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Research article

Phenological cycle and physicochemical characteristics of avocado cultivars in subtropical conditions

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Abstract: Avocado has a great potential as a commercial crop in southern Brazil, for its high productivity, rusticity, and multiple uses. Its high oil content can be explored for biodiesel production with advantages over other crops. This study comprised six avocado cultivars-Geada, Fortuna, Fuerte, Margarida, Primavera and Quintal-belonging to the Agronomic Institute of Paraná (IAPAR) collection, from Londrina, Brazil (23° 23′ S, 50° 11′ W). Analysis of fruit growth (length and diameter) allowed the classification of the cultivars into groups showing early, midseason, and late maturation, which were harvested in March/April, May/June, and July/August, respectively. The fruits were analyzed to assess their pulp, peel, and seed proportions, and their levels of oil (pulp) and starch (seed). Results showed these six cultivars are good alternatives for oil extraction. Fuerte stands out as the most adequate for biodiesel production from pulp and seed due to its higher yield of oil and starch. The fruit cycle diversity of these cultivars might allow combining them for prolonged fruit production, both for fresh fruit marketing and biodiesel supply, as well as possibly using fruit pulp for oil extraction and seed starch for alcohol production.

Keywords: biodiesel; growth curves; oil content; starch content; acidity; peroxide

1. Introduction

Avocado is considered a tropical fruit, although its genetic diversity allowed it to adapt to subtropical climate conditions. In Brazil, it is present in nearly every state; however, the commercial plantations that are concentrated in the South, Southeast, and Northeast were responsible for producing 157,000 t in 2013, according to the Brazilian Institute of Geography and Statistics [1]. Several avocado cultivars are found throughout the Brazilian territory presenting different shapes, colors, sizes, and chemical composition.

In Brazil, avocado is mainly consumed naturally (raw fruit), or used in confectionery, cosmetics, and oil productions [2]. Another potential use is the extraction of its pulp oil for the production of biodiesel. Given the diversity of cultivars, wide climate adaptation, and high oil content with adequate physical and chemical quality [3], avocado is a promising crop for biodiesel production. In addition, it has the advantage of enabling the extraction of two important raw materials, the oil from the pulp and the ethanol from seed [4]. The fruit oil content is third after palm and olive [5]. Previous studies showed a variation from 5 to 30% in pulp oil content, and from 8 to 26% in seed starch content between different avocado cultivars. This pointed out the need for a thorough evaluation of cultivars, in order to select those with high levels of pulp oil and seed starch for further uses of avocado's raw material [2,6].

Avocado has an extremely low fruit set, typically less than 0.3% under natural pollination to about 5% with manual pollination [7]. In the first month after flowering a large drop in potential fruit production occurs due to low fertility and abnormal flowers; later, a natural fertilized seed dropping also occurs due to competition for vegetative flux [8]. Therefore, evaluations of the development cycle of the fruits should only start after this natural seed dropping. Avocado also presents protogynous dichogamous flowers [9] i.e., female parts of flowers mature before the male parts separating cultivars into two groups (A and B). However, in order to ensure pollination it is necessary to mix cultivars from both groups in the same area.

Fruits (raw material) need to be produced for a prolonged period in order to supply the industry and/or the market, so that the processing structure and the consumer may have full access to the product. In this sense, species with diverse production cycles such as avocado have a great potential for industrial use.

Knowledge on the periodicity of climate conditions, on soil and environmental conditions and on the effects of these factors on plants biological cycle, including reproductive organs development and vegetative growth, is important for the study of avocado phenology [10]. Information on phenology then enables planning of planting and fruit harvesting and marketing, allowing taking advantage of market opportunities and maximizing industrial activity. According to [11], the different thermal conditions and cycles of avocado cultivars enable fruit harvesting throughout most of the year, providing favorable conditions for supplying the biodiesel production industries in Paraná state, Brazil.

Nevertheless, for the raw avocado materials to be used in the industry it is necessary to fulfill certain efficiency and quality requirements. In this work, we examine the production cycle, harvest season, and physicochemical properties of six avocado cultivars from Paraná, in order to determine their potential for biodiesel production.

2. Materials and Methods

The field study was based on data collected in the orchards of the experimental farms belonging to the Agronomic Institute of Parana State (IAPAR), in Paranavaí (23° 05′ S, 480 m of altitude) and Londrina (23° 23′ S, 566 m of altitude), Paraná, Brazil. The climate of this region is Cfa, according to Koeppen classification. The average annual temperature is 21 °C to 22 °C with precipitation from 1300 to 1600 mm, according to IAPAR historical records. The rainy season begins in September and ends in March-April [12]. Severe frosts are infrequent and do not constitute a risk factor for avocado [13]. Between 1986 and 1996, data of 24 avocado cultivars were collected from these two locations [11], from flowering to harvest, which was determined as the time when fruits started to fall naturally. These data allowed classifying the cultivars into three groups according to their phenological cycle: early, midseason, and late harvest. Based on the results of that study a more detailed research was carried out in Londrina between June 2011 and December 2012, comprising data from six cultivars belonging to the three harvest groups: Geada and Fuerte (early), Fortuna and Quintal (midseason), Primavera and Margarida (late). Geada and Fuerte fruits are pear-shaped whereas the others are rounded. The climatic conditions during the experimental period in Londrina are in Figure 1. The assessments in this study were as follows.

2.1. Fruit growth curves

Three representative plants were selected from each cultivar. Within each plant, four branches belonging to the middle third and distributed across the four quadrants of the tree canopy were selected. Every week, fruits from each tree were counted and their length and larger diameter were measured using a caliper; the average value per quadrant and per plant was obtained for each evaluation. As avocado fruits grow in both length and diameter, and this growth can occur unevenly, an index (Gi) corresponding to the sum of these two parameters was defined. Measurements were taken after the natural fruit dropping, starting November 4, 2011, and continuing until Gi values remained constant for each cultivar, when harvest time was assumed to start. The values of Gi for each cultivar were adjusted to sigmoid functions.

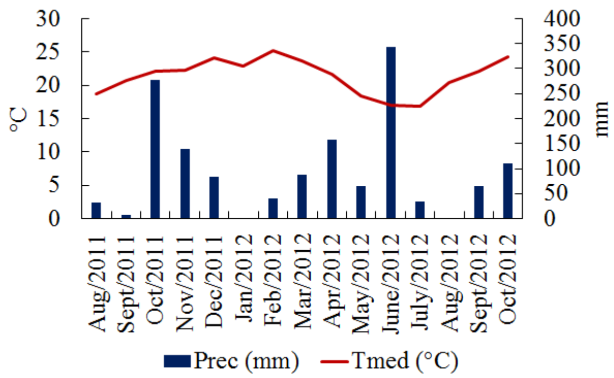

Figure 1. Monthly means of temperature and rainfall from August 2011 to October 2012 in Londrina, PR, Brazil.

2.2. Weight percentage of pulp, peel, and seed

After growth stabilization, four fruits of each cultivar were collected randomly, one from each quadrant, but avoiding the marked branches. The fruits were weighed and stored in a cool place until they were mature (indicated by their soft consistency). After maturation, fruits were weighed once again to determine the weight of the pulp, peel, and seed relative to the total weight of each fruit.

2.3. Seed starch content

A portion of seeds taken from four fruits aleatory chosen from each cultivar was dried in a forced circulation stove at 50–60 °C until it reached a constant weight. This seed portion was then ground and kept at –20 °C until further analyses. To hydrolyze seed starch, 50 mg of ground seed were transferred to a test tube with a screw cap; after adding 25 mL of 0.7 M HCl, this mix was stirred and placed in a 96 °C water bath for 90 min; following a cooling period, this solution was neutralized with 3M NaOH in a 500 mL volumetric flask to which distilled water was supplemented; the content of reducing sugars (RS) (RS fraction of the hydrolyzed starch) was determined after filtering this solution using Whatman NO.1 filter paper [14]. To perform the alcoholic extraction of glucose (RS fraction of the alcoholic extract), 200 mg of ground seed were added to 10 mL of 80% ethyl alcohol contained in a centrifuge tube. After thorough agitation, the mixture was placed in a shaking water bath at 80 °C for 30 min and then centrifuged at 804 × *g*; the supernatant was then collected in a beaker. This operation was repeated three times, resulting in 30 mL of alcoholic extract that was subsequently evaporated at 50 °C until there were only about 5 mL of extract left in the beaker. This residue was transferred into a 100 mL volumetric flask, the volume completed with distilled water, and the content of RS further determined. The acidic hydrolysis of sucrose (RS fraction of the hydrolyzed sucrose) was performed by adding 2.5 mL of concentrated HCl (purity of 37%) to 25 mL of the alcoholic extract diluted (to 100 mL volumetric flask) obtained during glucose extraction. This mixture was kept at room temperature for 16 h and neutralized with 3 M NaOH in a 100 mL volumetric flask to which distilled water was supplemented, before proceeding with the RS fraction determination. In all the fractions mentioned above, i.e., starch, glucose, and sucrose, the RS was determined employing the method of Somogyi and Nelson [15] as follows: 1 mL of Somogyi's reagent was added to 1 mL of each fraction, and this mixture was boiled for 10 min; after its immediate cooling on ice, 1 mL of Nelson's reagent was added and the mixture was stirred; 7 mL of distilled water was subsequently added and the mixture was again stirred. The RS percent content of each fraction was then determined by spectrophotometry at 535 nm. The standard curve was obtained with a glucose solution at 0.1% of benzoic acid (1 to 10 mg 100 g^{-1}). All determinations were performed in duplicate. Seed starch, sucrose, and glucose contents were expressed as a percentage, according to the following equations:

Starch content = 0.9 (% RS of the hydrolyzed starch – % RS of the sucrose hydrolyzate)

Glucose content = % RS of the alcoholic extract

Sucrose content = 0.9 (% RS of the sucrose hydrolyzate – % RS of the alcoholic extract)

2.4. Fruit pulp oil content

Oil content was determined using the Soxhlet method [16]. Sample preparation consisted of collecting a part of the pulp of four fruits aleatory chosen for each cultivar. The pulp was homogenized and macerated to obtain a paste and dried in a forced circulation oven at a temperature ranging from 50 to 60 °C, until constant weight. The dried pulp was then grounded in a blender and frozen at –20 °C. One gram of this pulp was transferred to filter paper cartridges and dried again at

60 °C for 12 h. The cartridge was subsequently transferred to a Soxhlet extractor where it was kept under reflux for 16 h, using petroleum ether as the solvent extractor. After extraction, the cartridges were dried at 60 °C and weighed. Oil content was expressed in 100 g^{-1} of dry weight of pulp. Three replicates were performed for each cultivar.

2.5. Acidity index (Ai)

The Ai of avocado oil for each cultivar was determined using the following solution: 0.5 g of avocado oil extracted as described above were dissolved in 10 mL of ethyl alcohol, previously neutralized by titration with 0.01 N NaOH. The oil titration was carried out using phenolphthalein as an indicator and until a pink color persisted for 30 seconds [17]. The oil acidity index was determined according to the equation:

Ai (%) = (mL NaOH 0.01N × 0.282)/avocado oil weight

2.6. Peroxide index (Pi)

The Pi was determined according to the methodology described in [17] and using 1 g of the avocado oil extracted as described above. This gram of oil was dissolved in 30 mL of acetic acid: chloroform solution (3:2 volume ratio), to which 0.5 mL of saturated potassium iodide were added; after stirring this solution for 1 min, 30 mL of distilled water and 0.5 mL of a 1% starch solution were added. The starch solution was prepared by dissolving starch in distilled water, heating this solution up to 80 °C, followed by cooling titration with 0.01 N sodium thiosulfate. The peroxide index value was calculated by the following equation:

Pi (mEq kg^{-1}) * = (mL sodium thiosulfate × 1000)/avocado oil weight

* milliequivalents of active oxygen per kg of oil

2.7. Refractive index (Ri)

The analyzes were performed employing a refractometer Astral bench (mod. EEQ9001). About 3 to 4 drops of avocado oil were transferred to the prism and performed refractometer readings of the refractive index at 20 °C.

2.8. Statistical analyses

To compare oil and starch contents between cultivars, data were subjected to an analysis of variance (ANOVA) followed by Tukey's tests at the 5% probability level in order to evaluate the significance of means' differences. Principal component analysis (PCA), applying the Waard clustering method, was used to determine the parameters associated with characteristics favorable to the production of biodiesel. Analyses were performed using the statistical software XLSTAT version 2008.4.02 [18].

3. Results and Discussion

The study of the characteristics of the phenological cycle of these avocado cultivars can give indications of the periods in which there will be supply of raw material for *in natura* consumption or production of biodiesel, ensuring the continuity of production and commercialization throughout the year. Fruit diameter plus length (Gi) growth curves adjusted to sigmoid functions are shown in Figure 2. Fuerte has the smallest Gi and Primavera and Quintal cultivars have the highest. Table 1 shows each cultivar's adjusted equation with its respective coefficient of determination (R^2) and number of days needed to complete a cycle (DCC). Considering that flowering started in August but mainly occurred in the beginning of September, the average duration of a complete cycle, from flowering to harvest, could be obtained by adding 60 days (measurements started to be taken in the beginning of November) to the values of DCC shown. The ideal commercial maturation point for avocado is not easy to be determined. Visual aspects and size of the fruit are mostly used in the field [19,20]. There is an uncertainty in using the start of ripening as a criterion, since it is not always accompanied by visual external changes of the fruit [21]. Periodical fruit size measurements in the orchard could be a good criterion to determine the exact harvesting time for each cultivar.

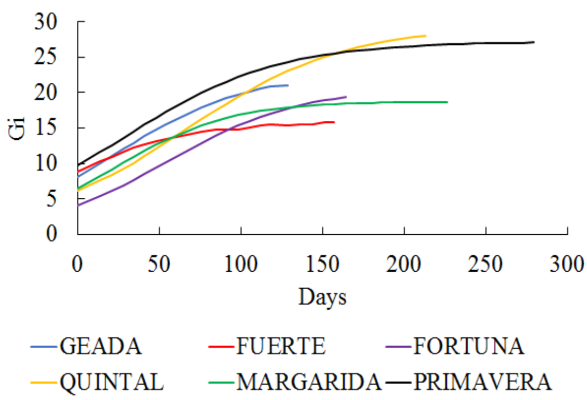

Figure 2. Sigmoid growth curves of the Growth index (Gi) for the six avocado cultivars. Measurements started on November 4th 2011, after the natural fruit dropping. Londrina, PR, Brazil.

Table 1. Sigmoid growth curves adjusted to each avocado cultivar with the respective coefficient of determination (R^2) and days to complete the fruit cycle (DCC).

Cultivar	Sigmoid Function	R^2	DCC
Geada	22.9982/(1 + exp (– (x – 24.4293)/40.9597))	0.9979	130
Fuerte	15.7753/(1 + exp (– (x + 8.3585)/35.6181))	0.9981	150
Fortuna	20.6347/(1 + exp (– (x + 55.6300)/40.0704))	0.9986	170
Quintal	20.6347/(1 + exp (– (x + 55.6300)/40.0704))	0.9986	215
Margarida	18.7321/(1 + exp (– (x + 22.4931)/34.7987))	0.9962	230
Primavera	27.1541/(1 + exp (– (x + 28.0527)/47.0111))	0.9968	280

Avocado fruits grew according to the genetic characteristic of each cultivar (Figure 1): Geada was the first to reach the maximum Gi in the beginning of March (120 days), followed by Fuerte and Fortuna in the middle of April (160 days); Quintal completed fruit growth in late May (200 days), therefore being classified as midseason. The fruits of Primavera and Margarida cultivars were the last to reach their maximum growth-late June for Margarida (240 days) and early August for Primavera (280 days).

These results are very important to the raw fruit production and market distribution (*in natura* consumption) as well as for the production of biodiesel from avocado. Thus, by combining the different cultivars in orchards, one can have avocado fruits available from mid-March to late September-October, considering that after reaching their maximum growth the fruits remain on the plant for an extended period. This is interesting for fresh fruit consumption, as this long supply period reduces the risk of getting a low market price associated with productions concentrated in short harvesting intervals. In addition, this longer harvest period is also important for the biodiesel production industry as it implies a shorter idle time of the processing facilities [2,11].

Yield of pulp, seeds and bark of avocado fruits as well as oil content of pulp and starch in seeds provide important information indicating potential cultivars for biodiesel production. Table 2 shows the percentages of pulp, seed, and peel of the fruits from the six cultivars. The pulp content ranged from 63.20 to 80.10%, with a coefficient of variation (CV) of 8%. The influence of the local thermal regime on the cultivars cannot be neglected, as different cultivars adapt differently to the environmental [11,22].

Table 2. Percentage of the components pulp, seed, and peel in fruits of six cultivars of avocado. Londrina-PR, Brazil.

Cultivar	Pulp (%)	Seed (%)	Peel (%)
Fuerte	63.20	24.30	12.49
Quintal	74.87	16.89	8.23
Fortuna	76.60	16.14	7.14
Geada	80.06	11.39	8.53
Primavera	72.30	15.73	11.95
Margarida	77.31	13.25	9.42
Mean	74.06	16.28	9.63
CV (%)	8%	27%	22%

The highest oil percentage was found in Fuerte, followed by Primavera (Table 3). Oliveira et al. (2013) obtained the highest percentage of oil in Fuerte and Haas in a comparison among eleven cultivars of avocado in the state of São Paulo, Brazil [20]. Found highest oil concentration in the pulp of the cultivars Hass and Fuerte and the lowest values in Fortuna and Margarida. Regarding the seed's starch content, this ranged from 45.37 to 62.22% but no significant differences were found between cultivars. The starch content values obtained in the present study for Fortuna, Fuerte, and Quintal are very close to those reported by [6]: 51.60% for Fortuna, 56.60% for Fuerte, and 37.20% for Quintal. These authors evaluated the oil (pulp) and starch (seed) contents in 24 cultivars and found that cultivars with high oil content showed a starch content above the average; our results also seem to agree with this, as the cultivar with the highest oil percentage-Fuerte-also presented the highest starch percentage (72.14 and 62.22%, respectively).

Table 3. Percentage of oil (pulp) and starch (seed) of avocado cultivars. Londrina-PR, Brazil.

Cultivars	Oil (pulp)	Starch (seed)
Fuerte	72.14 ± 2.8 [a]	62.22 ± 7.4 [a]
Quintal	55.25 ± 1.6 [c]	48.15 ± 3.6 [a]
Fortuna	55.64 ± 1.5 [c]	48.52 ± 6.7 [a]
Geada	55.84 ± 2.0 [c]	45.37 ± 4.5 [a]
Primavera	63.00 ± 1.0 [b]	45.58 ± 9.0 [a]
Margarida	57.10 ± 2.4 [b,c]	59.25 ± 1.4 [a]

Means followed by same letter vertically do not differ to each other by the Tukey test at 5% probability.
Values are on a dry basis.

In previews studies, the authors pointed out that the ethanol extracted from avocado seeds can be a secondary raw material for biodiesel production [4]. The avocado seed has potential for the production of ethyl alcohol due to its high total starch content that, when subjected to a hydrolysis process, raises the Brix content of the broth providing conditions for fermentation to occur.

Acidity index, peroxide index and refractive index are evaluations of avocado oil that may reveal the quality and stability to oxidation processes of this raw material (crude oil). The average values of the acidity, peroxide, and refraction indices obtained for the avocado oil extracted from each of the six cultivars are shown in Table 4.

Table 4. Acidity index (Ai), peroxide index (Pi), and refractive index (Ri) of the oil of six cultivars of avocado.

Cultivar	Ai (%)	Pi (mEq kg^{-1})	Ri
Fuerte	0.39 ± 0.01	1.79 ± 35.9	1.469 ± 0.0
Quintal	1.16 ± 0.01	1.01 ± 48.8	1.478 ± 0.0
Fortuna	0.87 ± 0.02	0.68 ± 15.5	1.468 ± 0.0
Geada	0.78 ± 0.00	0.76 ± 47.0	1.473 ± 0.0
Primavera	0.57 ± 0.01	0.56 ± 68.2	1.468 ± 0.0
Margarida	0.63 ± 0.01	0.70 ± 13.7	1.466 ± 0.0

3.1. Acidity index (Ai)

Quintal presented the highest Ai among cultivars (1.16%) while Fuerte presented the lowest (0.39%); the other cultivars had intermediate values ranging from 0.57 to 0.87%. Acidity values indicate the amount of free fatty acids present in the oil. According to [23], the oil conservation status is related to the nature and quality of the raw material, to the quality and degree of oil purity, and to oil processing. The Ai of vegetable oils should be the lowest possible. High levels of Ai indicate alterations that prevent its use as a fuel or for human consumption [24]. Storage conditions are also very important as heat and light accelerate the decomposition of glycerides (rancidity), and this is almost always accompanied by the formation of free fatty acids [25]. These free fatty acids form fatty acid salts (soap) in basic media, which is responsible for the formation of emulsions during the purification step of biodiesel production, leading to a reduction of the yield. High acidity may catalyze intermolecular reactions, which affect the thermal stability of the fuel in the combustion chamber, and have a corrosive action on the metallic components of the engine.

3.2. Peroxide index (Pi)

The peroxide content of Fuerte was the highest among cultivars. Average values ranged from 1.79 mEq kg^{-1} in Fuerte to 0.56 mEq kg^{-1} in Primavera, and can be considered low when compared to other species [26,27]. The peroxide content in vegetal oils estimates the degree of degradability of raw material. The values found in this work are much lower then the maximum accepted values of 10 mEq kg^{-1} [27], and indicate therefore a low possibility of oxidative deterioration in avocado.

3.3. Refractive index (Ri)

The Ri obtained for the oil of each of the six cultivars ranged from 1.466 to 1.478. The refractive index of oils and fats is widely used as identification and quality criteria, as this index increases with increasing iodine content and can thus be used for controlling unsaturated oils hydrogenation processes [28].

3.4. Principal components analysis (PCA)

Bilateral relations between the variables account for physiological events, but when the intention is to verify the possibility of discriminating a group of samples, the best choice is the analysis by multivariate techniques. Among the several multivariate techniques, Principal Component Analysis (PCA) was used to reduce the dimensionality of a data group, explaining the variance. In this process, the original variable data is linearly transformed into a small number of uncorrelated components. From these components, the samples are projected in the plane formed by the components and their proximity to the projection of the variables allows to infer the similarities between them [29]. The PCA performed allowed to analyze the simultaneous influence of pulp, seed, and peel percentages, as well as oil and starch contents, in the Ai, Pi, and Ri of each cultivar (Figure 3), in order to discriminate avocado cultivars and verify which components, or set of components, could be used for selecting cultivars for biodiesel production. The first two components explained 81% of the total variance according to the following equations:

$$F1 = 0.87\ Ai + 0.81\ Ri - 0.66\ Oil - 0.63\ Starch + 0.88\ Peel - 0.80\ Pulp$$

$$F2 = 0.85\ Pi + 0.61\ Oil + 0.71\ Seed.$$

Peel, seed, and pulp (Table 2), and oil and starch contents (Table 3) refer to percentages of these fractions.

The principal component F1 was positively correlated to the acidity index, refractive index, and percentage of peel, and negatively correlated with oil content and starch and pulp percentage; F2 was positively correlated with the peroxide index value, oil content, and percentage of seed.

As can be observed from Figure 2, the cultivars Quintal, Geada, and Primavera are characterized by high refractive and acidity indices, and high percentage of peel (F1 +) whereas

Fuerte, Margarida, and Fortuna differed from the others by their high oil and starch contents, and high percentage of pulp (F1 –). Regarding cultivars' differentiation according to F2 +, Fuerte and Quintal cultivars showed the highest levels of oil, peroxide index, and percentage of seed.

The cultivar Fuerte presented high oil and starch content, and high percentages of seed and peel (Tables 1, 2 and 3). Although its percentage of pulp was the lowest among all cultivars, it showed the highest oil extraction and peroxide values (value still within the allowable range). This profile is interesting for obtaining biodiesel through seed and pulp processing, because the highest oil content was obtained from the lowest percentage of pulp and the highest starch content was obtained from the highest seed percentage.

The cultivars Margarida and Fortuna showed very similar profiles, with intermediate levels of oil and starch, pulp, seed, and peroxide index.

The cultivars Primavera and Geada also performed similarly to each other. They were characterized by higher refractive and acidity indices and percentage of peel, and lower values of percentage of oil, peroxide index, and percentage of seed.

The cultivar Quintal separated from the others for its larger values of refractive, acidity and peroxide indices, percentage of peel, seed, and lower oil content (Tables 1, 2 and 3). This cultivar has the least favorable profile for biodiesel production due its higher acidity and peroxide indices. This fact would result in a greater risk of oil degradation, which would imply additional steps of oil pretreatment.

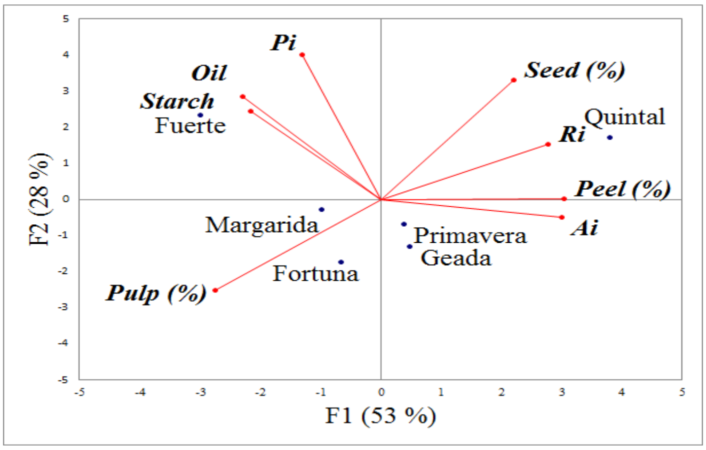

Figure 3. Biplot of the principal components 1 and 2 for oil yield, parameters analyzed in the oil, proportions of the fractions (pulp, peel and seed) and starch content in the seed of the cultivars. Pi = peroxide index, Ri = refractive index, Ai = acidity index.

In general, all cultivars showed potential to be used as a source of biodiesel given their physiochemical properties and the diversity of maturation cycles that would enable a long production period, but we can highlight the cultivar Fuerte that stood out for presenting the composition and physicochemical characteristics more suitable for potential use in the production of biodiesel. A study considering each cultivar's oil stability is thus necessary in order to combine this high potential of extraction with a higher oil quality, avoiding the use of additional steps in raw material treatment, which would increase biodiesel production costs.

4. Conclusions

The diversity of maturation cycles observed in this study shows the advantage of combining avocado cultivars with different harvest times in production orchards, enabling the market and biodiesel production supply at different times and for a longer period.

Although all avocado cultivars are potentially suitable for biodiesel production, Fuerte stands out as the most adequate for biodiesel production from pulp and seed due to its higher yield of oil and starch, when compared to the remaining five.

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Conflict of Interest

All authors declare no conflicts of interest in this paper.

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