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Chemico-technological parameters and maturation curves of sweet sorghum genotypes for bioethanol production

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The sweet sorghum has high potential for bioethanol production, especially for using the same industrial production complex and for being processed during sugarcane off-season. The objective of this study was to evaluate the chemico-technological characteristics of the following sorghum genotypes: CVSW80007, CVWS80147, and BRS610, which were grown in Jaboticabal – São Paulo, State, Brasil. The experimental design used was a completely randomized block in a split-split-plot desing. The main treatments were three genotypes, two stalk management systems (stalks with and without leaves and panicles), and the tertiary treatment were six harvesting times (100, 105, 110, 118, 135, and 160 days after sowing) with three replications. The soluble solids content (Brix), total reducing sugars (TRS), pH, total acidity, total phenolic compounds, and starch content of the juice extracted were evaluated. The results indicated that given the chemico-technological characteristics of the genotypes CVWS80147 and CVSW80007, they can be used as raw material for bioethanol production, with useful-period-of-industrialization (UPI) about 40 days, starting at 110 days after sowing. The better results with chemical-technological parameters is find at 118 to135 days after sowing.

Key words: *Sorghum bicolor*, technological quality, productivity, biometrics, bioethanol.

INTRODUCTION

The growing global concern about environmental pollution resulting from the use of non-renewable energy and the release of greenhouse gases has stimulated the search for alternative fuels. These new energy sources may lead to increased and improved energy efficiency besides increasing energy availability and reducing costs

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thus ensuring sustainability and less environmental impact. The use of plant biomass as renewable energy sources, although it will not replace petroleum in its entirety, should help reduce dependence on fossil fuel and, consequently, reduce negative impacts on the environment. This possibility highlights the importance of such change making the production of energy from modern biomass an alternative strategy for all nations.

According to Nassif et al. (2012), sugarcane provides economic, social and environmental contributions, especially as raw material, to Brazil supporting the bioethanol production process. Similarly, other countries have successfully adopted the national ethanol production model, using sugarcane, resulting in a paradigm shift in fuel production in these modern days.

According to Jank (2011), the growing demand for ethanol in the domestic and international market is an issue of major concern, especially considering the predicted increase in industry demand for raw materials in the coming years. By 2020, it will be necessary to process 1.2 billion tons of sugarcane for the production of 51 million tons of sugar, in addition to 69 billion liters of ethanol (anhydrous and hydrated).

Under this scenario, one can deduce that the processing of sugarcane alone will not be sufficient to meet such high demand. Therefore, sweet sorghum has been identified as a high potential crop for biofuel production (Almodares and Hadi, 2009), as it has a short life cycle fully mechanized planting and harvesting and stalks rich in fermentable sugars; besides, its bagasse can be used as forage, for the co-generation of power (Cutz et al., 2013), or for producing second generation ethanol (Heredia-Olea et al*.,* 2013). Data available in the literature also demonstrate the feasibility of its use during the sugarcane off-season in the center-south region of Brazil, allowing the sugarcane mills to anticipate and extend the period of grinding (Teixeira et al., 1997).

Nevertheless, the inherent agronomic characteristics of the deployment of industrial large-scale production, such as planting, management, and harvesting systems and the fermentation process conduction are still liable for characterization. In order to produce high levels of industrial production, it is essential that the raw materials to be processed have high levels of fermentable sugars. Thus, the knowledge of the chemico-technological characteristics of sweet sorghum genotypes and their suitability for the fermentation process are of utmost importance. However, there are few studies that address this topic and little information available in the literature. The objective of the present study was to investigate the behavior of three sorghum genotypes during harvest in terms of their chemico-technological characteristics to evaluate the possibility of using them as raw materials for bioethanol production, in the sugarcane pre harvest season.

MATERIALS AND METHODS

Experimental conditions

The experiment was carried out in an experimental area of the Department of Crop Production at UNESP/FCAV, 21°14'05''S and 48°17'09''W, during the 2011/2012 harvest season. Sowing took place on12/14/2011 using 90 x 70 cm row spacing. A surplus of seeds was used, and 15 days after sowing (DAS) the plants were thinned to 10 plants.m^{-1} in order to obtain a population of 100,000 plants.ha^{-1}. N-P_2O_5-K_2O fertilizer was added at the rate of 20-100-100 kg. ha^{-1}. The weeds were removed manually, and thiamethoxam plus lambda-cyhalothrin was applied in the grooves at the rate of 20+15 g.ha^{-1} for pest control. The area was sprayed at 30 and 45 DAS at the same rate using the same pesticide for fall armyworm (*Spodoptera frugiperda*) control. N-P_2O_5-K_2O fertilizer was added at 30 DAS at the rate of 40-10-40 kg.ha^{-1}.

Experimental design

A split-split-plot design in a completely randomized block with three replications was used. Each plot consisted of 10 rows of 10 m length. The main treatments consisted of the three genotypes (CVSW80007, CVWS80147, and BRS610); the secondary treatments consisted of the two stalk harvesting systems (stalks with and without leaves and panicles), and the tertiary treatment consisted of the six harvesting times (100, 105, 110, 118, 135, and 160 DAS).

Sweet sorghum harvest and juice extraction

For every genotype at each harvesting time, 25 whole stalks (with leaves and panicles) and 25 clean stalks (without leaves and panicles) were harvested. They were sent to the Laboratory of Sugar and Ethanol Technology and Fermentation Microbiology, UNESP/FCAV, where they were weighed and their juice was extracted using a laboratory hand mill. The juice obtained was used in the chemico-technological analyses.

Chemical-technologycal evaluation

The chemico-technological characteristics evaluated were: Brix, determined according to Scheneider (1979); pH, measured using a DMPH-2 pH meter (Digimed) with temperature compensation; Total Reducing Sugars (TRS), determined by the Lane and Eynon (1934) volumetric method; phenolic compounds, quantified according to Folin and Ciocalteau (1927); and total acidity and starch content, determined according to Icumsa (The International Commission for Uniform Methods of Sugar Analysis) (2013). Whereas the sweet sorghum is used as raw material at season early, we compared its characteristics as sugarcane available in region at same time. We used the cultivars RB966928 and RB855156, classified as early maturing (crop in April and May). However, we did not statistical analysis.

Statistics

The results were submitted to analysis of variance (F test), test of multiple comparison of mean (Tukey 5%), and polynomial regression when this was significantly analysed, using the Barbosa and Maldonado (2011) method.

Table 1. Soluble solids (Brix), pH, total acidity, total reducing sugars (TRS), phenolic compounds, and starch content of the juice extracted from sorghum and mean values of sugarcane (Jaboticabal, 2012).

Treatment	Brix (%)	pH	Total Acidity ($g.L^{-1}$ H_2SO_4/L)	TRS (%)	Phenolic compounds (ug/ml)	Starch (ug/ml)
Genotypes (G)	43.32**	0.85ns	2.22ns	89.51**	0.09ns	21.90**
CVSW80007	16.12A#	4.94 A#	1.54 A#	12.03 A#	705.02 A#	385.55 B#
CVWS80147	15.89 A#	4.96 A#	1.76 A#	11.70 A#	726.42 A#	496.86 A#
BRS610	11.97B	4.92 A#	1.72 A#	9.47 B#	690.16 A#	346.75 B#
LSD	1.78	0.09	0.40	0.74	303.27	83.91
CV	14.48	2.34	29.03	7.97	51.04	24.38
Harvesting system (HS)	1.31ns	15.20**	5.94ns	1.07ns	1.13ns	6.42*
Without leaves	14.75 A#	4.90 A#	1.58 A#	11.17 A#	730.14 A#	447.12 A#
With leaves	14.57 A#	4.98 A#	1.77 A#	10.96 A#	684.26 A#	372.32B
LSD	0.36	0.04	0.18	0.50	105.72	72.22
CV	5.32	2.05	23.54	9.70	31.74	37.43
Harvesting time (HT)	38.51**	14.84**	5.36**	12.28**	15.09**	22.23**
100	11.93 C#	5.09 A#	1.34 B#	9.03 D#	450.08 B#	205.66 D#
105	13.63 B#	5.03 A#	1.59 B#	9.70 CD#	316.62 B#	303.91 CD#
110	14.77 B#	4.91 B#	1.48 B#	11.84 AB#	440.37 B#	342.21 CD#
180	16.28 A#	4.90 BC#	1.63 B#	11.52 AB#	1499.12 A#	439.98 BC#
135	16.68 A#	4.91 BC#	2.27 A#	13.19 A#	508.88 B#	555.10 AB#
160	14.67 B#	4.80 C#	1.74 AB#	11.12 BC#	1028.14 A#	611.45 A#
LSD	1.17	0.11	0.57	1.78	493.32	136.87
CV	8.13	2.29	35.00	16.47	71.08	34.04
$F_{Gx HS}$ Test	1.68ns	3.05ns	0.15ns	3.10ns	3.92ns	0.33ns
F_{GxHT} Test	2.98 **	1.73 ns	0.95 ns	0.51 ns	0.99ns	1.85ns
F_{HSxHT} Test	0.24 ns	1.16 ns	0.81 ns	0.71 ns	1.75ns	2.73*
$F_{GxHSxHT}$ Test	1.12 ns	1.57 ns	0.39 ns	1.45 ns	0.61ns	1.20ns
Sugarcane		(Mean obtained at same dates of sweet sorghum harvest)				
RB966928	21.18	5.25	0.99	17.80	505.38	244
RB855156	18.00	5.00	1.72	10.32	655.00	450

**Significant at 1 % probability ($p < 0.01$), *significant at 5% probability ($p<0.05$), and ns - not significant ($p>0.05$). # Mean following with same capital letter, in vertical orientation, inside fator analysed (genotypes or harvesting system or harvesting time), did not differ significantly.

RESULTS AND DISCUSSION

The results obtained for the chemico-technological characteristics of the sweet sorghum juice are shown in Table 1. It was found that the genotype BRS610 had the lowest Brix and total reducing sugars (TRS) in comparison to those of CVSW80007 and CVWS80147, which were approximately the same. This result was expected since BRS610 is classified as a forage type of high biomass productivity, which is generally associated with low sugar. It was observed that the genotypes CVSW80007 and CVWS80147 showed significant increase in Brix values and TRS up to 135 DAS (Table 1, Figures 1 and 2), followed by a significant reduction up to the final harvest at 160 DAS. The harvesting systems evaluated did not significantly influence the raw material quality, where as Ribeiro Filho et al. (2008) found differences verifying Brix values of 12.4 and 11.6 and TRS of 9.66% and 9.81% in stalks without and with leaves, respectively.

According to Schaffert (2012) in order to achieve economic and sustainable production of ethanol from sweet sorghum, it is necessary to obtain a minimum of 12.5% of TRS, which is used to establish the useful-period-of-industrialization (UPI). The results obtained in this study indicate that CVSW80007 and CVWS80147

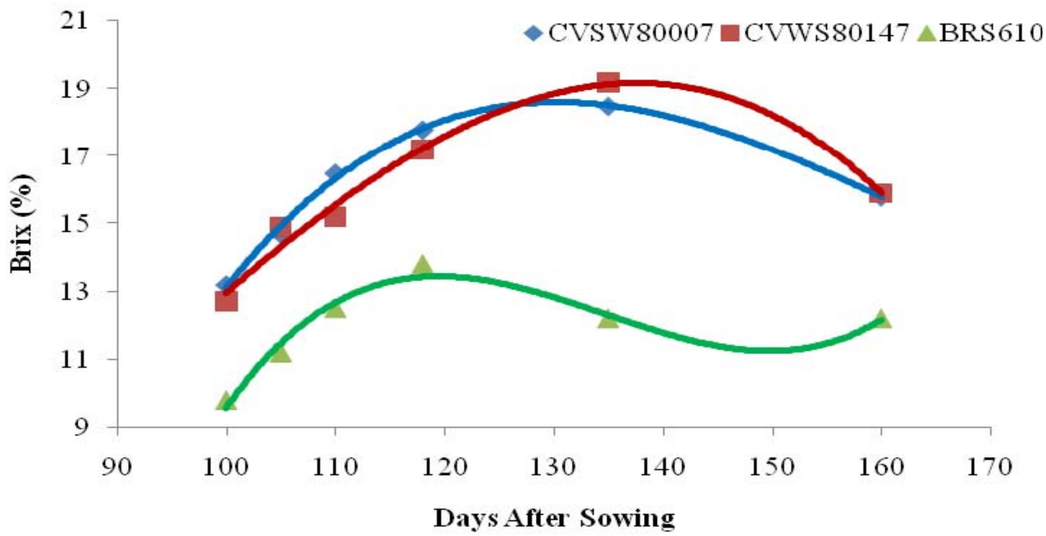

Figure 1. Polynomial regression of soluble solids (Brix) of the genotypes CVSW80007, CVWS80147 and BRS610 as a function of the harvesting time. Jaboticabal-São Paulo, State- Brazil, 2011/2012 harvest season.

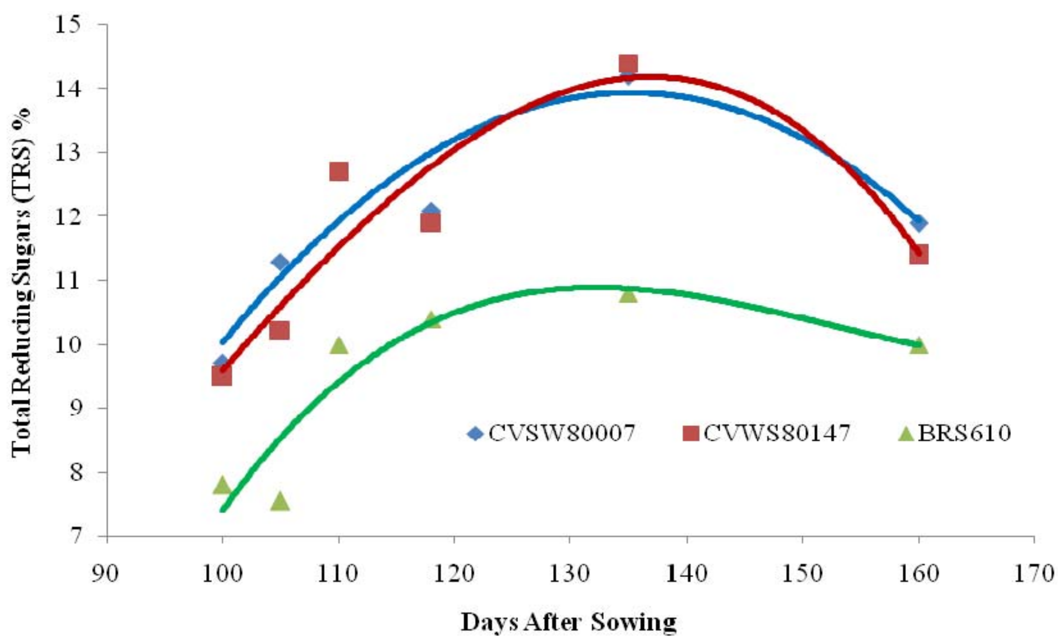

Figure 2. Polynomial regression of Total Reducing Sugars (TRS) for the genotypes CVSW80007, CVWS80147 and BRS610 as a function of the harvesting time. Jaboticabal- São Paulo, State- Brazil, 2011/2012 harvest season.

had a UPI of 40 days, which started around 110 days after sowing (Figure 2). The mean values of TRS obtained ranged from 11.7 to 12.0. These values are in accordance with those found by Teetor et al. (2011), who evaluated 24 different genotypes and found that only 10 had TRS levels between 10 and 11, and only 3 had TRS higher than 12. However, when the mean values of the genotypes of sorghum were compared with those of the sugarcane cultivars RB966928 and RB855156, harvested in April in Jaboticabal-SP, it was found that the Brix values of the juice extracted were lower than those found for the sugarcane cultivars (Table 1). Though the Brix were higher to sugarcane, it should be noted that this raw material was immature, once the acidity was higher than 0.8 g.L^{-1} H_2SO_4 (Ripoli and Ripoli, 2009). In this way, the sugarcane could stay in the field more time.

The levels of TRS in the sorghum juice were lower than those recommended by Amorim (2005), who considers values greater than 15% as optimal when sugarcane juice is used as raw material. The TRS values in the BRS610 genotype were significantly lower than those of CVSW80007 and CVWS80147. However, when compared to those of the sugarcane juice analyzed, it was found that the TRS levels in the RB855156 genotype was lower than the mentioned values, while for the RB966928, they were much higher. Considering the data gathered from the sugarcane producers in the state of São Paulo in the month of April during the 2010/2011, 2011/12, and 2012/2013 harvest seasons (UNIÃO DA INDÚSTRIA DE CANA-DE-AÇÚCAR (Unica), 2013), it can be seen that the TRS values in the juice obtained from the raw materials were in the range between 13.10 and 15.45, and the highest values were found at the end of the month. Thus, it appears that the sorghum materials evaluated (Figure 2) had better quality in the first half of April (135 DAS). For the parameter pH we observed there was no significant difference between the genotypes and the harvesting system (Table 1). The pH determined during the harvesting period from 110 to 135 DAS varied, but the mean values were significantly higher at 100 and 105 DAS. In a study on sorghum juice characterization, Ribeiro Filho et al. (2008) found pH values higher (5.31 for clean stalks and 5.46 for stalk with leaves) than those obtained in the present study. Considering the sugarcanes pH, it was observed values for the cultivar RB966928 higher than those of the sorghum genotypes (Table 1), which indicates it reached a mature stage of development. In general, the values obtained for the sorghum juice extracted are suitable for fermentation process, with optimal pH range for yeast growth from 4.5 to 6.5 (Amorim, 2005).The sorghum harvesting systems and genotypes did not influence the total acidity and phenolic compounds levels of the extracted juice (Table 1).

The mean levels of the phenolic compounds were significantly higher in the fourth (118 DAS) and the sixth period of time evaluated (160 DAS). This behavior may be due to the physiological response of the culture to the environmental conditions prevailing in these periods of time, namely low rainfall, low levels of water storage in the soil, and large water deficit combined with high average temperatures. Under these conditions, the sweet sorghum culture after reaching total metabolic activity started to allocate some photosynthates to produce defense biomolecules such as phenolic compounds (Taiz and Zeiger, 2004). According Dicko et al. (2006), sorghum has a high content of phenolic compounds, reaching up to 6% in some varieties, and the genetic characteristics and environmental conditions in which they are grown are determining factors for the production of these compounds by the plant. The phenolic compound values obtained for RB966928 and RB855156 were similar to those reported for the sorghum genotypes (Table 1). Taking into consideration the quality standard according to Amorim (2005), who recommends phenolic compounds levels in the juice below 500 ppm, it can be said that sorghum was suitable for industrial processing for production ethanol at 135 DAS.

Starch, a biomolecule characteristic of sorghum, is stored mainly in the panicle grains, but it can also be found in the stalk. According to Guiying et al. (2000), the level of starch in the plant depends on the stage of maturity and its genotype; the higher the stage of maturity, the larger the amount of starch stored in the grain. Nan et al. (1994) found contents of starch in the juice ranging from 300 to 9900 ppm and most of them were around 2000 ppm. The genotype CVWS80147 showed significantly higher levels of starch in the juice, about 25 to 30% more than that of CVSW80007 and BRS610, respectively, whose values did not differ significantly (Table 1). According to Ripoli and Ripoli (2009), these values are considered adequate. The cultivar RB855156 showed values similar to those, while the values of RB966928 were around 40% lower than those of BRS610, once it was immature.

The harvesting systems evaluated during the period of time studied indicated that the juice of the stalks without leaves and panicles showed the highest mean values of starch content at 105, 110, 118, and 160 DAS, and no significant differences between the two treatments were observed at 135 DAS (Figure 3). This behavior may be due to leaves, that contain high levels of moisture, and this water could dilute the phenolic compounds.It was found that the starch content increased significantly with time (Figure 4); Guiying et al. (2000) reported similar results, while Nan et al. (1994) found lower values with the growth and development of sorghum. Considering the behavior of the genotypes, starting at 118 DAS, it can be observed that CVSW80147 showed the highest starch content, followed by CVSW80007 and BRS610; the latter showed the lowest content, similar to those of RB855156.

We conclude that the genotypes CVSW80007 and CVWS80147 have the best chemico-technological characteristics. Harvesting the stalks with and without leaves does not influence the quality of the raw material, except for the starch content. In this way, the stalks harvested with leaves could be more economical to industry, and this leaves could be used to burn and generate power. The best technological quality of the raw material was found between 118 and 135 DAS, when

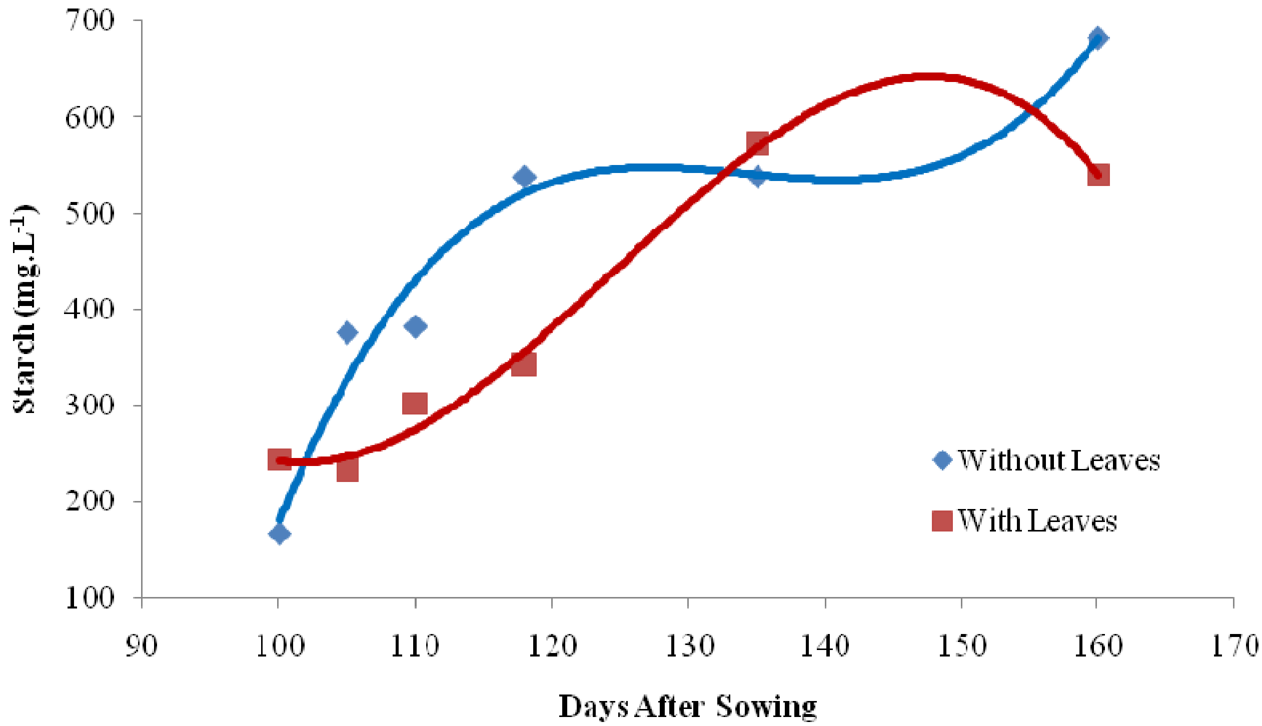

Figure 3. Polynomial regression of the starch content of the juice obtained in the harvest systems studied as a function of the harvesting time. Jaboticabal- São Paulo, State- Brazil, 2011/2012 harvest season.

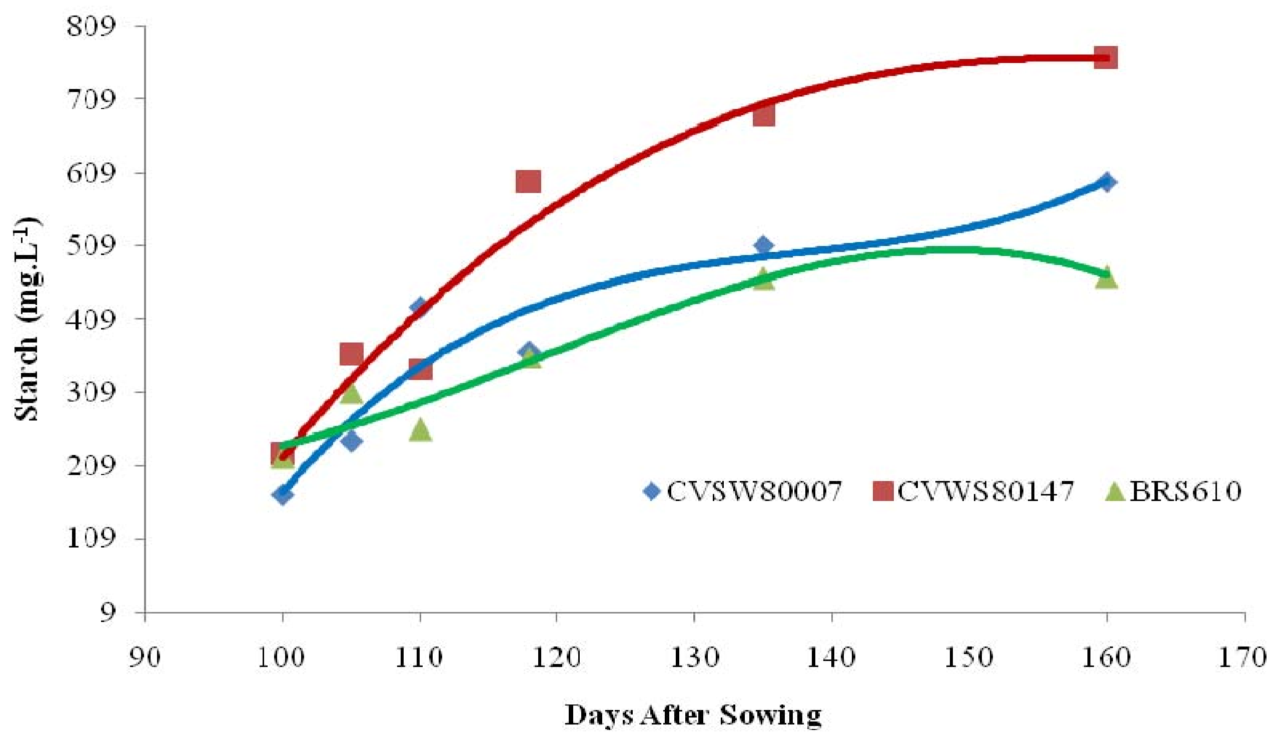

Figure 4. Polynomial regression of starch content of the juice in the genotypes CVSW80007, CVWS80147 and BRS610as a function of the harvesting time. Jaboticabal- São Paulo, State- Brazil, 2011/2012 harvest season.

these cultivars showed high levels of Brix and TRS, and low levels of Phenolic Compounds Total.

Conflict of Interest

The authors have not declared any conflict of interest.

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