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Effects of ethanol treatment on inhibiting fresh-cut sugarcane enzymatic browning and microbial growth

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ABSTRACT

The objective of this study was to investigate the effects of ethanol on the physicochemical properties and microbial quality of fresh-cut sugarcane. Ethanol (300 mL/L) treatment resulted in the lowest weight loss, electric conductivity, and browning and the best quality attributes in fresh-cut sugarcane. It also significantly reduced the activities of polyphenol oxidase, phenylalanine ammonia-lyase and peroxidase and decreased total phenols and quinines contents. The total bacterial count and yeast mold count showed that there was significant suppress of microbial spoilage in fresh-cut sugarcane with ethanol treatment. Ethanol treatment increased the CO_2 production and decreased O_2 level inside the package environment compared to treatments that combined ethanol (300 mL/L) and ascorbic acid (10 g/L) as well as the control (distilled water). Ethanol was the most efficient approach to inhibiting enzymatic browning and controlling microbial growth on fresh-cut sugarcane.

1. Introduction

Sugarcane (*Saccharum officinarum* L.) is one of the most important crops in the world, particularly in the tropical countries (Adelekan, 2013). In recent years, the demand for newly cut products, including fresh-cut sugarcane, has significantly increased in response to the advantages offered by such a product to consumers, such as novelty and convenience (Galus & Kadzińska, 2015). However, minimal processing for fresh produce, such as peeling, cutting and chopping, could cause the spoilage of the fruit tissues and initiate enzymatic reactions, resulting in cut surface discoloration, tissue softening, water loss, aroma and change in the flavors in addition to encouraging microbial growth (Xiao, Luo, Luo, & Wang, 2011). The maintenance of a high-quality level of sensory is the most important factor to the success of minimally processed fresh-cut produce (Artés, Gómez, & Artés-Hernández, 2007).

Color is an important quality imputation in the food industries for both aesthetic value and quality judgment that influences the choice and preference of the consumers. A significant consideration during fresh-cut fruit processing is to preserve the normal tissue color and control the discoloration or surface browning. Browning coloration has an influential effect on food handling and processing, affecting the appearance and quality (Manolopoulou & Varzakas, 2014). Because enzymatic browning usually limits the shelflife of fresh-cut sugarcane, so the prevention of enzymatic browning should be an important approach to extending for shelf life of fresh-cut sugarcane (Galindo, Sjöholm, Rasmusson, Widell, & Kaack, 2007). Enzymatic browning is generally related to a direct consequence of polyphenol oxidase (PPO) and peroxidase (POD) actions on polyphenols to form quinines (Queiroz, Mendes Lopes, Fialho, & Valente-Mesquita, 2008). Currently, there are many methods used to maintain the quality of the fresh-cut produce by inhibiting enzymatic browning and controlling spoilage and pathogen effects (Raybaudi-Massilia, Mosqueda-Melgar, Soliva-Fortuny, & Martín-Belloso, 2009), but they cannot meet the requirements of the fresh-cut produce industry.

Ethanol is a common food additive with the antimicrobial activity (Ji & Luo, 2008). In addition, it is considered as a Generally Recognized as Safe ("GRAS") substance (Dao & Dantigny, 2011); as a

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result it can be used in the food industry. It was used to effectively extend the shelflife of some fruits and vegetables, such as Chinese bayberry (Zhang et al., 2007), eggplant (Hu, Jiang, Tian, Liu, & Wang, 2010), mango (Gutiérrez-Martínez, Osuna-López, Calderón-Santoyo, Cruz-Hernández, & Bautista-Baños, 2012), white asparagus spear (Herppich, Huyskens-Keil, & Hassenberg, 2014) and lettuce (Yan, Yang, & Luo, 2015). There is a potential to use ethanol to maintain the quality of fresh-cut sugarcane and extend its shelflife during storage. The objective of this study was to evaluate the effects of the physicochemical properties and microbial quality of fresh-cut sugarcane as well as to investigate its ability to regulate its post-harvest shelflife during storage at 5 °C.

2. Materials and methods

2.1. Raw material

Commercially fresh sugarcanes (*Saccharum officinaum*.L.) were purchased from a market (Jing Zhou, Hubei, China) and used for the experiment without further storage. Fresh sample were selected, while damaged or diseased materials were thrown away.

2.2. Sample preparation

After washing with tap water, central part of fresh sugarcane was crosscut into discs that were 15 mm in diameter and 5 mm in thickness. Then, they were immersed into one of 3 different treatment solutions, 300 mL/L ethanol, 10 g/L ascorbic acid in 300 mL/L ethanol (combination) and distilled water (control) at the ambient temperature for 2 min (500 g sample/1000 mL solution). Then excess moisture was removed using a T65-388 spin-drier (Xiaoya Domestic Appliances Co. Ltd, Jinan, Shandong, China) with 100 rpm for 2 min. The samples were packaged into low density polyethylene zipper bags (medium size, 20 mm × 14 mm, Mingke Plastic Co. Ltd, Shanghai, China), and then stored in a refrigerator (FYL-YS-100LL, Beijing Fuyi Electric Appliance Co. Ltd, Beijing, China) at 5 °C for up to 28 days.

2.3. Weight loss

Samples of fresh sugarcane were weighed at the beginning and end of the storage on a digital balance. Weight loss was determined by the following equation.

$$\text{Weight loss} = (\boldsymbol{M_0}\text{-}\boldsymbol{M_1})/\boldsymbol{M_0} \times 100$$

Where $\boldsymbol{M_0}$ was the weight on Day 0, and the $\boldsymbol{M_1}$ was the mass on each sampling day.

2.4. Electrical conductivity (EC)

Relative conductivity was measured using a modified procedure that was developed by Chen et al. (2010). A disc sample (1.0 g) was placed in 50 mL of deionized water in a graduated test tube (50 mL volume), cut through with a glass stopper, and soaked for 30 min at a room temperature. The extract conductivity value (R_1) was assessed by a conductivity meter (Mettler-Toledo Instruments Shanghai Co. Ltd, Shanghai, China). The tubes were heated in a boiling-water bath for 15 min, cooled to room temperature and shaken; and then re-measured conductance extracts (R_2). The relative conductivity was calculated based on the following formula:

$$\text{Relative conductivity} = (\boldsymbol{R_1}/\boldsymbol{R_2}) \times \mathbf{100}\%$$

2.5. Texture analysis

Texture firmness of fresh-cut sugarcane discs was measured using a texture analyzer (TA-XT2, Stable Microsystems, UK) equipped with a cylindroid P6 probe (6 mm diameter) and a 1 kg load cell. Sugarcane disc samples were placed on the platform of the texture analyzer and measured with 10 mm/s pre-test speed, 5 mm/s test speed, 10 mm/s post-test speed, 4 mm/s rupture test distance, 5 mm distance and a 5 g trigger force strain at 25 °C.

2.6. Analysis of gas composition

The fractional pressures of O_2 and CO_2 in the packages were ascertained using an O_2 and CO_2 analyzer (PBI Dan sensor, Checkmate 9900, and Ringsted, Denmark). Without opening each bag, a gas sample was acquired by inserting a needle connected to the measuring apparatus through a rubber septum that adhered to the package.

2.7. Color measurements

The color values of the cut surfaces of sugarcane were measured using a colorimeter (CR-10, Minolta Co. Ltd, Tokyo, Japan) fitted with CIE illuminant C and 8 mm diameter measuring aperture, which was calibrated using a standard white plate ($L^* = 97.79$, $a^* = -0.38$, $b^* = 2.05$). The L*, a* and b* values were recorded.

2.8. Determination of the total phenolics and quinones

The quantification of the total soluble phenolic compounds was performed using the method proposed by Ke and Saltveit (1988) with slight changes. The sugarcane sample (3 g) was homogenized with 20 mL of methanol and then centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant was used to directly measure the browning potential of the fresh-cut sugarcane tissue. The absorbance at 320 nm represented the phenolic content, while the one at 437 nm was the quinone content.

2.9. Polyphenolic oxidase (PPO) activity

The PPO activity was extracted from the samples using a standard procedure developed by Du, Fu, and Wang (2009). Sugarcane sample was homogenized with pestle and mortar in extraction buffer (0.2 mol/L, pH 7.0) and sodium phosphate buffer, stored at 4 °C at the ratio of 2.0 mL: 1.0 g (buffer: sample) in an external ice bath for 3 min. The homogenates were centrifuged at 12,000 × g at 4 °C for 10 min. The supernatants were collected and their PPO activity was measured by assessing the increase in the absorbance at 410 nm for catechol at 25 °C using a UV-754N spectrophotometer (APL17 Inc, Shanghai, China). The reaction mixture contained 2.8 mL of substrate solution and 0.2 mL of enzyme extract. The substrate solution was 0.02 mol/L catechol dissolved in sodium phosphate buffer (0.2 mol/L, pH 7.0). The reference cuvette only contained the substrate solution. A unit of enzyme activity was defined as a change of 0.001 in the absorbance value per minute under the assay conditions.

2.10. Phenylalanine ammonia lyase (PAL) activity

The PAL was extracted according to the method described by