

Full Length Research Paper

## Physiological maturity of seeds and colorimetry of yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Degener)

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The passion fruit is among the major fruit crops grown in Brazil, being propagated by seeds due to ease of implementation. However, obtaining seeds of quality is associated with the proper time of collection. Thus, the objective was to determinate the physiological maturity of seeds of passion fruit (*Passiflora edulis* f. *flavicarpa* Degener) using the external color of the fruit. Seeds from passion fruit were collected from four plants. It was adopted a completely randomized design, separating the fruit into four stages of maturation, based on color of the epicarp and quantified as Munsell color chart and refractive index analysis using digital colorimeter. Seeds of completely green fruit did not germinate. Seeds from fruits with advanced stage of maturation tending to wilt, staining with 5 Y 7/10 according to the Munsell color chart and measures of refractive index falling, showed better physiological potential according to the integrity of their membranes, accumulation of dry matter and performance in tests of vigor. This stage was identified as the stage of physiological maturity of the passion fruit.

**Key words:** Maturation stage, germination, electric conductivity, vigor.

### INTRODUCTION

The passion fruit (*Passiflora edulis*) is among the major fruit crops that are grown in Brazil, making the country the world's largest producer, with production of 776,097 tonnes and 59,246 ha planted in 2012, with sales of the fruit in raw or processed for industrial extraction of pulp (IBGE, 2012). Highlighting the yellow passion fruit (*P.*

*edulis* f. *flavicarpa* Degener) and purple passion fruit (*P. edulis* f. *edulis* Sims.) due to a greater acceptance and commercial exploitation (Negreiros et al., 2006).

The propagation of these species can be performed sexually, by seed, or asexually, by budding, cuttings or tissue culture *in vitro*. Propagation by seed is widely used

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due to its greater facility of implementation, reduced time to obtain seedlings and the need of hand labor less specialized when compared to asexual methods (Ferreira, 2000).

In order to obtain good quality seeds, there must be considerations about the time of the fruit's collection. It is necessary to follow the development of the fruit and/or seed through their physical and physiological characteristics (Carvalho and Nakagawa, 2012). According to these authors, the maximum quality of the seed is associated with the accumulation of dry matter, also called mass maturity.

Mass maturity occurs close or at the same time of physiological maturity of the seeds, stopping the transfer of plant dry matter to the seeds, reaching maximum physiological potential or quite near of reaching it (Marcos Filho, 2005). According to Carvalho and Nakagawa (2012), seeds that are not completely mature also possess capacity of germination, however, do not generate plants as vigorous as those collected at the point of physiological maturity. Analyzing the process of physiological maturation in seeds of *P. edulis f. flavicarpa* Degener, Negreiros et al. (2006) found that the extraction of seeds should be done on fruits with epicarp color at least 5 to 50% yellow. Before the production of yellow passion fruit's seedlings, it is recommended to keep the fruits for 3 to 6 days in storage before the seed extraction.

However, the visual qualification of fruit's coloration even when based in diagrammatic scale is subjective and with limitation of precision, and when done by softwares demand time and deep training (Effendi et al., 2009). This way, standardized methods with fast execution to determinate physiological maturity can be elaborated. Effendi et al. (2009) and Dranski et al. (2010) presented the use of electronic colorimetry in cultures of jatropha (*Jatropha curcas* L.) to quantify the visual stage of maturity groups. Rodríguez-Pulido et al. (2012) also found a relation between the color of the fruit and the processes of grape maturation, finding reliable correlation between the disposition of phenolic compounds and berry epicarp color, indicating the harvest time to processing.

In general, the propagation of passion fruit by the seeds presents difficulties due to the low percentage of germination and disuniformity on seedling formation (Pádua et al., 2011). Therefore, knowing the aspects that affect seed germination, as those related to pre and post-harvest, morphological and physiological, assist in the solution of several problems related to the seminiferous propagation of the specie for seedling. In addition, stays evident the need of studies that determinate standard conditions and ideal harvest period of yellow passion fruit, to obtainment of seeds with maximum physiological potential, in order to obtain larger uniformity in germination and production of seedlings. Thus, the objective of the present study was to determinate the physiological maturity and ideal time of harvest of yellow

passion-fruit's seeds (*P. edulis f. flavicarpa* Degener), using the extern coloration of the fruit through digital colorimeter as the indicator of maturity.





## MATERIALS AND METHODS

The experiment was conducted in the Seed and Seedling Laboratory and Plant Physiology Laboratory, both located in State University of West Paraná – Unioeste, Campus Marechal Cândido Rondon, in 2013. The seeds were collected from fruits of yellow passion-fruit, specie *P. edulis f. flavicarpa* Degener, that came from four plants in rural propriety, situated in 24°33'20,68"S and 54° 02'36,32"W, in different stages of maturity, collected in one single moment. The experiment was conducted in entirely casualized lineation with four treatments. In the harvest process, occurred the separation of the fruits in four visual states of maturation (Figure 1). For each stage, were used five fruits, totalizing 20 experimental plots. For the segregation of the stages of maturity, were applied Munsell's color chart (Munsell, 1976) and analysis of the refractive index of the epicarp in the red, green and blue spectrum, with the assistance of digital electronic colorimeter (Instrutherm® ACR 1023). The values were obtained from three measurements in each fruit, taken at equidistant points in the equatorial region. After classification, the fruits were opened, with the aid of a scalpel, in the equatorial region of fruit and, supported by a spatula, had its seeds extracted along with the placental membranes.

In relation to the seeds, as stated in Figure 1, in the first stage of maturation of the fruits, the absence of yellow color was observed, as well as thin integument, without formed pulp, indicating the immature stage of the fruit. On stage two, the seeds presented in transition phase, with seeds both dark and yellowish, and has been verified the presence of characteristic pulp, although in low quantity. On stages three and four, all the seeds presented dark coloration of the integument and the fruits had high quantity of pulp. On stage four, the fruits presented external wilt. The seeds obtained from the fruits were washed in running water, on steel sieve, to remove the aryl, as described by Carvalho et al. (2012). Then, were accommodated in trays above paper towel and under shade for four hours. After processing the seeds, the electrical conductivity, dry matter, moisture content and seed vigor and germination were determined.

The electrical conductivity test occurred in five replicates of 25 seeds, weighed on an analytical balance and placed in polypropylene cups of 200 ml. The seeds were immersed in a volume of 50 ml, with distilled and deionized water, and kept at constant temperature of 25°C, for 24 h. With the assistance of a bench conductivity meter (Engineering® BEL W120), and according to the methodology of Vieira and Krzyzanowski (1999), the reading of conductivity were performed, and the results were expressed in  $\mu\text{S cm}^{-1} \text{g}^{-1}$ . Five samples of 100 seeds were used to quantify the mass of dry matter and moisture content of seed for each group, quantifying the fresh mass of the samples, placed in kraft paper bags properly identified and taken to forced circulation air oven, with a temperature of  $105 \pm 3^\circ\text{C}$  for 24 h. After this period, the samples were weighed again on analytical balance, measuring the dry mass of the sample. Simultaneously, it was determined the moisture content of seeds based on the mass of wet material, as described by Marcos Filho (2005), consisting on the ratio between the mass of water present in the seeds ( $W_m - D_m$ ) and dry mass ( $D_m$ ) of the sample, expressed in percentage.

The germination test was conducted under vermiculite substrate, accommodated in plastic polypropylene trays. The trays were placed in a germinator, type Biochemical Oxygen Demand (BOD), set at 25°C, as recommended by Brasil (2009), photoperiod 12:12 (light: dark). Were used five replicates of 20 seeds for each group,

Stage of maturation	Coloration	Visual characterization	Munsell's chart
1		Green fruit	7,5 GY 4/4
2		Fruit in pigmentation transition green/yellow	2,5 GY 6/8
3		Predominantly yellow fruit	5 Y 8/6
4		Yellowish fruit starting wilt	5 Y 7/10

**Figure 1.** Coloration of yellow passion fruit [*Passiflora edulis* f. *flavicarpa* (Degener)] in different stages of maturation. Marechal Cândido Rondon/PR, 2013.

separated by the color of the fruit. The count of normal emerged seedlings occurred daily from the test installation and finished at 50 days from sowing. With data from seedling emergence, determined the number of days until the start of emergence (EI), according to Labouriau (1983). Also, computed the Emergence Speed Index (ESI) proposed by Maguire (1962). After 50 days, it was determined the Percentage of Germination (PG) by counting the emerging seedlings.

Using the methodology proposed by Czabator (1962), it was stipulated the Peak Value of Germination (PV), corresponding to the ratio between the maximum value of the germination accumulated percentage and the number of days to obtain it. It was also determined the Daily Germination Average (DGA), which expresses the ratio between the total germinated seeds and the time (in days) of the duration of the test, and the Germinal Value (GV), obtained by the product between PV and DGA.

The data were subjected to analysis of variance. The treatment means were compared by Tukey's test at 5% probability of error, with the aid of SISVAR version 5.1 (Ferreira, 2011). Based on the average of nine characteristics: electrical conductivity, dry matter weight of 100 seeds, moisture content, EI, ESI, PG, PV, GV and DGA, in each maturation stage, the dissimilarity matrix was calculated using the Euclidean mean distance standardized, by the method of UPGMA (unweighted pair group method with arithmetic mean). Next, applied to the analysis of hierarchical agglomerative clustering (HAC) concocting dendrogram with the aid of  $\chi$ Lstat program®.

## RESULTS AND DISCUSSION

According to the data shown in Table 1, there was a decrease in the electrical conductivity, accompanying the ripening of the fruits, of 51.61, 75.76 and 83.25%,

comparing stage one to two, three and four, respectively. Thus, according to the advance in development and fruit ripening process, there is greater organization of the seed's membranes, and therefore less loss of electrolytes through these (Araújo et al., 2007), resulting in increased seed vigor when compared to seeds that have high electrical conductivity.

Studying the correlation between electrical conductivity test and vigor and seed viability tests of yellow passion fruit, Barbosa et al. (2012) verified that the conductivity test is effective to segregate seed lots at different levels of vigor, when correlated with seedling emergence. Evaluating different stages of maturation and storage conditions of the yellow passion fruit, Araújo et al. (2007) also observed that the seeds that presented lower values for the electrical conductivity showed highest germination, recommended parameter for the determination of seed vigor of this specie.

In the analysis of dry matter weight of 100 seeds, were also found differences between the stages of fruit maturation. Stage three of maturation showed higher absolute dry matter accumulation (0.486 g), however, being statistically similar to stage four (0.466 g). Stage one had the lowest values (0.266 g) differing significantly of the others, while stage two presented an intermediate value (0.394 g), also showing significant differences. This increase in dry matter accumulation may have correlation with seed vigor, since the greater the accumulation of reserves, the better the intrinsic ability to properly develop (Marcos Filho, 2005).

**Table 1.** Electrical conductivity, dry matter weight of 100 seeds and moisture content calculated on a wet basis of seeds of yellow passionfruit [*Passiflora edulis* f. *flavicarpa* (Degener)] at different stages of fruit ripening. Marechal Cândido Rondon/PR, 2013.

Stage of maturation <sup>(1)</sup>	Electrical conductivity ( $\mu\text{S cm}^{-1} \text{g}^{-1}$ )	Dry matter weight of 100 seeds (g)	Moisture content (%)
1	388.898 <sup>a</sup>	0.226 <sup>c</sup>	36.03 <sup>a</sup>
2	188.156 <sup>b</sup>	0.394 <sup>b</sup>	24.16 <sup>b</sup>
3	94.265 <sup>c</sup>	0.486 <sup>a</sup>	26.04 <sup>b</sup>
4	65.136 <sup>d</sup>	0.466 <sup>a</sup>	16.72 <sup>c</sup>
Mean	184.114	0.393	25.74
CV (%)	9.55	3.32	10.15

Different letters in the column indicate significant differences by Tukey test at 5% probability of error. (1) 1: Green Fruit; 2: Fruit in pigmentation transition green/yellow; 3: predominantly yellowish fruit; 4: yellowish fruit starting wilt.

**Table 2.** Days until the start of emergence (EI), emergence speed index (ESI), percentage of germination (PG), peak value (PV), daily germination average (DGA) and germinative value (GV) of seeds of yellow passion fruit [*Passiflora edulis* f. *flavicarpa* (Degener)] at different stages maturation. Marechal Cândido Rondon /PR, 2013.

Stage of maturation <sup>(1)</sup>	EI	ESI	PG (%)	PV	DGA	GV
1	50.00 <sup>a</sup>	0.000 <sup>c</sup>	0.00 <sup>c</sup>	0.000 <sup>b</sup>	0.00 <sup>c</sup>	0.000 <sup>b</sup>
2	39.00 <sup>b</sup>	0.593 <sup>b</sup>	25.50 <sup>ab</sup>	0.054 <sup>a</sup>	5.10 <sup>ab</sup>	0.289 <sup>ab</sup>
3	40.80 <sup>b</sup>	0.441 <sup>b</sup>	20.00 <sup>b</sup>	0.048 <sup>a</sup>	4.00 <sup>b</sup>	0.249 <sup>ab</sup>
4	22.60 <sup>c</sup>	1.312 <sup>a</sup>	37.50 <sup>a</sup>	0.078 <sup>a</sup>	7.50 <sup>a</sup>	0.611 <sup>a</sup>
Mean	37.95	0.668	20.75	0.045	4.15	0.287
CV (%)	4.91	12.44	18.93	1.58	16.66	11.81

Different letters in the column indicate significant differences by Tukey test at 5% probability of error. (1) 1: Green Fruit; 2: Fruit in pigmentation transition green/yellow; 3: predominantly yellowish fruit; 4: yellowish fruit starting wilt.

Similarly, Araújo et al. (2007) find no significant differences in dry matter accumulation in seeds derived from passion fruit harvested at intervals of 10 days, at 55, 60 and 65 days after anthesis (DAA). Also stated that treatment with higher average of dry matter accumulation showed a low percentage of germination when subjected to the accelerated aging test, indicating that larger seeds are more subject to degradation. The same authors also found that the decrease of the water content of seeds occurs at 55 and 60 DAA.

The moisture content of the seeds suffered a decrease, following the trend of electrical conductivity, due to the organization of the membranes during the process of seed and fruit maturation (Table 1). The stage four showed lower moisture percentage (16.72%) when compared to others; the second (26.04%) and third (24.16%) showed no significant differences, showing lower moisture content when compared to stage one (36.03%). These results indicate the superiority of the group four when compared to the other stages, showing greater amount of reserves in relation to water, being beneficial to the storage, germination and development process of the seed. According to Padua et al. (2011), seeds of *Passiflora setacea* are resistant to desiccation in levels close to 4% water, and low humidities and storage temperatures induce dormancy in seeds of this species. Fonseca and Silva (2005) found that 7% of moisture

reveled favoring the maintenance of the physiological potential of *P. edulis* f. *flavicarpa* Degener in between 31 and 7% moisture in seeds.

In Table 2, it was observed a difference between the phases of maturation to EI, in stage four showed shorter period for early emergence (22.60 days), while the first stage showed no seed emerged during the study period. Stages two and three showed an intermediary behavior when compared to similar treatments and to each other, 39 and 40.80 days, respectively. These responses follow the progress of the maturation process by reducing the number of days for the beginning of germination according with the course of the senescence process of the fruit. It can be observed that stage four also showed better performance for ISE and PG variables, resulting in the stage with greater speed emergency (1.312) and higher germination (37.50%). These results complement those obtained previously in the electrical conductivity and dry matter tests, giving these answers to a greater integrity of their membranes and higher accumulation of dry matter of stage four, providing better conditions for germination and survival of seeds. Highest germination of seeds of passion fruit are found in seeds from shriveled fruit (Lopes et al., 2007). Similarly, seeds derived from fruits harvested at 65 DAA showed higher germination percentage compared to fruit harvested at 55 and 60 DAA (Araújo et al., 2007).

**Table 3.** Refractive index mean values of the fruits of yellow passion fruit [*Passiflora edulis* f. *flavicarpa* (Degener)], obtained through digital colorimeter and qualitative characterization of the Munsell chart. Marechal Cândido Rondon/PR, 2013.

Stage of maturation <sup>(1)</sup>	Refractive Index Scale (nm)			Munsell
	Red	Green	Blue	
1	170.133 <sup>d</sup>	157.400 <sup>c</sup>	98.866 <sup>b</sup>	7.5 GY 4/4
2	238.933 <sup>c</sup>	206.000 <sup>b</sup>	113.000 <sup>b</sup>	2.5 GY 6/8
3	491.933 <sup>a</sup>	346.600 <sup>a</sup>	192.333 <sup>a</sup>	5 Y 8/6
4	386.066 <sup>b</sup>	246.600 <sup>b</sup>	116.533 <sup>b</sup>	5 Y 7/10
Mean	321.766	239.150	130.183	-
CV (%)	9.81	9.54	14.27	-

Different letters in the column indicate significant differences by Tukey test at 5% probability of error. (1) 1: Green Fruit; 2: Fruit in pigmentation transition green/yellow; 3: predominantly yellowish fruit; 4: yellowish fruit starting wilt.

Stages two and three presented similar results and intermediary among treatments for the ESI and PG variables. Corroborating with the results found, Negreiros et al. (2006) did not find significant differences in the ESI in three stages of maturation (unripe fruit starting to yellow, 5 to 50% yellowish and more than 50% yellowing) of yellow passion fruit. So when contrasted his description of stages of maturation with the present work, the green fruit starting to yellow equates to stage two, while the group with more than 50% yellowish equivalent to stage three. For the PV variable in Table 2, stages two (0.054), three (0.048) and four (0.078) were superior to stage one. For the analysis of DGA, the early stages showed lower values compared to the others, again being the fourth stage (7.50) the one that was successful for the variable, equivalent to stage two (5.10) and differing from stage three (4.00). Finally, to the GV test, only the fourth stage differed from the first, being equivalent to the others. These data are derived from mathematical relations that utilize directly the germination percentage and the test duration in days as the basis of calculation, being variables used to express the vigor, speed and uniformity of germination (Czabator, 1962), having high correlation and similarities between them (Santos et al., 2009). There is the possibility of pointing stage four as the best point to harvest the fruits for seed production.

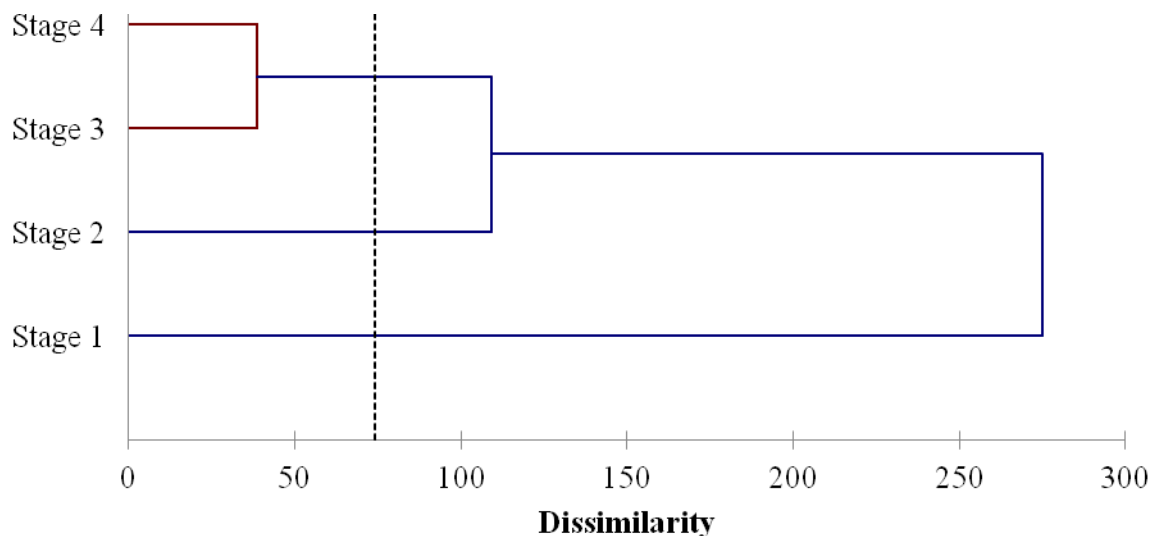
Refractive index measures made with digital colorimeter showed differences between the maturation phases of passion fruit for the three measurements made (Red, Green and Blue) as shown in Table 3. The readings showed increase in values following the maturation, reaching its highest values in stage three of maturation (491.933, 346.600 and 192.333 for red, green and blue, respectively), and coinciding with the scale 5 Y 8/6 of the Munsell chart (Munsell, 1976). After this stage, there was a decrease in the three evaluated colors.

Several studies have succeeded in relating the coloring, either of the fruit or seed, as an indicator of physiological maturity of seeds from many different cultures. Ragagnin et al. (1994) in seeds of maritime pine

(*Podocarpus lambertii* Klotzsch), Gemaque et al. (2002) in seeds of purple ipe (*Tabebuia impetiginosa* (Mart.) Standl.), Fonseca et al. (2005) with seeds of yellow ipe (*Tabebuia chrysotricha* (Mart. Ex DC.) Standl.) and Mendes et al. (2005) studied the process of maturation in annatto (*Bixa orellana* L.).

However, evaluations of tone require trained personnel and are mostly subjective, causing a reduction in the accuracy on practical application. Thus, it denotes the importance of a standardized method such as digital colorimeter, exemplified in studies conducted with jatropha (*Jatropha curcas* L.). Effendi et al. (2009), who developed a classification system to indicate the level of ripeness of the fruit system, helping to define the ideal harvest point for production of oil crop. Likewise, Dranski et al. (2010) related the epicarp color of the fruit to the physiological maturation of the seeds of Jatropha. The use of colorimetry is also applied to other cultures and with different purposes, as in grape (*Vitis vinifera* L. cv. Graciano), where Rodríguez-Pulido et al. (2012) related the epicarp color to the contents of phenolic compounds in the culture, making colorimetry an alternative, fast, and inexpensive tool for indicating the point of harvest for wine production. The passion fruit seeds presents greater germination power and dry matter accumulation when the fruit is fully yellowish (Negreiros et al., 2006), following the highest values of refractive index obtained in the study (Table 3). Thus, the use of digital colorimeter shown as an excellent tool to help with the identification of suitable harvest time, in order to obtain seeds for propagation purposes.

Finally, it is observed by the dendrogram (Figure 2) the formation of three distinct groups. It can be identified similarity between the stages three and four of passion fruit maturation, which although differ in most vigor tests, are grouped due to proximity to the mass of dry matter accumulated in the seeds and the integrity of their membranes (electrical conductivity). Stage two showed intermediary dissimilarity, while stage one, presented farthest of the other stages of maturation. Thus, stage four, followed by three, presented better characteristics to



**Figure 2.** Dendrogram made using cluster analysis of stages of maturation of yellow passion fruit based on the standardized Euclidean mean distances matrix calculated by the method of UPGMA (unweighted pair group method with arithmetic mean), using nine characteristics related to seed vigor and viability.

form vigorous plants and performance in vigor and seed viability tests, while stage two showed intermediate behavior, being stage one smaller than the others due to the inability of the seeds to produce viable seedlings.

## Conclusions

Seeds of yellow passion fruit (*P. edulis* f. *flavicarpa* Degener) obtained from yellowish fruits tending to wilt (stage four) showed better performances in tests of vigor, having higher speed, uniformity and emergence percentage. In the moment that refractive index values reach its peak (491.933, 346.600 and 192.333 nm for the red spectrum, green and blue, respectively) and go into decline, coincides with the higher physiological potential of the seeds. It is accompanied by the maximum accumulation of dry matter, lower water content and higher membrane integrity, indicating that the refractive index of the fruit's epicarp is a valid and easy method to use for determining the physiological maturity of seeds of passion fruit.

## Conflict of Interest

The authors have not declared any conflict of interest.

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