

Full Length Research Paper

Determination of macaw fruit harvest period by biospeckle laser technique

Anderson Gomide Costa^{1*}, Francisco de Assis de Carvalho Pinto², Roberto Braga Alves Júnior³, Sérgio Yoshimitsu Motoike⁴ and Luís Manuel Navas Gracia⁵

¹Departamento de Engenharia, Instituto de Tecnologia, Universidade Federal Rural do Rio de Janeiro/UFRRJ, Brazil.

²Departamento de Engenharia Agrícola, Universidade Federal de Viçosa/UFV, Brazil.

³Departamento de Engenharia, Universidade Federal de Lavras/ UFLA, Brazil.

⁴Departamento de Fitotecnia, Universidade Federal de Viçosa/UFV, Brazil.

⁵Department of Agriculture and Forestry Engineering, Universidad de Valladolid/ Uva, Spain.

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Macaw palm has been stood out as a raw material for the production of bioenergy, because it has high productivity of oil and less emission of polluting waste during combustion, meeting the worldwide demand for sustainable energy sources. The aims of this research were the evaluation of response of the biologic activity measured by the optical technique of the biospeckle laser applied to macaw palm fruits at different maturity weeks and develop a classifier in function of biologic activity to determine the harvest period related with oil content in the fruits. To perform the experiment, 10 weeks fruits different maturity stages were evaluated. The biospeckle laser images were obtained by illuminating the epicarp of each fruit. The biological activity was quantified by absolute value of difference algorithm applied to biospeckle images. A neural network was developed to classify the fruits which were closer to harvest in function of biologic activity. Biologic activity showed a significant linear ratio ($R^2 = 0.913$) with the maturation of fruits. Classification results have shown that fruits from 59th week after flowering are ideal for harvest and present the highest oil levels.

Key words: Biologic activity, optical sensors, maturity, oil content.

INTRODUCTION

Brazil has stood out in the worldwide energy scenario, being considered to be one of the biggest producers and consumers of biodiesel. In spite of the advances made in this sector, the Brazilian energy matrix faces biodiesel production bottlenecks, due to the reduced amount of

oilseed raw material. Currently, soybeans are the main source of biodiesel production, covering 74% of the raw material demand, corresponding to the production of 2.2 million m³ (ANP, 2014). This production is insufficient to sustain the demand for biodiesel projections in the

*Corresponding author. Email: acosta@ufrj.br Tel: (55)02126821864.

coming years. The diversification of the raw materials used for the production of biodiesel is important for the continuous increase of the Brazilian bioenergy sector.

In this context, the macaw palm stands out because of the high production potential of oil from its fruits (Conceição et al., 2016), and the possibility to be used in a consortium with other species into agrosilvopastoral systems (Henderson et al., 1995; Viana et al., 2011). The estimate of oil productivity in the mesocarp is of 5000 kg.ha⁻¹ palm-kernel type, needed for biodiesel production, and of 1400 kg.ha⁻¹ of oil in the kernel is lauryl type, used in cosmetics (Clement et al., 2005; Barreto et al., 2016).

Despite the high productive potential of biodiesel, the palm tree presents some characteristics that hinder its exploitation in a large-scale production system (Motoike et al., 2013; Mota et al., 2011). Due to the variation in the maturity stage of the fruits, the period of harvest is still seen as a point that requires further study and development of specific technologies for the culture. The harvest realized with the fruits in different maturity stage reflects in oil quantity and quality (Hiane et al., 2005). Currently, the period to start macaw palm harvest is determined by the natural fall of fruits from the bunch. However, the contact of the fruit with the soil results in reduced oil quality due to an increase in oil sourness caused by microorganisms (Queiroz et al., 2015).

To determine physiologic maturity, direct methods of measuring are used, which take into account the physical and chemical features of fruit and vegetable (Kluge et al., 2010; Pinto et al., 2013). Despite the reliability, these analyzes generally present disadvantages such as sample deterioration, high analysis time and high costs (Chitarra and Chitarra, 2005; Santos et al., 2013).

Regarding the estimate on macaw palm oil content, one of the main analytic methods applied is the ASE (Accelerated Solvent Extraction) which uses a combination of temperature, pressure and solvent to extract the oil. However, this method is considered laborious, and requires the destruction of the fruit in the process, using up a great amount of the sample to perform analysis (Elfadl et al., 2010). The use of magnetic resonance significantly reduced the time spent during analysis; however, this method requires a prior preparation of the samples, which makes it difficult to use it (Panford and Deman, 1990).

Noninvasive measurement methods appear as an alternative to minimize these disadvantages. Optical instruments are being applied as an alternate to classify and control fruit and vegetable quality, with the use of spectral reflection data (Saeed et al., 2012; Viegas et al., 2016), the use of fluorescent sensors to determine maturity (Hazir et al., 2012), the use of digital images (Tan et al., 2010) and the use of biospeckle laser for quality and monitoring of biologic activity (Rabelo et al., 2005; Zdunek and Herppich, 2012).

Optical instruments have been used in the maturation analysis and oil content of the macaw palm. Matsimbe et

al. (2015) used the spectroscopy of infrared and near infrared (VIS-NIR) applied to the macaw palm fruit mesocarp to estimate oil content. Although promising, these results still limit the measurement of the oil content without destroying the fruit, once the VIS-NIR models did not obtain significant results when applied directly in the epicarp.

The biospeckle laser technique has been successful as an alternative to traditional optical analysis for indirect measurement of biological material characteristics. Biospeckle images are formed when a biological material is illuminated by laser light, generating an interference pattern due to the scattering caused by structural cell tissue (Rabal and Braga, 2008). Observed through time, the interference patterns will be dynamic, once cell tissue activity result in a variation in the spread of the light reflected. This way, the intensity of the biospeckle pattern is associated to dynamic properties of the material analyzed. One of the main analysis methods of biospeckle is based on the quantity of dispersion of points around the diagonal of co-occurrence matrix by the calculation of the inertia moment (Arizaga et al., 1999) and the absolute value of difference (Cardoso and Braga, 2014). Biospeckle laser presents as an advantage the simple handling and low cost (Rabal and Braga, 2008; Zdunek et al., 2014).

The biospeckle laser technique has been applied to quantify and differentiate regions of different activities in several studies related to agriculture as in the analysis of leaf tissues (Ansari and Nirala, 2015), quality of meat (Amaral et al., 2015), root growth (Braga et al., 2009; Ribeiro et al., 2013), incidence analysis of parasites (Ansari et al., 2016; Grassi et al., 2016) and bacteria (Ramírez-Miquet et al., 2015).

In the analysis of fruit quality in specific, the biologic activity of fruit and vegetable obtained through biospeckle activity can be correlated with maturity (Chargot et al., 2012; Retheesh et al., 2016; Skic et al., 2016), senescence (Alves et al., 2013; Costa et al., 2017) and effect of storage temperature (Kurenda et al., 2012).

The objectives of this research were to analyze the response of the biologic activity measured by biospeckle laser technique applied to macaw palm fruits in different maturity weeks and develop a classifier in function of biologic activity, to determine the harvest period related with oil content in the fruits.

MATERIALS AND METHODS

Fruit collection

The fruits used in the experiment were collected in an area located in the Zona da Mata region, Minas Gerais state, located at 20° 23 '33' ' of latitude south and 47° 07 '31' ' of longitude west, with altitude of 601 m. The trees used were from a single population of the *Acrocomia aculeata* species with more than 10 years old, in reproductive state, cultivated into extractive system, with no commercial use and without prior soil preparation.

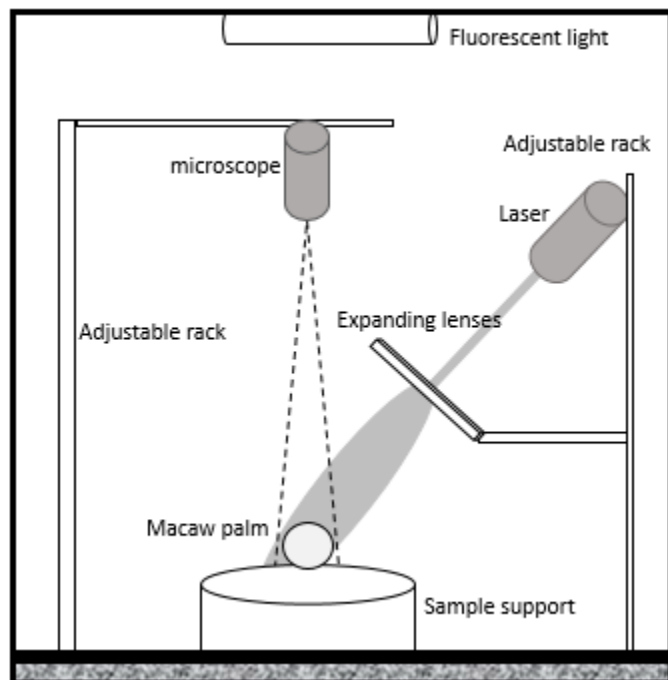


Figure 1. Experimental setting for image acquisition from biospeckle laser.

During the beginning of flowering, a bunch in each tree was marked randomly. After flowering 20 bunches from 20 different trees were selected. Ten fruit collections were performed between the 40th and the 61th week after flowering (WAF). In each collection, 100 fruits were acquired, being composed of 5 fruits of each bunch. The fruits were collected randomly disregarding fruit position within each bunch.

The collections were performed at intervals with progressive reduction, since maturity occurs more rapidly in the weeks approaching physiological maturation. Thus, between the first and the third collection there was a break of four weeks. Between the third and the eighth collection there was a break of two weeks. Finally, between the eighth and tenth collection there was a break of a week.

Analysis of fruit biologic activity using biospeckle laser technique

The analysis of the biologic activity obtained by biospeckle laser was performed in each fruit in all 10 weeks of maturity evaluated. The illuminations were made at the epicarp (peel) of each fruit, with no previous sample preparation and in an intermission of approximately 4 hours after fruit collection in the field.

To get images, an experimental unit was used, constituted by portable microscope (AM413ZT, 1280 x 1024 pixel resolution) for image acquisition connected straight to the USB port of a computer, a He-Ne laser (632 nm, 50 mW), movable rack and a set of lenses and intensity reduction filters. The laser beam was filtered with a neutral filter to reduce the intensity of light the beam was expanded using a converge lens to generate homogeneous illumination of samples. The field of view consisted of a window of radius of 2.5 cm. Laser has been positioned at 15 cm high from sample with an approximate inclination of 60° in relation to sample. The microscope was positioned 25 cm high from the position and with an approximate inclination of 20° in relation to sample and a 10x zoom.

Figure 1 shows the disposition of the experimental settings of equipment in the experimental mobile unit.

The experimental setting that was used is backscattering, which records the light reflectance reflected by the fruit (Rabal and Braga, 2008). In each light session, for each fruits, 128 images (1280 x 1024 pixel resolution) were collected in 8 bits, relating to the biospeckle patterns in intermissions of 0.08 s. Activities measured in the experiment were allotted in frequency waves between 0 – 12.50 Hz. The software Speckle Tools (Godinho et al., 2012) was used to perform the image acquisition into biospeckle pattern collected by the portable microscope.

To quantify intensity variation from biospeckle patterns, the indexes of biologic activity were calculated in the fruit epicarp, using the algorithm of the absolute values of difference (Cardoso and Braga, 2014), according to Equation 1.

$$AB = \sum_{ij} (M_{ij} \frac{MOC_{ij}}{\sum_{ij} MOC_{ij}} |i - j|) \quad (1)$$

Where M_{ij} is the normal matrix value of co-occurrence in coordinates i and j , and MOC_{ij} is the real matrix value of co-occurrence in line i and column j .

An analysis of the colorimetric property hue was made with the purpose of demonstrating that the biological activity obtained in the different weeks of maturation reflect in quantitative colorimetric changes in fruits.

Determining oil content in fruits

Oil extraction was performed using n-hexane solvent into Soxhlet extractor by the method O32/IV from Analytical Norms from Adolfo Lutz Institute (IAL, 2005). Mesocarp was dried in a fanned heater at 65°C for 72 h. After drying, 5 g samples were placed in filter paper cartridges, and were arranged into the Soxhlet extractor immersed in 150 ml of n-hexane, for 8 h. In sequence, the extract was transferred to a heater at 105°C for 24 h for the evaporation of n-hexane and water contained in the oil. Finally, cooling to ambient temperature was performed followed by weighing. Oil extraction was performed for each fruit individually, which generated an oil content value for each evaluated sample (Equation 2).

$$OC = \left(\frac{M_o}{M_s} \right) 100 \quad (2)$$

Where OC stands for oil content in percent, M_o stands for oil mass in grams and M_s stands for sample total mass in grams.

RESULTS

The analysis of variation values for biologic activity was performed by boxplot, grouping five fruits in a bunch in each maturity week. Values that were out of inferior or superior limits were considered outliers and eliminated.

After elimination of outliers, the average biologic activity value was calculated for each of the 10 weeks of collection. From these values, a simple linear regression was applied to evaluate the relation between the number of weeks after flowering and biologic activity of fruits. A variation analysis was used to verify the significance of the regression model used at 5% significance level.

To evaluate biospeckle technical capacity in differing maturity weeks to determine the harvest period of fruits, a cluster analysis was carried out with a non-supervised

Table 1. Number of fruits constituted in each class.

Classification		Number of fruits per WAF	Total of fruits in each class
Immature Class (A)	41°WAF	94	181
	45°WAF	87	
Mature Class (B)	60°WAF	70	139
	61°WAF	69	

Where, WAF= weeks after flowering.

classifier which used k-means algorithms. The biologic activity averages in each harvest week were grouped into two classes, from the shortest distance to the center of each class. Ten interactions were performed, until no values has been incorporated into a different class that was in the former interaction.

It was determined that the biological activity values of the first class indicated that fruits were immature and not suitable for harvest. The biological activity values of the second class indicated that fruits were ripe and ready for harvest.

Correlation between oil content and biologic activity were analyzed during fruit maturity by Pearson coefficient at 1% significance.

Fruit classification to determine harvest moment in function of biologic activity

For the development of the classifier, two classes were defined according to the fruit maturity stage. The Immature class (A) was comprised of fruits collected in 41°WAF and 45°WAF. These fruits were chosen to represent the class of immature, for they were gathered in the first two collections, which are considered inappropriate for harvest. The mature class (B) was comprised of fruits gathered in 60°WAF and 61°WAF, that is, in the last two weeks of harvest, when the fruits were considered closer to physiologic maturity and therefore, good for harvest. In Table 1, it is presented the number of fruits for each class.

A feedforward backpropagation neural network was trained to classify the fruits in the immature and mature classes according to biologic activity measured by biospeckle laser, using Levenberg-Marquadt variable to accelerate training time and improve the performance during classification (Demuth et al., 2008).

Two architectures have been evaluated. The neural network architectures 1 (NN1) was comprised by an input layer, an intermediate layer with two neurons, an output layer with two neurons (Figure 2a). The neural network architectures 2 (NN2) was comprised by an input, two intermediate layers and one output layer with two neurons (Figure 2b). Biologic activity was used as an input descriptor. The intermediate and output layers used hyperbolic tangent as activation function. A binary system

was used in the output layer, being 0 related to fruits classified into Immature class (A) and 1 refer to fruits classified into Mature class (B), considered able for harvest.

It was used 50% of the fruits to train, 20% for validation and 30% for tests the trained neural networks. Each structure was trained 10 times, since in the beginning of the training the network parameters are generated randomly and these values influence. Training was interrupted by the early stopping, when error increase in validation samples occurred in 6 consecutive cycles. The neural network architecture that presented each hit on the test classification was selected.

Performance in the chosen nets was determined through confusion matrix collected from the classification of the sample test (Congalton, 1991). Accuracy and comparison were determined by overall accuracy coefficient and Kappa index. Significance from Kappa indexes was analyzed through Z testing, which enabled to verify if classification in the neural networks was considered better than a random classification (De Leeuw et al., 2006).

The selected neural network was applied to fruits in the 10 maturity weeks evaluated, and the percentage of classified fruits pertaining to Immature class (A) and Matured class (B). The predominant classification of fruits taken from a bunch, determined the class for each bunch.

RESULTS AND DISCUSSION

Relation between biologic activity and fruit maturity

Figure 3 depicts a model for the response of biologic activity measured in the fruit epicarp during maturation. R^2 of 0.9127 for biologic activity adjustment in the epicarp shows that biospeckle laser has the capacity of being an efficient technique in pointing out maturity level, which can be applied directly to the peel of macaw palm fruit. Zdunek and Herppich (2014) used the biospeckle technique to predict in a non-destructive way the maturity stage for apples. Similar to the results observed for macaw palm maturity, an increase was observed in biologic activity during the period of fruit development, being more evident in periods of more advanced maturity.

When penetrating in plant tissues the laser light

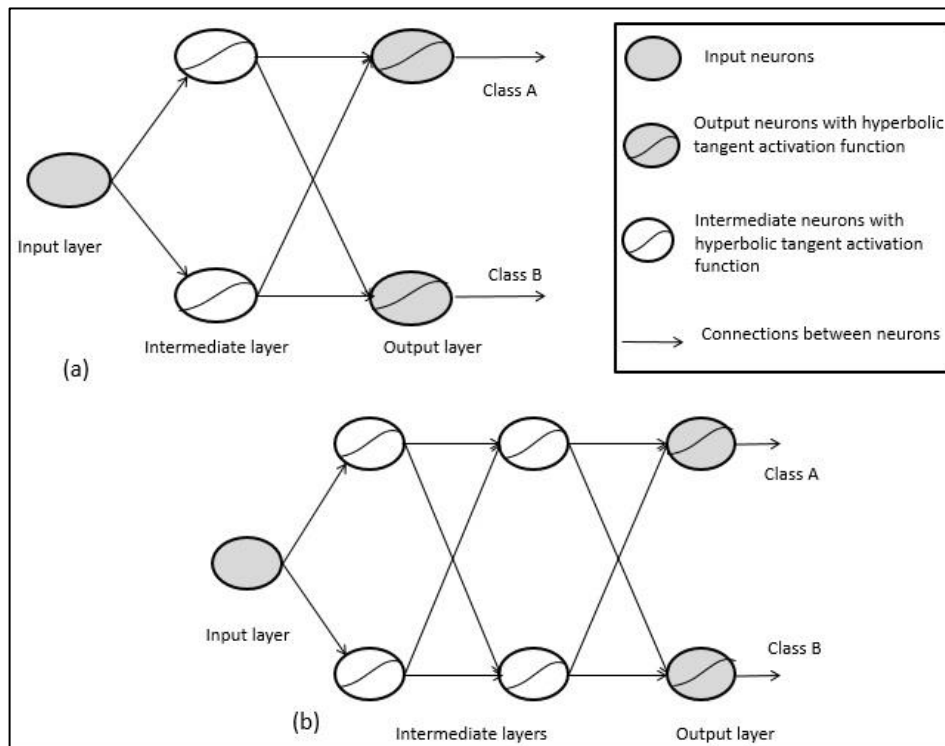


Figure 2. Representation of architectures Neural Network 1(a) and Neural Network 2(b).

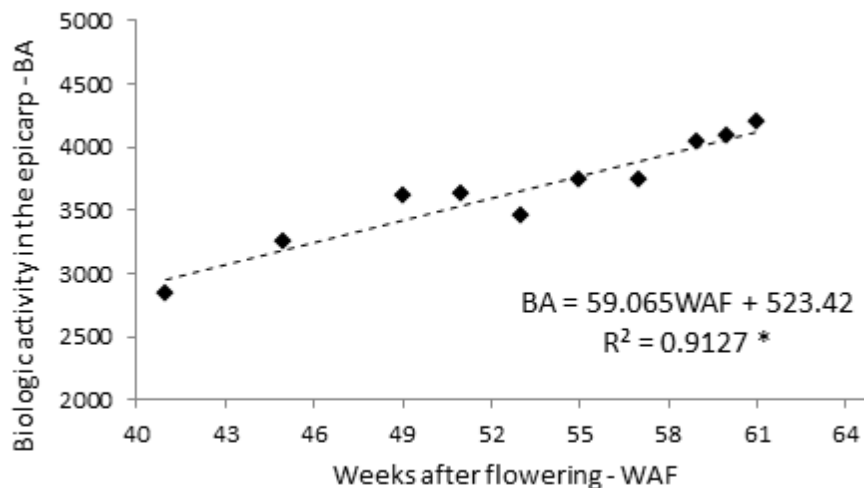


Figure 3. Analysis of simple linear regression related to biologic activity in the epicarp and number of weeks after flowering significance at level 5%.

undergoes scattering in different directions due to Cytoplasmic movement existing in the superficial and subsurface layers of tissues (Rabelo et al., 2005). The cells that compose the plant tissue act as scattering centers of the light beam, generating multiple refractions and reflections which produces the laser biospeckle patterns. In plant tissues the exchange of materials between organelles and the transport of nutrients

and enzymes happens near the cell walls, place where it occurs the interaction light and biological material. Changes in cellular characteristics result in alteration of the scattering centers of the light beam resulting in quantitative variation of the biological activity measured by biospeckle laser (Rabal and Braga, 2008).

During fruits maturation, cytoplasmic flows, breath, growth and cell division are factors responsible for

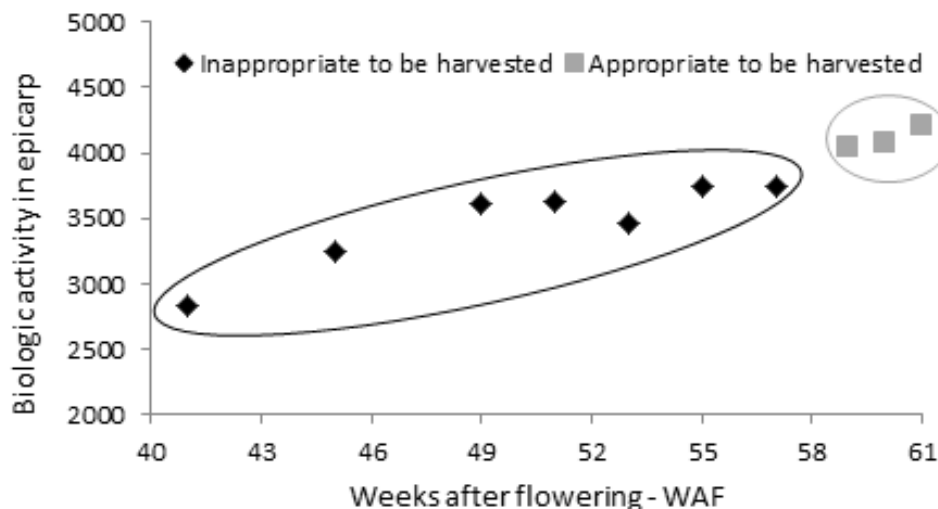


Figure 4. Grouping in classes (K-means) to distinguish fruits in moments considered inappropriate to be harvested and in moments considered appropriate to be harvested having biologic activity in the epicarp as a basis.

determine the activity of biospeckle (Braga et al., 2009). Thus, quantified biological activity by biospeckle can be understood as an index related to the mobility and vitality of a tissue.

The complexity of plant tissues makes it difficult to understand the cause and effect related to biospeckle activity. Zdunek and Cybulska (2011), demonstrate that the degradation of the starch during ripening of apples affects the optical properties of the fruit tissues. According to the authors, the starch particles do not move along with the cytoplasmic flow, forming scattering centers of laser light. Thus, the more starch particles within the cells, higher the biospeckle activity.

Another aspect to be considered, is the absorption of laser light by plant tissue. In this case chlorophyll has an important role, since this type of material efficiently absorbs light in the visible spectrum (Rabal and Braga, 2008). Chlorophyll levels tend to decrease during maturation, decreasing the absorption of laser light and consequently increasing the scattering intensity and the values of biological activity. Nassif et al. (2012) and Hu et al. (2013) demonstrate a significant correlation between chlorophyll and biological activity for various fruits. In the case of macaw palm, more in-depth studies are necessary with the objective of obtaining a relation between the biological activity and physiological factors during a maturation.

When analyzing the grouping into classes (Figure 4) it was possible to observe the distinction of fruits considered immature, and thus, inappropriate for harvest, and fruits considered mature and ready for harvest having biologic activity in the epicarp in the 59th WAF, showing that from this point on fruits are ready to be harvested.

The analysis of the colorimetric property hue reinforce

the results obtained by biospeckle laser technique demonstrated that the biological activity reflects quantitatively the physiological changes of the fruits in the different weeks (Figure 5). Fruits near the harvest period tended to lower values of the hue when compared to fruits in lesser degree of maturation. The fruits between 41st and 49th WAF had a concentration in the value of the hue close to 60°, indicating a medium tonality close to yellow. The fruits from the 59th WAF presented a hue with an average value of 45°, indicating a yellowish red tonality.

Analysis of oil content throughout maturity weeks

The closer it gets to fruit physiologic maturity, the higher were oil contents found in bunches (Figure 6). In advanced maturity stages, it was noticed a variation in oil content between 47 and 66% among the observed bunches. Bunches b1, b10, b13, b16 and b18 presented outliers in at least one of the maturity stages.

The correlation of 0.959 (significant at level 0.01) between the biological activity and the oil content showed that biologic activity could also be used as a parameter indicator of oil content in the fruits, which allows for the generation of a relationship between both parameters.

The synthesis and accumulation of oil in the fruits are directly connected to availability of starch reserves in the form of sugars (Chitarra and Chitarra, 2005). Montoya et al. (2016), demonstrated that in the fruits of macaw palm the reduction of starch content coincides with the increase in oil content. As highlighted, the particles generated by the degradation of the starch in the formation of sugars form laser light beam scattering centers, which increases the biological activity measured

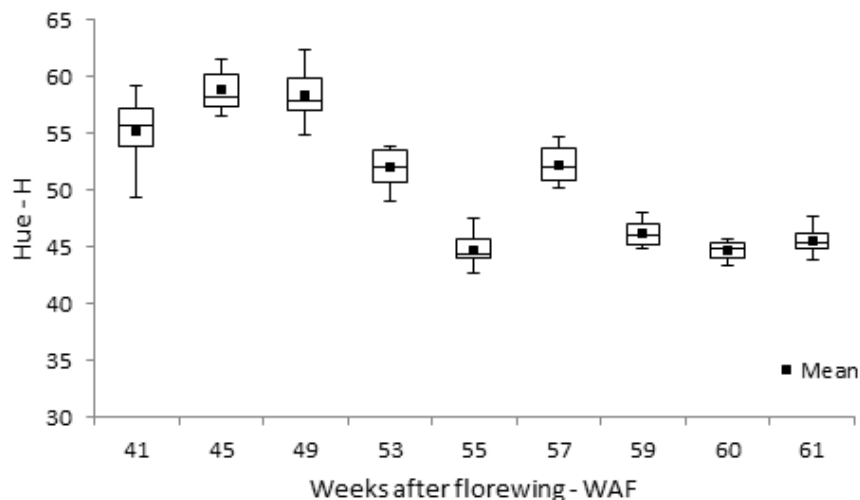


Figure 5. Analysis of the colorimetric property hue in the different weeks of maturation of macaw palm.

Table 2. Parameters for accuracy verification in the net used in the test group.

Parameter	NN 1	NN 2
Global Efficiency (GE%)	80.21	82.29
Kappa Coefficient (K)	0.60	0.65
Zc	7.2922*	8.8047*

*Significance at level 0.01.

by laser biospeckle. In this way, the hypothesis is suggested that the degradation of the starch during maturation of fruits macaw palm can be one the factors responsible for the correlation between oil content and biological activity.

Fruit classification in the moment of harvest related to biologic activity

Two classes were established according to the maturation stage of the fruit. Immature class was comprises of fruits gathered in 41°WAF and 45°WAF and considered inappropriate for harvest. The Mature class was comprised of fruits in 60°WAF and 61°WAF, period considered close to the ideal moment to be harvested.

The Kappa coefficient showed that classifications performed by both neural networks were better than a random classification at significance level 0.01 (Table 2). NN2 presented a higher global efficiency and it was chosen as the most appropriate net where fruit classification can be performed.

When analyzing fruit classification in the 10 maturity weeks, it has been observed that fruits harvested until 57°WAF were predominantly classified into immature class, presenting average oil content of up to 45.50%

(Table 3). The percentage of classification of harvested fruit in the 55th WAF and 57th WAF (close to 50.00%), showed that these dates the number of immature fruits to be harvested and fruits ready to be harvested are close by, making classification variable. From the 59th WAF fruits were classified predominantly into mature class, being considered ready to be harvested, when biologic activity was used as an evaluation parameter for the maturity stage. In these stages, the fruits show the average oil content higher (above 45.96%).

In extreme maturity stages, in 41th WAF and 61th WAF, the lowest errors in classification were detected, 17.02 and 11.59%. In these cases, classification error can be associated to experimental imprecisions during the application of biospeckle technique. The presence of damage in the lightened region of the epicarp such as micro chinks, cracks and darken spots May also have influenced the biological activity and chlorophyll levels in the fruit epicarp.

The period between the 55th WAF and 57th WAF presented the smallest difference between the percentage of classified fruits in the Immature and Mature classes, showing that in stages close to harvest, the classification by biologic activity has its accuracy reduced. The non-uniform maturity, mainly in fruits coming from different bunches, might have reflected in

Table 3. Fruits classified as Immature (A) and Mature (B) through neural network NN2.

Activity	NF	Immature Class %	Mature Class %	OC (%)	PC
41 WAF	94	82.97	17.02	9.68	A
45 WAF	87	72.41	27.59	22.56	A
49 WAF	90	58.89	41.11	31.02	A
51 WAF	95	57.95	42.05	32.63	A
53 WAF	71	71.84	28.16	39.58	A
55 WAF	67	50.74	49.25	44.68	A
57 WAF	88	56.82	43.18	45.50	A
59 WAF	78	20.51	79.49	45.96	B
60 WAF	70	20.00	80.00	50.67	B
61 WAF	69	11.59	88.41	53.02	B

Where: NF = number of fruits; OC (%) = Oil content; PC = predominant class.

Table 4. Predominant Classification (PC) for each bunch through neural network NN2 in the analyzed maturity weeks. Immature Class (A) and Mature (B).

Classification	WAF (Week After Flowering)									
	41	45	49	51	53	55	57	59	60	61
Bunch 1	A	A	A	B	B	A	A	A	--	--
Bunch 2	A	A	A	A	A	A	B	B	B	B
Bunch 3	A	B	B	A	--	A	A	B	B	B
Bunch 4	A	A	A	A	A	A	B	--	--	--
Bunch 5	A	A	A	A	A	--	B	B	B	B
Bunch 6	A	A	B	A	A	B	B	B	--	--
Bunch 7	B	A	B	B	B	A	A	B	B	B
Bunch 8	B	A	A	A	A	A	A	B	B	B
Bunch 9	A	A	A	B	A	--	A	A	B	B
Bunch 10	A	B	B	B	A	--	A	B	B	B
Bunch 11	A	A	A	A	A	B	B	B	--	--
Bunch 12	A	A	B	B	A	A	A	B	B	B
Bunch 13	A	A	B	B	B	--	B	B	B	B
Bunch 14	A	A	A	B	A	B	A	B	--	--
Bunch 15	A	A	A	A	--	B	B	B	B	B
Bunch 16	A	A	A	A	A	--	A	B	B	B
Bunch 17	A	B	A	B	A	B	A	B	B	B
Bunch 18	A	A	A	A	A	A	B	B	B	B
Bunch 19	A	A	A	A	A	--	A	A	B	B
Bunch 20	A	A	A	B	A	A	A	B	B	B
PC	A	A	A	A	A	A	A	B	B	B

the variant of biologic activity in this period.

It is important to point out that although anticipated harvest allows for loss reduction of the mature fruits through natural fall and of the number of times in the performance of field harvest compared to selective harvest (Skic et al., 2016), it will demand a careful handling in the post-harvest phase so that the fruit reach the highest levels of quality and quantity of oil content.

In specific situations where the classification of each

fruits becomes unfeasible, a general evaluation of the bunch might be an option for analysis in the harvest time. In Table 4 the general classification per bunch in each maturity week is depicted. Bunches were classified predominantly in the immature class, being considered inappropriate to be harvested up to the 57th WAF. From the 59th WAF the bunches were predominantly classified in the Mature class, it is a period they are considered ready by the classifier to perform macaw palm fruit

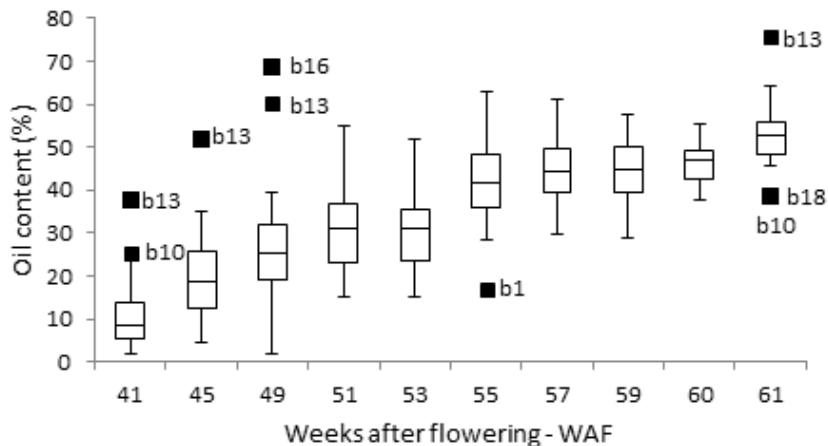


Figure 6. Variation in oil content in bunches in analyzed maturity stages.

harvest, with higher oil levels, when biologic activity is used as a parameter to evaluate maturity stage.

Based on the results, biologic activity measured through biospeckle laser can be considered a viable parameter for maturity evaluation and a promising tool when it comes to indicating the harvest period for macaw palm fruit. Sensors built from biospeckle laser technique might be used in automated selection systems to evaluate fruit conditions in relation to its oil content and to verify bunch condition at harvest directly in field.

Conclusions

An increase in biologic activity was observed throughout maturity weeks after flowering for macaw palm fruits when the epicarp was analyzed, presenting a significant linear relation between biologic activity and maturity weeks. These results showed the effectiveness of optical capacity from biospeckle laser technique in following the development of maturity for macaw palm fruits.

Oil level obtained from macaw palm bunches presented an increase throughout fruit maturity, whose higher oil levels were found closer to physiologic maturity of fruits. The developed classifier showed that the 59th week after flowering was considered the ideal period for harvest, when biologic activity was used as a parameter to set maturity, showing the applicability of biospeckle laser to determine the harvest period for macaw palm fruits.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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