (L-ascorbic acid), carotenoids (primarily lycopene) and polyphenols. Assays of the micronutrients were performed on frozen puree after thawing overnight at +4°C.

2.7.1. Total polyphenol content (TPC)

TPC was determined using the Folin-Ciocalteu method (Folin-Ciocalteu reagent, Sigma-Aldrich) optimized by [21]. Reducing substances other than polyphenols - in particular, vitamin C - were removed by solid-liquid extraction. The phenolic substances were then reduced using Folin-Ciocalteu reagent. Determination of the concentrations in the crude extract (total reducing substances) and in the washed extract (reducing substances other than polyphenols) was performed by reading the absorbance at 760 nm on a Fenway 6715 UV/Vis spectrophotometer. The calibration series was prepared using gallic acid (Sigma-Aldrich) for concentrations ranging from 50 to 600 mg/100 ml (slope = 0.0014, R^2 = 0.99). The results are expressed in mg/100 g of fresh material, gallic acid equivalent (GAE).

2.7.2. Ascorbic acid content (AAC)

The AAC was determined using two methods: the enzymatic method (Boehringer Mannheim Kit, ref 409.677) and the Folin-Ciocalteu method.

2.7.2.1. Enzymatic kit method

Guava puree was centrifuged (Rotanta 460R centrifuge) at 11,500 rpm for 20 minutes at 4°C and the supernatant was recovered for the assay. A colorimetric kit for determination of ascorbic acid in foodstuffs was obtained from Boehringer Mannheim. Ascorbic acid reduces the yellow-colored tetrazolium salt 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium methosulfate to orange-yellow-colored formazan at a pH of 3.5 in the presence of an electron carrier 5-methylphenazinium methosulfate. The difference in absorbance between the sample and the reference is proportional to the amount of ascorbic acid present in the sample. The absorbance is read on a Fenway 6715 UV/Vis spectrophotometer at 578 nm. The results are expressed in mg/100 g of fresh material.

2.7.2.2. Folin-Ciocalteu method

AAC was also determined using the Folin Ciocalteu method [21]. The washed extract obtained as described for the assay of total polyphenols was heated at 85°C for 2 hours in order to remove heat-labile ascorbic acid. The difference in absorbance between the washed extract and the heated washed extract makes it possible to determine the ascorbic acid concentration. The absorbance was measured at 760 nm on a Fenway 6715 UV/Vis spectrophotometer. The calibration series was prepared using ascorbic acid for concentrations ranging from 50 to 500 mg/100 ml (slope = 0.0009, R^2 = 0.98). The results were expressed as ascorbic acid equivalent in mg/100 g of fresh material.

2.7.3. Lycopene content (LC)

Lycopene was extracted and quantified by HPLC using the method of [22]. Fifteen grams of distilled water were added to a sample of 10 g of puree. Extraction of total carotenoid content (TCC) was performed by crushing the guava fruit in an ULTRA-TURRAX – IKA T18 blender in 50 ml of petroleum ether (Sigma-Aldrich) for 5 minutes. The orange-yellow supernatant (TCC + TPC) was recovered after centrifugation (ROTANTA 460R centrifuge) for 10 minutes at 10,000 rpm, at 20°C. Three successive extractions were required for optimum extraction of TCC. The TPC was removed by liquid-liquid extraction with methanol (Sigma-Aldrich), leading to a TCC extract. The TCC was then obtained in powdered form following evaporation under vacuum (Heidolph- HEI VAP rotary evaporator), resuspended in 2 ml of petroleum ether and then dried in a desiccator. The resulting powder was taken up in 1 mL of tetrahydrofuran (THF). LC was quantified by the HPLC method on TCC obtained as indicated above. The mobile phase consisted of a ternary solvent of THF/Acetonitrile/Methanol (10:55:35 v/v/v) and the samples were injected into a C18 column at a flow rate of 2.5 ml/min and a detection wavelength of 470 nm [23]. The peaks were identified by comparing their retention times with those of a commercially available tomato lycopene standard (Sigma-Aldrich). The results are expressed in mg/100 g of fresh material.

2.8 Measurement of antioxidant activity using the DPPH test

The antioxidant activity was measured using the DPPH method of [24] and modified for apolar solvents [22].

2.8.1. Sample preparation

The samples were prepared as described in section 2.7.3 up to the TCC + TPC stage, except that the quantities of distilled water and puree used were doubled. The TCC + TPC powder was obtained following evaporation under vacuum, re-suspension in 2 ml of THF and then drying in a desiccator. The antioxidant activity due to these two micronutrients was determined. In addition, in order to obtain the antioxidant activity due to the three micronutrients quantified in the guava puree (TCC + TPC + AAC), commercially available ascorbic acid (Sigma-Aldrich) was added to the TCC + TPC powder, in an equivalent amount in 20 g of guava puree.

2.8.2. DPPH assay

The free radical DPPH and antioxidant solutions were prepared with a THF/ethanol (2:1 v/v) mixture (Sigma-Aldrich). The THF/ethanol mixture was also used to calibrate the spectrophotometer. For each type of solution: control (THF/ethanol + DPPH) and samples (antioxidant solutions + DPPH), a blank was prepared: blank for the control solution (THF/ethanol) and blank for the sample solutions (antioxidant solution + THF/ethanol). Powdered DPPH (Sigma-Aldrich) was diluted at 39.4% in THF-ethanol (2:1 v/v) to give a 10^{-3} M solution. Stock solutions at 300 mg/L were prepared from dilute powdered antioxidant standards and samples in THF/ethanol (2:1 v/v). The dilute solutions are prepared by serial dilutions (for concentrations ranging from 240 to 5.05 mg/L). DPPH (1 ml) was added to the tubes containing the control and sample solutions at 3-minute intervals. The mixtures were thoroughly vortex-mixed and kept in the dark for 30 min. The results were read on a Fenway 6715 UV/Vis spectrophotometer. The zero for the apparatus is set with the blank for the control solution and then the absorbance of the control solution is read at t = 30 min. The zero for the stock solution is set with the blank for the stock solution and the absorbance of the stock solution is read at t = 33 min. The absorbance of the dilute solutions was read under the same conditions every 3 min, starting from 36 min. The ($Abs_{control\ mixture} - Abs_{antioxidant\ mixture}$) value of DPPH made it possible to determine the reaction kinetics of the antioxidant mixtures tested. The percentage inhibition of DPPH (I%) was determined using the following equation : $I\% = (Abs_{control\ mixture} - Abs_{antioxidant\ mixture}) / Abs_{control\ mixture} \times 100$.

These percentages were plotted on a graph against the sample concentrations selected on the basis of a reliable linear relationship between antioxidant concentration and calculated absorbance values. The line equations were obtained by linearization of the trend curves. Thus, the EC_{50} – which is defined as the amount of sample necessary to reduce the initial DPPH concentration by 50% - could be calculated. The antioxidant activities of the guava puree extracts were compared with ascorbic acid as a natural antioxidant.

III. Results and Discussion

3.1 Physical characterization of fruit batches

The data for the physical characterization of the fruit were subjected to statistical analysis as described in section 2.6. First of all, the histograms for each physical measurement and for each batch were estimated by bootstrapping. The sample size selected was 20,000. The skewness and kurtosis values were determined for all sampled data. Since these values were zero for all batches, the Gaussian distribution was proved.

3.1.1. Batch homogeneity

After confirming the normality of the data, we analyzed the homogeneity of each batch by examining the box plot diagram. This diagram confirmed the normal distribution of the data and enabled quantitative assessment of batch homogeneity. ANOVA provided quantitative information concerning the batches, while multiple comparison analysis identified batches that were statistically different. For the sake of simplicity, we only show the results obtained with the RSR variety using the weight parameter.

Figure 1(a) shows the box plot diagram for the weight distribution of each batch for both harvests. This diagram confirms that the data are normally distributed, with the median values roughly centered within their boxes, the whiskers extending approximately the same distance on either side of the boxes (first and third quartiles) and very few outliers being present. Having confirmed the normality of our data, we examined the homogeneity of the batches. The ANOVA results for Harvest 1 (F = 0.4, df = 149, p = 0.8082) presented in Figure 1(b) indicate that the batches were homogeneous. The p-value was above the significance level, indicating that the null hypothesis that batches were homogeneous was confirmed. The first five boxes in the box plot diagram in Figure 1(a) visually confirm this conclusion. The median values are very close and although some quartiles are slightly dissimilar, the diagram as a whole describes homogeneous batches. These results validate the harvest fields and demonstrate the expertise of the fruit pickers who harvested the fruit on the basis of sensory criteria. This means that for each variety, we could perform physicochemical measurements on any batch constituted from a given population. Consequently, for Harvest 2, we formed only one batch using the same methodology and the same manipulator.

On adding the weight parameter for Harvest 2, i.e. the sixth batch, it could be seen on the far right of Figure 1(a) that the corresponding box is significantly different. The ANOVA results shown in Figure 1(c) are clearly different from those obtained previously. From a probability of 0.8082, for F=0.4, we have moved to a probability of $2.61*10^{-9}$, for F=11.12. This indicates that the differences between some batches are statistically significant this time. However, it can be seen that there is a slight overlap between the sixth batch and the others. To complete this analysis and determine whether this batch was indeed different, multiple comparison analysis was applied. The results shown in Figure 1(d) clearly indicate that the sixth batch relating to Harvest 2 was significantly different from all the other batches for Harvest 1. Variations in soil and climate conditions may explain the intra-varietal differences observed between harvests.

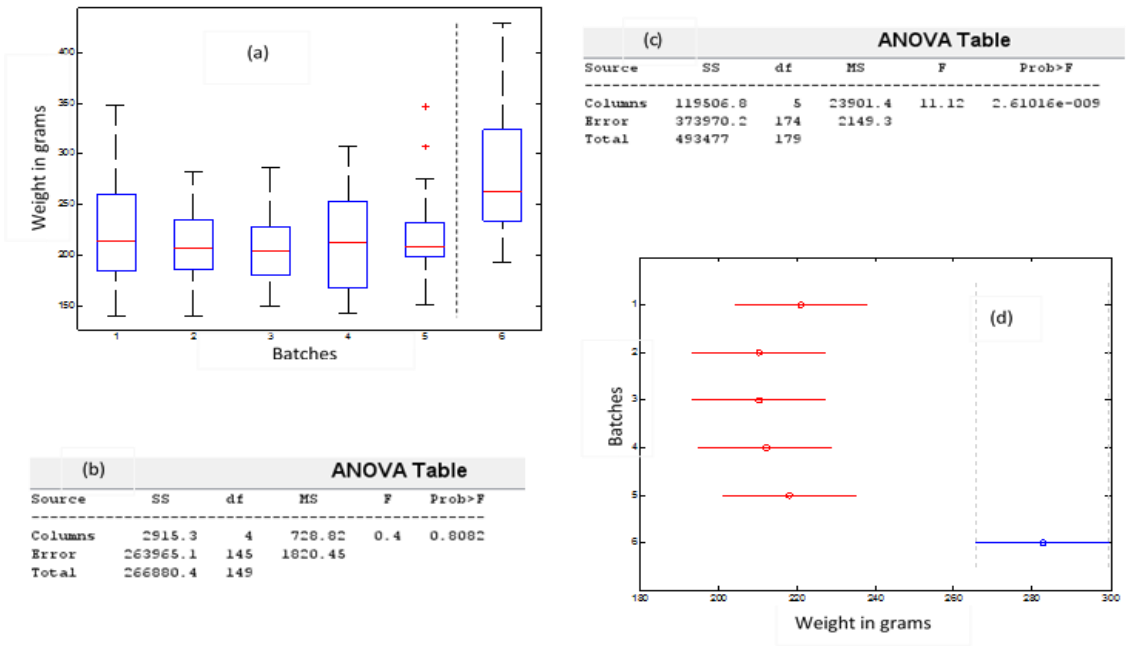


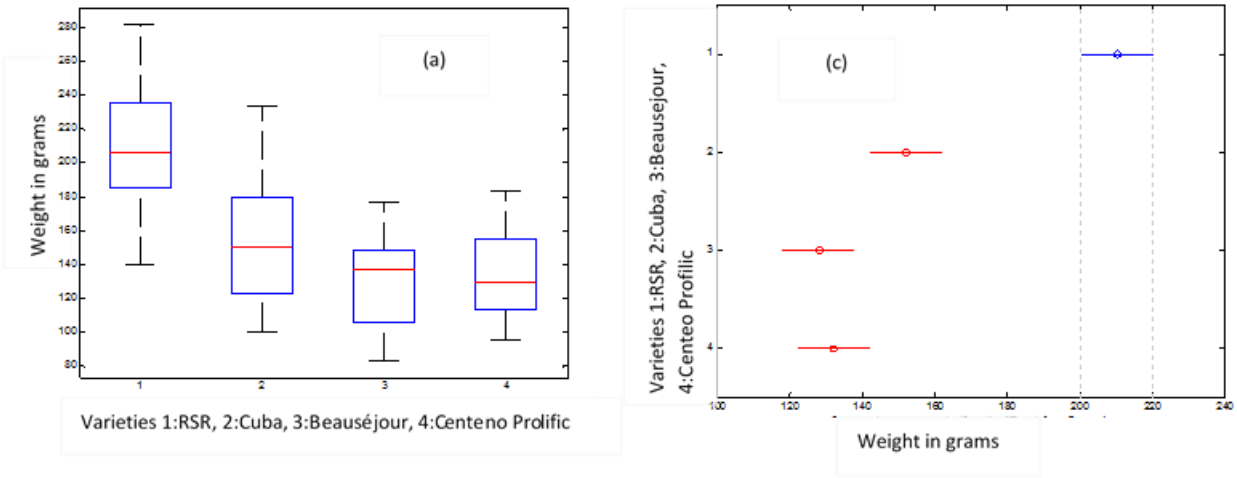
Fig. 1. Results for the RSR variety using the weight data. Batches 1 to 5 are from Harvest 1 while Batch 6 is from Harvest 2. (a) Box plot representation of the weight distribution for each batch. (b) ANOVA results for the

5 batches of Harvest 1. (c) ANOVA results for the 5 batches of Harvest 1 as well as the 6th batch of Harvest 2. (d) Results of the multiple comparison analysis for all 6 batches.

3.1.2 Inter-varietal comparison

We applied the methodology outlined above to analyze the similarities or differences between varieties using only one batch for each variety. Since the batches for each variety are homogeneous, we chose to present below the results obtained with the second batch of each variety for Harvest 1 using the weight parameter.

The box plot diagram for the weight distribution for each variety presented in Figure 2(a) shows differences between varieties. This qualitative analysis was confirmed by the ANOVA result in Figure 2(b); F = 48.29, df = 119, p = $2.49*10^{-20}$, indicating that the differences between at least two varieties were statistically significant. The multiple comparison analysis shown in Figure 2(c) indicates that there are significant differences between RSR, EC and BS, while CP is relatively similar to EC and BS. This result was confirmed by selecting other batches for each variety. The results obtained for Harvest 2 confirmed the same profile, thus indicating that physical characteristics are intrinsic characteristics of the varieties.



(b) ANOVA Table

Source	SS	df	MS	F	Prob>F
Columns	129166.8	3	43055.6	48.29	2.49088e-020
Error	103423.4	116	891.6		
Total	232590.2	119			

Fig. 2. Results for the RSR, EC, BS and CP varieties using the weight data of the 2nd batch of each variety of Harvest 1. (a) Box plot representation of the weight distribution for each variety. (b) ANOVA results. (c) Results of the multiple comparison analysis for all 4 varieties.

The results of the physical measurements performed on the fruits are presented in Table 1. For Harvest 1, the BS variety presented the lowest mean weight, followed, in increasing order, by CP, EC and RSR. The average weight of the fruits was between 124.2 g and 214.4 g. Broadly comparable results were observed for Harvest 2, the average weight of the fruits being between 96.0 and 282.7 g. Our results were higher than those reported for Indian guava by [25]. On the other hand, [26] reported average weights higher than those for CP and BS. If the number of fruits per tree is considered to be identical irrespective of variety, the variation in weight directly reflects the variation in fruit yield for a given orchard, surface area and cultivation conditions. The RSR and EC varieties appear to present the best fruit yields for the two harvests. The average diameters ranged from 56.9 to 77.5 mm. These values are comparable to those obtained by [26].

The mean values for the Lab color space parameters, and the standard deviations were comparable for the four varieties and the two harvests. This demonstrates that estimation of skin color for picking guava fruit is a valid criterion for determining maturity stage. These values are comparable to those reported by [26]. Parameter **a** has negative values characteristic of greenness that is increasingly intense for increasing negative values of a, whereas parameter **b** has positive values characteristic of yellowness which becomes brighter as b increases. By correlating parameters **a** and **b** on the Lab color space diagram, we showed that all the fruits collected had a green-yellow color characteristic of the maturity stage selected. Detailed analysis of the measurements of Lab made it possible to quantify physical color differences that were undetectable by the naked eye and to reveal differences between the two harvests. For Harvest 1, the RSR variety had the darkest and dullest green/yellow color, tending towards green. The BS and CP varieties had equivalent green/yellow

colors, lighter and brighter than the RSR variety. The EC variety had the green/yellow color, tending towards yellow, as dull as but lighter than the RSR variety. For Harvest 2, the RSR variety also presented the darkest and dullest green/yellow color, tending towards green, followed, in increasing order of green/yellow colors tending increasingly towards yellow and increasingly bright and light, by EC, CP and BS.

For Harvest 2, the average skin firmness is presented in Table 1. The mean values are between 705.0 and 971.4 g. For the same maturity stage, the varieties present varying degrees of sensitivity to handling after picking. RSR is the least firm variety and must be handled with greater care than the EC and BS varieties, while CP is the variety that will best tolerate impact.

Table 1 : Physical characteristics of four Martinique pink guava varieties

Varieties	**Weight**[1] **(g)**	**Diameter**[1] **(mm)**	**Firmness**[1] **(g)**	**Hunter color values**[1]		
				L	a	b
Harvest 1						
Beauséjour	124.2 (± 7.8)	58.9 (± 1.2)	nd	80.3 (± 0.4)	-4.6 (± 0.4)	54.8 (±0.5)
Centeno Prolific	133.8 (± 6.0)	63.4 (± 1.0)	nd	79.9 (± 0.5)	-4.1 (± 0.3)	45.5 (± 0.9)
Enana Cuba	147.5 (± 10.0)	64.3 (± 1.3)	nd	77.7 (± 0.5)	-2.5 (± 0.6)	41.6 (± 0.7)
Red Suprême Ruby	214.4 (± 4.9)	70.0 (± 0.5)	nd	73.4 (± 0.8)	-7.0 (± 0.8)	40.1 (± 0.4)
Harvest 2						
Beauséjour	144.4 (± 19.4)	62.6 (± 3.0)	782.7 (± 199.5)	79.0 (± 2.4)	-5.7 (± 1.9)	57.7 (± 3.1)
Centeno Prolific	96.0 (± 14.2)	56.9 (± 3.1)	971.4 (± 378.0)	77.6 (± 2.3)	-7.2 (± 2.6)	45.7 (± 3.5)
Enana Cuba	233.2 (± 38.5)	73.7 (± 4.2)	780.9 (± 188.1)	72.0 (± 3.0)	-10.6 (± 3.3)	44.5 (± 2.6)
Red Suprême Ruby	282.7 (± 60.6)	77.5 (± 5.1)	705.0 (± 113.1)	64.9 (± 4.1)	-14.7 (± 2.6)	40.0 (± 2.7)

[1]Mean ± SD obtained from analysis of three independent samples

3.2 Physicochemical characterization of guava puree

The results for the physicochemical characterization of puree are presented in Table 2. The mean values for all the parameters measured were comparable for the two harvests.

The CP and RSR varieties had the lowest average Brix values, at 8.1-9.3 and 8.9-9.3, respectively but very different acidity profiles; CP being the most acidic variety while RSR had a low acidity. The EC and BS varieties had the highest Brix values, at 9.4-9.9 and 11.3-12.3, respectively, with differing acidity profiles. The Brix value of the BS variety is higher than that reported in the literature for fruit grown in Brazil, India or Colombia [25], [26], [27], [28], [29], [30]. It is comparable to that of the guava varieties grown in Sudan [31]. The sugar/acid ratio is used to indicate the predominance of sweetness or sourness and is conventionally used in the fruit processing industry. It is an effective indicator of the sensory perception of a food product. CP stands out from the other three varieties with a sugar/acid ratio of 5.7-6.7, equivalent to that found by [25] for an Indian guava variety. The other three varieties had ratios of between 21.4 and 31.4, comparable to Brazilian and Indian varieties [26], [27], [28]. The BS variety, which has the highest Brix value and an intermediate acidity level, offered an interesting compromise in terms of the sensory perception of the sourness of guava for fresh consumption and for processing. The pH and the acidity varied in an inversely proportional manner, with average pH values of between 3.09 and 4.22. Comparable pH values were recorded for guava from Brazil, India and Colombia [26], [28], [30].

The TDE values for all the varieties were between 11.3 and 15.5%, lower than the 16.7% reported by [27] for Brazilian guava. The trends were similar to those for the Brix values, the soluble sugars accounting for between 70 and 80% of the TDE.

With viscosity values between 1275.3 and 1383.5 cP, the BS variety had the highest viscosity while the CP variety had the lowest viscosity with values between 629.2 cP and 972.3 cP. EC had viscosity values of between 984 and 992.6 cP. As with the CP variety, the RSR guava from Harvest 1 has a lower viscosity (745.3 cP) than RSR from Harvest 2 (1023.3 cP). These intra- and inter-varietal differences could suggest differences in the qualitative and quantitative profiles of the parietal polysaccharides including cellulose, hemicellulose and pectin and are therefore worth studying, as has been the case for the Supreme variety [1], [2]. This basic knowledge helps explain the impact of bioprocessing, such as enzymatic treatment, of puree [3], [4], [11].

Table 2 : Physicochemical analysis of puree of four Martinique pink guava varieties

Varieties	TDE[1,2] (%)	TSS[1,3] (°Brix)	Acidity[1] (%citric acid)	Sugar/Acid Ratio[1]	pH[1]	Viscosity[1] (cP)	Hunter color values[1]		
							L	a	b
Harvest 1									
Beauséjour	15.5 (± 0.1)	12.3 (± 0.7)	0.52 (± 0.02)	23.8 (± 0.04)	3.92 (± 0.02)	1383.5 (± 26.3)	52.9 (± 0.4)	14.8 (± 1.3)	16.1 (± 1.3)
Centeno Prolific	11.3 (± 0.4)	8.1 (± 0.1)	1.48 (± 0.06)	6.7 (± 0.39)	3.18 (± 0.02)	629.2 (± 13.8)	52.6 (± 0.7)	17.4 (± 0.4)	9.9 (± 0.3)
Enana Cuba	13.5 (± 0.4)	9.4 (± 0.2)	0.32 (± 0.01)	29.6 (± 0.09)	4.23 (± 0.01)	992.6 (± 22.3)	48.3 (± 0.6)	19.8 (± 0.4)	10.1 (± 0.2)
Red Suprême Ruby	12.2 (± 0.2)	8.9 (± 0.1)	0.34 (± 0.01)	26.0 (± 0.06)	4.06 (± 0.03)	745.3 (± 30.2)	52.9 (± 0.41)	17.8 (± 1.0)	9.1 (± 0.4)
Harvest 2									
Beauséjour	15.5 (± 0.2)	11.3 (± 0.2)	0.53 (± 0.01)	21.4 (±0.6)	3.93 (± 0.04)	1275.3 (± 32.7)	53.1 (± 0.7)	14.3 (± 1.0)	20.2 (± 0.6)
Centeno Prolific	13.0 (± 0.2)	9.3 (± 0.1)	1.63 (± 0.02)	5.7 (± 0.1)	3.09 (± 0.02)	972.3 (± 39.3)	52.1 (± 0.2)	14.9 (± 0.5)	11.9 (± 0.5)
Enana Cuba	13.7 (± 0.2)	9.9 (± 0.0)	0.32 (± 0.01)	31.4 (± 1.1)	4.20 (± 0.0)	984.0 (± 13.7)	44.8 (± 0.4)	15.5 (± 0.1)	11.5 (± 0.5)
Red Suprême Ruby	13.1 (± 0.1)	9.3 (± 0.0)	0.32 (± 0.01)	29.5 (± 1.1)	4.22 (± 0.02)	1023.3 (± 14.0)	47.2 (± 0.2)	13.0 (± 0.2)	9.8 (± 0.2)

[1]Mean ± SD obtained from analysis of three independent samples [2]TDE = Total Dry Extract
[3]TSS = Total Soluble Solids

For puree prepared from the four varieties, the Lab values gave positive values on the Lab color space diagram and were of comparable values. [30] and [26] measured equivalent values for the L and b parameters but lower values for the **a** parameter for Colombian and Indian guava. Visual observation showed that all the purees had shades of color between salmon and pink.This information, taken together with the Lab measurements, reveals varietal differences undetectable by the naked eye. The EC guava puree had the most intense pink color (highest value of a) but the darkest color (lowest L value). The pink color of the BS variety, although slightly less intense, was almost twice as bright (highest value of b) as the other three varieties. This information is of interest in the context of the processing of the fruit, when varieties with the most intense puree color will be preferred, especially if the process involves heat treatment which promotes non-enzymatic browning. In this regard, the BS variety once again exhibited an interesting compromise.

3.3 Functional characterization of guava puree

The results obtained for Harvest 2 are presented in Table 3.

The TPC values were comparable for the varieties, ranging between 119.7 ± 1.8 mg GAE /100 g puree (RSR) and 141.1 ± 4.9 mg GAE /100 g puree (BS). These values were similar to those observed in guava from Malaysia, Brazil, Thailand or Mauritius [29], [32], [33], [34], [35] while higher values were found for cultivars from USA and Ecuador by [5] and [36]. In contrast, a lower content was found by [37] in a Brazilian variety.

The results for AAC obtained using two different methods (enzymatic kit and Folin-Ciocalteu) were similar. CP and BS were the varieties with the highest AAC content (106.7 ± 5.0 - 176.4 ± 2.8 mg/100 g), close to the values obtained in guava from USA, Malaysia, Thailand and Brazil [5], [27], [34], [38]. EC and RSR contained substantially less AAC (36.0 ± 2.0 - 43.9 ± 7.2 mg/100 g) than CP and BS, with values comparable to Nigerian cultivars [39]. The Folin Ciocalteu method is easy to implement for quality control in the fruit processing industry in Martinique and is also less expensive than the enzymatic kit protocol. The latter nevertheless offers the advantage of greater specificity. The results obtained in our study using the two methods are similar. This offers a less expensive solution for industry for routine quality control.

The EC puree had the highest lycopene content (7.7 ± 0.4 mg/100 g), close to the values obtained for Brazilian and Indian varieties [26], [29], and has a much higher lycopene content than the CP puree (4.6 ± 0.3 mg/100 g). The BS and RSR purees had identical values of lycopene content (6.1 mg/100 g). [40] found a lower lycopene content for Japanese guava varieties and they showed that it is the principal carotenoid in this fruit, accounting for up to 80% of total carotenoids. Lycopene is also the predominant carotenoid (86%) found in Brazilian guava varieties [27], [41]. The higher the LC, the higher the antioxidant activity of the extracts.

Table 3: Content of total polyphenols (TPC), lycopene (LC) and ascorbic acid (AAC) of puree from four Martinique pink guava varieties for Harvest 2

Varieties	TPC (mg GAE[4]/100g)[1]	LC (mg/100g)[1]	AAC (mg/100g)[1]	
Beauséjour	141.1 ± 4.9	6.1 ± 1.1	118.3 ± 1.8[2]	106.7 ± 5.0[3]
Centeno Prolific	134.7 ± 7.1	4.6 ± 0.3	176.4 ± 2.8[2]	126.1 ± 13.9[3]
Enana Cuba	128.8 ± 3.9	7.7 ± 0.4	36.0 ± 2.0[2]	43.9 ± 7.2[3]
Red Suprême Ruby	119.7 ± 1.8	6.1 ± 0.4	38.4 ± 0.9[2]	37.8 ± 2.8[3]

[1]Mean ± SD obtained from analysis of three independent samples [2]Enzymatic kit method
[3]Folin Ciocalteu method [4]Gallic Acid Equivalent

3.4 Antioxidant activity

The antioxidant activity determined in our research study was related to the combination and interaction of the total carotenoid content (TCC), total polyphenol content (TPC) and ascorbic acid content (AAC) contained in guava puree. Table 4 summarizes the EC_{50} values for antioxidant samples from guava puree and ascorbic acid standard.

Table 4 : EC_{50} obtained from measurements in the linear range for antioxidants from guava puree and ascorbic acid standard

	Samples	**EC_{50} (mg/L)[1] of TCC[2] + TPC[3]**	**EC_{50} (mg/L)[1] of TCC + TPC + AAC[4]**
Standard	Ascorbic acid	13.0 (± 1.1)	13.0 (± 1.1)
Antioxidants in guava puree	Centeno Prolific	165.0 (± 29.9)	9.3 (± 0.8)
	Enana Cuba	116.7 (± 22.0)	22.4 (± 1.4)
	Red Suprème Ruby	143.3 (± 26.4)	18.5 (± 4.0)
	Beauséjour	130.6 (± 33.2)	16.2 (± 3,1)

[1]Mean ± SD obtained from analysis of three independent samples [2]TCC = Total Carotenoid Content
[3]TPC = Total Polyphenol Content [4]AAC = Ascorbic Acid Content

Whatever the guava variety, the EC_{50} of TCC + TPC was ten times higher than for the ascorbic acid standard. The EC variety had a higher antioxidant activity than the other three varieties.

For all the varieties, the EC_{50} values of TCC + TPC + AAC were of the same order of magnitude as the ascorbic acid standard. The EC_{50} value for the CP variety was lower than the ascorbic acid standard. In order to demonstrate the potential contribution of the three phytochemicals to total antioxidant activity, we analyzed the correlation between the EC_{50} for TCC + TPC + AAC on the one hand and TPC, LC and AAC on the other using the Enzymatic Kit method and the Folin Ciocalteu method. The results indicate that there is a statistically significant correlation between EC_{50} and LC. The Pearson correlation coefficient is 0.660 and the Sig (2-tailed) value is 0.020. There is also a statistically significant correlation between LC and both methods of determination of AAC. The Pearson correlation coefficients are 0.700 and 0.674 and the Sig (2-tailed) values are 0.011 and 0.016 for the Enzymatic Kit and Folin Ciocalteu methods respectively. As indicated previously (Table 3), the TPC values of all the guava varieties were of the same order of magnitude. We were therefore able to draw a parallel between variations in AAC and LC and variations in antioxidant activity. Thus, the CP variety has the highest AAC and the lowest LC. Consequently, the antioxidant activity of the combination TCC + TPC + AAC appears to be primarily related to the AAC content. This conclusion is also valid for the BS variety, although it contains a higher LC content. The RSR and EC varieties had higher EC_{50} values (Table 4) than CP and BS, the AAC content being three to four times lower than for CP and BS, while the LC content is of the same order of magnitude. However, the EC_{50} values for the RSR and EC varieties remained comparable to the BS variety. This shows the synergistic action between ascorbic acid and lycopene demonstrated by Pearson's correlation coefficient. Consequently, the combination of these two phytochemicals and their synergistic mode of action in guava fruit extracts may be primarily responsible for the high antioxidant activity of guava puree.

Previous studies have demonstrated that ascorbic acid contributes to the antioxidant activity of guava puree from Thailand, USA and Malaysia [5], [34], [38], whereas total carotenoids play a minor role [5]. However, [42] and [32] demonstrated a low correlation coefficient between vitamin C content and antioxidant activity for Mauritian and Colombian guava varieties, respectively. Although our study has shown a contribution of vitamin C toward total antioxidant activity, this was not statistically demonstrated by Pearson's correlation coefficient. In addition, several authors ([5], [29], [32], [37], [42]) have demonstrated that total polyphenols make a major contribution toward the antioxidant activity of Colombian, Brazilian and Mauritian guava extracts although this was not demonstrated in the present study.

IV. Conclusions

Our study was intended to fill a significant knowledge gap for the four main varieties of guava grown in Martinique for consumption in unprocessed or processed form. This has been achieved by the physical, physicochemical and functional characterization of the guava varieties. Based on the antioxidant properties reported in this study, these varieties can already be marketed as offering health benefits. On the basis of our results, it is now possible to identify the stage of maturity for harvesting guava fruits and this knowledge was transmitted to farmers during training sessions. For all the physical characteristics measured, we have demonstrated intra-varietal differences from one harvest to the other, possibly due to variations in local soil and weather conditions. These conditions should be taken into consideration by farmers and processing industries at each fruit harvest since they have an influence on fruit and puree yields. Moreover, the physicochemical characterization of guava puree demonstrated that the BS variety exhibited a good compromise in terms of the sensory perception of the sour taste of guava intended for consumption in the processed or unprocessed form. The combination, with a high content of the three major antioxidants (total polyphenols, lycopene and ascorbic

acid) in guava puree, is a phenomenon rarely observed in fruits and which is one of the distinguishing features of this study. Once more, the BS variety appears to be the best compromise because of the simultaneously high content of all three micronutrients. The antioxidant activity determined in the context of our research study shows that samples containing the three phytochemicals (total carotenoids, total polyphenols and ascorbic acid) had a free radical scavenging action similar to that of the ascorbic acid standard, the CP variety being even more effective than the ascorbic acid standard which is recognized for its antioxidant properties. The combination of the two phytochemicals, lycopene and ascorbic acid, and their synergistic modes of action in the guava fruit may be primarily responsible for the high antioxidant activity of guava puree.

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