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*Research article*

## **Free choice profiling sensory analysis and principal component analysis as tools to support an apple breeding program**

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**Abstract:** The challenge for genetic improvement of apples is to develop varieties that have appealing physicochemical and sensorial qualities, good adaptation to different environments, and resistance to pests and diseases. The present study evaluated the physicochemical parameters and sensory attributes of promising genotypes and of a commercial apple variety in the breeding program of the Paraná Rural Development Institute IAPAR-EMATER in two cultivation localities in Paraná state, Lapa and Palmas. We proposed the use of free choice profiling (FCP) sensorial analysis and principal component analysis (PCA) as tools to aid in the genetic improvement of apples. The genotypes analyzed from Palmas were PR2.40, PR2.13, PR2.21, PR2.31, PR2.51, PR2.26, PR2.5, PR2.70, PR2.60, IAPAR75-Eva, and from Lapa were PR2.40, PR2.13, PR2.21, and IAPAR75-Eva. The physicochemical parameters analyzed were weight, height, diameter, skin and pulp firmness, color, pH, titratable acidity (TA), total soluble solids (SST), and SST/TA ratio. In FCP, 10 assessors described the appearance, aroma, taste, and texture of apples. In Palmas, genotypes PR2.13, PR2.21, PR2.26, PR2.40, PR2.60, and IAPAR75-Eva showed sensory and physicochemical characteristics that were appealing to consumers, showing promise for launch as varieties. PR2.5, PR2.31, PR2.51, and PR2.70 presented low concordance between physicochemical characteristics and sensory attributes requiring more detailed study of these genotypes. Comparison between from two localities PR2.13, PR2.21, PR2.40, and IAPAR75-Eva the genotypes indicated a relationship between physicochemical and sensorial characteristics, and the presence of a higher number of indicative attributes of good quality. In Palmas, however, the apples presented physicochemical characteristics

of and sensory attributes of immature fruit. The application PCA contributed to the evaluation of a greater number of parameters of apple quality and showed the genotypes with high quality parameters. In addition, FCP allowed identify the attributes of genotypes grown in same local as well as identify attributes that separated same genotypes grown in two local. Therefore, these multivariate analyses were appropriated to apply in apple breeding program and aiding the breeder's decision to recommend new varieties of apples.

**Keywords:** *Malus domestica* BORKH; breeding; physicochemical; sensory analysis

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## 1. Introduction

The apple (*Malus domestica* Borkh.) has its origin in the region between the Caucasus and eastern China. Its modern commercial exploitation in Brazil began in the 1960s, in Santa Catarina State. Brazil, a traditional importer of apples until the 1980s, has now become an exporter to the European Union and North American markets. Currently, the states of Santa Catarina and Rio Grande do Sul are the main producers in the country [1].

Brazil produced approximately 1.02 million tons of apples in the 2018/2019 [2]. Fruit production aims to serve the consumer market, which is increasingly demanding with regard to quality [3,4]. Fruit consumption has decreased in relation to the growth of fast and ready-to-eat foods. To encourage a healthy diet through fruit consumption, it is necessary to associate the nutritional aspect with sensory pleasure. For this, the fruit must meet the sensory expectations of the consumer through its characteristic tastes/flavors and aromas.

The harvest of apples should be carried out at the ideal moment when the fruit have good visual characteristics and chemical parameters, which reduces the possibility of quality losses during storage and commercialization, due to physiological disorders inherent to immature or very ripe fruit. Fruits harvested too early have their ripening process compromised, which means they do not reach the desired quality for consumption, the taste and aroma are compromised, and they dehydrate more easily [5,6].

The growing conditions of apple trees influence the quality of the fruits produced because the temperature affects the growth regulator substances, and these substances control the start and finish of the dormancy metabolism [1]. The apple quality is influenced by solar incidence, temperature, precipitation and winds, and these factors contribute to the color of the fruit, sugar content, shape, and apple size [6]. As apples are plants of temperate climatic conditions, cultivars with low cold requirements are recommended for regions with mild and cold winters. In addition the adoption of artificial dormancy breaking techniques helps to increase productivity. In Brazil appropriate conditions are found in the southern states (Paraná, Santa Catarina and Rio Grande do Sul) and new varieties adapted in these conditions are continually developed for breeding programs.

Genetic improvement has been conducted to select genotypes with superior agricultural and sensory quality and greater genetic variability [7]. Conventional breeding methods, where targeted hybridizations are used to select superior genetic combinations for launching a new apple variety, can last up to 12 years. Pre-selection can take two years, with another four years for the selection of the crossed materials, and finally, a further six years for the evaluation process.

A new variety must be well adapted to climatic conditions of growth and high productivity

together physicochemical characteristics such as color, weight, size and firmness [4]. The physicochemical evaluations carried out for the launch of new varieties of apples take in account both the physical (weight, size, firmness, and color) and chemical aspects (titratable acidity (TA), total soluble solids (SST), pH, and SST/TA ratio). These evaluations identify the cultivars that better fit the commercial categories, allowing the commercialization of quality assured products [4,8].

However, the breeding process can be facilitated using molecular markers and statistical tools, such as multivariate analyses. The principal component analysis (PCA) is a multivariate analysis that allows the simultaneous evaluation of the variables resulting in better interpretation of the physicochemical and sensory data.

Sensory analysis is a methodology designed to assess the acceptance of the product for the consumer market and it is essential to ensure the good quality of the genotypes developed in the genetic improvement, evaluating the specific characteristics of each product (color, texture, aroma, and taste/flavor) to meet the requirements of the consumer [9].

In the FCP sensory technique, assessors have the freedom to use their own vocabulary to describe and evaluate the samples. However if at any point in the evaluation of the samples a new attribute appears, the assessor may add it to his in the evaluation form. These may sometimes appear in only one sample, thus identifying characteristics that distinguish that sample from another sample. The FCP employs the generalized Procrustes analysis (GPA) that is a statistical analysis that interprets the sensory perceptions of appearance, aroma, taste/flavor and texture chosen by the assessors to describe each sample. Although the FCP test is exploratory, it is the first step towards the application of more specific and complete tests such as quantitative descriptive analysis in breeding processes of fruits.

In this way, the influence of several variables on apple quality can be better interpreted using both PCA and FCP, and contribute to reliable information in databases and technical bulletins since currently these data are based only on the observations of the breeders. With these tools, the breeder can be give more confident and complete information in moment of the launch of a new variety.

The objective of this study was to evaluate the physicochemical and sensory characteristics of promising genotypes and a commercial variety of apple from the breeding program of the Paraná Rural Development Institute IAPAR-EMATER. The PCA and FCP were applied to study the physicochemical and sensory characteristics and the influence growing environments in order to help with launch decisions in the breeding program.

## **2. Material and methods**

### *2.1. Plant materials and field experiments*

The apples were collected from the two experimental orchards of the Paraná Rural Development Institute IAPAR-EMATER, Brazil in Palmas and Lapa during the 2018 harvest season. Palmas (Figure 1A) is located at coordinates 26°27'56" S and 51°58'33" W, 1088 m.a.s.l., with a temperate oceanic climate (Figure 1A), with low-temperature winters, cool summers, and no defined drought period. The experimental station of Lapa (Figure 1B) is located at 25°46'11" S and 49°42'57" W, 800–900 m.a.s.l., with a humid mesothermal subtropical climate, with low-temperature winters, cool summers, and no defined drought period.



**Figure 1.** Experimental Station of Palmas (A) and Experimental Station of Lapa (B) at IAPAR.  
Image from: Google Earth (2018).

Nine genotypes PR2.40, PR2.13, PR2.21, PR2.31, PR2.51, PR2.26, PR2.5, PR2.70, and PR2.60 and the commercially produced variety IAPAR75-Eva, grown in Palmas, were harvested in the maturation stage according to the background color and firmness compatible with the particularity of each genotype. The fruit were stored at 6–10 °C in plastic bags and covered until analysis.

Among these genotypes studied, the genotypes PR2.13, PR2.21, PR2.40, and IAPAR75-Eva were chosen for an evaluation in two localities in order to comparing the environmental effects in the physical properties and sensory attributes.

For both experiments 15 to 20 fruits were harvested for each genotype, depending on production in this year. Among these, ten fruits were chosen for sensory analysis and 5 for physicochemical analysis.

Meteorological data were collected at the Paraná Rural Development Institute IAPAR-EMATER meteorological stations of Palmas and Lapa. The climatic variables analyzed were average annual temperature and average annual precipitation, corresponding to the period from January 2018 to December 2018.

## 2.2. Measurement of physical properties

Five fruit of each genotype with uniform size and ripeness were selected and weighed in an electronic balance, and the diameter and height measurements in millimeters were obtained with a digital caliper (799a-12/300). Color parameters were measured using CIE illuminant C, with a 10 ° angle and a CIE standard observer (Minolta CR-410, Japan). A reading from the skin of five whole fruits of uniform ripening at diametrically opposite sides was performed [10]. The color determination was performed measuring the colorimetric coordinates  $L^*$ ,  $a^*$ , and  $b^*$  to the CIELab system. The reflectance spectra were recorded using the standardized CIE  $L^*a^*b^*$  chromaticity system as a function of wavelength. This system estimates the value of three variables: coordinate  $L^*$  for lightness, representing the position on the black-white axis ( $L^* = 0$  for black,  $L^* = 100$  for white), coordinate  $a^*$  for the position on the red-green axis (+100 = positive values for red, -80 negative values for green) and coordinate  $b^*$  for the position on the yellow-blue axis (+70 = positive values for yellow, -80 = negative values for blue).

Texture analysis was performed using a Texture Analyzer (model TAX-T2–Stable Micro Systems, UK), with a P/6 probe. The parameters of the determinations were pre-test speed = 1.5 mm/s, test speed = 1.5 mm/s, post-test speed = 1.5 mm/s, target mode = distance, distance = 5.0 mm, trigger force = 0.98 N. The samples consisted of apple slices of 30 × 30 × 30 mm (length, width, thickness). The penetration force was evaluated to estimate the peel and pulp resistance to rupture.

### 2.3. Measurement of chemical properties

Five whole fruits were ground in a blender and filtered through a fine mesh cloth to obtain homogenized juice. The TA, pH, and SST were determined. The pH was determined using a potentiometer (Digimed DM-20). The TA was determined by titrating 5 mL of juice and 10 mL of distilled water with 0.1 N NaOH until reach pH 8.2 using an autotitrator (Titroline-easy) [11]; the percent acidity was expressed as the malic acid equivalent [12]. The SST was measured using a hand refractometer (RT-90 ATC) at 20 °C (°Brix). Once the SST and TA contents were measured, the SST/TA ratio was determined.

### 2.4. Sensory Analysis step-by-step

Sensory evaluations were carried out by a panel of ten who were students and employees between 20 and 50 years old and who had taken at least one college course. Assessors had already participated in sensory evaluations of other foods using the FCP. Even so, in this study, assessors were given an explanation about apple evaluations.

For FCP analysis, the first step was the selection of the assessors over orientation of the team leader who coordinated and guided the participants. The selection of assessors considered the availability of time and sensory aptitude. Sensory aptitude was assessed by a test of recognition of basic taste and odors according to the methodology described by Kitzberger [13]. In the second step, the attribute terms for the evaluation of the apple samples were developed by the panel using the repertory grid method [14]. The assessors were instructed to record the similarities and differences between a pair of apples in order to describe the sensory attributes of appearance, aroma, flavor, and texture of the samples, which were selected for their distinct sensory characteristics [14].

The third step was the creation of individual score sheets and a glossary based on the individual descriptors that were prepared by the team leader. Individual score sheets ranged from 1 to 10, anchored at the ends with intensity expressions for each attribute [13]. The final step was the application of the core sheets and the glossary to the apples of the experiment. In each session, apple samples were evaluated based on the period of harvest and maturation point determined for each genotype.

For the evaluation of the fruits, visually similar and standardized apples in terms of color, size, and absence of defects, were selected and were evaluated as described by Kitzberger [12]. Each assessor received a fruit from each cultivar coded with three-digits, presented on plastic plates and served at 25 °C  $\pm$  1.5 °C. The team leader guided the assessors to remember the attributes and descriptions according to the glossary. The assessors then tasted the fruit and in each attribute of the score sheet assigned a mark on the scale according to the intensity of the attribute. Between tests, mineral water was used to rinse the mouth and clean the palate. Sensory evaluations were carried out in individual sensory booths at room temperature and ambient daylight in a tasting room. Sample preparation and sensory analysis were conducted in the Physiology Laboratory of the Paraná Rural Development Institute IAPAR-EMATER.

### 2.5. Data analysis

Analysis of variance (Anova) and Tukey test were employed for the physicochemical data. PCA

allows evaluating a set of samples, based on the components formed from the original variables and the samples are projected in the space formed by these components. Considering this point of view, PCA was applied to the original matrix formed by the physicochemical evaluations of apples. In the FCP technique, the data obtained in the evaluations of the apples were organized in a spreadsheet, where the columns showed the scores for each sample and in the rows showed the attributes evaluated by each assessor. The values of the attributes of each assessor for the same samples were initially subjected to translation, scaling, and rotation procedures by applying GPA. The results of GPA are presented as a consensus graph and a table of the attributes that present correlation greater than  $|0.25|$  with the dimensions and high number of quotation by the assessors. All statistical analyses were performed using the XLStat statistical software [15].

### 3. Results and discussion

#### 3.1. Physicochemical evaluation of the apple genotypes

Nine genotypes of apples were evaluated for their physicochemical and sensory characteristics in order to verify their potential for launch compared to a variety already well established in the market.

For launching a new variety, genotypes must showed good responses to climate and soil adaptations, as well as good resistance to pests and diseases. In this schedule of genetic improvement all evaluated genotypes in the present study showed stability for these characteristics. The next step was evaluated the physicochemical and sensory characteristics which are extremely important for acceptability of the consumers. Table 1 and 2 shows the physicochemical parameters linked to the quality of promising genotypes.

**Table 1.** Physicochemical parameters of the quality of genotypes.

Genotype	Skin Firmness	Pulp Firmness	SST	pH	TA	SST/TA ratio
IAPAR-75 EVA	24.29 $\pm$ 1.3abc	18.96 $\pm$ 0.9ab	16.38 $\pm$ 0.0a	3.8 $\pm$ 0.0b	0.40 $\pm$ 0.0b	41.45 $\pm$ 0.2d
PR2.13	25.90 $\pm$ 1.3abc	14.27 $\pm$ 3.4abc	14.18 $\pm$ 0.1c	4.01 $\pm$ 0.0a	0.21 $\pm$ 0.0h	67.34 $\pm$ 0.6a
PR2.21	29.22 $\pm$ 6.2a	17.53 $\pm$ 4.5ab	13.58 $\pm$ 0.3cd	3.76 $\pm$ 0.0b	0.28 $\pm$ 0.0f	49.17 $\pm$ 0.8c
PR2.40	20.86 $\pm$ bcd	15.06 $\pm$ 0.1abc	13.18 $\pm$ 0.3d	3.82 $\pm$ 0.0b	0.35 $\pm$ 0.0d	37.49 $\pm$ 0.9e
PR2.5	12.00 $\pm$ 2.8cd	9.19 $\pm$ 1.2cd	14.38 $\pm$ 0.3c	3.57 $\pm$ 0.0d	0.27 $\pm$ 0.0f	52.56 $\pm$ 0.8c
PR2.51	11.35 $\pm$ 0.2d	6.96 $\pm$ 0.3d	13.18 $\pm$ 0.3d	3.54 $\pm$ 0.0de	0.41 $\pm$ 0.0a	31.89 $\pm$ 0.7f
PR2.31	17.37 $\pm$ 1.9bcd	9.92 $\pm$ 2.2bcd	15.38 $\pm$ 0.3b	3.51 $\pm$ 0.0e	0.41 $\pm$ 0.0a	37.39 $\pm$ 0.6e
PR2.70	18.69 $\pm$ 1.1cd	10.42 $\pm$ 1.4cd	14.18 $\pm$ 0.3c	3.31 $\pm$ 0.0g	0.37 $\pm$ 0.0c	37.81 $\pm$ 0.8e
PR2.60	21.70 $\pm$ 2.9bc	14.91 $\pm$ 0.2abc	12.98 $\pm$ 0.3d	3.42 $\pm$ 0.0f	0.33 $\pm$ 0.0e	39.71 $\pm$ 2.3de
PR2.26	27.86 $\pm$ 4.3ab	20.92 $\pm$ 1.9a	13.78 $\pm$ 0.3cd	3.73 $\pm$ 0.0c	0.23 $\pm$ 0.0g	60.07 $\pm$ 1.4b

Note: Legend: Firmness (N), SST (°Brix), TA (%). \* Different small letters in the same column indicate significant differences among genotypes  $p < 0.05$  (Tukey test).

Fruit firmness is mainly used to decide the optimal harvest time and determine stage of maturity [8]. The firmness is a critical factor that can influence storage or handling, transport, and attack from microorganisms. The skin firmness ranged from 11.35 to 29.22 N (PR2.51 and PR2.21, respectively), and the pulp firmness varied from 6.96 to 20.92 N (PR2.51 and PR2.26, respectively).

The pH values ranged from 3.31 to 4.01 (PR2.70 and PR2.13, respectively), that are similar to values that were found in the literature for the several apple varieties (pH3.79–3.92) [16].

**Table 2.** Physical parameters of the quality of genotypes.

Genotype	Diameter	Height	Weight	L*	a*	b*
IAPAR-75 EVA	64.47 ± 22.6a	59.03 ± 2.3ab	103.57 ± 3.3b	56.8 ± 12.7a	21.3 ± 2.9ab	33.2 ± 8.7ab
PR2.13	71.30 ± 4.7a	64.87 ± 0.8ab	163.09 ± 18.3ab	60.3 ± 5.8a	8.3 ± 11.1b	39.8 ± 1.8a
PR2.21	73.17 ± 1.1a	65.83 ± 2.1ab	167.61 ± 8.3ab	59.6 ± 4.1a	6.1 ± 5.4ab	35.6 ± 4.0ab
PR2.40	68.33 ± 5.8a	69.31 ± 2.9a	151.83 ± 37.3ab	61.4 ± 3.2a	10.1 ± 5.4ab	34.3 ± 2.5ab
PR2.5	70.76 ± 1.1a	67.10 ± 0.9ab	142.73 ± 7.2b	52.4 ± 5.6a	27.4 ± 0.8a	30.8 ± 6.0ab
PR2.51	69.63 ± 10.1a	65.34 ± 6.9ab	152.38 ± 51.7ab	43.3 ± 6.2a	22.8 ± 2.7ab	22.0 ± 4.7b
PR2.31	61.00 ± 0.6a	55.59 ± 3.6a	96.55 ± 3.0b	47.1 ± 1.9a	31.8 ± 3.8a	27.0 ± 0.8b
PR2.70	88.97 ± 7.3a	75.86 ± 11.3a	284.53 ± 77.3a	59.7 ± 1.7a	12.6 ± 2.8ab	35.9 ± 2.7ab
PR2.60	62.26 ± 9.8a	52.75 ± 5.0b	99.12 ± 21.9b	67.0 ± 0.7a	10.2 ± 4.3ab	39.0 ± 2.4ab
PR2.26	64.13 ± 3.2a	62.86 ± 2.5ab	131.80 ± 6.3b	63.7 ± 1.9a	4.5 ± 4.4ab	38.3 ± 0.7ab

Legend: Diameter (mm), height (mm), weight (g). \* Different small letters in the same column indicate significant differences among genotypes  $p < 0.05$  (Tukey test).

Consumer preference indexes for apples are correlated positively with the SST content, and are observed rejection of apples with SST content below 12 °Brix [17]. All genotypes in the present study showed high SST values, ranging from 12.98 °Brix to 15.38 °Brix (PR2.60 and PR2.31, respectively), which indicated a probable sensory acceptability.

Ideal TA for apple consumption must be in the range of 0.20% to 0.70% expressed in malic acid equivalents [16]. The genotypes varied from 0.21% (PR2.13) to 0.41% (PR2.51 and PR2.31). Similar results were also found for IAPAR75-Eva and others commercial varieties in early studies [18,19].

The SST/TA ratio is considered a parameter of importance for harvest and industrial processing. Apples with an SST/TA less than 20 are more suitable for industrial processing (juices and ciders), while those with a higher ratio are considered sweet and suitable for *in natura* consumption. In the present study, the SST/TA varied from 31.89 to 67.34 (PR2.31 and PR2.13, respectively), demonstrating that fruits were suitable for *in natura* consumption and that the fruits showed complete ripeness [19].

For the fruit weight, the genotypes, with the exception of PR2.31 and PR2.60, presented fruit of good weight, over 100g, in agreement with Santos et al., [20], who obtained fruit of the Fuji Suprema variety with a weight greater than 100g. In general, the fruits showed good characteristics in this parameter (Table 2), meeting the similar quality requirements to cultivars already launched in the market.

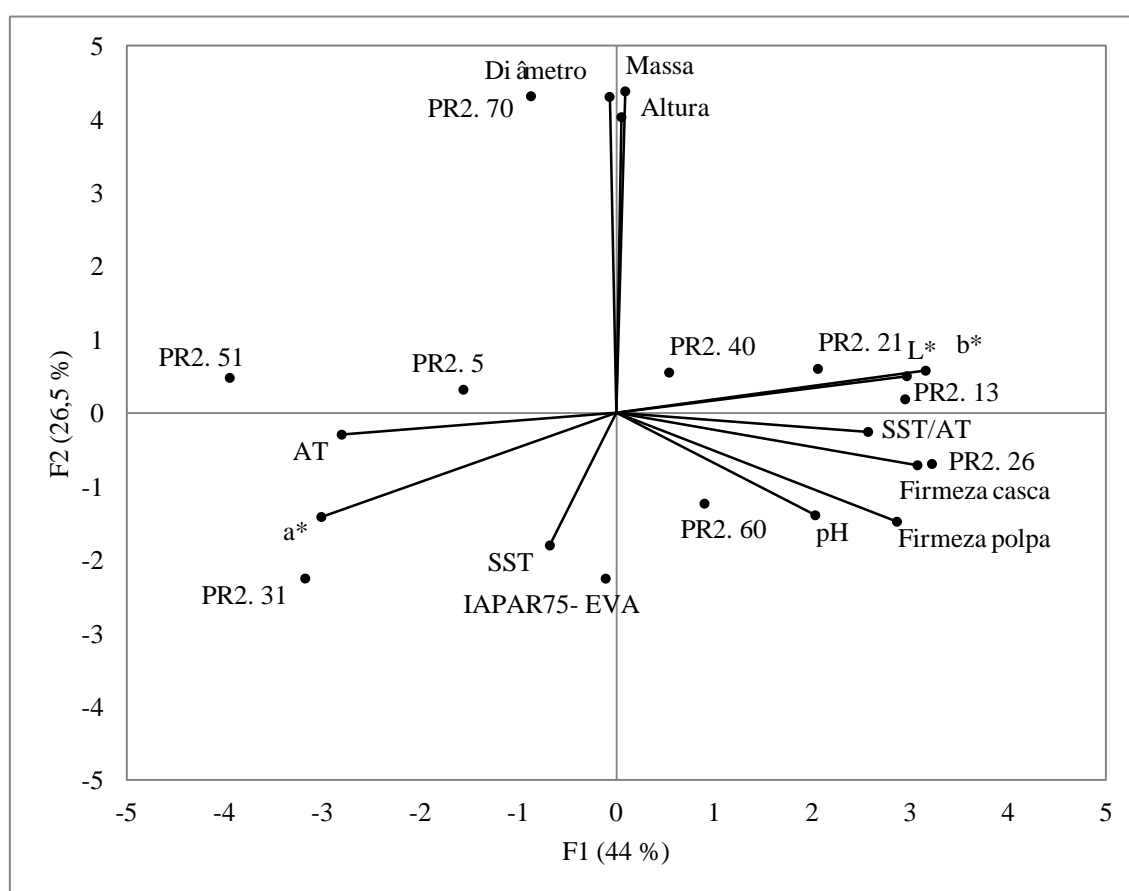
Fruit smaller than 65 mm and larger than 85 mm in diameter are considered out of the commercial standard in the Brazilian market. The IAPAR75-Eva, PR2.13, PR2.21, PR2.40, PR2.5, PR2.26, and PR2.51 genotypes were agreed with this measurement range.

The epidermis color of apples is not considered an index of ripeness because it varies according to environmental factors and apple varieties [21]. But for apples the visual aspect of fruit, mainly the color of the skin, has a great influence at the time of purchase. The L\* (luminosity) color parameter varied from 43.3 to 67.0 (PR2.51 and PR2.60, respectively), where a higher L\* value described lighter apples. The a\* color parameter, that indicate the red color of the apple skin, varied from 4.5 to 31.8 (PR2.26 and PR2.31, respectively). The b\* color parameter ranged from 22.0 to 39.8 (PR2.51

and PR2.13, respectively) and represented the yellow color. The genotypes IAPAR75-Eva, PR2.5, PR2.51, and PR2.31 had the darkest, reddest, and least yellow color. The other genotypes showed the opposite characteristic, that is, lighter, with more yellow, and less red.

The characterization physicochemical of genotypes from Palmas using PCA is showed in Figure 2. The genotypes were dispersed according to the first two components (F1 and F2) that explained 70.5% of the total variance. The F1 component formed by skin firmness (0.88), pulp firmness (0.82), pH (0.58), TA ( $-0.81$ ), SST/TA ( $-0.74$ ),  $L^*$  (0.86),  $a^*$  ( $-0.87$ ) and  $b^*$  (0.91). This component separated the genotypes horizontally in this dispersion. F2 component was formed by SST ( $-0.4$ ), diameter (0.96), height (0.89), and mass (0.9) separated the genotypes vertically in the plane of the dispersion.

The PR2.13, PR2.21, PR 2.26, PR2.40, and PR2.60 genotypes located on the right side of the biplot (separated by F1+) were light-colored and more yellow, more mature (higher SST/TA), less acidic (higher pH), and firmer in the skin and pulp. The PR2.5, PR2.31, PR2.70, and PR2.51 genotypes separated by F1 ( $-$ ) had a redder color, darker, and were more acidic (higher TA) with a softer texture in the skin and pulp. The IAPAR75-Eva variety showed intermediate characteristics between the two groups mentioned above. The F2 (+) promoted the separation of the genotypes by greater size and mass, it is interesting highlight the PR2.70 genotype as the largest (high height) and heaviest (75.86 mm in height and 284.53 g). Others genotypes (PR2.51, PR 2.5, PR2.40 PR2.21, and PR 2.13) had similar characteristics.



**Figure 2.** PCA of physicochemical analyses of apple genotypes.



### 3.2. Sensory analysis of apple genotypes

Sensory descriptions of the apples (Figure 3) were obtained by FCP through the projection of dimensions F1 and F2. These dimensions are formed by attributes mentioned with correlation of  $|0.25|$  in the respective dimension and by assessor's quotations. In Supplementary Table 1 were showed the frequency of quotation by the assessors and the correlation of attributes with these dimensions.

PR2.13, PR2.21, PR2.26, PR2.40, PR2.60, and IAPAR75-Eva genotypes, allocated on the right side of the Figure 3, were described as having green skin, yellow spots, characteristic apple aroma, immature fruit, skin bitter, sweet pulp and skin tastes, characteristic apple flavor, immature fruity flavor. In addition, these fruits of genotypes had a crunchy, fibrous, and succulent textures.

The apple color indicated by the assessors in FCP matched with instrumental evaluation. The genotypes that showed the highest  $L^*$  and  $b^*$  values were also interpreted by the assessors as a more intense green color, with the presence of yellow spots (Table 2, Table 3).

The immature fruit flavor did not relate with any physicochemical data. However, the crunchy, fibrous, and succulent textures of these genotypes can be associated to a higher pulp and skin firmness value (N) according texture analysis.

The PR2.5 and PR2.31 apples allocated to the upper left side of the Figure 3 had small size and they presented red color, smooth skin, characteristic apple aroma, sweet aromas; sweet pulp and skin tastes, acid taste, and characteristic apple flavor, and the texture was with a soft skin. These attributes could be associated to physicochemical data (low values to skin and pulp firmness). However the sweet taste and aroma were not associated with the high SST and SST/TA values in these apples, although they are described as such (Figure 2, Table 1).

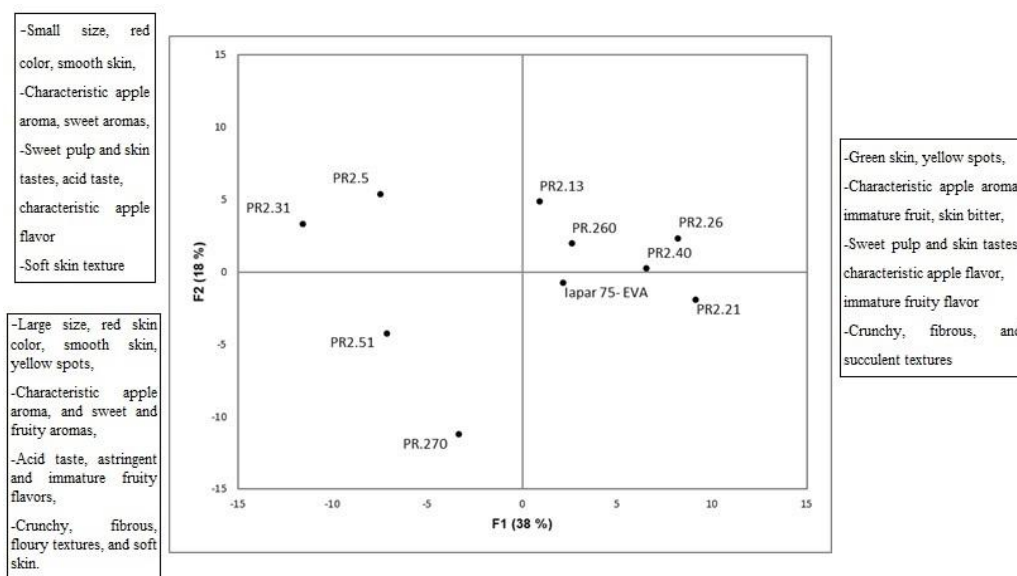
The PR2.51 and PR2.70 apples allocated to the lower left side of the Figure 3 (F1– and F2–) were described as large size, red skin color, smooth skin, yellow spots, characteristic apple aroma, and sweet and fruity aromas, acid taste, astringent and immature fruity flavors. These genotypes had yet, crunchy, fibrous, floury textures, and soft skin.

Acid taste and green flavor were correlated to physicochemical data for these genotypes (Figure 2) because these genotypes had low pH and high TA. We can verify that PR2.51 and PR2.70 presented less instrumental firmness of the skin and pulp. In this group, the size attribute was sensory perceived because these genotypes (especially PR2.70) have the largest size compared to the others (Figure 2, Table 2).

Despite of the genotypes from different crossings and presented variability in the physicochemical parameters, they presented characteristics of acidity and SST in the range recommended for *in natura* consumption (acidity  $< 0.45$  g/100 g, SST  $> 12$  °Brix, and SST/TA  $> 20$ ). Among the genotypes allocated on the left side of Figure 2, it was observed a greater acidity amplitude of 0.14 g/100 g and amplitude of 3.2 °Brix for SST. On the other hand the genotypes allocated on the right side presented greater SST/TA, and the acidity amplitude was 0.12 g/100 g and 1.2 °Brix for SST.

However, it was not possible found a relationship between sensory and instrumental evaluations because there are many sensory interactions among these characteristics.

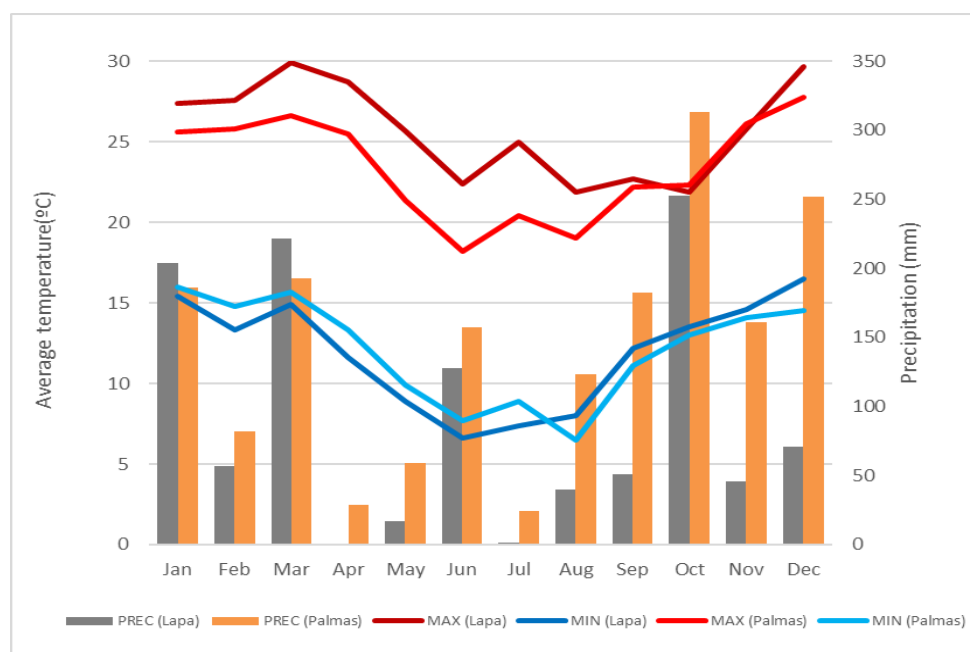
The difference between the sensory perception of sweetness and acidity and the results of the physicochemical analysis may be due to a balance between these parameters, where the most acids genotypes had the highest SST and the sweetest genotypes had the least acidity (Table 1).



**Figure 3.** Consensus of sensory attributes of apple genotypes.

### 3.3. Physicochemical evaluations of genotypes grown in the two localities

The climatic conditions of average temperature and precipitation at Lapa-PR and Palmas-PR in 2018 were presented in Figure 4 and shows that the temperature started to rise in both locations in October. The rainfall in Palmas was higher than in Lapa, with 160.8 mm in November and 251.8 mm in December in Palmas, and 45.6 and 70.8 mm in Lapa, respectively.



**Figure 4.** Monthly averaged temperature and precipitation at Lapa-PR and Palmas-PR in 2018.

The physicochemical parameters related to the quality of genotypes IAPAR-75 EVA, PR2.40, PR2.21 and PR2.13 grown in Palmas and Lapa showed significant differences, suggesting great diversity in the characteristics evaluated (Tables 3 and 4). The effect of the location was observed for all variables, with the exception of skin firmness and color parameters L\*, a\* and b\*.

**Table 3.** Physicochemical parameters of genotype quality grown in Palmas-PR and Lapa-PR.

	Skin	Pulp	SST	pH	TA	SST/TA
Palmas						
IAPAR-75 EVA	25.71 ± 1.3ab	18.79 ± 0.9ab	16.38 ± 0b	3.80 ± 0c	0.4 ± 0a	41.45 ± 0.2e
PR2.40	19.99 ± 1.4b	14.74 ± 3.4b	12.99 ± 0.1c	3.81 ± 0c	0.35 ± 0b	36.95 ± 0.6f
PR2.21	34.71 ± 6.2a	18.84 ± 4.5ab	13.39 ± 0.3c	3.77 ± 0cd	0.28 ± 0d	48.48 ± 0.8d
PR2.13	26.35 ± 2.8ab	15.87 ± 0.1b	14.09 ± 0.3c	4.00 ± 0b	0.21 ± 0e	66.91 ± 0.9b
Lapa						
IAPAR-75 EVA	22.15 ± 0.3ab	17.37 ± 1.0ab	14.19 ± 0.3c	3.71 ± 0e	0.32 ± 0c	45.16 ± 0d
PR2.40	25.97 ± 0.6ab	16.06 ± 0.1b	17.79 ± 0.3a	3.73 ± 0de	0.30 ± 0c	59.40 ± 1.0c
PR2.21	31.62 ± 0.6ab	28.68 ± 0.4a	13.99 ± 0.3c	3.81 ± 0c	0.31 ± 0c	46.21 ± 0.3d
PR2.13	26.07 ± 0.6ab	26.18 ± 0.1ab	13.99 ± 0.3c	4.08 ± 0a	0.18 ± 0f	80.99 ± 0.3a

Legend: Firmness (N), SST (°Brix), TA (%). \* Different small letters in the same column indicate significant differences among genotypes,  $p < 0.05$  (Tukey test).

**Table 4.** Physical parameters of genotype quality grown in Palmas-PR and Lapa-PR.

	Diameter	Height	Weight	L*	a*	b*
Palmas						
IAPAR-75 EVA	62.53 ± 2.6b	61.56 ± 2.4c	112.34 ± 3.3e	59.590.6 ± a	34.23 ± 2.9a	42.82 ± 1.6a
PR2.40	64.88 ± 4.7ab	68.33 ± 0.8bc	176.60 ± 1.5bcd	45.75 ± 1.5b	23.97 ± 0.9ab	33.20 ± 1.8ab
PR2.21	70.15 ± 1.1ab	63.68 ± 2.1bc	156.67 ± 4.1d	48.37 ± 4.1ab	21.57 ± 5.4ab	29.55 ± 4.0bc
PR2.13	70.87 ± 1.0ab	62.88 ± 2.9bc	170.62 ± 3.2cd	59.82 ± 3.2a	10.28 ± 5.4a	35.74 ± 2.5ab
Lapa						
IAPAR-75 EVA	75.64 ± 0.7a	71.35 ± 1.6ab	196.95 ± 1.0ab	53.18 ± 1.0ab	24.3 ± 1.7ab	27.55 ± 0.4bc
PR2.40	75.00 ± 1.1a	78.03 ± 0.2a	211.03 ± 3.5a	53.3 ± 3.5ab	15.75 ± 4.1ab	20.14 ± 3.4c
PR2.21	75.52 ± 0.7a	71.84 ± 0.5ab	196.33 ± 1.8ab	55.2 ± 1.8ab	22.50 ± 1.4ab	26.01 ± 0.8bc
PR2.13	72.20 ± 0.9a	68.03 ± 0.1bc	177.45 ± 1.3bc	47.05 ± 1.3ab	32.80 ± 0.4a	23.85 ± 1.5bc

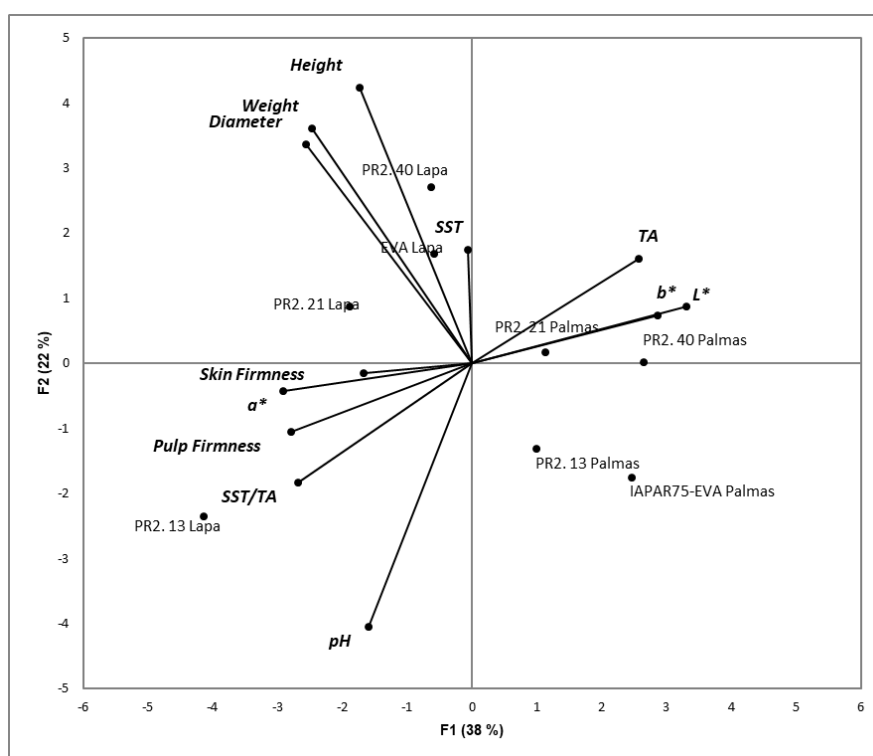
Legend: Diameter (mm), height (mm), weight (g). \* Different small letters in the same column indicate significant differences among genotypes,  $p < 0.05$  (Tukey test).

Due to the large number of variables used in the study, the description of the genotypes of each location can be better achieved using a multivariate analysis such as the PCA than analyzing the variable alone.

PCA was applied to the physicochemical characteristics of the fruit from two localities. The first two components (F1 and F2) of PCA explained 60.5% of the total variance among the genotypes from these locations. F1 component was formed mainly by the following variables: skin firmness (−0.43), pulp firmness (−0.72), TA (+0.66), SST/TA (−0.69), L\* (+0.85) a\* (−0.74) and b\* (+0.73). This component separated the cultivation localities: All genotypes from Palmas were located on the right side while the genotypes from Lapa on left side of the biplot graph (Figure 5).

The (F2 component) was formed basically by SST (+0.34), pH (−0.79), diameter (+0.66), height (+0.83), and weight (+0.70) and promoted the separation of genotypes of each local.

The genotypes from Palmas presented a light color (higher  $L^*$ ), intense yellow color (higher  $b^*$ ), little reddish color and higher acidity (higher TA). These same genotypes cultivated in Lapa were more reddish, darker, and less acidic. The environmental conditions of higher temperatures and low incidence of light have a great influence on the development of the redder and darker color of the epidermis at the early fruiting stage, which may explain the predominant color of Lapa apples [19].



**Figure 5.** Principal component analysis of the physicochemical characteristics of apple genotypes grown in Palmas-PR and Lapa-PR.

The genotypes grown in these local present also differences in the skin and pulp firmness; genotypes from Lapa had higher skin and pulp firmness than those grown in Palmas (Figure 5).

The acidity of genotypes was higher in Palmas than Lapa. The lower temperatures increased the synthesis of organic acids and consequently their use was also reduced in respiration, resulting in fruit with greater acidity in the final stage of their development [23]. As the temperature in Palmas was lower than in Lapa throughout the year (Figure 4) the apples from Palma had more acidity. The SST/TA value was employed as maturation indicator. It is possible observe that fruits from Lapa were ripen because they high values of SST/TA.

In addition, when analyzing Fig 5, it is observed that the genotypes of the same location differ and are separated by the F2 component. Among the genotypes harvested in Lapa PR 2.40, IAPAR75-Eva, and PR 2.21 genotypes, and PR 2.21 and PR2.40 grown in Palmas showed the highest value SST.

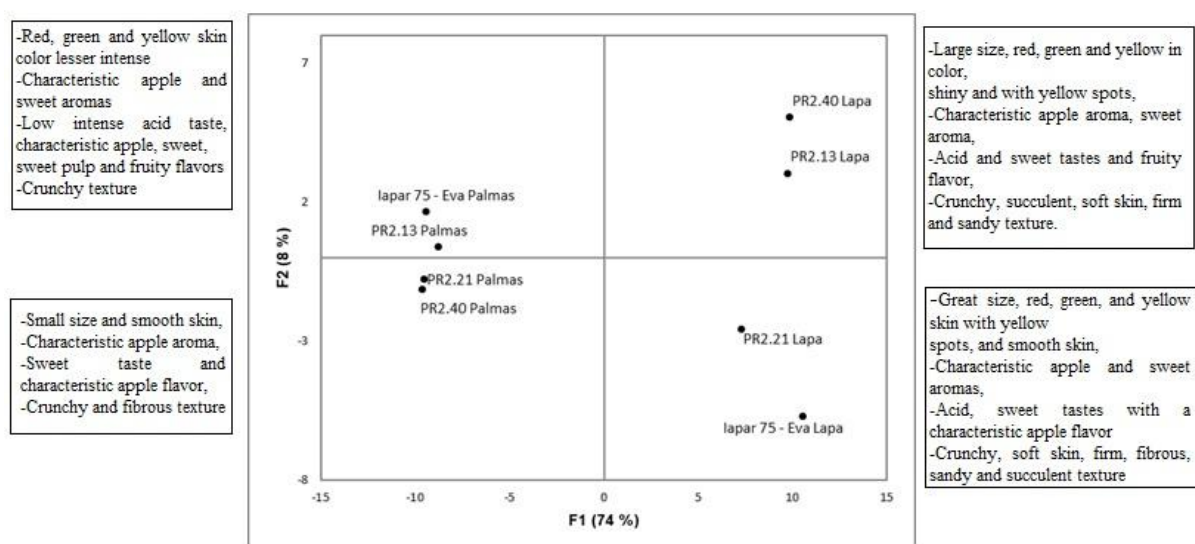
This fact is due to among climatic variables, radiation is one of the most important factors in the production and accumulation of sugar in apples, because it influences the synthesis of photo-assimilates during photosynthesis, generating an increase in the SST content [22].

The PR2.40 genotype from Lapa had the highest SST content (17.98 °Brix) of all, which can be directly linked to ideal climatic condition in Lapa in the months of October to December (Figure 4), possibly favoring the greater deposition of SST in the fruit. This fact may also have contributed to the greater diameter, height and weight of this genotype (Table 3).

The PR2.13 genotype had low acidity in both localities while IAPAR75-Eva variety showed great variability of acidity in both local (Table 3).

### 3.4. Sensory evaluations of genotypes grown in the two localities

The sensory evaluation of these genotypes showed difference of attributes between the localities and separated the genotypes grown in these localities. The dimensions are formed by the number of citations of the attributes (frequency of quotation) and by the correlation of these attributes with the respective dimension. In the present study, the dimensions F1 and F2 were formed by attributes shown in Supplementary Table 2. Then, the formation of dimensions and the projection of the genotypes in the plane formed by them (Figure 6) allow to describe the sensory characteristics of the genotypes.



**Figure 6.** Consensus configuration of the sensory attributes of the apple genotypes produced in Palmas-PR and Lapa-PR.

The genotypes PR2.40 and PR2.13 genotypes from Lapa on the upper right side (F1+ and F2+) were described as large size, red, green and yellow in color, shiny and with yellow spots. In addition, they have characteristic apple aroma, sweet aroma, acid and sweet tastes and fruity flavor. The texture of these genotypes was described as crunchy, succulent and they have also soft skin and firm and sandy texture.

Analyzing the color and size characteristics of these genotypes (Table 4) it was verified that these genotypes presented compatible physical parameters: PR2.13 Lapa was the darkest and reddest, and PR2.40 Lapa was the largest (Figure 5). The attributes of sweetness in aroma and taste

for these genotypes were highlighted because they presented the highest SST/TA values for this location (Table 3).

Besides that, PR.2.21 and the IAPAR75-Eva grown in Lapa (F1+ and F2-) were described as having great size, red, green, and yellow skin with yellow spots, and smooth skin. In these genotypes was found characteristic apple and sweet aromas, and also acid, and sweet tastes with a characteristic apple flavor. The main attributes of texture shown by these genotypes were crunchy, soft skin, firm, fibrous, sandy and succulent texture.

The sensory descriptions of Lapa apples a greater number of descriptive attributes were noted. The color, sweetness, and acidity parameters were found in the physicochemical data describe in PCA (Figure 5). However, the instrumental texture of IAPAR75-Eva and PR2.21 were different from sensory texture probably because sensory evaluations involved other characteristics that are not measured by the instrumental determination of texture.

All genotypes grown in Palmas were smaller size than respective genotypes from Lapa (Figure 6) but they presented other different attributes. IAPAR75-Eva and PR2.13 genotypes grown in Palmas (Figure 6, F1- and F2+) had red, green and yellow skin color lesser intense than respective genotypes cultivate in Lapa. These genotypes had also characteristic apple and sweet aromas as well as low intense acid taste, characteristic apple, sweet, sweet pulp and fruity flavors, and crunchy texture.

PR2.21 and PR2.40 genotypes from Palma (Figure 6; F1- and F2-) were described as having a small size and smooth skin and sweet taste and characteristic apple aroma and flavor. The texture was noted as crunchy and fibrous.

#### 4. Conclusion

The genotypes PR2.13, PR2.21, PR2.26, PR2.40, and PR2.60 were considered promising because they had both physicochemical and sensory characteristics appreciated by consumers.

The genotypes cultivated in Lapa and Palmas presented significant differences, suggesting great diversity in the characteristics evaluated and PCA separated the genotypes from two locals according respective physicochemical characteristics. A greater number of descriptive attributes with positive connotation of quality for the appearance, taste/flavor, and texture categories were found in genotypes grown in Lapa and Palma. These attributes were efficient to separated genotypes from each local and in the same local and showed the influence of the climatic conditions in the attribute formation.

The application PCA contributed to the evaluation of a greater number of parameters that indicated the quality of apples and showed the genotypes with better quality parameters. In same way, FCP allowed identify the attributes of genotypes grown in same local as well as identify attributes that separated same genotypes grown in two local.

Therefore, these multivariate analyses were appropriated to apply in apple breeding program and aiding the breeder's decision to recommend new varieties of apples.

#### Conflict of interest

All authors declare no conflicts of interest in this paper.

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