Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 98 (2006) 338–342
Food Chemistry
www.elsevier.com/locate/foodchem

Sugar metabolism and involvement of enzymes in sugarcane (*Saccharum officinarum* L.) stems during storage

Linchun Mao *, Fei Que, Guoze Wang

Department of Food Science and Nutrition, Zhejiang University, Hangzhou 310029, PR China

Received 10 January 2005; received in revised form 30 May 2005; accepted 30 May 2005

Abstract

Sugarcane (*Saccharum officinarum* L. cv. Badila) was harvested at the mature stage and stored at 2, 10, and 20 °C for 30, 90, and 120 days, respectively. Metabolic changes in the contents of sucrose and reducing sugar in relation to the activities of soluble acid invertase (SAI), neutral invertase (NI) and sucrose-phosphate synthase (SPS), in sugarcane juice, were studied. Extractable juice, sucrose and vitamin C declined significantly with increasing storage temperatures, while respiration rate increased. There was a rapid increase in titratable acidity during storage, with a more rapid rate at higher temperatures. A sharp increase in reducing sugar was observed within 20 days at 20 °C and 70 days at 10 °C, followed by a rapid decrease. Both SAI and NI activities showed a sharp increase within 15 days at 20 °C, followed by a rapid decrease, while a moderate increase occurred within 40–60 days at 10 °C. Slight increases were observed in SPS activity within 20 days at 20 °C and 50 days at 10 °C. Enzyme activities remained steady or underwent a small change in canes stored at 2 °C. Enzyme activities were significantly correlated with reducing sugar content.

Keywords: *Saccharum officinarum*; Sucrose; Soluble acid invertase; Neutral invertase; Sucrose-phosphate synthase

1. Introduction

"Badila" of the purple sugarcane variety is the most popular cultivar grown for fresh consumption as fruit in China. It produces abundant juice and less fibre and has good flavour compared to normal varieties for sugar production. High juice yield and sucrose content are the most important qualities, not only for sugar industry, but also for fresh consumption. Unfortunately, rapid decreases in juice and sucrose contents in harvested stems are always the inevitable problem during storage (Mao & Liu, 2000). Many studies have shown that soluble acid invertase (SAI), neutral invertase (NI), sucrose synthase (SS) and sucrose-phosphate synthase (SPS) are key regulators for sugar accumulation and degradation in sugarcane stem storage parenchyma (Bosch, Grof, & Botha, 2004; Gayler & Glasziou, 1972; Hatch & Glasziou, 1963; Lingle, 1997; Rose & Botha, 2000; Sacher, Hatch, & Glasziou, 1963; Sehtiya & Densay, 1991). Invertases cleave sucrose to glucose and fructose. SPS synthesizes sucrose-6-phosphate, which is dephosphorylated by sucrose-phosphate phosphatase to form sucrose. SS can either cleave sucrose to UDP-glucose and fructose or catalyze the reverse synthetic reaction. It is widely believed to act in the cleavage direction in vivo. Sucrose concentration in individual sugarcane internodes was correlated with a decrease in soluble acid invertase activity with maturation, and a difference between sucrose-phosphate synthase and soluble acid invertase activities (Zhu, Komor, & Moore, 1997). However, the biochemical basis for the regulation of sucrose level in sugarcane is still poorly understood and requires further investigation.

To date, there are few published reports regarding the physiological and chemical changes in sugarcane stem after harvest (Mao & Liu, 2000; Zeng, Chen, Zhang,

* Corresponding author. Fax: +86 571 86091584.
E-mail address: linchun@zju.edu.cn (L. Mao).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.05.076

& Lin, 1997). Since sugars and acidity are the main flavour components in sugarcane stem, and are important for maintaining high quality of fresh juice, we studied the changes in these quality parameters during storage at different temperatures. Activities of SAI, NI and SPS were also determined to evaluate their involvement in these metabolic changes in harvested sugarcane stems.

2. Materials and methods

2.1. Sampling

Mature stems of sugarcane, about nine months old, were harvested from a plantation in Hangzhou during November 2003. Stems were excised with rootstalk and leaves were removed. Five stems were bundled together and 50 bundles were stored at 2, 10, and 20 °C, respectively. Samples (5 stems each) were taken every 5 or 10 days. Stems were peeled and cut into three equal portions, according to the number of internodes. Middle portions were used for analysis. The experiment was performed in triplicate.

2.2. Extraction of juice

A self-made stainless steel crusher was used to extract juice. The juice was then filtered through a four-layer muslin cloth and chilled immediately at 0 °C.

2.3. Titratable acidity

Titratable acidity (TA) was determined by titrating 10 ml of juice with 0.1 N NaOH using phenolphthalein as an indicator. The result was expressed as percent of citric acid.

2.4. Sugar analyses

Reducing sugar was determined as described by Miller (1959), and sucrose was determined by the anthrone method (van Handel, 1963): 70 μl of reaction solution was added to 70 μl of 30% KOH, boiled for 10 min, and cooled to room temperature; 1 ml of freshly prepared anthrone reagent, containing 76 ml of H_2SO_4, 30 ml of H_2O, and 150 mg of anthrone, was added and the reaction was incubated at 37 °C for 20 min. A650 was measured immediately. Sugar concentrations were calculated as mg per ml of juice.

2.5. Vitamin C and respiration rate

The 2,6-dichlorophenolindophenol method was used to assay vitamin C content in juice by spectrofluorometer. A wavelength of 350–430 nm was used to determine total vitamin C content (AOAC, 1995).

Five sugarcane stems, each, were placed in a plastic container with an air flow at 500 ml min^{-1}. CO_2 concentration in the effluent air stream was analysed with an infrared gas analyzer (Mao, Ying, Xi, & Zhen, 1995).

2.6. Enzyme assays

SAI and NI in juice were determined according to Sehtiya and Densay (1991). SAI activity at 37 °C was assayed by adding 50 μl of juice to 50 μl of 1 M sodium acetate (pH 4.5). Enzyme reaction was started by the addition of 100 μl of 120 mM sucrose solution. Reaction was stopped at 60 min by adding 30 μl of 2.5 M Tris base and boiling the mixture for 3 min. NI activity assay was similar to that for SAI, except that the reaction was conducted at pH 7.5 and no Tris base was added. The concentration of glucose liberated was determined by the DNS method (Miller, 1959).

SPS assay was conducted at 37 °C in the direction of synthesis at pH 7.5 (Hubbard, Huber, & Pharr, 1989). Fifty microlitres of juice were added to 50 μl of assay solution containing 100 mM Hepes (pH 7.5), 20 mM glucose-6-P, 4 mM fructose-6-P, 3 mM UDP-glucose, 5 mM $MgCl_2$, and 1 mM EDTA. For the control, UDP-glucose was not added in the assay solution. Reactions were incubated at 37 °C for 0, 30, and 60 min and then stopped by boiling for 3 min. Sucrose produced by these reactions was assayed using the anthrone assay (van Handel, 1963).

3. Results and discussion

3.1. Changes in extractable juice and vitamin C

Maximum juice yield (69.92%) was obtained when stems were harvested. It decreased over the entire storage period, with a faster rate at higher temperatures (Fig. 1a). After 30 days of storage at 20 °C, juice yield decreased by 36.11%, and stems showed obvious wilting and thus severely lowered customer acceptance. Similar problems appeared in stems stored at 10 °C for 90 days, when juice decreased by 31.8%. However, juice in stems stored at 2 °C decreased slightly during storage. Vitamin C in sugarcane juice was as high as 4.75 mg l^{-1}. It was found to decrease rapidly during storage. Degradation of vitamin C was strongly accelerated by higher storage temperatures, where the concentration of vitamin C decreased by 64.6% at 2 °C for 120 days, 79.6% at 10 °C for 90 days, and 94.5% at 20 °C for 30 days (Fig. 1b).

Generally, fruits and vegetables show a gradual decrease in vitamin C content as storage temperature or duration increases (Adisa, 1986). Many harvesting and postharvest handling procedures influence the nutritional quality. Much of the available information is about vitamin C, which appears to be the most sensitive

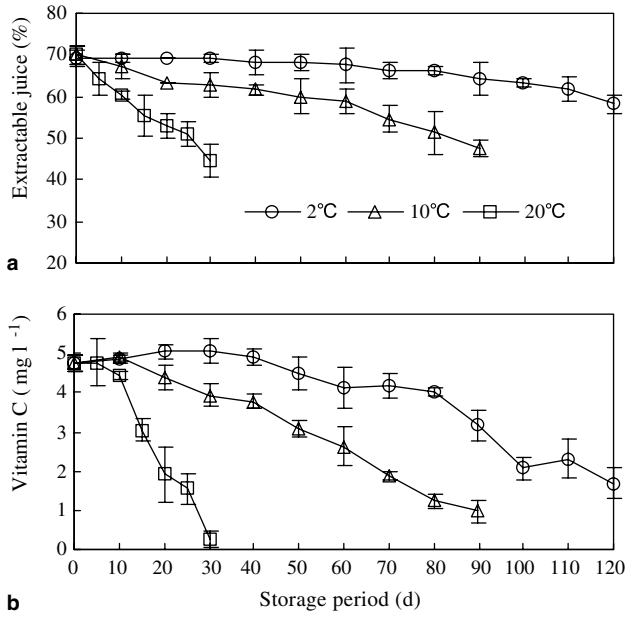

Fig. 1. Contents of extractable juice (a) and vitamin C (b) in sugarcane stems stored at 2 °C (○), 10 °C (△) and 20 °C (□). Data are the means from triplicate samples, each of five stems. Vertical bars represent S.E.

to postharvest losses (Lee & Kader, 2000). Oxidation can occur in the presence of catalysts and/or oxidase enzymes, causing loss of vitamin C, especially at high temperatures.

3.2. Changes in titratable acidity and sugars

At harvest, titratable acidity (TA) in sugarcane juice was 0.47 mg l^{-1}, and increased rapidly during storage with greater increases at higher storage temperatures (Fig. 2a). There was little change within 5 days at 20 °C, but this was followed by a very fast increase. A rapid increase was observed after 30 days at 10 °C. However, TA in stems stored at 2 °C remained stable and only a slight increase appeared after 80 days. Almost opposite to the pattern of TA, sucrose declined rapidly after 15 days at 20 °C and 30 days at 10 °C, respectively, while TA increased (Fig. 2b). Rate of decrease was much greater with increasing storage temperatures. However, in stems stored at 2 °C, a moderate decrease in sucrose content was observed after 60 days. On the other hand, a rapid increase in the content of reducing sugar was observed after 5 days at 20 °C and 30 days at 10 °C (Fig. 2c). Reducing sugar increased by 4.9-fold after 15 days at 20 °C and 3.3-fold after 70 days at 10 °C. After that time, reducing sugar declined significantly. However, reducing sugar in stems stored at 2 °C increased slightly over the entire storage time.

The increase in content of reducing sugar may be the result of sucrose hydrolysis, releasing glucose and fructose. After 15 days at 20 °C or 70 days at 10 °C more reducing sugars could have been depleted in the process

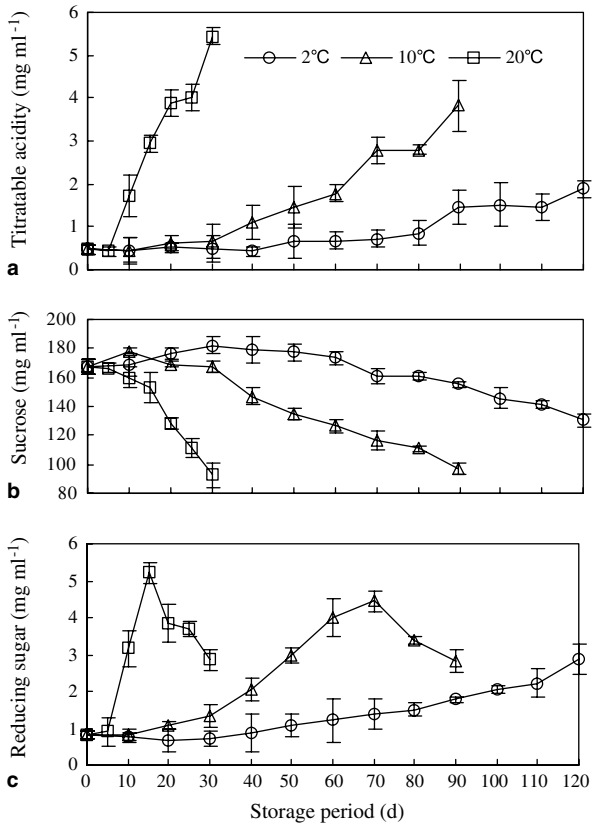

Fig. 2. Changes in titratable acidity (a), contents of sucrose (b) and reducing sugar (c) in sugarcane stems stored at 2 °C (○), 10 °C (△) and 20 °C (□). Data are the means from triplicate samples, each of five stems. Vertical bars represent S.E.

of respiration and other intermediates formation than was yielded from sucrose hydrolysis, resulting in the decline in content, which coincided with the occurrence of maximum respiration rate (Mao & Liu, 2000). Temperature had a significant influence on the metabolism of sugars. Storage of sugarcane internodes seems to coincide with a redirection of carbon from sucrose to reducing sugars and organic acids, and respiration. This is significantly influenced by temperature, with the highest rate at 20 °C and the lowest at 2 °C. An interesting result was observed in the significant negative relationship between sucrose and TA levels at 2 °C ($r = -0.92965$), 10 °C ($r = -0.96987$), and 20 °C ($r = -0.9441$).

3.3. Changes in respiration rate

Respiration rate in harvested sugarcane stem was 11.7–12.8 mg CO_2 kg^{-1} FW h^{-1}. It increased rapidly during storage at 20 °C, especially after 15 days. In stems stored at 10 °C, a rapid increase in respiration rate was also observed after 50 days. However, CO_2 production in stems stored at 2 °C remained stable with a slight decrease early within 50 days (Fig. 3). Generally, respiration rates in sugarcane stems were strongly influenced

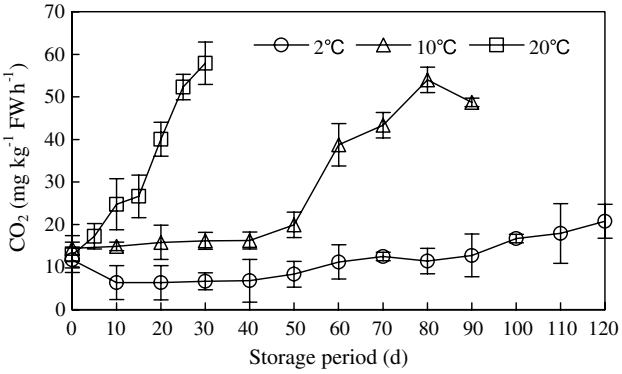

Fig. 3. Changes in respiration rate in sugarcane stems stored at 2 °C (○), 10 °C (△) and 20 °C (□). Data are the means from triplicate samples, each of five stems. Vertical bars represent S.E.

by temperature, with the highest rate at 20 °C and the lowest at 2 C.

3.4. Changes in activities of SAI, NI and SPS

Activity changes in SAI and NI exhibited a similar pattern after sugarcane stems were harvested, with higher activities at higher temperatures (Fig. 4a,b). In stems stored at 20 °C, activities of both invertases increased rapidly, reaching top levels after 15 days, as stems accumulated the highest level of reducing sugars (Fig. 2c). After that they declined rapidly with a sharp decrease in content of reducing sugar. In stems stored at 10 °C, there was a gradual increase in activities of SAI and NI over 60 days and 40 days, respectively, followed by a gradual decrease. However, SPS activity showed a gradual increase, although higher activities were found at higher temperatures (Fig. 4c). Harvested internodes had much higher activities of cleaving enzymes than that of SPS. When enzyme activity and sugar level were compared simultaneously, a significant overall relationship between activities of SAI, NI and reducing sugar levels did exist. However, no significant relationship was found between enzyme activity and sucrose content.

The final sucrose concentration should be correlated with the difference between rate of sucrose synthesis and sucrose hydrolysis. However, little sucrose could be synthesized in stems during storage because of the limitation of substrates when photosynthesis ceases. In fact, sucrose hydrolysis could be more active for increased hexoses used in respiration and other ripening processes during storage. It might, therefore, be concluded, that as stems ripen after harvest, respiration and other intermediates formation would be the primary cause of the sharp decline in sucrose because both invertases (SAI and NI) responsible for sucrose hydrolysis, and SPS responsible for sucrose synthesis, showed an increased trend. Respiration rates in harvested sugarcane increased as high as 62.3 mg

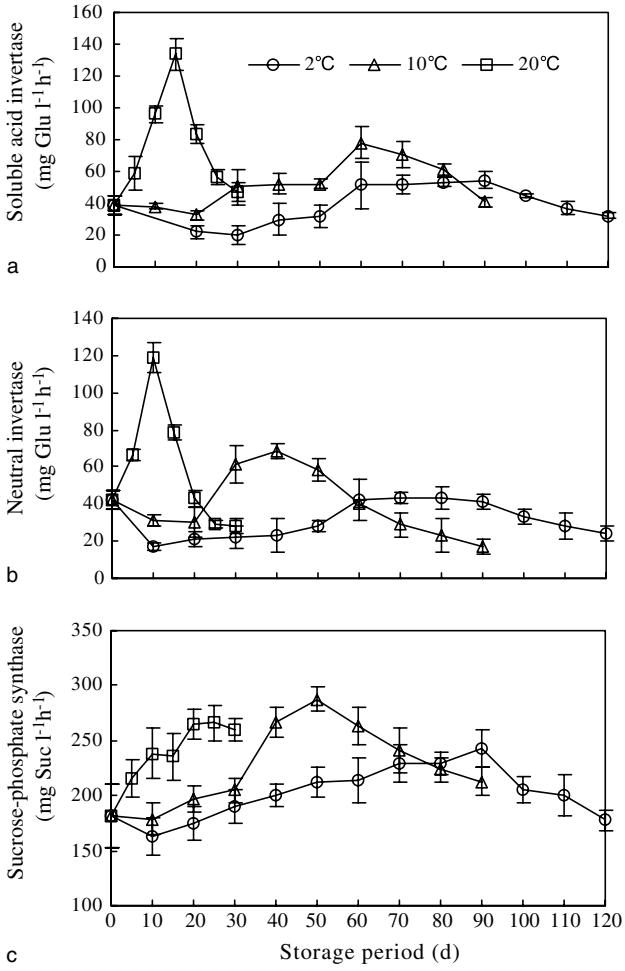

Fig. 4. Changes in soluble acid invertase (a), neutral invertase (b) and sucrose-phosphate synthase (c) in sugarcane stems stored at 2 °C (○), 10 °C (△) and 20 °C (□). Data are the means from triplicate samples each of five stems. Vertical bars represent S.E.

CO_2 kg^{-1} FW h^{-1} after 30 days at 20 °C and 54 mg CO_2 kg^{-1} FW h^{-1} after 80 days at 10 °C (Fig. 3). Whittaker and Botha (1997) summed ^{14}C recovered in CO_2, amino acids, organic acids, and lipids as an indicator of the total carbon partitioned into the respiratory pathway in sugarcane. They found that, of ^{14}C-glucose metabolized, 27, 19, and 15% entered respiration in internode nos. 2, 3, and 7, respectively.

Although the sucrose level in sugarcane decreased continuously during storage, SPS activity remained significantly greater than zero and showed a slight increase. Thus, low sucrose content was concurrent with significant sucrose synthesis activity. However, if little sucrose were unloaded into stems from assimilates produced during photosynthesis in leaves, this would cause the consumption of the entire stored sucrose pool in internodes. This is clearly the case in harvested sugarcane. Therefore, factors delaying sucrose hydrolysis, such as low storage temperature, should be the primary considerations during storage of sugarcane stems.

4. Conclusion

It is evident that harvested sugarcane stems possessed high SAI and NI activities and such a high degree of sucrose hydrolysis is known to provide hexoses for such oxidation processes as respiration. Storage of sugarcane stems coincided with an increase in the flux of hexoses from sucrose into organic acids, phosphorylated intermediates, and respiratory pathway, thus causing the inevitable decrease in sucrose and increase in reducing sugar and TA. Temperature had significant influences on metabolic changes of sugar and enzyme activities. Direct losses due to water loss and decay and indirect losses, such as sucrose hydrolysis and vitamin C degradation, limit the shelf-life of sugarcane stems. Temperature management is effective for maintaining the fresh quality of sugarcane stems.

Acknowledgements

The authors are grateful to the Department of Science and Technology of Zhejiang Province for providing funds to carry out this study.

References

Adisa, V. A. (1986). The influence of molds and some storage factors on the ascorbic acid content of orange and pineapple fruits. *Food Chemistry, 22*, 139–146.

AOAC. (1995). Vitamin C (total) in vitamin preparations. Official methods of analysis (Vol. 2, p. 967.22).

Bosch, S., Grof, C. P. L., & Botha, F. C. (2004). Expression of neutral invertase in sugarcane. *Plant Science, 166*, 1125–1133.

Gayler, K. R., & Glasziou, K. T. (1972). Physiological functions of acid and neutral invertases in growth and sugar storage in sugar cane. *Plant Physiology, 27*, 25–31.

Hatch, M. D., & Glasziou, K. T. (1963). Sugar accumulation cycle in sugar cane. II. Relationship of invertase activity to sugar content and growth rate in storage tissue of plants growth in controlled environments. *Plant Physiology, 38*, 344–348.

Hubbard, N. L., Huber, S. C., & Pharr, D. M. (1989). Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (Cucumis melo L.) fruits. *Plant Physiology, 91*, 1527–1534.

Lee, S. K., & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology, 20*, 207–220.

Lingle, S. E. (1997). Seasonal internode development and sugar metabolism in sugarcane. *Crop Science, 37*, 1222–1227.

Mao, L., & Liu, W. (2000). Study on postharvest physiological changes and storage techniques of sugarcane. *Scientia Agriculre Sinica, 33*, 41–45.

Mao, L., Ying, T., Xi, Y., & Zhen, Y. (1995). Respiration rate, ethylene production, and cellular leakage of fig fruit following vibrational stress. *HortScience, 30*, 145.

Miller, G. L. (1959). Use of dinitro salicylic acid reagent for determination of reducing sugar. *Annals of Chemistry, 31*, 426–428.

Rose, S., & Botha, F. C. (2000). Distribution patterns of neutral invertase and sugar content in sugarcane internodal tissues. *Plant Physiology and Biochemistry, 38*, 819–824.

Sacher, J. A., Hatch, M. D., & Glasziou, K. T. (1963). Sugar accumulation cycle in sugar cane. III. Physical and metabolic aspects of cycle in immature storage tissues. *Plant Physiology, 39*, 348–354.

Sehtiya, H. L., & Densay, J. P. S. (1991). Internodal invertase and stalk maturity in sugarcane. *Journal of Agricultural Science, 116*, 239–243.

van Handel, E. (1963). Direct microdetermination of sucrose. *Analytical Biochemistry, 22*, 280–283.

Whittaker, A., & Botha, F. C. (1997). Carbon partitioning during sucrose accumulation in sugarcane internodal tissue. *Plant Physiology, 115*, 1651–1659.

Zeng, X., Chen, R., Zhang, M., & Lin, D. (1997). Physiological bases of quality deterioration in sugarcane. *Journal of Fujian Agriculture University, 26*, 390–396.

Zhu, Y. J., Komor, E., & Moore, P. H. (1997). Sucrose accumulation in kthe sugarcane stem is regulated by the difference between the activities of soluble acid invertase and sucrose phosphate synthase. *Plant Physiology, 115*, 609–616.