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Botanic and physiology

Sample size for the evaluation of 'BH-65' papaya fruits under protected cultivation

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Abstract - The cultivation of papaya under greenhouse has become recently a profitable alternative for horticultural crops in different regions of the world, among them South East Spain. The objective of this work was to determine the sample size to evaluate fruit quality features in mature fruits of 'BH-65' papaya cultivated under greenhouse in Almería (Spain). With this aim, fruits were harvested at stage 2 in July 2013, and at the end of the production cycle in March 2014. On each occasion and when fruits reached maturation phenological stage 5, 26 fruits were evaluated for physical characteristics (weight, length, diameter, cavity width), total soluble solids content, and color attributes of skin and pulp. The optimal sample size was calculated using a deterministic method. The physical parameters and the skin and pulp color attributes of fruits of 'BH-65' papaya cultivar present different experimental accuracy among themselves and between harvest seasons, requiring different sample sizes. Higher sample size is required for evaluating fruit weight. 25 fruits were necessary at harvest performed in July, but only 7 fruits in March, considering an error of 15% around the average.

Index terms: *Carica papaya* L.; experimental planning; sampling.

Tamanho ótimo de amostra para avaliação de frutos de mamoeiro 'BH-65' em cultivo protegido

Resumo - O cultivo de mamoeiro em estufa vem tornando-se, nos últimos anos, uma alternativa de cultivo em diversas partes do mundo. Objetivou-se com este trabalho determinar o tamanho de amostra para avaliar frutos maduros de mamoeiro 'BH-65' cultivado em estufa no sul da Espanha. Foram colhidos 26 frutos no estádio 2 de maturação, em julho de 2013, e outros 26 frutos, também no estádio 2, no final do ciclo produtivo, em março de 2014. Os frutos foram avaliados quando atingiram o estádio 5 de maturação, para características físicas (massa, comprimento, largura, diâmetro da cavidade), química (teor de sólidos solúveis) e atributos de cor da casca e da polpa. O cálculo do tamanho da amostra foi feito por método determinístico. As características físicas de frutos e dos atributos de cor da casca e do fruto de mamoeiro 'BH-65' apresentam diferentes precisões experimentais entre si e entre as épocas de colheita, requerendo diferentes tamanhos de amostra. O maior tamanho de amostra é requerido para avaliar a média de massa de frutos, sendo necessário avaliar 25 frutos na colheita de julho e 7 frutos na colheita de março, considerando erro de 15% em torno da média.

Termos para Indexação: *Carica papaya* L.; planejamento experimental; amostragem.

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Introduction

Papaya (*Carica papaya* L.) is one of the main tropical fruits produced in the world. Global papaya production reached 13.05 million tons in 2016, being India and Brazil the main producers (FAO, 2016), and Europe one of the more important import market. Although commercial cultivation is traditionally performed in open field, more recently papaya cultivation under greenhouses is generating interest in Brazil (MARTELLETO et al., 2011), India (PRAKASH et al., 2015) and Mediterranean regions, mainly Spain and Turkey (GUNES and GÜBBÜK, 2012; GALÁN, 2014; SALINAS et al., 2017, 2018). Growing papaya under protected cultivation near European markets offers the possibility of harvesting fruits ripen on the tree, and therefore with a higher quality. Ruggiero et al. (2011) mention that the Brazilian market already values more the called 'fruit ripened in the plant', whose fruits are sweeter.

Papaya fruits should be carefully selected according to the target market criteria. Fagundes and Yamanishi (2001) report that several physical (weight, length, diameter, form, color, firmness) and chemical (total soluble solids, pH, titrable acidity) characteristics, can be measured to evaluate the quality of papaya fruits. These features, in turn, can be influenced by edaphoclimatic conditions, genotype, season, cultural practices and harvest and postharvest handling, which vary depending on market requirements, especially maturity stage at harvest that depend on the distance to consumer market (CHITARRA and CHITARRA, 2005).

The sample size to evaluate fruits is influenced by several factors, among them, the intrinsic variability of fruits, physical or chemical (SCHMILDT et al., 2017), the maturation stage (SILVA et al., 2017), harvest date (TONINI, 2013) and sample precision defined by the researcher (KRYSCZUN et al., 2018). According to Krysczun et al. (2018), factors that lead to variability of experimental error interfere in the statistics of hypotheses tests and in the comparison of treatments, leading to erroneous interpretations and conclusions (KRYSCZUN et al., 2018). Regarding precision level, it is well known that the lower the established error by the researcher for parameter estimation, such as averages and coefficients of variations, the sample required for accurate estimates will be higher (TOEBE et al., 2014b). In fact, determining sample size is fundamental in any scientific experiment since a sample size lower than required lead to inaccurate estimations. On the contrary, excessively large samples suppose unnecessary use of resources and time (COELHO et al., 2011).

Several studies show the adequate sample size to describe fruit characteristics in several crops as *Citrus sinensis* (AVANZA et al., 2010), *Passiflora* spp. (COELHO et al., 2011; BANDEIRA et al., 2016; SCHMILDT et al., 2017), *Prunus persica* (TOEBE et al., 2012), *Litchi chinensis* (ANDRADE and JASPER, 2012), *Ananas comosus* (KRAUSE et al., 2013), *Bertholletia excelsa* (BORGES et al., 2016), *Carica papaya* (FERREIRA, 2014; SILVA et al., 2017), *Malus domestica* (TOEBE et al., 2014a), *Carya illinoinensis* (CARGNELUTTI FILHO et al., 2015; POLETTO et al., 2018), and *Spondias purpurea* (SILVA et al., 2016).

In papaya, different studies using fruits of 'Golden THB' establish sample size for fruits harvested in the field (Ferreira, 2014), and for fruits subjected to a postharvest treatment (Silva et al., 2017). However, there are no reports about the correct sample size for papaya fruits produced under protected cultivation, despite the importance acquired in recent years (MARTELLETO et al., 2011; PRAKASH et al., 2015; SALINAS et al., 2017, 2018). In this regard, greenhouse cultivation imposes harsh conditions and additional constraints, besides the reduced area of most plantations. This limitation is even greater considering that today several genotypes are often cultivated in the same greenhouse in order to select the most appropriate for these conditions, requiring biometric studies and the establishment of the adequate sample size.

Considering the importance of papaya, the objective of this work was to determine the sample size required for the measurement of physical and chemical characteristics of mature fruits harvested in two consecutive seasons in plants grown under greenhouses.

Materials and methods

The work was carried out with papaya fruits (*Carica papaya* L.) from 'Solo' group, specifically 'BH-65' cultivar, grown in a low height greenhouse in Almería, South East Spain. 'BH-65' cultivar was selected for its indication to be cultivated in greenhouses due to the small size of plants (SAÚCO, 2014). The plantation was initiated on September 14th, 2012. Fruits were collected in two moments, 10 months after planting (July 2013) and 23 months after planting (March 2014), collecting 26 fruits in each season. The fruits were harvested at stage 2 of maturation, that is, fruits with up to 25% of the skin yellow, and evaluated at maturation stage 5, at full yellow color, according to Reis et al. (2015).

The fruits carefully handled were transported in plastic boxes to the Fruticulture Laboratory of the University of Almería and stored at room temperature until reaching maturation stage 5, when fruit quality was evaluated. The parameters analysed were weight (g), length (mm), diameter (mm), cavity width (mm), total soluble solids content (TSS); determined by a direct reading using a portable digital refractometer Atago Pal 1, model PR-101 (Atago Co., Japan), provided with automatic temperature compensation, expressed in ºBrix,

skin and pulp color (L*, C and h parameters) according to *CIELAB 1976* (CIE, 2004); determined using a digital colorimeter model CR-400 (Konica-Minolta Co., Japan), which uses the CIELAB three-dimensional color system, being measurements expressed by L*, C and h. L* value represents lightness, varying from black (L* = 0) at the base of the vertical axis of the three-dimensional scale, to white (L* = 100) at the top of the same axis. Chroma (C) represents the saturation intensity of color, having zero value in the middle of the three-dimensional scheme and increasing as it moves away from this point. Hue (h) is defined as the color location angle in the diagram, showing the hue of color, in which 0º angle represents pure red, 90º represents pure yellow, 180º represents pure green and 270º represents pure blue.

For each quality parameter, we calculated the descriptive statistics of minimum and maximum values, arithmetic average, standard deviation and coefficient of variation. We also verified the normality of data, using Shapiro-Wilk normality test, with the aim of characterizing the sample data and verify its suitability for a deterministic method based on Student-t distribution for each measured parameter (SCHMILDT et al., 2017).

Sample size (η) was calculated for the half-amplitudes of the confidence interval, applying the following expression, according to Resende (2007):

$$\eta = \frac{S^2(t_{\alpha/2})^2}{e^2m^2}$$

Where: S is the estimate of standard deviation; $t_{\alpha/2}$ is the critical value of Student t-distribution, whose right-hand area is equal to α/2, with (n-1) degrees of freedom, α = 5% probability of error; while e is the error in the average estimate (5; 6; 7; 8; 9; 10; 15 and 20%); m is the sample arithmetic average.

Data were processed using R analytical software (R Development Core Team, 2018).

Results and Discussion

Fruits of 'BH-65' papaya cultivar, harvested and measured in July 2013, as well as of those produced in 'BH-65' plants in March 2014, are represented in Figure 1.

Descriptive statistics evaluations, using the minimum, maximum, arithmetic average, standard deviation (SD) and coefficient of variation (CV), and Saphiro-Wilk normality test for the characteristics of 'BH-65' fruits harvested in July 2013 and in March 2014, are presented in Table 1. Regarding fruit physical parameters, a significant statistical difference was found in weight, length, diameter and cavity width measurements between fruits harvested in July 2013 versus those collected in March 2014, being higher the values of fruits harvested in March. In July 2013, average fruit weight was 220.96 g, similar to the values found by Gunes and Gübbük (2012) for 'BH-65' plants grown in similar conditions in Turkish greenhouses. Average fruit weight in March 2014 was 598.73 g, value close to that observed in 'BH-65' cultivated in open fields of Cuba (Alonso et al., 2008). Martelleto et al. (2001), working with 'Baixinho de Santa Amalia' cultivated in greenhouses in Brazil, also found differences between fruits of different seasons.

Regarding skin color, hue angle (h) values were close to 90º (Table 1), indicating a yellow color (CIE, 2004), in both harvest seasons. However, there were significant differences between fruits harvested in different seasons for lightness (L*) and chroma (C), with fruit harvested in March showing higher values and therefore greater luminosity and more intense yellow color. The appearance of a fruit is the combination of its geometric and chromatic attributes which directly interferes with its acceptability by consumers (OLIVEIRA et al., 2015; REIS et al., 2015).

Regarding to pulp tone (h), a significant difference was also found: fruits harvested in July presented average h values= 68.35 while March fruits have a h= 83.56. These values represent a reddish and a yellowish color, respectively. No statistical differences in L* or chroma values of the pulp were found. L* values were similar to those reported by Gunes e Gübbük (2012) for 'BH-65' cultivated under greenhouse in Turkey.

The variability estimated by the coefficient of variation (CV) was higher, for all parameters, in fruits collected in July 2013. Therefore, sampling in July requires a larger sample size than in March. The greatest variability was found in fruit weight for pieces harvested in July (CV=36.2%), a value considered high by Ferreira et al. (2016). In the evaluation of fruit weight in 'Golden THB', Silva et al. (2017) measured a CV<15.0%. However, it should be noted that these fruits were previously selected for postharvest treatments, and in this case, fruit lots were grouped according to weight range. Fruit mass was also a characteristic of noticeable variability in pineapple (KRAUSE et al., 2013).

Regarding cavity width, a CV=21.5% was found in July, the third highest. On the contrary, fruits harvested in March only presented a CV=6.5%, one of the lowest values in this evaluation. Considering that 'BH-65' is a cultivar with no genetic variability among experimental plants, we confirm that environmental effect is more important for fruits harvested in July, reflecting severe climatic conditions during this period.

All parameters showed a normal distribution of the data, as Shapiro-Wilk normality test confirms. This permits sample sizing by deterministic methods. Considering that the fruits harvested in 2013 were more heterogeneous than the fruits harvested in 2014, and that statistical differences were found between the averages of the two harvest seasons in most parameters, we decided

to perform an analysis of the two harvest dates separately, since the measurements of central tendency (average) and variability (standard deviation and coefficient of variation), interfere in sample size (RESENDE, 2007; TOEBE et al., 2014).

The sample size estimated for each parameter of quality, according to the assumed error, are shown in Table 2. Sample size was lower in fruits harvested in March than those harvested in July for all parameters. Characteristics demanding smaller sample sizes were fruit length and diameter, total soluble solids content and skin and pulp color attributes L* and h. To evaluate the average length of fruits, with a 10% error around the average, it was necessary to evaluate 7 fruits in harvest carried out in July and only 3 in March. Fruit length and diameter were also the parameters that required smaller sample size to evaluate pineapple fruits (KRAUSE et al., 2013), plum (SILVA et al., 2016) and 'Golden THB' papaya (SILVA et al., 2017).

The higher sample size was required for fruits weight, as predicted for its greater variability (Table 2). Thus, to evaluate the weight of fruits, with a 5% error, sample should be made of 223 and 55 fruits, respectively, for July and March harvests. As expected, sample size decreases when the error allowed around the average increases (for instance from 5 to 10%) (RESENDE, 2007; TOEBE et al., 2014a; SILVA et al., 2017). Thus, if the researcher wants to evaluate fruit weight considering a 10% error around the average, only 56 and 14 fruits are needed, for July and March harvests, respectively. If the error is increased up to 15%, sample size will be only 25 and 7 fruits, respectively.

Consequently, for an experiment planned on 'BH-65' papaya fruits under conditions similar to those experimented in this study, in a completely randomized experimental design, 25 fruits per treatment should be evaluated when fruits are harvested in July to estimate the average of each treatment with 15% accuracy. If the experiment were planned considering five replicates per treatment, five fruits per replicate would be sampled (25/5 = 5), that it is, five fruits per plot. Besides, if four treatments were evaluated in the experiment, the researcher have to use 100 fruits to perform such experiment (25 fruits per treatment).

In this study, sample size was different for the different parameters evaluated. Similar results were verified by other researchers in yellow passion fruit (COELHO et al., 2011), peach (Silva et al., 2008), pineapple (KRAUSE et al., 2013), apple (TOEBE et al., 2014a), plum (SILVA et al., 2016) and 'Golden THB' papaya (SILVA et al., 2017).

With the aid of Table 2, the researcher can verify the sample size needed for different parameters of interest, with different precisions, and for different harvest seasons. However, if the researcher needs to evaluate the average of physical parameters, soluble solids content and skin and pulp color, should assume sample size needed for fruit weight, since this parameter requires the highest sample size.

Figure 1. Fruits of 'BH-65' papaya cultivar harvested in July 2013 (left) and 'BH-65' papaya plant with mature fruits, in March 2014 (right).

Table 1. Minimum, maximum, arithmetic average (average), standard deviation (SD), coefficient of variation (CV%) and normality by Shapiro-Wilk (S-W) test, for weight, length, diameter, cavity width, total soluble solids content (TSS), and skin and pulp lightness (L*), color intensity (C) and color tone (h), in fruits of 'BH-65' cultivar harvested in two seasons of papaya plants grown under greenhouse in Almería, Spain.

Parameter	Unit	Minimum	Maximum	Average [1]	SD	CV (%)	S-W [2]
Fruits harvested in July 2013 (fruits developed in spring)							
Weight	g	80.00	380.30	220.96 b	79.91	36.20	0.7457
Length	mm	84.44	130.84	107.70 b	13.24	12.29	0.5906
Diameter	mm	42.85	82.80	64.60 b	11.12	17.21	0.1426
Cavity width	mm	18.72	39.97	27.52 b	5.92	21.50	0.4146
TSS	°Brix	9.00	12.50	10.41 b	0.97	9.35	0.5123
Skin color – L*	Un.	45.04	65.97	56.31 b	5.62	9.98	0.7471
Skin color - C	Un.	33.23	74.00	47.41 b	10.99	23.19	0.0574
Skin color - h	°	74.61	128.43	100.09 a	14.54	14.54	0.6156
Pulp color - L*	Un.	56.17	71.38	63.33 a	3.97	6.27	0.5615
Pulp color - C	Un.	36.98	59.49	49.88 a	6.81	13.65	0.2393
Pulp color - h	°	56.85	80.07	68.35 b	5.05	7.39	0.0722
Fruits harvested in March 2014 (fruits developed in winter)							
Weight	g	426.00	878.00	598.73 a	107.72	17.99	0.2589
Length	mm	128.00	155.00	140.00 a	10.70	7.64	0.5032
Diameter	mm	81.91	104.43	91.31 a	5.84	6.40	0.5534
Cavity width	mm	43.69	52.03	48.81 a	3.17	6.49	0.8785
TSS	°Brix	12.90	14.70	13.39 a	0.6976	5.21	0.4325
Skin color – L*	Un.	56.86	73.84	67.84 a	4.03	5.93	0.1322
Skin color - C	Un.	50.27	72.09	64.21 a	5.65	8.80	0.1093
Skin color - h	°	87.38	107.46	93.56 a	5.35	5.72	0.0551
Pulp color – L*	Un.	63.33	69.06	65.61 a	2.38	3.62	0.3671
Pulp color - C	Un.	44.73	53.04	49.17 a	3.27	6.65	0.9230
Pulp color - h	°	80.05	85.96	83.56 a	2.63	3.15	0.1736

[1] The average of the parameters measured in two harvest seasons followed by the same letter differ by the bilateral t test, at 5% of error probability.
[2] $p > 0.05$ indicates normal distribution of sample data.

Table 2. Number of fruits to evaluate weight, length, diameter, cavity width, total soluble solids content (TSS), and skin and pulp lightness (L*), color intensity (C) and color tone (h), in fruits of 'BH-65' cultivar harvested in two seasons of papaya plants grown under greenhouse in Almería, Spain.

Parameter	Amplitude of the 95% confidence interval								
	5	6	7	8	9	10	15	20	25
Fruits harvested in July 2013 (fruits developed in spring)									
Weight	223	155	114	87	69	56	25	14	9
Length	26	18	14	11	8	7	3	2	2
Diameter	51	35	26	20	16	13	6	4	3
Cavity width	79	55	41	31	25	20	9	5	4
TSS	15	11	8	6	5	4	2	2	1
Skin color – L*	17	12	9	7	6	5	2	2	1
Skin color - C	92	64	47	36	29	23	11	6	4
Skin color - h	36	25	19	15	12	9	4	3	2
Pulp color – L*	7	5	4	3	3	2	1	1	1
Pulp color - C	32	22	17	13	10	8	4	2	2
Pulp color - h	10	7	5	4	3	3	2	1	1
Fruits harvested in March 2014 (fruits developed in winter)									
Weight	55	39	29	22	17	14	7	4	3
Length	10	7	6	4	4	3	2	1	1
Diameter	7	5	4	3	3	2	1	1	1
Cavity width	8	5	4	3	3	2	1	1	1
TSS	5	4	3	2	2	2	1	1	1
Skin color – L*	6	5	4	3	2	2	1	1	1
Skin color - C	14	10	7	6	5	4	2	1	1
Skin color - h	6	4	3	3	2	2	1	1	1
Pulp color – L*	3	2	2	1	1	1	1	1	1
Pulp color - C	8	6	4	3	3	2	1	1	1
Pulp color - h	2	2	1	1	1	1	1	1	1

Conclusions

Physical characteristics and skin and pulp color measured in mature fruits of 'BH-65' papaya cultivar grown under greenhouse present different accuracy among them and among harvest seasons, requiring therefore different sample sizes.

The highest sample size is required to evaluate fruit weight, where is necessary to evaluate 25 fruits for July harvest but only 7 fruits for March, assuming an error of 15% around the average.

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