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Effect of Different Sweet Sorghum Storage Conditions on Ethanol Production

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Abstract

Preservation of the sugar has been a major concern in the bioprocessing of sweet sorghum. The present study attempted to establish a simple and feasible storage method for sweet sorghum by testing different storage temperature with/without the additive of nitrogen (N_2). The effects of temperature and N_2 on the changes of the fermentable sugars during the sweet sorghum storage process were investigated. Three temperatures including Room Temperature (RT), 4°C, 20°C with/without N_2 were tested. The fermentable sugar content and the ethanol yield were used to evaluate the storage condition. The ANOVA shows that temperature is the more predominant factor in inhibiting the sucrose degradation compared to N_2 for a longer preservation. After 112 days' storage, 126.75 mg/g DW (Dry Weight) and 121.2 mg/g DW sucrose were obtained in the sweet sorghum which was stored at20 with/without N_2, respectively, much higher than those at RT and 4 with/without N_2. A similar trend was observed on the variation of glucose and fructose content in the sweet sorghum during the storage. The remarkable increase of glucose and fructose content was observed due to the rapid degradation of sucrose in sweet sorghum within the first two weeks. The ethanol production of 16.54 g/100g DW was achieved in the feedstock stored at -20°C for 112 days, corresponding to 85.4% of that from the fresh feedstock.

Keywords: Sweet sorghum; Storage; Glucose/fructose content; Sucrose Degradation; Fermentation; Bioethanol

Introduction

More emphasis has been given on the conversion of biomass to bioethanol because of the increasing demand for alternative fuels [1-3]. Sweet sorghum (*Sorghum bicolor* L.) is viewed as a very important feedstock for bioethanol production due to its high-yield, tolerance to abiotic stresses, and short growth season etc. Despite the great agronomic flexibility and productivity, some challenges should be overcome before sweet sorghum can be used as the feedstock for the bioethanol production. One of the primary challenges is how to store the fresh stalk or juice of sweet sorghum effectively in order to meet the needs for the feedstock supply chain in the bioethanol plant. After harvest, the sugars in the fresh stalk or juice can be deteriorated easily under the ordinary conditions. Therefore the storage condition is of significance for protecting the sugars within sweet sorghum. So far, the storage technologies of various kinds of crops including corn stove, sugarcane, grape pomace, sugar beet have been reported. The effects of some important factors, such as temperature, particle size, moisture content etc., on the production of ethanol have been studied [4-12].

Some investigations with variable results to reduce sugar losses on the storage of sweet sorghum have been tried. It was found in Eliand's study that chopped sweet sorghum lost 49% of its fermentable sugars after 1 week [13]. Schmidt used the enzyme-assisted ensiling with the additive of celluase to preserve sweet sorghum and the sugar loss of 28.6% was found after one month [14]. Although these methods are easy to handle, they are not effective to preserve the sugars in the sweet sorghum. Eiland et al. [15] and Eckhoff et al. [16] found that there was no sugar deterioration when SO_2 at 4000 μg g^{-1} or above was added in preserving chopped stalks of sweet sorghum for about three to four months. However, another study showed that sweet sorghum stalks stored in sealed containers with 2% (wet basis) SO_2 for 200 days, only 19% of the original sugar left [17]. These results indicate that SO_2 was favourable for remaining the sugars for a certain period of time. However, using large amounts of SO_2 would be not feasible since SO_2 is classified as toxic material. Therefore, just as stated as Walter and Monti [18] that the research on storage options of sweet sorghum is still in its infancy and more studies need to be carried out to make the sweet sorghum production chain operational and sustainable.

The present study tried to establish an effective and feasible storage solution for sweet sorghum by simply adjusting the storage temperature with/without N_2, a commonly used and easily achieved gas in the industry. The effect of temperature, storage time, with/without the additive of nitrogen during the storage of sweet sorghum on the sugar preservation and ethanol production were carried out. The results obtained are anticipated to provide the basic data for large scale storage on sweet sorghum.

Material and Methods

Raw material

Sweet sorghum was harvested by reaper in September, 2010 and the stalk (without leaves) of sweet sorghum was then cut with knife into 15-20 cm.

Storage of sweet sorghum and preparation of feedstock

The cut sweet sorghum was put into plastic bags with 300 g/bag. The plastic bag was then sealed with the Vacuum Packaging Machine (DZ400/ZLC, Beijing Zhongyige Automation Equipments Co., Ltd,

Beijing, China). Some of the bags were filled with N_2 (99.5% with H_2O ≤ 15ppm). The sealed bags were then stored at six different conditions as following: Room Temperature (RT) with/without N_2; 4 with/without N_2; -20 with/without N_2. Duplicates were run for each condition.

After being stored for 14 days, 28 days, 56 days, 84 days and 112 days, the sweet sorghum was taken out and milled by a grinder (FZ102, Tianjin Taisite Instruments Co., Ltd, Tianjin, China) and the milled sweet sorghum was used for the ethanol fermentation test.

Fermentation

Yeast activation: The fermentation was carried out with 20 g (Dry Weight, DW) milled feedstock from 2.2, 0.5% (W/DW) activated ADY (Angel Yeast Co., Ltd. Yichang City, Hubei Province, China), 0.1 g $(NH_4)_2SO_4$, 0.1 g $CaCl_2$, and supplementation of tap water which brought the total weight to 100 g. Nitrogen was filled and fermentation locks pre-filled with glycerol were mounted on the 100 ml fermentation bottle. The fermentation was performed at 32. The amount of ethanol produced was determined as weight loss caused by CO_2 release. All the fermentations were done in triplicate.

After fermentation, 10 times of the distilled water was added and then incubated at 80 for 1h. The supernatant was used to determine the sucrose, glucose and fructose.

Analysis methods

Dry matter content: Dry matter content was determined by the moisture analyzer, Mettler Toledo HR83. Duplicate experiments were run for each sample.

Chemical composition analysis: The sucrose, glucose, and fructose of stored/fresh sweet sorghum were determined by HPLC.

HPLC analysis: The amounts of sugar monomers were measured by HPLC (Agilent technologies, 1260 Infinity) using a Hi-Plex-Pb Column (Strong cation exchange resin consisting of sulfonated cross-linked styrene-divinyl benzene copolymer in the lead form) at 80°C and Millipore water as eluent at a flow rate of 0.6 ml min^{-1} with operation pressure at 12-13bar. A Refractive Index Detector (RID) was used.

Ethanol yield: The ethanol yield (Y_E) was calculated based on 100 g dry raw material.

$$Y_E = \frac{MCO_2 \times \frac{46}{44}}{20} \times 100 \qquad (1)$$

MCO_2: Mass of CO2 released during the fermentation process, g;

46/44: The conversion constant of CO_2 to ethanol;

20: Mass of feedstock used in the fermentation, g;

100: Mass of feedstock, g.

Statistical Analysis: All data were analyzed using Statistical Product and Service Solutions (SPSS, version 16.0). One-way Analysis of Variation (ANOVA) was carried out to compare the means of results at different runs. The significant F values were obtained, and Duncan's multiple range tests were applied to determine the significant differences among the different conditions.

Results and Discussion

Changes on sucrose content in sweet sorghum stored at different conditions

The changes on sucrose content in the storage process at different conditions were investigated and shown in Figure 1.

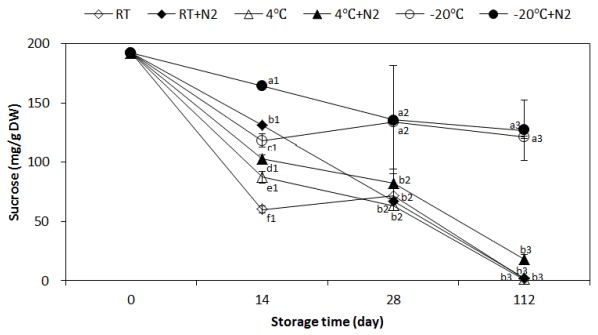

Figure 1: Changes on sucrose in sweet sorghum storage process: data points within a group (with the same Arabic numbers) followed by the same letter are not significantly different according to the Duncan's multiple range test.

The initial sucrose content in the fresh harvested sweet sorghum was 192 mg/g Dry Weight (DW). As the storage time was prolonged, the sucrose content reduced and the reduction was directly related to the condition employed. In the first 14 days, there was a drastic decrease found for all the storage conditions. The decrease was then weakened in the subsequent 14 days. Thereafter, the sucrose content dropped to a larger extent for the feedstock stored at Room Temperature (RT) and 4 with/without addition of N_2 (data not shown for 56 days and 84 days since the trend for them was consistent with the overall one). However, for the storage condition at -20, the sucrose content in the feedstock did not decrease dramatically. After 112 days storage, the sucrose content of 121.20-126.75 mg/g DW was observed in the feedstock stored at -20 with/without N_2, much higher than that from the other four conditions. The ANOVA analysis shows that there were significant differences ($p<0.01$) in terms of sucrose content among the six storage conditions in the first two weeks as shown in Figure 1. However, the difference was no longer significant among RT with/without N_2 and 4 with/without N_2 ($p>0.05$) when the storage time was extended to 112 days, neither between -20 with and without N_2. However, the significant difference was observed among -20 with/without N_2 and the other four conditions as far as the sucrose content was concerned. It is worthy to stress that the addition of N_2 was helpful in restraining the degradation of sucrose in the first 14 days. As the storage proceeded to 112 days, there was almost no difference in sucrose content between the storage with and without N_2 at a constant temperature. The reason might exist in that the metabolism of its own cells and microorganisms in sweet sorghum was still active which caused the relatively quick sucrose degradation in the first 14 days [19]. However, as the storage time was extended from 14 to 112 days anaerobic microorganism and endogenous enzyme became the main factors that degraded sucrose, and the relatively gentle changes of sucrose between the 14 to 28 days revealed the adaptation of some anaerobic microorganism [12,20].

Changes on glucose and fructose content in sweet sorghum stored at different conditions

Figure 2A and 2B present the changes on glucose and fructose content in the sweet sorghum stored at different conditions, respectively.

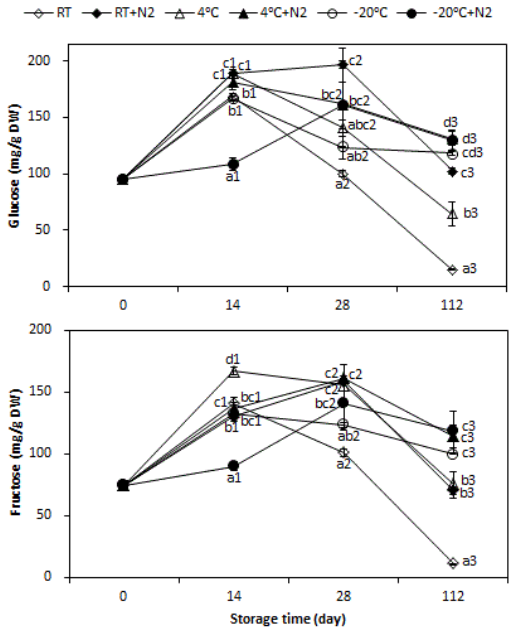

Figure 2: Changes on glucose and fructose in sweet sorghum storage process: data points within a group (with the same Arabic numbers) followed by the same letter are not significantly different according to the Duncan's multiple range test (A=glucose, B=fructose).

The trend for glucose and fructose content change in the storage process was related to the degradation of sucrose. In the first 14 days, the glucose and fructose content in the feedstock increased except for that in the feedstock stored at -20 with N2 which showed a minor decrease. As the storage was extended from 14 to 28 days, the glucose content in the feedstock stored at -20 with N2 showed a sharp increase, while it decreased for the other storage conditions. Compared with glucose content, fructose content reached its peak at 28 days when the N2 was introduced to the storage process. For the feedstock stored without N2, the fructose content achieved its maximum value at 14 days and then it began to decrease afterwards. After 28 days, both the glucose and fructose content in the feedstock stored at all the six conditions were showed a certain decrease. A decrease in glucose and fructose was found to be 90.9% and 92.3% respectively for the feedstock stored at RT when the storage time was increased from 14 days to 112 days. It was followed by the storage condition at 4 and the reduction of glucose and fructose content was 65.9% and 55%, respectively. The ANOVA analysis indicates that there was significant difference ($p>0.05$) in terms of glucose and fructose content between the storage condition with and without N2 at RT and 4. When the temperature went down to -20, the difference in glucose and fructose content between the storage with and without N2 was no longer significant. This indicates that the addition N2 was positive in retaining higher glucose/fructose content at a relative higher storage temperature (RT and 4). When the storage was carried out at a lower temperature such as -20 employed in the present study, there was no need to add N2 in the storage process which made the storage process more economically feasible.

Ethanol production from sweet sorghum stored at different conditions

The ethanol production potential was investigated on the feedstock stored at different conditions. Figure 3A-F present the ethanol production calculated based on Eq. (1).

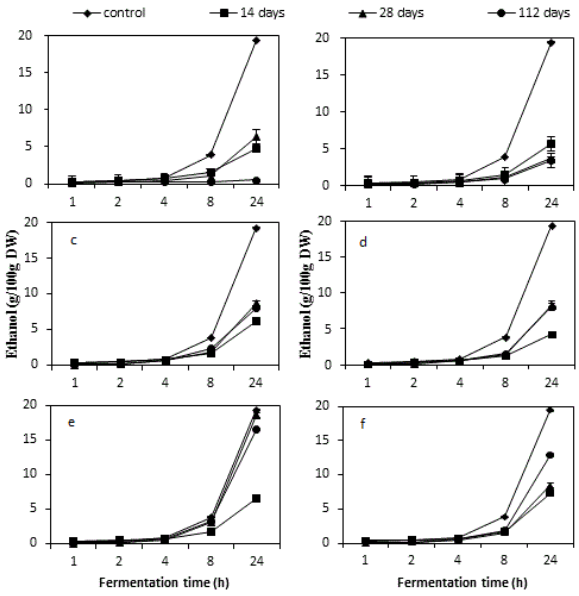

Figure 3: Ethanol production from sweet sorghum stored at six different conditions: A= RT, B= RT with N_2, C= 4, D= 4 with N_2, E= -20, F= -20 with N_2.

The ethanol production from the feedstock newly harvested was also tested and used as the reference. In the first 4 hours, there was almost no ethanol produced for all the feedstock including the reference. A small increase was then found on the ethanol production for all the feedstock when the fermentation time was prolonged to 8 hours. After 8 hours, the ethanol production increased sharply for the fresh feedstock and 19.4 g/100g DW was achieved at 24th hour. Meanwhile, the ethanol production of the feedstock stored at -20 with/without N_2 showed the same trend as the reference and 12.89 g/100g DW and 16.54 g/100g DW was obtained, respectively. Although the ethanol production was also accelerated after 8 hours for the feedstock stored at RT with/without N_2 and 4 with/without N_2, the ethanol production was much lower than the reference. This indicates that the storage of the sweet sorghum at a lower temperature was more helpful in ethanol production.

Conclusions

For storage of sweet sorghum for a short period (14 days), the addition of N_2 was helpful in inhibiting the sucrose degradation at a constant temperature. However, the temperature is the determinant for storing sweet sorghum for a long time in order to provide the

feedstock for the biorefinery plant. A lower temperature is satisfactory to conserve the sucrose even without N_2 in the storage process. The suitable temperature for sweet sorghum was -20°C and the total sugar remained as high as 93.7% of the original after 112 days' storage. The maximum ethanol production of 16.54 g/100g DW was obtained in the feedstock stored at -20°C for 112 days, corresponding to 85.4% of that from the fresh feedstock.

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