Production of Bioethanol from Sweet Potato Tubers with Different Storage Times

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To meet the demands for screening sweet potato tubers for bioethanol production, 12 genotypes of sweet potato tubers were collected from Henan, Shandong, Anhui, and Jiangsu Provinces, China. Based on the optimized determination method of the percent dry content, the nutritional composition and fermentation properties were studied. There were differences in the compositions and their correlations among the sweet potato varieties. The results showed that the starch content was weakly correlated with other ingredients, while the percent dry content and fermentable sugars contents had a close correlation with starch content. The percent dry content significantly and positively correlated with the flour and fermentable sugars contents. The percent dry content and starch contents had a significantly positive correlation, with a correlation coefficient that reached 0.96.

Keywords: Percent dry content; Lignocellulose; Bioethanol fermentation; Fermentable sugars

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INTRODUCTION

The limitation of fossil energy storage space and increasing concerns about CO_2 emissions have resulted in biomass energy becoming an alternative renewable fuel to gasoline (Malcata 2011; Khoo *et al.* 2013; Liu *et al.* 2013). Bioethanol is the ideal form of biomass energy. Production of bioethanol as a new and reproducible energy source to supplement fossil fuel energy has gained attention in many countries (Brennan and Owende 2010). Lignocellulosic biomass is the most abundant renewable feedstock for the industrial production of bioethanol (Olofsson *et al.* 2008). Because of the complex structure of biomass, pretreatment is necessary to make lignocellulose more susceptible to enzymatic attack (Park *et al.* 2010; Ruiz *et al.* 2012). Overdue sweet potato is not intended for use as a food source, and therefore it is considered a promising substrate for bioethanol fermentation. It has a higher starch yield per unit of cultivated land than lignocellulose, and fermentable sugars have been found to be closely correlated with starch content. (Srichuwong *et al.* 2009; Ziska *et al.* 2009; Lee *et al.* 2012; Duvernay *et al.* 2013).

Sweet potato has many advantages for growth on non-mainstream lands, such as drought resistance, saline-alkali tolerance, and the ability to grow on poor soils (Xu *et al.* 2003; Huang *et al.* 2006). The sweet potato starch content in its tubers is approximately 20% to 30% on a wet basis, which makes the tubers an ideal source of fermentable sugars for several applications (Zhang *et al.* 2010). In China, sweet potato tubers have been chosen as the main raw material for bioethanol fermentation, as the country produces approximately 85% of the total global output of sweet potato tubers (Li *et al.* 2009; Guo *et al.* 2014). Recently, there has been increasing interest in using sweet potato as a substitute for bioethanol production, rather than using sugar cane bagasse or corn grains.

Transformation of sweet potato tubers into chips or powder can be done to facilitate their transport and/or resource conservation (Ishiguro *et al.* 2003; Srichuwong *et al.* 2012).

The storage of sweet potato tubers causes many biochemical changes in the carbohydrate polymer fraction of the tubers (Şengül *et al.* 2004; Jusuf and Ginting 2014). The composition of the polymers in the sweet potato tubers dramatically affects the fermentation quality and processing traits. Generally, longer storage times of sweet potato tubers prior to processing results in loss of firmness (Dziedzoave *et al.* 2010). Significant variation in the starch digestibility has been observed among stored sweet potato tubers (Hansen *et al.* 2010). Zhang *et al.* (2002) studied the changes in the carbohydrate polymers, digestibility, α-amylase, and pasting properties of six stored sweet potato genotypes (*Ipomoea batatas* (L.) Lam) with differing percent dry contents. Their results showed that most of the genotypes exhibited a slight decrease in the percent dry content when they are stored.

In this study, based on the optimized determination method of the starch content and fermentation technology, the nutritional composition, including the moisture, starch, soluble sugars, and fermentable sugars contents, and fermentation properties were studied. Then, according to the correlation analysis between the quality traits and fermentation results, indicators for assessing the fermentation quality of sweet potato are presented.

EXPERIMENTAL

Materials

All of the varieties of sweet potato tubers (*Ipomoea batatas*) used in this study were obtained from different provinces in China. Twelve sweet potato genotypes, which were Ji 21, Ji 23, Shang 108, Yu 8, Yu 12, Luo 0402, Yu 7, Yan 24, Luoxu 8#, Xu 27, Shang 19, Wansu 31, and Wan 3, were used in this study. The sweet potato genotypes were grown in Henan, Shandong, Anhui, and Jiangsu Provinces, and were mainly grown in the middle or lower reaches of the Yellow River in China, where they can widely adapt. After harvesting, the sweet potatoes were stored at 15 °C to 20 °C and 80% to 90% relative humidity at the Academy of Agricultural and Forestry Sciences in Zhengzhou, China. The tubers were removed from storage after 1 month, 2 months, 3 months, 4 months, and 5 months for analysis. The sweet potato tubers were washed thoroughly, peeled, sliced into thin chips (5 cm × 0.5 cm × 0.5 cm), dried at 40 °C for 3 h in an oven, and then dried in a freeze-dryer. Samples were ground with a shredding machine (JYL-C020, Taisite Instrument Co., LTD, Tianjin, China), and then processed into 100-mesh flour for analysis.

Analytical Methods

Analysis of the percent dry content

Sweet potato tubers were grown and harvested under different growth conditions to obtain a large variation in the percent dry content. After harvest, the sweet potato tubers were stored at 15°C to 20 °C and 80% to 90% relative humidity until the day before analysis. The moisture content (MC) of the sweet potato slurry was determined for three samples by destructive grading using heating at 105 °C for 3 h in an oven, and then obtained for moisture content (MC) calculation. Using the MC of the sweet potato tubers, the percent dry matter content was evaluated using Eq. 1,

$$FC(\%) = 100 - MC(\%) \quad (1)$$

where *FC* is the percent dry matter content and *MC* is the moisture content of the sweet potato slurry.

Analysis of the starch content

According to the standard method of AACC 76.13.01, the soluble sugars were removed. The determination of the starch content was done using a Megazyme test kit (including thermostable α-amylase, glucoamylase, *etc*.; Wicklow, Ireland). In strict accordance with the procedures and methods in the Megazyme test kit, thermostable α-amylase and glucoamylase completed the hydrolysis of the sweet potato starch into glucose. However, Novozymes (Suzhou, China) commercial Liquozyme SC DS and Spirizyme Fuel were used instead of liquefaction enzymes, glucoamylase was used in the corresponding Megazyme total starch content assay kit, and the additive amount and reaction time were changed.

Analysis of the glucose content

The glucose was analyzed isocratically with a Rezex RCM column (5 μm, 300 mm × 7.8 mm; Phenomenex, Torrance, USA) at 80 °C using an RI detector, which was maintained at a constant temperature of 40 °C. Ultrapure water was used as the mobile phase with an injection volume of 20 μL and flow rate of 0.6 mL/min.

Analysis of the fermentable sugars content

The concentrations of the fermentable sugars glucose, fructose, and sucrose were determined by high performance liquid chromatography (HPLC). The HPLC analysis was performed on a liquid chromatograph (Welch Materials Inc., Concord, USA) equipped with a vacuum degasser, quaternary pump (G1311B), and RID connected to an ultimate XB-NH2 sugars column (250mm × 4.6mm × 5.0μm; Welch Materials Inc., Concord, USA). An RI detector was used, and was maintained at a constant temperature of 40 °C. The XB-NH2 sugars column temperature was 40 °C, and the mobile phase was ACN/ultrapure water (75/25, v/v) with an injection volume of 20 μL and flow rate of 1.0 mL/min.

Analysis of the bioethanol content

After fermentation, the contents of each fermentation broth were completely transferred to a 1-L volumetric flask. Samples were taken from the broth after homogeneous mixing, and then 2 mL were poured into a centrifuge tube and centrifuged for 5 min at a speed of 10000 rpm. The HPLC analysis of the fermentation products determined the bioethanol content in the supernatant aqueous phase with 0.45-μm membrane filtration.

An HPLC system is typically used to profile the carbohydrate polymers, bioethanol, and organic acid contents of fermentation liquors. A bioethanol fermentation standard was run on a 300-mm × 7.8-mm H^+ ion Rezek ROA column (FLM Scientific Instrument Co.Ltd, Guangzhou, China). To make this Rezek ROA column work effectively in this process, 20-μL filtered aliquots were injected while the HPLC was operating. The mobile phase was a dilute solution of H_2SO_4 (0.005 N) at a flow rate at 0.6 mL/min, with a column temperature of 65 °C. An RI detector was used, and was maintained at a constant temperature of 40 °C.

Bioethanol Fermentation

Microorganisms

Yeast strains were maintained on agar plates, which contained 20.0 g/L yeast extract, 20.0 g/L peptone, 20.0 g/L glucose, and 10.0 g/L agar. Multiplication of the culture was performed in a sterilized enrichment YPG medium (3.0 g/L yeast extract, 5.0 g/L peptone, 50 g/L glucose, 1.0 g/L KH_2PO_4, and 0.5 g/L $MgSO_4 \cdot 7H_2O$) at 38 °C and 200 rpm for 30 min.

Fermentation

After cleaning the fresh sweet potato tubers, the tubers were peeled and sliced into thin chips (5 cm × 0.5 cm × 0.5 cm). The materials were beaten with a juice grinder, and 100 g of the tubers pulp was placed into a 250-mL Erlenmeyer flask. A volume of water and liquefying enzyme, which was preheated to 60 °C, were added and the flask was placed in a rotary water bath shaker.

The flasks were incubated in a GYROMAX orbital shaker (Amerex Instruments Inc., Concord, USA) and agitated at 170 rpm. After the temperature reached 80 °C, the flask was transferred to a GYROMAX water bath cycle shaker (Amerex Instruments Inc.), agitated at 100 rpm at 86 °C, and then incubated for 90 min. After cooling the liquefied sample to room temperature, a saccharifying enzyme was added to the liquefying hydrolysate for vaccination.

One milliliter of liquid yeast was added after activation. Simultaneous saccharification and fermentation was performed in an Innova 40 constant shaker (New Brunswick Instruments, Inc., Edison, USA), which was agitated at 200 rpm and 30 °C. The quality of the bottles was recorded every 6 h.

During the process of fermentation, the bioethanol fermentation loss of mass yield was calculated from the quality of the bottles before and after fermentation with Eq. 2,

$$\text{Loss of mass} = \frac{m_1}{m_2} \times 100 \quad (2)$$

where m_1 and m_2 are the dry mass within the bottles before and after fermentation, respectively.

The bioethanol fermentation efficiency was calculated using the actual and theoretical bioethanol production yields with Eq. 3,

$$\text{Fermentation efficiency} = \frac{m_1}{m_2} \times 100 \quad (3)$$

where m_1 is the actual bioethanol production yield (ml) and m_2 is the theoretical bioethanol production yield calculated by the starch content in the sweet potato tubers (ml).

Statistical Analysis

All of the experiments were performed in triplicate and were statistically analysed using OriginLab 8.5 (Origin Lab Corporation, Hampton, USA) and SPSS for Windows (Version 16.0, SPSS Inc, Chicago, USA). The results were reported as their means with the standard deviation, and the significance p-levels were set at 0.05.

RESULTS AND DISCUSSION

Effect of the Storage Time on the Percent Dry Content

For the sweet potato tubers, the texture is of great importance for perception of the quality, and it is well-established that the percent dry content can be converted into bioethanol. However, the percent dry content mainly depends upon the maturity of the tubers and soil components (Lu *et al.* 2011; Chung *et al.* 2014). The sweet potato percent dry content refers to the percentage of dry matter in the sweet potato tubers. The percent dry content exhibits a downward trend for tubers with a high MC. It was proposed that the tubers keep a high MC during initial storage, and moisture is lost from the tubers as the storage time increases. Therefore, it was expected that the percent dry content has an upward trend over time. The analysis results of the percent dry content of the 12 genotypes is shown in Table 1. The percent dry content ranged between 19.7% and 33.4%. Considerable changes in the percent dry content with the various genotypes occurred, and the content was constrained within relatively large bounds. Luoxu 8# had the highest dry solids content and Xu 27 had the lowest percent dry content among the 12 genotypes. Therefore, it was verified that the percent dry content differs among genotypes. The analysis of variance showed that the differences among the sweet potato tubers had a 0.05% significance level. The results suggested that the percent dry content is influenced not only by the genotypes and growing environment, but also by the storage time.

Table 1. Changes about MC content with Different Storage Times

Genotype	Storage Time (month)				
	1	2	3	4	5
Ji 21	25.9 ± 0.13^{d}	26.7 ± 0.26^{c}	29.5 ± 0.34^{a}	29.9 ± 0.08^{a}	29.4 ± 0.02^{b}
Shang 103	32.2 ± 0.04^{a}	30.2 ± 0.01^{c}	30.9 ± 0.16^{b}	30.2 ± 0.16^{c}	30.1 ± 0.08^{c}
Ji 23	21.39 ±0.1^{e}	26.69 ± 0^{b}	27.6 ± 0.1^{a}	24.98 ± 0.1^{c}	24.06 ± 0.3^{d}
Luo 0402	27.8 ± 0.15^{c}	27.8 ± 0.13^{c}	29.2 ± 0.05^{a}	28.6 ± 0.02^{b}	29.2 ± 0.05^{a}
Yu 7	25.1 ± 0.07^{e}	29.1 ± 0.07^{d}	30.6 ± 0.08^{b}	30.8 ± 0.07^{a}	29.3 ± 0.07^{c}
Yan 24	20.6 ± 0.17^{c}	27.6 ± 0.06^{b}	27.3 ± 0.11^{b}	27.6 ± 0.14^{b}	29.5 ± 0.1^{a}
Luoxu 8#	32.4 ± 0.02^{c}	33.4 ± 0.07^{a}	32.7 ± 0.03^{b}	31.4 ± 0.05^{d}	33.3 ± 0.21^{a}
Yu 12	25.61 ± 0.1^{c}	25.34 ± 0.06^{c}	26.85 ± 0.12^{b}	24.41 ± 0.06^{d}	27.84 ± 0.45^{a}
Shang 19	26.1 ± 0.08^{d}	25.9 ± 0.08^{d}	28.2 ± 0.03^{b}	27.9 ± 0.07^{c}	28.5 ± 0.19^{a}
Wan 3	25.8 ± 0.08^{b}	25.8 ± 0.07^{b}	27.6 ± 0.06^{a}	22.5 ± 0.23^{e}	25.4 ± 0.05^{d}
Yu 8	24.46 ± 0.06^{e}	27.83 ± 0.06^{a}	25.06 ± 0.25^{c}	24.78 ± 0.06^{d}	26.53 ± 0.21^{b}
Wansu 31	28.1 ± 0.06^{c}	28.06 ± 0.12^{d}	30.73 ± 0.06^{a}	34.66 ± 0.26^{a}	28.52 ± 0.06^{b}
LSD(0.05)	4.0	4.2	2.9	3.6	2.8

LSD – least significant difference; Values represent the means and standard deviations, n = 3; Values in a column with different superscripts are significantly different ($p < 0.05$)

Effect of the Storage Time on the Starch Content

As starches are the most valuable components in sweet potato, changes in the starch content during storage time directly influences the development of the sweet potato industry. During bioethanol production, the starch content is an important characteristic that affects the fermentation efficiency (Ramasamy *et al.* 2014). A wide variation in the starch content in the sweet potato tubers was observed, with Wansu 31 containing the

highest starch content (70.23%) and Yu 8 containing the lowest (41.47%) after 5 months (Fig. 1). During storage, all of the genotypes showed slight decreases in the starch content, except for Shang 19, Yu 12, and Luo 0402, which demonstrated slight increases after the fifth month. The results showed that the highest starch content at the time of harvest decreased among the 12 genotypes after 5 months. Therefore, when screening genotypes for bioethanol fermentation, the stability of these properties in unprocessed sweet potato tubers over time should be taken into account. Fresh sweet potato tubers contain a lot of water and a variety of enzymes and microorganisms. The starch content and transformation processes of various enzymes highly correlate. Different tubers undergo different conversions of various chemical components during storage because of the activity of various enzymes. The results of this study indicated that the size and volume of the potato tubers significantly affected the starch content. However, there was no specific testing to determine the activity of starch synthases and amylolytic enzymes for each month.

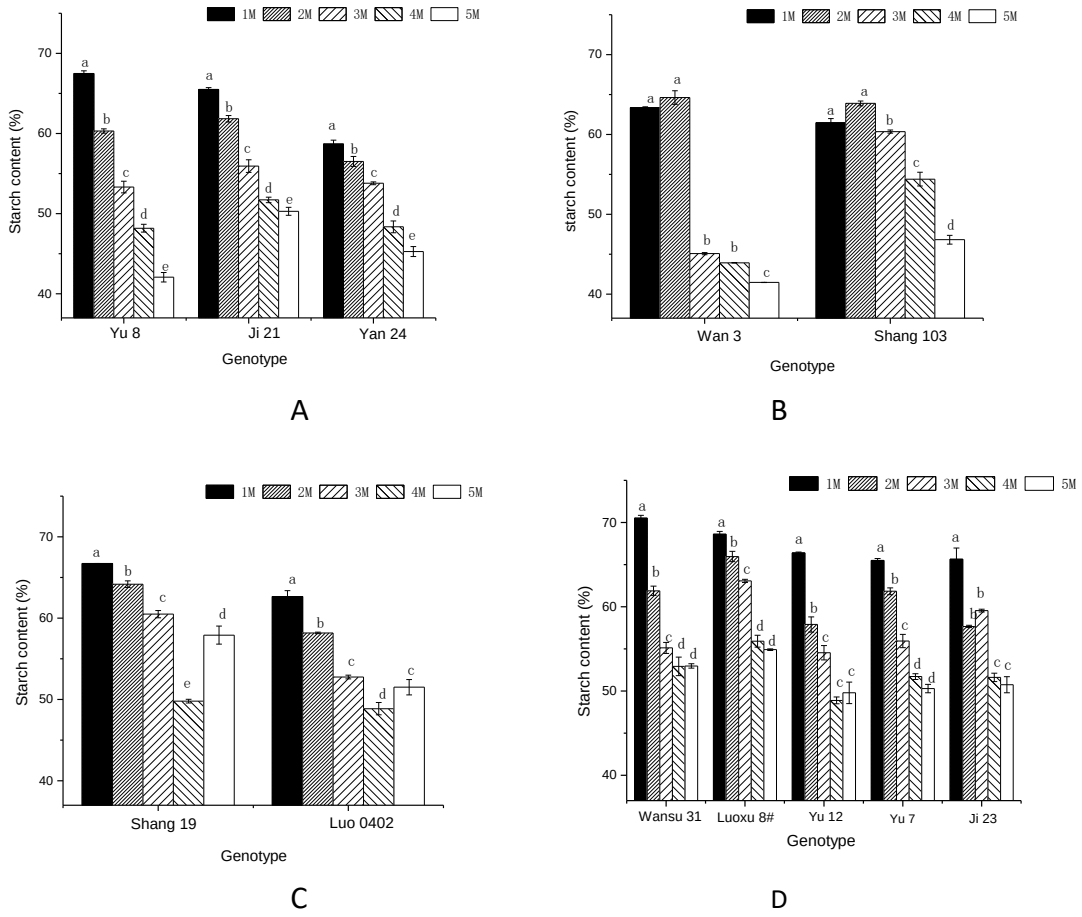

Fig. 1. Influence of the storage time on the starch content for different sweet potato genotypes: (A) Yu 8, Ji 21, and Yan 24, (B) Wan 3 and Shang 103, (C) Shang 19 and Luo 0402, and (D) Wansu 31, Luoxu 8#, Yu 12, Yu 7, and Ji 23; Values represent the means and standard deviations, n = 3; Values in a column with different superscripts are significantly different ($p < 0.05$)(M =months).

Correlation Analysis of the Percent Dry Content and Starch Contents

The main component in the sweet potato tubers is starch. Theoretically, the starch content at different storage times should vary with different percent dry contents. The

correlation analysis of the starch and percent dry contents over the entire storage duration is shown in Table 2. The results showed that the starch and percent dry contents had a highly significant correlation during storage, especially during the first month. However, this correlation weakened by the fifth month of storage. Over the course of storage, the percent dry content and starch contents had a significantly positive correlation, and the correlation coefficient reached 0.96.

Table 2. Correlation between the Starch and Percent Dry Contents for Different Storage Times

Storage Time (month)	1	2	3	4	5
R	0.961**	0.985**	0.893**	0.952**	0.836**

** indicates the value is significant for $p < 0.01$; * indicates the value is significant for $p < 0.05$

Effect of the Storage Time on the Fermentation Loss of Mass

Fermentation loss of mass is measured by monitoring the change in the weight of the fermentation flasks during the simultaneous saccharification and fermentation process. The reaction of the fermentation reflects the amount of CO_2 gas produced during fermentation. During the course of simultaneous saccharification and fermentation processes, the fermentation loss of mass and amount of bioethanol are closely related. Table 3 shows that different fermentation loss of mass values were measured for different storage times.

For all of the sweet potato tubers, the fermentation loss of mass ranged from 8.95 g/100 g of potato pulp to 13.06 g/100 g of potato pulp. The analysis of variance showed a significant difference between the different storage times for the same genotype. However, the different storage times did not significantly affect the fermentation loss of mass for Shang 103. The other genotypes had various degrees of significant differences, and different genotypes were very significant at the same storage times.

Table 3. Change in the Fermentation Loss of Mass in the Sweet Potato Tubers with Different Storage Times

	Storage Time (month)				
Genotype	1	2	3	4	5
Ji 21	10.43 ± 0.01^{c}	10.89 ± 0.04^{bc}	11.91 ± 0.13^{a}	11.93 ± 0.03^{ab}	11.72 ± 0.09^{ab}
Shang 103	13.06 ± 0.01^{a}	12.43 ± 0.03^{a}	12.39 ± 0.01^{a}	11.82 ± 0.08^{a}	12.2 ± 0.03^{a}
Luo 0402	11.08 ± 0.04^{b}	10.72 ± 0^{c}	11.29 ± 0.08^{a}	11.05 ± 0.09^{b}	11.23 ± 0.02^{ab}
Yu 7	9.55 ± 0.06^{e}	11.20 ± 0.02^{d}	12.02 ± 0^{b}	12.27 ± 0.06^{a}	11.78 ± 0.02^{c}
Shang 19	10 ± 0.03^{d}	10.09 ± 0.05^{d}	10.80 ± 0.04^{c}	10.97 ± 0.04^{b}	11.35 ± 0.11^{a}
Ji 23	8.95 ± 0.18^{e}	10.68 ± 0.04^{b}	11.35 ± 0.01^{a}	9.62 ± 0.04^{c}	9.4 ± 0.01^{b}
Yu 12	9.68 ± 0^{b}	9.79 ± 0.01^{b}	10.64 ± 0.09^{a}	9.96 ± 0.54^{b}	11.01 ± 0.04^{a}
Yu 8	9.39 ± 0.04^{d}	10.75 ± 0.04^{a}	9.67 ± 0.13^{c}	9.45 ± 0.08^{d}	10.22 ± 0.08^{b}
Wansu 31	10.74 ± 0.09^{cd}	10.6 ± 0.01^{d}	11.99 ± 0.04^{a}	11.01 ± 0.1^{b}	10.86 ± 0.05^{bc}

Values represent the means and standard deviations, n = 3; Values in a column with different superscripts are significantly different ($p < 0.05$)

Figure 2 shows the loss of mass in the flasks during bioethanol fermentation of Ji 21 for different storage times. The results reflected the bioethanol production output rate throughout the fermentation process. The results showed that the yeast content was not high because there was a slower propagation rate of yeast during the first 6 h, which meant the carbon dioxide production was lower; therefore, the loss of mass increased slowly. However, between 6 h and 24 h of bioethanol fermentation, the fermentation rate increased relatively quickly, which can be seen in the figure where the curves went almost straight up. The loss of mass then remained basically unchanged after 30 h. At the 0.05 significance level, the storage time had significant effects on the fermentation loss of mass.

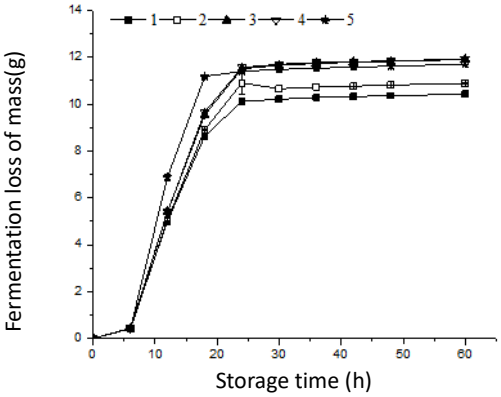

Fig. 2. Relationship of the fermentation loss of mass and storage time for Ji 21

Effect of the Storage Time on the Bioethanol Production

Bioethanol production is the ultimate expression of fermentation quality in sweet potato tubers. During storage and the fermentation process, there are many factors that lead to a change in the bioethanol yield from the different sweet potato genotypes with the storage time (Koga *et al.* 2013).

This study selected 12 different genotypes of sweet potato tubers and investigated the impacts of the storage time on the bioethanol fermentation from sweet potato tubers with different dry matter contents (Fig. 3). The bioethanol production ranged from 6.6 g/100 g of potato pulp to 13.43 g/100 g of potato pulp. Figure 3 shows that the sample with the highest bioethanol yield was Luoxu 8# and the sample with the lowest bioethanol yield was Yan 24 after 1 month.

For Ji 21, Yu 7, and Shang 19, Fig. 3A shows that the fermentation bioethanol yields progressively increased with longer storage times, and the bioethanol production reached maximum yields after the last month. However, the bioethanol production yields from Yu 12, Yu 8, and Yan 24 showed wavy curves that were not regular with the storage time (Fig. 3B).

Figure 3C shows that the bioethanol production of Shang 103, Luoxu 8#, and Wan 3 slowly decreased with the storage time. However, the bioethanol production of Shang 103 and Wan 3 exhibited slight increases after the last month. The fermentation bioethanol production was not regular for the other genotypes. The effect of this experiment was not remarkable over the whole storage time for Luo 0402. The bioethanol production yields of Wansu 31 and Ji 23 irregularly changed, and their maximum yields occurred after the second month of storage (Fig. 3D).

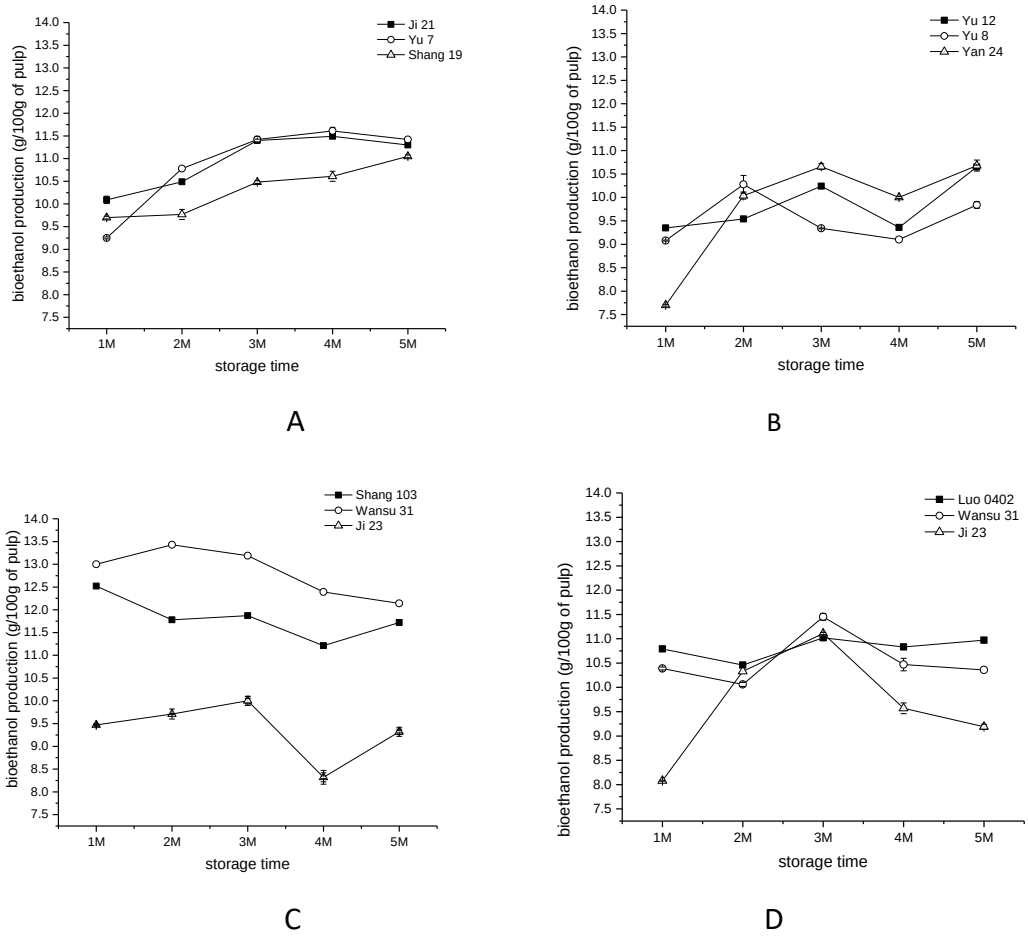

Fig. 3. Influence of the storage time on the bioethanol production for different sweet potato genotypes: (A) Ji 21, Yu 7, and Shang 19; (B) Yu 12, Yu 8, and Yan 24; (C) Shang 103, Luoxu 8#, and Wan 3; and (D) Luo 0402, Wansu 31, and Ji 23

The results of the analysis of variance showed that there were significant differences in the bioethanol production at the same storage time for the different sweet potato genotypes, and there were significant differences in the bioethanol production after different storage times for the same sweet potato genotype, except for Luo 0402. It was determined that the bioethanol production of sweet potato tubers was affected significantly by the storage time and genotype.

CONCLUSIONS

1. The results suggested that the percent dry content was readily influenced by not only the sweet potato genotype and growing environment, but also by the storage duration.
2. Over the entire storage duration, there was a significantly positive correlation between the percent dry content and starch content, and the correlation coefficient reached 0.96.
3. The analysis of variance revealed that there were significant differences in the bioethanol production with the same storage time and different sweet potato genotypes.

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