

Chemical, bioactive and color analysis in three varieties of guava

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Abstract

Guava (*Psidium guajava* L.) is a fruit highly prized for its nutritional value and antioxidant capacity. In Mexico, the state of Zacatecas is the third largest guava producer. In the south of the state is the region of Santiago el Chique, which contributes to this production. This work aimed to determine the moisture, °Brix, titratable acidity, pH, ascorbic acid content, color, as well as the content of total polyphenols and antioxidant capacity of three varieties of guava (Blanca, China and Fresa), acquired in 2022 with producers in the locality of Santiago el Chique, Zacatecas. The analyses were conducted in the Food Research and Safety Laboratory of the nutrition academic program of the Autonomous University of Zacatecas. The results showed significant differences in practically all the parameters analyzed, except in the case of the percentage of moisture. Compared to the other varieties, the high concentration of ascorbic acid in the 'Fresa' guava stands out.

Palabras clave:

Psidium guajava L., fitoquímicos, vitamina C.



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Introduction

Consuming antioxidant-rich fruits helps prevent long-term health-related diseases; they can act as therapeutic agents in preventing chronic diseases, including diabetes, cancer, obesity, and hypertension (Patel *et al.*, 2016). Polyphenols are secondary plant metabolites with antioxidant activity beneficial to human health (Marquina *et al.*, 2008).

The health benefits associated with these polyphenols are based on their antioxidant properties, as they are the main determinants of the fruit's total antioxidant capacity; this property is defined as the ability of antioxidant compounds to protect a biological system against the potentially harmful effect of processes or reactions caused by reactive oxygen and nitrogen species (ROS and RNS) (Cervantes *et al.*, 2020).

Ascorbic acid (AA) or vitamin C is an essential nutrient often used as an indicator of the nutritional value of fruits and to estimate spoilage due to processing (Aguilar *et al.*, 2017). It belongs to the group of water-soluble vitamins and is considered a powerful antioxidant (Castro-López *et al.*, 2016).

It should be included daily in the diet through fruits and vegetables because it is necessary to maintain the health of blood vessels, skin, teeth, bones, and cartilage; it is essential in anti-allergy treatments, strengthens the immune system, prevents flu and respiratory infections (Porto *et al.*, 2019) and acts synergistically with tocopherol to preserve antioxidant function in chronic disease states (Andarwulan *et al.*, 2012).

Guava (*Psidium guajava* L.) is a plant of the Myrtaceae family, which includes about 133 genera and 3 800 species of trees and shrubs; the genus *Psidium* contains about 150 species, presents a high level of variability in populations, with different fruit sizes, differences in the color of the pulp and the skin or peel, and differences in the number of seeds and other morphological characteristics (Angulo-López *et al.*, 2021).

Originally from the Americas but introduced to other regions of the world, it is cultivated for its appreciable nutritional properties, especially its high content of ascorbic acid, vitamins, and minerals such as calcium, iron, and phosphorus, for the abundance of antioxidant compounds such as polyphenols and flavonoids, as well as for the derivatives that are produced from it (Fajardo-Ortíz *et al.*, 2019).

According to official data, in 2020, the state of Zacatecas consolidated its position as the third largest guava producer nationwide, with a volume of 32 252 tonnes, only below Michoacán and Aguascalientes, which rank first and second with 172 729 and 62 897 t, respectively (SIAP, 2022).

Different guava varieties are produced in the region of Santiago el Chique (Zacatecas); however, there is no information available on their nutritional and functional value and, consequently, on the possible differences between them. For this reason, this work aimed to physicochemically characterize three varieties of guava (Blanca, China, and Fresa) and to determine their functional value by analyzing their antioxidant capacity and total phenolic content.

Materials and Methods

Three varieties of guava (China, Blanca, and Fresa), produced and acquired in the region of Santiago el Chique, Zacatecas (22° 03' 11.8" north latitude, 102° 51' 58.6" west longitude), were used, to which the following was determined: pH, °Brix, moisture content (% Xw), titratable acidity (TAc), ascorbic acid (AA) content, total phenolic content (TPC), antioxidant capacity (AC), and color from CIEL b coordinates. The analyses were conducted in the research and food safety laboratory, belonging to the academic nutrition program of the Autonomous University of Zacatecas, in 2022.

Ascorbic acid content

It was performed using the volumetric method 967.21 described by the Aoac (2000). To analyze the samples, 10 g of crushed fruit and 10 ml of metaphosphoric acid (25%) were mixed and then



adjusted to 50 ml with deionized water. From the previous solution, three aliquots of 10 ml were taken and titrated with the indicator 2.6 dichlorophenol-indophenol, previously titrated with an AA standard (250 ppm), in both cases until the appearance of a persistent pink color for 30 s. Results were expressed as mg AA 100 g⁻¹.

Extraction of bioactive compounds

The extraction of phytochemicals for the quantification of TPC and AC was carried out using the method described by Tomás-Barberán *et al.* (2001). Twenty grams of fruit were crushed with 20 ml of MeOH, 5 ml of 6N HCl, and 2 mg of NaF, mixed with continuous stirring for 30 min at room temperature, and then centrifuged (Sigma 3-16KL, Germany) for 10 min at 4°C and 4 500 rpm.

Total phenols

Total phenols (TPC) were quantified using the Folin-Ciocalteu test (Li *et al.*, 2006). Two hundred fifty microliters of extract were mixed with 15 ml of deionized water and 1.25 ml of Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, Missouri, USA). After 5 min, 3.75 ml of Na₂CO₃ (7.5%) was added; it was made up to 25 ml with deionized water and left to react for 2 h in darkness; then, absorbance was measured at 765 nm on a UV-Vis spectrophotometer (Thermo Scientific 10S, Thermo Fisher Scientific Inc, USA). Results were expressed as mg of gallic acid (mg GAE 100 g⁻¹).

Antioxidant capacity (AC)

Antioxidant capacity (AC) was quantified using the spectrophotometric techniques of ABTS⁺⁺ (Re *et al.*, 1999) and DPPH (Brand-Williams *et al.*, 1995).

ABTS^{•+} method

The 7 mM ABTS^{*+} radical (Sigma-Aldrich, St. Louis, Missouri, USA) was generated by 2.45 mM potassium persulfate ($K_2S_2O_8$). This mixture was previously left to react for 16 h in darkness and at room temperature (~ 20 °C). The above solution was diluted to obtain an absorbance of 0.7 ±0.1 at 734 nm. Once the desired absorbance of the ABTS^{*+} reagent was reached, 100 µl of extract from each fruit was mixed with 900 µl of the diluted ABTS^{*+} solution, left to react for 2.5 min at 20 °C, then the absorbance was measured at 734 nm. Results were expressed as µmol of Trolox equivalents (TEAC)/100 g of fresh fruit. All experiments were replicated three times.

DPPH method

Regarding the measurement of AC by the DPPH method, 100 μ l of fruit extract was added to 1 ml of 2.2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, St. Louis, Missouri, USA) (3 mg 100 ml⁻¹ in ethanol solution). The absorbance of the samples was determined at 515 nm in the spectrophotometer after a 2.5-minute reaction at 20 °C. The results were expressed as μ mol of Trolox equivalents/100 g of fresh fruit.

Color characteristics

The data of the CIEL a b coordinates were taken in the outer layer of the guava using an AMT506 colorimeter (SMI, Mexico) with a 10° observer and a D65 illuminant previously calibrated. L (luminosity) was obtained, with values ranging from 0= black to 100= white, a is negative for green and positive for red, and b values are negative for blue and positive for yellow. The hue results from the two coordinates, a and b, and is determined as arctan b/a, where 0°= bluish red, 90°= yellow, 180°= green, and 270°= blue; for its part, the chroma is a measure of intensity or saturation and is calculated as (a Cb)1/2 (Wrolstad *et al.*, 2005).

The experimental design was completely randomized with three replications. All analyses were performed in triplicate, and results were expressed as mean and standard deviation. To determine

the significant differences in the variables between the data in the fruit varieties, a one-way Anova was performed; when significant, a Tukey test was applied (*p*# 0.05). All statistical analyses were performed using Statgraphics[®] Centurion XV (Statpoint Technologies Inc., Warrenton, VA, USA).

Results and discussion

Table 1 shows the values in the physicochemical parameters analyzed for the three guava varieties. Except for water content, there were significant differences ($p \le 0.05$) among the varieties; Fresa guava stood out for its high value in degrees Brix (15.1 °Brix), for its acidic pH (3.64), and total acidity (177.1 mg AC 100 g⁻¹), and above all, for its high ascorbic acid content (629 mg AA 100 g⁻¹ FF). Compared to other research, Panayampadan *et al.* (2022) reported values of 87.6% moisture in Indian guava, while Rojas-Barquera and Narváez-Cuenca (2009) reported values of 85.3 to 91.8% in four Colombian varieties.

Table 1. Physicochemical characterization of three guava varieties.							
Guava variety	(%) moisture	°Brix	рН	Total acidity (mg 100 g ⁻¹ FF)	Ascorbic acid (mg 100 g ⁻¹ FF)		
Blanca	81.6 (0.8) a [#]	13.1 (0.1) b	3.87 (0.03) c	127.4 (3.3) a	237.8 (14.7) b		
China	81.8 (2.7) a	12.1 (0.1) a	3.77 (0.02) b	141.8 (5.1) b	214.1 (11.8) a		
Fresa	79.5 (0.6) a	15.1 (0.1) c	3.64 (0.06) a	177.1 (1.3) c	629.1 (22.4) c		

Regarding degrees Brix, Vargas-Madriz *et al.* (2018) obtained 9.12° in Mexican guava, Fajardo-Ortíz *et al.* (2019) a range of 6.46 to 9.43° Brix in six genotypes of Colombian guava, Rojas-Barquera and Narváez-Cuenca *et al.* (2009) from 5.9 to 9.5 in four Colombian varieties, Musa *et al.* (2015) recorded values of 7.5 to 8.57 °Brix in pink guava from Malaysia and Kanwal *et al.* (2018) 8.8 °Brix in guava from Pakistan; all these data are lower than those obtained in this work.

On the other hand, Kumari *et al.* (2020) presented similar values of degrees Brix (9.98 to 13.1) in Indian guava. Total soluble solids (TSS) play an essential role in improving fruit quality, giving an estimate of their sweetness (Kumari *et al.*, 2020).

Variations in TSS content may be due to season, soil, climatic conditions, and phenotypic and genetic constitution of cultivars, which, at some point in their development, may have needed to consume nutrients, causing a reduction in the concentration of carbohydrates in the fruit, in order to obtain larger fruits with higher TSS (Kumari *et al.*, 2020).

Regarding pH, Fajardo-Ortíz *et al.* (2019) obtained higher values (4.2 to 4.68) in six Colombian guava genotypes, while Rojas-Barquera and Narváez-Cuenca *et al.* (2009) presented similar pH values (3.6 to 4) in four Colombian varieties. In total acidity, Panayampadan *et al.* (2022) quantified values of 0.4%, Kumari *et al.* (2020) a range of 0.42 to 0.77%, Kanwal *et al.* (2018) 0.34% and Musa *et al.* (2015) 0.46 to 0.5% in pink guava; all of these concentrations higher than those of this research.

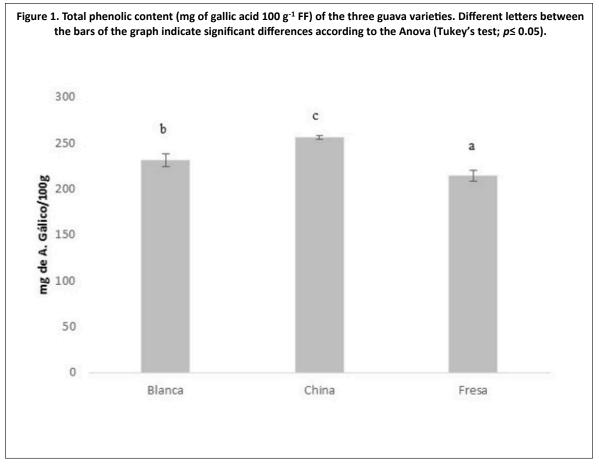
Fruit acidity is directly related to fruit growth and development, which tends to vary during growth and development (Kumari *et al.*, 2020). Regarding vitamin C content, Vargas-Madriz *et al.* (2018) obtained 11.71 mg AA 100 g⁻¹ in guava of the 'Media China' variety. Fajardo-Ortíz *et al.* (2019) obtained a range of 124.63 to 201.61 mg AA 100 g⁻¹ in six Colombian guava genotypes; Rojas-Barquera and Narváez-Cuenca *et al.* (2009) presented values of 78 to 268 mg 100 g⁻¹ of vitamin C in four Colombian varieties; Panayampadan *et al.* (2022) quantified values of 170 mg AA 100 g⁻¹; Musa *et al.* (2015) reported values of 135 to 202 mg AA 100 g⁻¹ in pink guava; finally, Kanwal *et al.* (2018) showed values of 155.5 mg AA 100 g⁻¹; all these values much lower than that obtained in the 'Fresa' variety, which stood out widely in this parameter.



AA plays a crucial role as a signaling molecule in many metabolic pathways, so its concentration must be controlled by precise metabolic regulation. Its synthesis depends on the specific cellular specialization. Thus, the AA content in plants changes depending on the light intensity, time of day, age, and type of plant tissue and cell compartment (Orsavová *et al.*, 2019).

In addition, the variability of AA content has been related to factors such as the particular genotype, which affects it by 50%, the locality by 17.1%, and the different growth conditions by 9.3% (Vagiri *et al.*, 2013). According to Patel *et al.* (2016), the AA content of guava is almost six times higher than that of an orange, which is why it is considered a very nutritious and attractive fruit to be consumed consistently. Its deficiency can cause a disease called scurvy (Porto *et al.*, 2019).

In relation to total polyphenols (TPC) (Figure 1), values of 231.5 mg of GAE 100 g were obtained in 'Blanca' guava, 255.8 mg GAE 100 g⁻¹ in 'China' guava, and 214.3 mg GAE 100 g⁻¹ in 'Fresa' guava. Statistical analysis showed significant differences between all varieties (Tukey's test; p# 0.05). According to the results of different authors, Rojas-Barquera and Narváez-Cuenca (2009) presented a range of values in TPC of 258 to 508 mg GAE 100 g⁻¹ in four Colombian varieties, Kanwal *et al.* (2018) reported 185.46 mg GAE 100 g⁻¹, Musa *et al.* (2015) 193.1 to 383.3 mg GAE 100 g⁻¹ in pink guava and Patel *et al.* (2016) 415.69 mg GAE 100 g⁻¹.



TPC in fruits could be significantly influenced by a particular step in the metabolism of individual phenolic compounds during fruit ripening. The composition of individual phenolic compounds is normally formed as an antioxidant protection that responds to environmental conditions (Orsavová *et al.*, 2019).

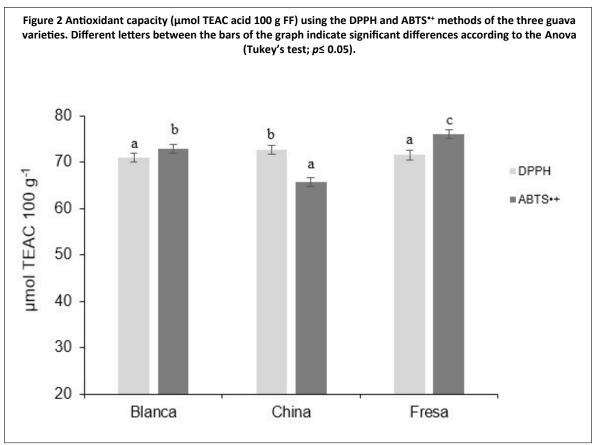
Harvest time, genotype, locality or geographic area, and cultivation technique are important factors affecting TPC (Orsavová *et al.*, 2019). The different methods of obtaining their phenolic extracts also have an impact on their determination or quantification (Rojas-Ocampo *et al.*, 2021). Currently,



due to the health benefits already mentioned and their antimicrobial effect, phenolic compounds are used in both traditional and modern medicine in the design and development of new medicinal agents (Rasouli *et al.*, 2017).

It is important to consider how much of these compounds one needs to consume to obtain the positive effects. Dietary intake of phenols is closely related to people's eating habits and preferences. The average daily consumption of polyphenols is approximately 1 g per person, with the main sources being beverages and fruits, and, to a lesser extent, vegetables and legumes (Shahidi and Ambigaipalan, 2015).

Nonetheless, the amounts of polyphenols needed to produce health benefits must be within the ranges present in commonly consumed foods to avoid toxicological hazards (Fraga *et al.*, 2021). With respect to AC (Figure 2), a significantly higher value ($p \le 0.05$) was observed for 'China' guava (72.6 µmol TEAC 100 g⁻¹ FF) compared to the 70.9 and 71.5 µmol TEAC 100 g⁻¹ FF of the 'Blanca' and 'Fresa' varieties, respectively, when analyzed with the DPPH method; in contrast, when measured with the ABTS technique, 76 µmol TEAC 100 g⁻¹ FF was quantified in the 'Fresa' variety against the 72.9 and 65.7 µmol TEAC 100 g⁻¹ FF of 'Blanca' and 'China' varieties, respectively. Rojas-Barquera and Narváez-Cuenca (2009) presented higher figures in four Colombian varieties, probably due to differences in extraction methods, as well as the variety of samples.



AC considers the complexity of interactions between all antioxidant compounds present in a food matrix (Li *et al.*, 2017). On the other hand, the antioxidant properties of fruits can be modified after ingestion by the digestion process (Ariza *et al.*, 2018), which is why the amount of polyphenols in raw fruits does not necessarily coincide with the AC of the fruit and with the health effects associated with its consumption for several reasons (Cervantes *et al.*, 2020).

The release (bioaccessibility) and absorption (ie., bioavailability) of polyphenolic compounds after digestion have been shown to affect the health properties of fruits (Ariza *et al.*, 2018). According to Saura-Calixto and Goñi (2006), the AC of each fruit is different, this depends on its phenolic and



vitamin content and the action and interaction of the different antioxidant compounds present in the fruits (Castro-López *et al.*, 2016), such as ascorbic acid, tocopherols, carotenoids, some of them related to pigmentation and the characteristic color of foods (Pennington and Fisher, 2009).

One of the most crucial conclusions today is that the risk of cancer decreases when consuming a diet rich in multiple antioxidants, and especially if they come from a combination of fruits (Shahidi and Ambigaipalan, 2015). Phenolic compounds contribute to the overall antioxidant activity of plant foods due to their high redox potential, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen suppressors (Kadžanoska *et al.*, 2011).

Nevertheless, due to the distinct antioxidant potential of the compounds and their polarity, methods aimed at assessing the AC of foods are greatly affected by the solvents used during extraction (Verma *et al.*, 2018), as it is the critical point in the determination and quantification of polyphenols since it dictates the nature and amount of polyphenols that will be transferred to the extract and subsequently characterized (Kadžanoska *et al.*, 2011). In relation to the analysis of color characteristics (CIEL a b coordinates), significant differences (*p*# 0.05) were observed between the samples in all parameters (Table 2).

Table 2. Color characteristics of the three guava varieties analyzed.								
Guava variety	Ľ	a	b	Chroma	Hue			
Blanca	69.66 (3.4) b	4.37 (3.54) a	43.5 (3.5) b	44.2 (3) b	83.7 (5.5) b			
China	72.6 (3.8) b	3.6 (0.3) a	51.6 (3.2) c	51.7 (3.2) c	85.9 (0.6) b			
Fresa	53.1 (3.9) a	31.4 (3.2) b	19.01 (1.77) a	36.9 (1.8) a	31.4 (4.8) a			

The coordinate a (negative for green and positive for red) coincides with the pink hue of the 'Fresa' guava. With respect to the coordinate b (negative for blue and positive for yellow), the 'China' guava showed the highest value, which would incline it towards yellow tones. Regarding the purity or intensity of the color (chroma), the highest data were obtained in 'China' guava. Regarding the hue, the values were closer to the yellow tone in the 'Blanca' and 'China' varieties, while the 'Fresa' guava was closer to the red tone.

In pink guava, Musa *et al.* (2015) recorded lower values in the CIEL a b coordinates, with a range of 48-49 in L, 11.25-16.06 in a, and 10.17-13.77 in b. Panayampadan *et al.* (2022) obtained color values of 76.81 in L, -4.85 in a, and 28.13 in b. The pigments present in fruits belong to various groups of chemicals that differ in color, stability, solubility, and sensitivity to environmental conditions in the presence of other substances.

These pigments can change with the light, the temperature used in processing, the effect of pH, or oxygen capacity (Kutlu *et al.*, 2022). The color of fruits and vegetables allows consumers to identify the product, assess its safety, quality, and ripeness, or even make inferences about its sensory properties (Schifferstein *et al.*, 2019).

However, it can also generate false expectations for the consumer; many of them intuitively relate the intensity of the colors of the food with the flavors; for example, green with an acidic flavor or red with sweetness (Ammann *et al.*, 2020) or when the color of the food has brown spots, it is likely that the consumer can assume that the product is in the decomposition phase and will consider that the product does not have the texture desired, that it may be soft, more bitter, or even have an unpleasant smell and taste (Schifferstein *et al.*, 2019).

One or more of these pigments can be found in foods; in addition to providing color, these pigments also greatly influence the health properties of foods, as they act as bioactive compounds with antioxidant and health-promoting properties (Kutlu *et al.*, 2022).

Conclusions

Guava from the region of Santiago el Chique (Zacatecas) represented a good source of antioxidants (polyphenols and vitamin C). Even though guavas have been cultivated under the same climatological conditions and geographical area, there were significant compositional differences (p# 0.05) between them.

Despite the fact that the 'Fresa' guava is less commercially attractive due to its size, from a nutritional point of view, of the three varieties, it stood out as the best source of vitamin C and the one with the highest antioxidant capacity when evaluated with the ABTS⁺⁺ method; this could indicate the influence of ascorbic acid and the pigments with antioxidant activity, specifically in this technique.

The antioxidant capacity was dependent on the analytical method; in ABTS^{*+}, the 'Fresa' variety presented the highest value, while the 'China' variety did so in the DPPH technique, which does not allow us to highlight any particular variety in this parameter. For sensory reasons or nutritional knowledge, including different fruits or vegetables or different varieties, but with different colors, in the diet will allow greater access to nutrients and phytochemicals for the benefit of health.

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