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Chemical composition of forage watermelon fruit at different maturity stage or storage length

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ABSTRACT. This study aimed to assess the chemical responses of forage watermelon fruit at different maturity stages or storage lengths, performing two experimental tests. In the first test, four maturity stages were assessed: 30, 45, 60, and 75 days after anthesis, with four replicates. In the second test, fruits were maintained under three storage lengths: T1D (harvest day), T3M (3 months after harvest), and T6M (6 months after harvest), with eight replicates. Experimental design was completely randomized in both experimental tests. Fruit maturity stage did not affect crude protein, total carbohydrate, neutral detergent fiber, *in vitro* dry matter digestibility (IVDMD), pulp firmness, soluble solids content and total pectin content, but increased acid detergent fiber content from 45 days after anthesis. Storage length up to six months after harvest increased ash, crude protein and IVDMD, and reduced the content of soluble solids. Forage watermelon fruit can be harvested from 30 to 75 days after anthesis equivalent to 75 - 120 days after planting, and they can be stored under tree shade up to 6 months after harvest.

Keywords: *Citrullus lanatus* var. *citroides*; fruit quality; forage plant; nutritional value.

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Introduction

Citrullus lanatus var. *citroides* has multiple uses as human and animal food and as source of genes for commercial watermelon breeding programs (Mashilo, Shimelisa, Odindo, & Amelework, 2017). Genotypes of this plant species are considered tolerant to water deficit (Mo et al., 2016) and have been identified as a potential forage resource in several regions, especially drylands (Souto et al., 2005; Silva et al., 2009; Santos, Melo, & Fonseca, 2017, Ribeiro et al., 2021).

Fruits are used for animal feeding especially in family farming-based systems. However, despite the potential, this forage resource requires management practices to increase production efficiency. Fruits after harvest have waived the traditional methods for forage conservation (silage or hay). Additionally, Kavut, Geren, and Simić (2014) reported weight loss of fruit may exceed 20% along 180 days after harvest. Strategies to reduce post-harvest fruit losses of forage watermelon (FW), such as the process of harvesting and storing fruits in an appropriate place and the adequacy of storage length are important to increase the amount of nutrients and water for animals.

In addition, food quality has a great importance to animal performance. Forage watermelon fruit have approximately 16.0% crude protein (CP) and 60.00% *in vitro* dry matter digestibility (IVDMD) (Souto et al., 2005; Silva et al., 2009; Santos et al., 2017). Moreover, Almeida, Silva, Rocha, Morais, and Sarmento (2010) observed that fruit size, color, and soluble solids contents are important variables to determine the harvesting point.

The determination of the adequate harvest point for forage watermelon fruit is relevant to provide better chemical composition. Santos et al. (2017) harvested FW fruits at 100 days after planting. The early harvest can be a management strategy to avoid greater fruit losses due to scarce an erratic rainfall.

We hypothesized that fruit of forage watermelon can be harvested early 100 – 120 days after planting and kept for up to six months post-harvest under tree shade, without losing their nutritional value. Thus, the aim of this study was to evaluate the effects of maturity stage and storage length on nutritional value of forage watermelon fruit.

Material and methods

This study consisted of two experimental tests, both carried out at Campo Experimental da Caatinga in Embrapa Semiárido, Petrolina, State of Pernambuco, Brazil (09°09′ S, 40°22′ W, and 376 m altitude). Fruit maturity stages were assessed in the first test and the storage length was evaluated in the second test.

Soil in the area is classified as Oxisol (Embrapa, 2013). Soil samples were collected at a depth of 0–20 cm for analyzing their physical and chemical properties, presenting pH 5.6, P 6.35 mg dm^{-3}, K 0.28 $cmol_c\ dm^{-3}$, Na 0.04 $cmol_c\ dm^{-3}$, Mg 0.70 $cmol_c\ dm^{-3}$, Al 0 $cmol_c\ dm^{-3}$, H+Al 1.8 $cmol_c\ dm^{-3}$, sum of bases (SB) of 2.8 $cmol_c\ dm^{-3}$, and cation exchange capacity (CEC) 4.6 $cmol_c\ dm^{-3}$. Soil physical properties were 42.82% porosity and clay, silt, and sand fractions of 131.2, 35.8, and 833.0 g kg^{-1}, respectively.

Forage watermelon was initially sown in expanded polystyrene trays containing a commercial substrate. Seedling transplanting to the field was done 15 days after planting. Planting rows were spaced every 3 m, with 1 m between plants. Additional water was supplied by drip irrigation with emitters spaced 1 m apart, nominal flow of 2,400 L h^{-1} and lateral rows were spaced 3.0 m apart. Irrigation was performed three times a week (Monday, Wednesday, and Friday) in order to meet 100% ETc (crop evapotranspiration), obtained by ETo (reference evapotranspiration) of the Class A tank (ECA) and an average Kc of commercial watermelon.

Composition of irrigation water was Ca^{2+} 0.43 mmol L^{-1}, Mg^{2+} 0.52 mmol L^{-1}, Na^{+} 0.14 mmol L^{-1}, K^{+} 0.05 mmol L^{-1}, CO_3^{2-} 0.00 mmol L^{-1}, HCO^{3-} 0.62 mmol L^{-1}, SO_4^{2-} 0.34 mmol L^{-1}, Cl^{-} 1.60 mmol L^{-1}, pH 8.05, EC 0.06 dS m^{-1}, and hardness 4.76 mg L^{-1}.

Fertilization was done using 0.5 kg $plant^{-1}$ sheep manure. Manure composition was EC (electric conductivity) of 2.20 mS cm^{-1}, pH 8.10; C 120 g kg^{-1}, N 9.30 g kg^{-1}, Ca 5.50 $cmol_c\ dm^{-3}$, Mg 5.9 $cmol_c\ dm^{-3}$, Al 0 $cmol_c\ dm^{-3}$, H+Al 3.0 $cmol_c\ dm^{-3}$, SB 11.40 $cmol_c\ dm^{-3}$, CEC 14.40 $cmol_c\ dm^{-3}$, and base saturation (V) 79%.

The first test (maturity stage) was conducted from June to November and the second test (storage length) from November to May. Meteorological data registered during both tests are listed in Table 1.

Table 1. Meteorological data registered during experimental tests (maturity stage and storage length) of forage watermelon fruit.

Climatic element	Maturity stage	Storage length
Average temperature, °C	26.5	23.3
Maximum temperature, °C	34.7	33.7
Minimum temperature, °C	18.7	28.4
Average relative humidity, %	53.67	53.32
Maximum relative humidity, %	88.58	81.92
Minimum relative humidity, %	28.03	27.93
Global radiation, MJ	21.75	22.82
Reference evapotranspiration, mm	4.96	5.07
Cumulative rainfall, mm	43.9	98.6

Source: Agrometeorology laboratory of Embrapa Semiarid.

To evaluate fruit maturity, treatments consisted of four maturity stages: 30, 45, 60, and 75 days after anthesis (floral bud opening), equivalent to 75, 90, 105, and 120 days after planting. The experimental design was completely randomized with four treatments and four replicates of five fruits each, totaling 80 fruits. Fruits were identified during anthesis and harvested at the corresponding maturity stage, and they presented an average weight of 6.25 ± 2.47 kg, longitudinal diameter of 85.60 ± 13.26 cm, vertical diameter of 58.22 ± 10.49 cm, length of 31.10 ± 9.17 cm, and width of 17.41 ± 4.65 cm.

Samples of forage watermelon fruit were dehydrated in a forced-ventilation oven at 55°C to constant weight and ground through a 1 mm screen in a mill. Contents of dry matter (DM), ash, and crude protein (CP) were determined following the methods 967.03, 642.05, and 981.10, respectively, according to AOAC (1990). Ether extract was determined using *Ankom* equipment. Neutral detergent fiber (NDF) (INCT-CA F-002/1), corrected for ash (INCT-CA M-002/1) and protein (INCT-CA N-004/1), according to Detmann et al. (2012). Lignin was determined according to the methodology described by Van Soest and Wine (1967) and total carbohydrates (TC) were calculated according to the methodology described by Sniffen, O'Connor, Van Soest, Fox, & Russell (1992).

IVDMD was determined according to Tilley and Terry (1963) modified by Holden (1999); an adult bovine weighting approximately 350 kg body weight fed grass hay and forage watermelon was used as a donor of ruminal fluid. This procedure was approved by the CEUA (Ethics Committee on Animal Use) of Embrapa Semiarid (process 12/2017). Pectin was analyzed using dry sample for determination according to McCready

and McComb (1952), using a spectrophotometer (Spetronic Genesys 2) at 520 nm, according to Blumenkrantz and Asboe-Hansen (1973) technique.

Pulp firmness was assessed by a manual penetrometer. Fruit was divided longitudinally performing three readings in each half. The soluble solids content (SSC) was determined using a digital refractometer. Samples were taken from a longitudinal slice of fruit pulp and homogenized in a blender.

For the fruit maturity stage assessment, harvest was made at the end of the first test (120 days after planting) and fruit were weighted and kept in groups of three fruits under the shade of adult mesquite trees (*Prosopis juliflora* (SW.) DC.), with soil covered by a plastic canvas of 200 microns. Incidence of solar radiation in full sun was 1,500 ± 23 µmol S^{-1} m^2, while under shade, it was 346±126 µmol S^{-1} m^2, with 77% radiation interception.

Treatments evaluated were T1D (harvest day) T3M (three months after fruit harvest), and T6M (six months after fruit harvest). The experimental design was completely randomized with three treatments and eight replicates of three fruits each, totaling 72 fruits. Fruit had an average weight of 3.56 ± 1.94 kg, longitudinal and vertical diameters of 71.54 ± 14.54 cm and 52.22 ± 9.96 cm, respectively, average length of 26.65 ± 7.08 cm, and width of 16.23 ± 3.21 cm. Fruit weight at the harvest day (T1D) was 4.13 ± 2.64 kg, while in the treatment of 6-month storage (T6M), 3.22 ± 1.69 kg.

Chemical composition, IVDMD and pulp firmness was determined applying a similar methodology to the first test. In this evaluation, pulp firmness and SCC analyses were done on the harvest day and six months later.

Data were tested for normality by the Univariate procedure. The influence of fruit maturity stages was tested by analysis of variance and regression, while for fruit storage length, analysis of variance and Tukey's test, considering 5% probability ($p < 0.05$) as significant level using the Statistical Analysis System - SAS (2009).

Results and discussion

Different maturity stages of FW fruit influenced DM content (Table 2). A quadratic effect for maturity stage was found on DM content, presenting the lowest concentrations (6.79%) 50 days after anthesis.

Table 2. Chemical composition and pulp firmness (Newton – N) of forage watermelon fruit harvested at different maturity stages after anthesis, in % dry matter (DM).

Component	Harvest days after anthesis (DAA)				Equation	R^2	CV (%)	p-value
	30	45	60	75				
Dry matter[1]	7.50	6.70	7.00	7.70	$Y = 0.0017x^2 - 0.17x + 11.04$	0.96	6.10	0.01
Ash	7.53	8.95	8.19	8.03	-	-	8.08	0.08
Crude protein	17.63	18.08	17.24	16.85	-	-	9.27	0.47
Ether extract	12.27	11.08	11.61	11.55	-	-	9.81	0.70
NDF	28.79	32.42	30.82	30.10	-	-	10.46	0.09
ADF	18.95	23.43	22.08	21.84	$Y = 0.0052x^2 + 0.60x + 6.03$	0.77	9.71	0.02
Lignin	3.58	4.98	3.79	5.22	-	-	13.20	0.20
TC	62.57	61.88	62.95	63.56	-	-	4.18	0.75
IVDMD	83.27	81.24	81.36	80.97	-	-	3.89	0.57
Pectin, %	8.22	8.64	9.55	9.53	-	-	23.77	0.52
SSC, °Brix	3.22	3.18	3.03	3.38	-	-	9.80	0.42
Pulp firmness, N	60.28	42.90	45.36	58.70	-	-	27.21	0.16

[1] = as fed, NDF = neutral detergent fiber, ADF = acid detergent fiber, TC = total carbohydrate, IVDMD = *in vitro* dry matter digestibility, SSC = soluble solids content, N = Newton, CV = coefficient of variation.

The lowest DM levels at 50 days after anthesis (DAA) compared to values at 60 and 75 DAA is attributed to the greater accumulation of components (carbohydrate and pectin), considering the maturity stage of the fruit. High water concentration in fruit reinforces the great potential of FW fruit to supply water to herds.

Fruit maturity stage did not affect ash, CP, and EE. Concentrations found were similar to those reported by Silva et al. (2009), who evaluated forage watermelon meal in lamb diets, and observed 8.13%, 10.39%, and 18.73% for ash, EE, and CP, respectively. Ether extract and protein are essential nutrients to determine the quality of a forage species.

The IVDMD found was higher compared to those observed by Souto et al. (2005) and Santos et al. (2017) (68.00 and 74.86%), respectively, what can be attributed to the forage watermelon genotype.

Fruit maturity stage did not influence NDF, TC, and lignin. However, it affected ADF content, which showed a quadratic effect with a point of maximum concentration at 58 DAA, presenting 23.35% ADF. The increase in ADF from 58 DAA may be due to the accumulation of cell wall components in fruit or seeds. While

the average NDF and lignin at 30 DAA were 28.79 and 3.58% respectively; from 45 to 70 DAA, values ranged from 30.10 to 32.48% for NDF and 3.79% to 5.22% for lignin, respectively. The average NDF at different fruit maturity stages was lower than those obtained by Souto et al. (2005) (41.00%) in FW fruit and by Silva et al. (2009) using FW meal (38.82%). Soluble solids content, total pectin and pulp firmness were not affected by maturity stages (Table 2), indicating similarity in SSC, pectin, and pulp firmness equivalent to older fruits between fruit harvested at 30 days after anthesis and older fruits.

Dry matter, EE, NDF, ADF, lignin, and TC contents were not influenced by storage length. On the other hand, storage length influenced ash, CP, and IVDMD. Fruit subjected to 6 months of storage presented highest ash and CP (Table 3). This increase may be due to the occurrence of losses of other organic components, such as SSC (Figure 1), as well as a water reduction, leading to a relatively increased participation of ash and CP.

Table 3. Chemical composition and pulp firmness of forage watermelon fruit subjected to different post-harvest storage lengths.

Component (% dry matter)	Storage length (Months)			CV (%)	p-value
	T1D*	T3M	T6M		
Dry matter, %	7.88	7.71	8.20	11.93	0.68
Ash	6.84b	7.51b	10.84a	27.83	0.01
Crude protein	14.83c	16.46b	17.52a	11.16	0.03
Ether extract	10.89	11.33	10.93	32.17	0.98
Neutral detergent fiber	33.02	37.59	34.31	12.12	0.09
Acid detergent fiber	24.56	28.66	25.86	16.64	0.09
Lignin	6.33	7.41	4.51	14.22	0.16
Total carbohydrate	68.79	67.33	65.10	6.32	0.54
IVDMD	75.22b	72.43c	81.80a	8.19	0.049
Pectin, %	6.27a	4.39ab	2.42b	40.44	0.02
Soluble solids content, °Brix	3.39a	-	2.19b	15.84	0.0008
Pulp firmness, N	31.28	-	12.45	77.50	0.10

Means followed by different letters on the same row are significantly different by Tukey's test ($p < 0.05$). T1D* = day of harvest, T3M = three months of storage, T6M = six months of storage, *In vitro* dry matter digestibility (IVDMD), N = Newton, coefficient of variation (CV); P = probability.

The average fruit weight on the harvest day (4.13 kg) and at six months of storage (3.22 kg), even in fruit placed in the shade suggests a loss of water and nutrients in fruit during the storage period, especially considering no difference in DM content. In addition, Kavut et al. (2014) assessed fruit storage length from 30 to 210 days after harvest, and verified a fruit weight loss up to 30%, depending on storage length, with a concomitant increase in fruit dry matter.

The higher IVDMD at 6 months of storage may be associated with solubilization of components of the cell wall (pectin, hemicellulose, and cellulose) of fruit and seeds in fruits. Lignin is one of these components of the cell wall, and it presented an average of 4.51% at 6 months of storage and 6.33% on the harvest day.

Pectin was affected by storage length. A reduction in total pectin was found for T6M compared to the harvest day (Figure 1), suggesting a degradation of cell wall components. Pectins are found in the cell wall of all living plants, acting as the adhesive material between cells (Petkowicz, Vriesmann, & Williams, 2017), and is present in various forms, such as protopectins, pectinic acids, and pectic acids. Protopectin is abundant in green fruit that have already reached a full development. During ripening, protopectin is hydrolyzed into pectin by enzymes and, during rotting or ripening, it can be decomposed and form methyl alcohol and pectic acid (Trape & Jain, 2014).

A reduction in pulp firmness was observed with advancing storage months considering the harvest day (Figure 2). Firmness were higher when compared to commercial watermelon, which, according to Ramos, Dias, and Aragão (2009), can vary from 2.92 to 10.75 N at different spacings and cultivars, evidencing that forage watermelon is firmer than commercial watermelon.

Lower fruit firmness may be related to the reduction in cell wall components. Lignin and pectin contents in fruit on the harvest day were 6.33% and 6.27%, respectively, and 4.51% and 2.42% after 6 months of storage, respectively. Both lignin and pectin are present in the cell wall and although there was no significant difference along the storage length, suggesting solubilization of cell wall components, contributing to a reduction in fruit firmness.

Soluble solids concentration was influenced by fruit storage length (Figure 3). In this case, we observed that at 6 months of storage, soluble solids content was lower (2.19 °Brix) compared to the concentration determined on the harvest day (3.39 °Brix). This indicates that soluble solids were used during the period in which fruits were stored. Kavut et al. (2014) found a value of total soluble solids of 3.46 for forage watermelon, within the range of values found in our study.

Conclusion

Forage watermelon fruit can be harvested from 30 to 75 days after anthesis equivalent to 75 - 120 days after planting. Fruit can be stored under tree shade up to 6 months after harvest.

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