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Water content of pomegranate seeds subjected to storage and packaging periods

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Abstract

The worldwide demand for unconventional fruits, such as pomegranate (*Punica granatum* L.), has grown due to their nutraceutical characteristics. Given the social and industrial importance of the crop, information about the seed storage conditions should be elucidated to technicalize its cultivation. The objective of this work was to evaluate, by determining the water content, the behavior of pomegranate seeds when stored in different periods and packages. The seeds were separated into two lots: seeds with sarcotesta and without sarcotesta. The storage periods used were: 30, 60, 90, and 120 days, in 3 types of packaging (polyethylene, paper bags, and glass bottles), stored in BOD at 5 ± 1°C, and relative humidity 35 ± 2%. After storage periods, the seed water content was evaluated. Throughout the storage periods, the polyethylene and glass packages maintained the water content of the pomegranate seeds with and without sarcotesta. The paper bag packages did not retain the water content of pomegranate seeds with sarcotesta during the storage periods.

Keywords: conservation, fruit, *Punica granatum* L., sarcotesta

Introduction

The demand for quality fruits and seedlings of pomegranate (*Punica granatum* L.) has been growing in parallel with the consequent search for production techniques due to its multifunctionality and high nutritional capacity (Matityahu et al., 2015).

However, despite its long history of cultivation, information about the technology of pomegranate seeds is incipient in the literature (Fawole & Opara, 2013), especially regarding the cultivars used by Brazilian producers, highlighting the cruciality of more in-depth knowledge about the physiological potential of the seeds of this fruit species.

Seed storage essentially integrates the process for maintaining their physiological quality. Besides being cost-effective compared to preservation *in situ*, it also assures plant breeding programs studies on species, aiming to obtain new cultivars resistant to pests and diseases. Additionally, it propagates the material regardless of the season, and thus make available to the productive sector materials with a better agronomic performance adapted to the growing regions (Oliveira et al., 2012; Suszka et al., 2014).

The lack of extensive information on adequate seed storage protocols frustrates the attempts to preserve genetic resources (Suszka et al., 2014). Also, research has shown that the efficiency of seed conservation is directly related to the storage conditions, and in controlled situations, preservation has been more outstanding when compared to natural environments (Forti et al., 2010).

Seed storage in drier or colder conditions can prolong their lifespan, whereas, at lower temperatures, the molecular movements are decreased, reducing the rate of biochemical reactions, and slowing their deterioration (Carvalho et al., 2014).

Temperature is an essential factor in seeds'

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storage, and their deterioration is inevitable. When there is adequate knowledge of the association of thermal aspects and water content during the conservation period, biological activities such as breathing can be controlled, since both factors directly influence the extent and speed of the decrease in seed quality, consequently interfering in their longevity (Harman & Mattick, 1976; Marcos Filho, 2015). Because of this, the success in the seed storage process requires in-depth knowledge about mechanisms such as quantification of water content (Goldfarb & Queiroga, 2013).

The available studies regarding the storage conditions of pomegranate seeds are scarce, which can hinder the correct determination of the water content of the seeds, causing deterioration in the quality of the product through microbiological and enzymatic reactions, and later, the formation of orchards with reduced life period due to the absence of demanding selection criteria (Catunda et al., 2003).

In this context, the objective of this work was to evaluate, by determining the water content, behavior of pomegranate seeds when stored in different periods and packaging.

Material and Methods

The experiment was carried out in the Laboratory of the Department of Phytotechnics, Food Technology, and Socioeconomics, at UNESP - Campus of Ilha Solteira/SP, from August to November 2015. Physiologically mature fruits of *cv. Valenciana* were collected from healthy adult pomegranate plants, from a commercial orchard located in Presidente Prudente/SP (latitude 22° 3' 21.24" S, longitude 51° 21' 35.16" W, and 477. 6 m altitude).

The experimental design used was completely randomized, in a 2 x 3 x 4 factorial scheme (types of seeds x types of packaging x storage periods) with four replications, each consisting of 25 seeds.

Factor 2 refers to seeds with and without sarcotesta. Therefore, after removing the seeds from the fruits, one batch remained with sarcotesta (Figure 1A), and in the other batch, the sarcotesta was removed with the aid of a 2.36 mm sieve (mesh smaller than the size of the seeds) (Figure 1B). Then, the seeds of the second batch remained in the shade for 12 hours to eliminate the externally adhered water. Both seed lots were immersed in a 1.5% active chlorine solution for 30 minutes for disinfestation.

Since the physiological quality of the seeds is the purpose in the evaluation of their storage potential, the sarcotesta of the pomegranate seeds was removed because, in addition to presenting phenolic compounds, anthocyanins and tannins that compromise the germination of the species (Noda et al., 2002), absorption and dehydration processes are favored by the absence of this structure, when the integument is exposed (Silva et al., 2015).

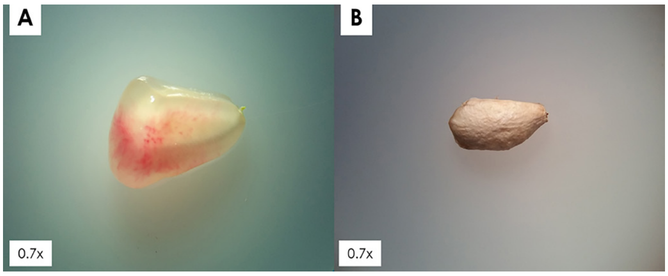

Figure 1. Pomegranate seeds (*Punica granatum L.*) with sarcotesta (A) and without sarcotesta(B). Increase = 0.7x. Ilha Solteira/SP, 2015.

The storage periods used consisted of 30, 60, 90, and 120 days, in 3 types of packaging, being transparent polyethylene bags (PL) (semi-permeable), white-colored paper bags (PP) (permeable), and glass bottles with cover (VI) (waterproof). All packages were properly closed and stored in a germination chamber of the BOD type at 5 ± 1 °C with a relative humidity of 35 ± 2%.

According to the Rules for Seed Analysis (Brasil, 2009), the water content of the pomegranate seeds was determined, where they remained in an oven with forced air circulation, regulated at a temperature of 105 ± 3 ° C, for 24 hours. Two repetitions were used with subsamples of 5.0 g of seeds each, and the results were expressed in percentage based on the equation: Water content (%) = 100 (*Pp*) / *P* - *t*, where *P* is the weight of wet seed, determined after the storage period, *p* is the weight of the dry seed, determined after the drying period in the oven, and *t* is the tare weight of the container with a lid.

The data were analyzed using SISVAR software (Ferreira, 2019). The analysis of variance was performed using the F test at 5% probability to detect the differences between treatments. When a significant difference was found for each variable, the Scott-Knott test (5% probability) and analysis of variance in the regression were performed. Adequate regression was verified from the p-value of the regression deviation (not significant) and the selected polynomial regression models based on the higher determination coefficients (R^2), among the significant regressions by the F test.

Results and Discussion

The initial water content of pomegranate seeds with sarcotesta was higher (78.57%) than in those without this structure (54.80%) (Figure 2). According to Scalon et al. (2012), high values of the water content of the seeds may indicate sensitivity to desiccation. The species present this period in a varied way, since dissection intolerance can be related to high levels of insoluble reserves, sugars, and proteins and the effectiveness of antioxidant and repair systems during rehydration (Leonhardt et al., 2010).

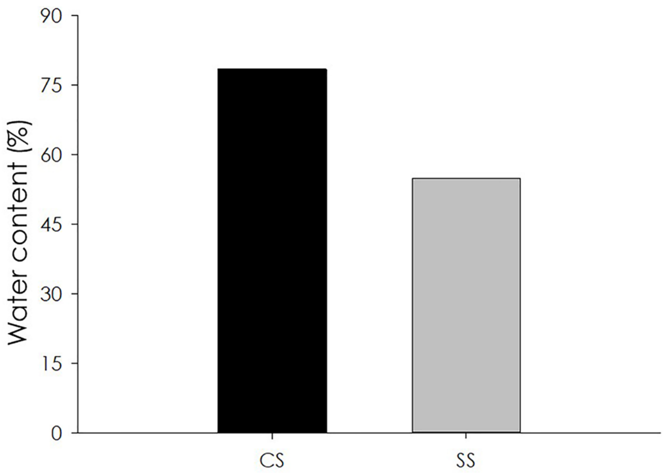

Figure 2. Initial water content of pomegranate seeds (*Punica granatum L.*) according to the presence (CS) or absence of sarcotesta (SS). Ilha Solteira/SP, 2015.

In the case of sarcotesta, the presence of this structure gave pomegranate seeds a significant difference in water content compared to seeds in which there was no such condition. This situation, according to Moreiras et al. (2013), occurs since the sarcotesta of pomegranate seeds has a large amount of water: 91.5 g of 100 g of an edible portion is composed of water.

Table 1 shows the square mean and average values about the water content of pomegranate seeds. The data display a significant interaction among all factors evaluated.

Regarding the packages, the data highlighted that pomegranate seeds stored in polyethylene bags and glass jars had similar water contents, while paper bags provided lower contents, with the types of packaging being statistically different (Table 1).

The low water content displayed by the seeds stored in the paper bags is because this type of hygroscopic packaging presents a greater exchange of moisture between the seed and the external environment of conservation, which increases the activity of microorganisms and the metabolic rate of the seed itself, consuming more reserves and, consequently, reducing its quality (Silva et al., 2010). The proper use of seed storage packages is crucial since they directly associate with the conservation of their viability and vigor (Souza et al., 2011).

Time is an essential factor and directly influences the viability of the stored pomegranate seeds. When observing the initial water contents in the seeds with or without sarcotesta (Figure 2), after 90 days of storage, the observed moisture losses were lower than in the other evaluated periods. The water losses did not display significant differences among the groups at 120 days (Table 1).

Table 1. Analysis of variance for the water content of pomegranate seeds (*Punica granatum* L.) with and without sarcotesta according to packaging and storage periods. Ilha Solteira/SP, 2015.

Variation Source	Water content (%)
	Square mean
Sarcotesta (S)	9381.7602 *
Package (E)	16734.3320 *
Period (P)	170.0798 *
S x E	72.1117 *
S x P	156.7572 *
E x P	136.2154 *
S x E x P	899.2975 *
V.C. (%)	4.18
Overall average	47.99
Sarcotesta	**Overall average**
Presence	59.41 A[1]
Absence	36.58 B
Package	**Overall average**
Polyethylene	61.93 B
Paper	17.55 C
Glass	64.52 A
Period (days)	**Overall average**
30	52.28 A
60	48.10 B
90	46.25 C
120	45.36 C

* Significant NS Not significant by F test at 5% probability. [1] Equal capital letters between rows in the column do not differ by the Scott-Knott test at 5% probability. C.V. = Variation Coefficient

The water contents of the seeds stored with sarcotesta were statistically higher than the values found for seeds without sarcotesta, in all storage periods of each evaluated package (Table 2). These more significant results are due to the sarcotesta, as already mentioned, which is a structure composed of water. However, this maintenance of high humidity levels can foster the deterioration of the seeds, providing a decrease in germination due to the reduction in the physiological quality of the stored seed (Goldfarb & Queiroga, 2013; Morais et al., 2014).

Regarding seeds storage with sarcotesta in polyethylene bags and glass bottles, there was no statistical difference for the water contents observed in all the evaluated periods (Table 2). As the conditions and type of packaging used have a direct influence on the physiological quality of the stored seeds, the data displayed that polyethylene and glass, at the low temperatures during the storage periods of the present study, were efficient in preserving the water content of the pomegranate seed similar to the initial (Figure 2).

For gabiroba seeds (*Campomanesia adamantium*), Melchior et al. (2006) observed a direct relationship between the decrease in water content and the low percentage and speed of germination, because as the seeds lose water over time, there is a decrease or delay in seed germination. Probably, the pomegranate seeds with sarcotesta stored in paper bags would have the same behavior since these packages did not conserve the moisture of the seeds, and there was a decrease in the water content over the storage periods (Table 2).

Table 2. Water content (%) of pomegranate seeds (*Punica granatum L.*) according to packaging, presence or absence of sarcotesta, and seed storage periods. Ilha Solteira/SP, 2015.

Treatment	PL		PP		VI	
	SS	SW	SS	SW	SS	SW
30	75.69 Aaa*	49.35 Aba	51.64 Aab	7.71 Abb	76.32 Aaa	52.96 Aba
60	75.31 Aaa	49.66 Aba	26.57 Bab	8.01 Abb	76.44 Aaa	52.63 Aba
90	74.94 Aaa	49.73 Aba	15.78 Cab	8.14 Abb	75.40 Aaa	53.51 Aba
120	73.83 Aaa	46.94 Abb	14.47 Cab	8.03 Abc	76.57 Aaa	52.33 Aba
Regression (equations)						
PL – SS	Not significant					
PL – SS	Not significant					
PP – SS	$y = 0.0066x^2 - 1.3975x + 97.3858$ $R^2 = 0.9987$ $F = 10.244^*$ $x_{max} = 105$					
PP – SW	Not significant					
GL – SS	Not significant					
GL – SW	Not significant					

* Capital letters in the column compare periods in the same treatment. Lower case letters, on the same line, compare types of seeds, in the same package. Underlined lower case letters, on the same line, compare the same treatment (type of seed), in the different packages. PL = polyethylene, PP = paper, GL = glass, SS = seeds with sarcotesta, SW = seeds without sarcotesta.

In all evaluated periods and packages, pomegranate seeds without sarcotesta presented lower water contents than seeds with sarcotesta. However, regardless of the presence or not of sarcotesta, storage in paper bags provided more significant water loss in the seeds to 30 days (Table 2), presenting an alternative to being used in short periods. As mentioned previously, paper packaging allows moisture exchanges with the environment, which causes the seeds to change in their water content during the storage period (Carvalho et al., 2014).

However, pomegranate seeds without sarcotesta stored in paper bags did not display significant alterations of the water contents during the storage periods, suggesting that the absence of this structure when associated with the packaging that allows humidity variation according to external conditions, favored the rapid dehydration of the seeds until 30 days of storage (Table 2).

The water content of pomegranate seeds without sarcotesta stored in polyethylene and glass packages remained around 50% throughout the experimental periods, with no significant difference between them (Table 2). Corroborating the results, in studies with genipap (*Genipa americana L.*), Bezerra et al. (2015) found a water content of 51% when the seed mucilage was removed with a sieve, the same method used in the present study to remove the sarcotesta.

Regarding the pomegranate seeds without sarcotesta stored in glass, there was no variation in their water content, as it did for seeds without yellow passion fruit sarcotesta (*Passiflora edulis Sims f. Flavicarpa Deg.*), in studies by Catunda et al. (2003).

The associations of types of packaging and seeds showed no adjustment for regression, except when pomegranate seeds without sarcotesta were stored in paper bags, where their equation was adjusted for a quadratic model, and the maximum point was obtained at 105 days packaging (Table 2).

As refers to microbial contamination, the presence of fungi was not observed in any kind of packaging and at any time, pointing out the effectiveness in disinfecting the seeds with 1.5% active chlorine solution. Besides, the low-temperature conditions of pomegranate seeds storage also favored the presence of fungi, as according to Pezzini et al. (2005), these are microorganisms sensitive to temperature changes. As the temperature is preserved below 15 °C, the incidence of fungi decreases, and when the temperature is around 30 °C, it provides better conditions for their development.

Conclusions

Throughout the storage periods, polyethylene and glass packaging maintained the water content of pomegranate seeds with and without sarcotesta.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.