Changes in the sugar content of sweet sorghum stems under natural conditions during winter in saline soil of the Yellow River Delta

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Abstract. In order to investigate the maximum storage period during their natural growth state, the sweet sorghum (Sorghum bicolor L. Moench) stems of four cultivars were analyzed to determine changes in contents of water, total sugars, main soluble sugars and the enzyme activity. From early November 2016 to late January 2017, the decrease in the total sugar content and the contents of sucrose, glucose and fructose slowed down, and the enzyme activities (sucrose synthase and sucrose phosphate synthase) involving sucrose metabolism in the stem remained stable. However, these indicators decreased significantly after the end of January 2017. Low temperatures and a dry environment were conducive to the storage of the sweet sorghum stems. During the winter (from early November 2016 to late January 2017) in northern China, the sweet sorghum plants can be stored naturally in the field via regulating sowing dates, which saves a lot of storage space and production costs for bioethanol company.

1. Introduction
Sweet sorghum (Sorghum bicolor L. Moench), which is a variant of sorghum with high biomass, high sugar content, salt tolerance, drought resistance and other biological characteristics compared with ordinary sorghum, can be used to produce sugar, wine and fuel ethanol as well as feed with a wide range of application values. Among annual herbaceous crops, sweet sorghum is regarded as one of the leading bioenergy crops because it contains large amounts of soluble sugar in its stalks along with other substances that can be used for alcoholic fermentation (Zegadalizarazu et al., 2012). As energy issues become increasingly prominent, efforts to produce clean, renewable biomass energy have significantly increased over the last decade (Iniyan and Sumathy, 2003). Sweet sorghum is an energy crop known as "the most powerful competitor in the bioenergy system". In a previous study, sweet sorghum stalks were found to be abundant in sugar. The use of sugar from sweet sorghum stalks to make fuel ethanol has broad application prospects (Ariyajaroenwong et al., 2012). Therefore, sorghum has been widely recommended for use as a bioenergy feedstock. China has a large number of barren saline lands, beaches and other marginal land resources. These types of marginal lands can be used to grow sweet sorghum to produce fuel ethanol, which is of great significance to address food security and energy shortages. Nearly 20 million hm² of saline land is not fully developed in the Yellow River...
Delta of northern China. These land resources have unique advantages to plant sweet sorghum to make up for these deficiencies.

However, sucrose, the main form of sugar in the sweet sorghum stems, can be converted into acids after harvest, leading to considerable sugar loss (Eiland et al., 1983). Various studies have confirmed that the sugar in these stems degrades easily. Therefore, the harvested stems must be quickly processed so that the sugar content is not affected. It is well known that processing a large volume of harvested sweet sorghum within a short time is very difficult. The crucial issue that needs to be resolved is how to harvest and store sweet sorghum stalks or juice in order to maximize its end-use quality as a feedstock for industrial fuel production (Zhao et al., 2012). Thus, it is necessary to find an appropriate way to store the sweet sorghum stems. During the storage period the storage method, the maximum storage time, changes in the weight of the stems and how to maximize the sugar content of the stems are crucial issues that affect the full use of the sugar, the business schedule processing times and the economic benefits obtained from sweet sorghum. Many researchers have studied the storage of sweet sorghum stalks, including methods of semi-horizontal stacking, keeping the whole bundle standing under natural air storage, wet storage, freezing storage, cold storage, antiseptic storage (Schmidt et al., 1997), tectorial storage with sulfur dioxide (Eckhoff et al., 1985), enzyme storage (Schmidt et al., 1997, Mei, 2008), and condensing stalk juice (Mei, 2008) to determine how to preserve the sugars in the sweet sorghum stalks. However, these methods have disadvantages that result in the loss of sugar (Eiland et al., 1983). In northern China in the winter, is it possible that sweet sorghum plants continue to grow in the field during the winter via suitable sowing time and cultivars?

In this study, we examined four different salt-tolerant sweet sorghum cultivars that grow in the Yellow River Delta. The objectives of the study were to determine sowing time, the water content of the stalks, the total sugar content, the changes in the main sugars and the changes in enzyme activities participating sucrose metabolism to investigate the maximum storage period during the natural growth state. Thus, finding an economical and suitable storage method of the sweet sorghum plants is of great significance for bioethanol company to harvest, store and ferment the stems to produce fuel ethanol.

2. Materials and methods

2.1. Experimental materials

A field study was conducted at the Dongying Experimental Station in the Yellow River Delta China. Four salt-tolerant sweet sorghum cultivars were used as the experimental materials, namely, Jitianza-early, Jitianza-late, Jincao10 and Jincao11. Different sowing dates have an important effect on the yield and quality of sweet sorghum. By adjusting the sowing period leading the varieties of sweet sorghum just to reach the maximum mature. The four sweet sorghum cultivars were planted in early July, and reached the maturity period of about 130 days, which was the best time for the utilization of sweet sorghum stalks because the plant height, fresh weight and sugar content reached the maximum (Table 1). The plants were grown in the saline soil of the Yellow River Delta, and each sweet sorghum cultivar was sampled approximately once every two weeks on November 13, 2016; November 25, 2016; December 9, 2016; December 23, 2016, and March 30, 2017 from November 2016 to March 2017. Five uniform and health plants of each cultivar were randomly sampled. The internodes were sampled by cutting on the third, seventh and eleventh node of the stalk for further analysis of physiological parameters.

| Sowing period | Plant height(m) | Fresh weight(g) | Brix(%) |
|---------------|----------------|----------------|---------|
| 2016.5.27     | 3.26           | 389            | 17.5    |
| 2016.6.28     | 3.45           | 406            | 18.9    |
| 2016.7.27     | 3.08           | 366            | 15.6    |
2.2. Experimental methods
Changes in local temperature, and the precipitation amount were monitored during the experimental period.

Determination of the water content: Fresh sweet sorghum stalks were collected to determine the fresh weight (FW), recorded as m1, and then placed in an oven at 105°C for 15 minutes and dried for 48 h at 70°C to a constant mass. Then, the dry weight (DW) was measured and recorded as m2, and then the water content was calculated:

\[
\text{Water content } \% = \frac{m_1 - m_2}{m_1} \times 100\%
\]

Determination of the sugar content in the stem: The sugar content of sweet sorghum stems from different growth periods was determined with a hand sugar meter (type of WYT-5 produced from Quanzhou ZhongYou Optical Instrument Co, China) according to (Audilakshmi et al., 2010).

Different sugars in the sweet sorghum stems: The sucrose and fructose were determined using the anthrone method (Halhoul and Kleinberg, 1972). Reducing sugars were determined by the 3,5-dinitrosalicylic acid method (Fan et al., 1996). The glucose content was determined by reducing the fructose with reducing sugar, represented as mg g-1DW.

Enzyme activities in sweet sorghum stalk: Resorcinol colorimetry was used to assay sucrose synthase (SS) and sucrose phosphate synthase (SPS) activities (Liu et al., 2014).

3. Results

3.1. Change in stalk water content of four sweet sorghum cultivars during the storage period
The stalk water content of the four sweet sorghum cultivars gradually decreased as the storage time. Two phase decrease in the water content in the stems during storage period was shown as Fig. 1. A slow phase decrease between early November and late December, the water content in the four cultivars of Jitianza-early, Jitianza-late, Jincao10 and Jincao11 decreased by 12%, 11.2%, 12.3% and 17.8%, respectively. A fast phase decrease during the period from late December 2016 to early March 2017, the water content in these four cultivars decreased by 50.1%, 48.3%, 43.6% and 56.3%, respectively.

![Figure 1](image1.png)  
**Figure 1.** Changes in stalk water content of four sweet sorghum cultivars during storage time.

![Figure 2](image2.png)  
**Figure 2.** Changes in stalk sugar content of four sweet sorghum cultivars during storage time. Data are means of 3 replicates (n=3) ± SD. The different letters indicated significant difference in sugar content between different dates of the same cultivar sweet sorghum at the level of P<0.05.
3.2. Change in stalk sugar content of four sweet sorghum cultivars during the storage period

The sugar content of sweet sorghum stems is expressed as Brix. In this study, the sugar content of the four cultivars was generally between 12% and 21%. Jincao10 had the highest sugar content of 21%, while Jincao11 had the lowest 12% (Fig. 2). Taking Jincao10 as an example, the sugar content in stems increased significantly (P<0.05) from early November to late November and early December to late December, from 17.5% to 18.6%, from 18.7% to 19.8%, respectively. Interestingly, the sugar content of the stems of the four cultivars did not significantly change during the storage period.

3.3. Change in stalk sucrose, glucose and fructose contents of four sweet sorghum cultivars during the storage period

In previous studies, sweet sorghum was typically rich in soluble sugars in its stems, and the main components were sucrose, glucose and fructose. All four sweet sorghum stems contained a larger proportion of sucrose and the sucrose content slightly declined as storage time, in particular after the 23rd of December (Fig.3). Notably, the sucrose content of Jincao10 was the highest among the four cultivars, and the lowest was that of Jincao11. The concentration of sucrose in Jitianza-early, Jitianza-late, Jincao10 and Jincao11 decreased by 13.1%, 14.2%, 11.1%, 15.2%, respectively. The glucose and fructose levels of Jincao10 were also higher than those of the other three varieties. Surprisingly, the contents of glucose and fructose exhibited increased significantly (P<0.05) from early November 2016 to late December 2016; the glucose content of Jitianza-early, Jitianza-late, Jincao10 and Jincao11 increased by 7.9%, 6.5%, 9.6%, 6.1%, respectively, and the fructose content increased by 3.6%, 6.9%, 3.2%, 7.8%, respectively. There were no significant (P<0.05) differences in either glucose or fructose during December. However, the contents of glucose and fructose significantly (P<0.05) decreased after the 23rd of December; the glucose content of Jitianza-early, Jitianza-late, Jincao10 and Jincao11 decreased by 32.2%, 36.7%, 37.4%, 30.8%, respectively, and the fructose content decreased by 37.6%, 36.9%, 29.4%, 34.3%, respectively (Fig.4 and Fig.5).

![Figure 3. Changes in stalk sucrose content of four sweet sorghum cultivars during storage time. Data are means of 3 replicates (n=3) ± SD. The different letters indicated significant difference in sucrose content between different dates of the same cultivar sweet sorghum at the level of P<0.05.](image)

![Figure 4. Changes in stalk glucose content of four sweet sorghum cultivars during storage time. Data are means of 3 replicates (n=3) ± SD. The different letters indicated significant difference in glucose content between different dates of the same cultivar sweet sorghum at the level of P<0.05.](image)
3.4. Changes of enzyme activities involving sucrose metabolism in the stems of the cultivars during different periods

The sucrose synthase (SS) and sucrose phosphate synthase (SPS) activities of the different cultivars of sweet sorghum stem differed, but they exhibited the same trend during the storage time. With the prolongation of storage time, the activity of SS and SPS decreased and the activity of the enzymes in the stem slightly decreased as the storage time from early November 2016 to late December 2016. However, the activities were reduced sharply after the late December 2016 (Fig. 6 and Fig. 7).

3.5. Changes in the local temperature and rainfall
The temperature and rainfall were recorded to examine their effects on the physiological parameters in stalks of four sweet sorghum cultivars during 5 months of the experiment. As shown in Fig. 8, the temperature changed greatly. From the beginning of November 2016 to the mid-November 2016, the temperature decreased from about 10 °C until below 0°C. The lowest temperature was observed in early January 2017, and then the temperature gradually rose to above 0°C from February 2017 until to about 10 °C at the end of experiment (March 2017). The rainfall also changed greatly. From the beginning of November 2016 to the January 2017, the rainfall amount significantly decreased and then gradually increased until to the end of experiment (Fig. 9).

4. Discussion
Because the local average temperature was above 0°C with some precipitation during the first period (from the early November to the late December), the plants respired vigorously. Therefore, the water content within the stem decreased faster during this time. In mid-November to late December 2016, the water content of the four sweet sorghum cultivar stems decreased slightly. This trend was relatively consistent because the minimum temperature dropped below 0°C during this period. Thus, the stems were frozen, resulting in a decrease in plant respiration, and the water content in the stems decreased slightly during this period.
From early November 2016 to late December 2016, the temperature gradually decreased. The stems lost water, resulting in a decrease in the water content and a concurrent increase in the sugar content. Between late December 2016 and early March 2017, the sugar content of the four varieties of sweet sorghum rapidly decreased, and most of the stems withered and changed conditions. The temperature gradually rose to above 0°C in this period, precipitation increased, and an increase in microbial activity led to the consumption of sugar and other compounds. Thus, the amount of sugar inside the stems dropped significantly. Therefore, we hypothesize that sucrose was converted into glucose and fructose during storage. By comparison, the contents of sucrose, glucose and fructose in the stems of the four cultivars decreased significantly from late December 2016 to early March 2017. At this time, the temperature increased, the frozen stems melted, and an increase in microbial activity caused the inside of the stems to roots. Fermentable sugars in sweet sorghum are mainly sucrose, glucose, and fructose (Deesuth et al., 2012). Increasing the juice yield or making proper use of sugars is crucial for realizing the high ethanol yield of sweet sorghum and is of important economical value (Wu et al., 2010). The sweet sorghum stems are well known to have a greater water content and high amounts of sugars that make them exceptionally vulnerable to microbial invasion, resulting in considerable sugar loss and deterioration that would affect the subsequent processing if the stems were stored improperly after harvest (Zhao et al., 2012). Low temperatures was considered to be the pivotal factor for the storage of the sweet sorghum stems after harvesting (Kamiyama et al., 2009). However, this storage process needs large room and cost. In the current study, a natural storage method was found to be suitable for the storage of sweet sorghum plants in saline soil of the Yellow River Delta because water content and main sugars of the stems were only slightly reduced during the four months storage period from the beginning of November 2016 to the end of February 2017. The main factors controlling stalk water content and sugar content are air temperature and the activity of sucrose synthase (SS) and sucrose phosphate synthase (SPS).

Sucrose synthase is known to play a role in sucrose synthesis using uridine diphosphate (UDP)-glucose and fructose as substrates, but the degradation reaction dominates in vivo (Geigenberger and Stitt, 1993, Botha and Black, 2000). A lack of correlation of SPS and sucrose accumulation had been reported earlier in sweet sorghum (Zhu et al., 1997), while a positive correlation between SPS activity and sucrose accumulation was reported in sugarcane stems, the evidence suggests that SPS may play a more important role in sucrose synthesis and accumulation (Botha and Black, 2000) and Botha & Black (2000) found that SPS activity is higher in mature internodes than in immature internodes. SS can also be involved in sucrose synthesis, but the equilibrium is usually in the direction of degradation. The correlation coefficient between temperature and the activity of the SS and the SPS, between the sucrose, glucose and fructose contents and the activity of the SS and the SPS (P<0.05), was analyzed to obtain the correlation coefficient (Table 2). The results demonstrated a positive correlation between the content of the three sugars in the stem and the activity of SS, and there was a significant positive correlation with the activity of SPS. As the temperature decreases, the activity of SS and SPS is reduced, but it is estimated that it will maintain a high activity under natural conditions from the beginning of November 2016 to the end of February 2017. Previous studies have shown that the enzyme activities involving sucrose metabolism maintain a certain activity at low temperatures (Guy et al., 1992).

**Table 2.** The correlation coefficient between the temperature and the activity of the SS and the SPS and between the sucrose, glucose and fructose contents and the activity of the SS and the SPS (P<0.05).

| Enzyme                  | Temperature | Sucrose | Glucose | Fructose |
|-------------------------|-------------|---------|---------|----------|
| Sucrose synthase        | 0.932       | 0.965   | 0.729   | 0.498    |
| Sucrose phosphate synthase | 0.918     | 0.983   | 0.854   | 0.706    |

Most of the literature on the relationship between sweet sorghum enzyme activity and glucose metabolism also demonstrated that the correlation between sucrose accumulation and enzyme activity...
of different varieties of sweet sorghum straw was not exactly the same (Lingle, 1987). The results of this study showed that the water content, total sugar content and main sugar content such as sucrose, glucose and fructose in the stalks of four sweet sorghum cultivars plants slightly decreased during the winter time from the early November 2016 to the March 2017, the lowest temperature was below 0°C, and the average temperature was near 0°C. This led to the freezing of the sweet sorghum stems, the decrease in the total sugar content and in the sucrose, glucose and fructose contents was minimized, and the enzyme activities in the stem were also stable. However, after the end of January 2017, the temperature gradually increased, resulting in the rapid loss of water in the stem. This resulted in a dramatic loss of sugar inside the stem, along with a significant reduction in the enzyme activities inside the stem. Thus, decay and metamorphism occurred in most of the stems.

5. Conclusion
Based on the results of this study, from early November 2016 to late January 2017, during the winter in northern China, if the temperature drops below 0°C, the sweet sorghum stems can be stored naturally in the field until the temperature rises above 0°C at the beginning of February. During this period, the sorghum can be harvested from the field at any time, thereby reducing storage space and production costs for bioethanol company.

Acknowledgements
This work was supported by the Major Projects of Science and Technology in Shandong Province (2015ZDJS03002) and Shandong Province Key Research and Development Plan (2016GNC113012).

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