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Analyses of quality and metabolites levels of okra during postharvest senescence by ^{1}H-high resolution NMR

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ABSTRACT

Okra were stored for 19 days at 25 °C and nuclear magnetic resonnance spectroscopy was used to monitor the changes of metabolite levels. Seventeen metabolites were identified and their relative levels were analysed by principal component analysis. The first principal components were isoleucine, fatty acids, γ-aminobutyrate, glutamine, asparagine, unsaturated lipids, choline, phosphocholine and cinnamic acid. Decreases of glucose and sucrose levels were responsible for the quality reduction. Accumulation of cinnamic acid was involved in the lignification of okra tissue in the late storage period. Amino acids and γ-aminobutyrate levels increased during storage, which indicated the degradation of proteins. Increased electrolyte leakage and chlorophyll loss were also observed. The results indicated that NMR technique could be a good choice for metabolomic analysis of okra.

1. Introduction

Okra (*Abelmoschus esculentus* L.) is a monocotyledonous herbaceous plant of the *Malvaceae* family. It is commonly referred to “lady’s finger”, “bhindi” and “gumbo”. Okra is widely cultivated in tropical, subtropical and temperate regions. It has attracted much attention due to the health benefits and delicious taste. Okra polysaccharides possess immunomodulatory activities by inducing production of nitric oxide and secretions of tumor necrosis factor (TNF)-α, interferon (IFN)-γ and interleukin (IL)-10 (Chen et al., 2016). Okra peel and seed powder show good antidiabetic activities. Blood glucose levels are being reduced in diabetic rats, increasing body weight and maintaining lipid profile levels (Sabitha et al., 2011).

Okra deteriorates after harvest, mainly due to water loss, color fading and decay, resulting in loss of commercial value. Epidermal tissue senescence plays an important role in quality deterioration during storage as membrane damage acts as the key event leading to a cascade of biochemical reactions in tissue degradation (Lester and Bruton, 1986; Maalekuu et al., 2006). Few publications have published the quality changes in okra during postharvest senescence (Boonyaritthongchai et al., 2013; Huang et al., 2012), and they only introduced the biochemical changes.

Metabolomics has been utilized to gain insight into the chemical composition of biological materials (Oms-Oliu et al., 2013; Yang et al., 2013). The application of nuclear magnetic resonance (NMR) spectroscopy is a promising tool for rapid determination of metabolomic profiles (Yuan et al., 2016). NMR spectroscopy allows for the analysis of intra- and extra-cellular metabolites, providing valuable insights into biochemical processes in plants. The advantages of NMR test in metabolomics investigation are as follows: Few samples are required, no derivatization is required, with short analysis time, good reproducibility, and sufficient quantitative and structural information.

In this work, NMR was used to determine the change of metabolites during postharvest senescence. The results would be helpful to understand the natural chemical composition of okra and its quality change during postharvest senescence.

2. Materials and methods

2.1. Plant materials and treatments

Okra seed pods were harvested at the commercial maturity from a local farm in Guangzhou, China. The okra pods were harvested seven days after flower fall, and 15 kg of okra pods were selected for

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uniformity of size (10–15 cm). Those having visible wounds or decay were excluded. Fifty okra pods were packed in a polyethylene bag (0.03 mm of thickness) with three replicates. They were stored at 25 °C and 75% relative humidity for 19 days. Three replicates were sampled at 0, 3, 6 and 19 days, for determinations of weight loss, respiration rate, color, chlorophyll fluorescence, electrolyte leakage and metabolite profiles.

2.2. Determination of weight loss rate and respiration rate

Five okra seed pods at each storage time were selected for weight loss rate determination. The weight loss was determined by the following formula (Chen et al., 2011):

$$\text{The weight loss}=\frac{W1 - Wn}{W1} \times 100\%,$$

Where W_1 is the weight of sample at the first day and W_n the sample weight at n day (n = 0, 3, 6, 19).

Respiration rate was determined according to Huang and Jiang (2012). Five okra pods were chosen randomly, weighed and sealed into a box that connected the CO_2/H_2O Analyzer (LI-6262, LI COR, America) before the amount of CO_2 was recorded for 5 min. The pods were divided into five replicated groups to measure the respiration. Respiration rate was expressed as the rate of CO_2 production on a fresh weight basis (mg kg^{-1} s^{-1}).

2.3. Measurements of color and chlorophyll fluorescence

The color of five randomly selected pods was determined by using a Minolta Chroma Meter CR-400 (Minolta Camera Co. Ltd., Osaka, Japan). Each okra was measured at three equidistant points around the middle position of the surface. Color was recorded by using the L*, a* and b* values. L means lightness. a* indicates the colour between green and red, and b* indicates the colour between blue and yellow. Numerical values of L*, a* and b* were converted into the hue angle [$h^0 = \tan^{-1}(b^*/a^*)$].

Chlorophyll fluorescence of ten pods was determined with a portable chlorophyll fluorometer (FAM 2100, Walz, Germany). Pods were incubated in dark for 30 min prior to measurement. The photochemical efficiency was determined and expressed as the ratio of the maximum variable fluorescence to the maximum yield of fluorescence (*Fv*/*Fm*).

2.4. Determination of electrolyte leakage

The rate of electrolyte leakage was determined according to Jiang and Chen (1995). The pod peels were rinsed with distilled water three times before transferring them into a 25-mL beaker with 15 mL of distilled water and incubated at 25 °C for 30 min. The weight of beaker together with okra peels was recorded and the electrolyte conductivity (L0) was measured by a conductivity meter. The beaker was boiled at 100 °C for 30 min. When cooled down to room temperature, the electrolyte conductivity (L1) was measured. The rate of electrolyte leakage was calculated as L0/L1.

2.5. Metabolites analysis by NMR spectroscopy

Fifty milligrams of tissues were sampled from pods at different storage times and were extracted with 1.1 mL methanol-*d4* by vortexing for 1 min, followed by ultrasound-assisted extraction at 40 °C for 1 h. The extract was centrifuged at 15,000*g* for 10 min and the supernatant was collected and subjected to NMR analysis. The ^{1}H NMR spectra were recorded on a BRUKER AVANCE III 500 spectrometer (Bruker, Karlsruhe, Germany) at 500.13 MHz proton frequency, equipped with a Z-gradient system at 25 °C.

For signal assignment, 2D NMR spectra were acquired at 25 °C, including ^{1}H-^{1}H correlation spectroscopy (COSY), selective total correlationspectroscopy (1D-TOCSY), J-resolved spectroscopy (JRES), ^{1}H-^{13}C heteronuclearsingle quantum coherence spectroscopy (HSQC) and heteronuclear multiple bond correlation (HMBC). For COSY and 1D-TOCSY experiments, 128 transients were collected into 1024 data points for each of the 160 increments with a spectral width of 12.0 ppm for both dimensions. Phase insensitive mode with gradient selection was used for the COSY experiments and MLEV-17 was employed as the spin-lock scheme in the phase sensitive 1D-TOCSY experiment with a mixing time of 80 ms. For JRES spectra, 128 transients were collected into 4096 data points for each of the 80 increments with a spectral width of 6000 Hz in the acquisition and 60 Hz in the evolution dimensions. ^{1}H-^{13}C HSQC and HMBC experiments were recorded by using the gradient selected sequences with 512 transients and 2048 data points for each of the 128 increments. The spectral widths were set at 6000 Hz for ^{1}H, 20625 Hz for ^{13}C in HSQC experiments and 27500 Hz for ^{13}C HMBC experiments, respectively. The data were zero-filled to a 2000 × 2000 matrix with appropriate window functions prior to Fourier transformation.

2.6. Statistical analysis

The results are expressed as means ± standard deviations. Significant differences were tested by one-way analysis of variance. Statistical differences with p value < 0.05 were considered to be significant.

2.7. NMR data processing and multivariate data analysis

At each storage stage, three samples were selected for NMR analyses. The samples were named as below: 1#, 2# and 3# from okra pods at 0 day storage; 4#, 5# and 6# from 3 day storage; 7#, 8# and 9# from 6 day storage; 10#, 11# and 12# from 19 day storage. ^{1}H NMR spectra of okra extract was manually corrected for phase and baseline distortions, and referenced to MeOD-d_4 (3.31 ppm) by using software package TOPSPIN (v3.2, Bruker Biospin, Germany). Seventeen metabolites were identified by 1D and 2D NMR spectra. The relative contents of these compounds were defined by the peak area. A linear baseline scaling normalization approach was used. The baseline was constructed by calculating the median of each feature over all spectra. The scaling factor was computed for each spectrum as the ratio of the mean intensity of the baseline to the mean intensity of the spectrum. The intensities of all spectra were multiplied by their particular scaling factors. Principal component analyses were carried out to find the general trend and possible outliers. The results were visualized in the score plots in which each point represented an individual sample. The quality of models was evaluated by R^2X and Q^2 values, reflecting the explained variables and the model predictability.

3. Results and discussion

3.1. Changes of weight loss, electrolyte leakage, respiration rate, Fv/Fm and hue^o

The appearance of okra pods at four senescence stages are shown in Fig. 1. The appearance turns dark after long time storage (at 19th day). Weight loss increased during storage (Fig. 2). The weight losses of okra seed pods stored for 6 and 19 days were 1.42 and 2.6 times of that for 3 days, respectively.

Okra pod at day 0 had the highest respiration rate (Fig. 2B). However, the respiration rate of okra pod showed a significant decrease after storing for 3 days, and subsequently it increased markedly. The possible reason was that okra had instant response to wound stimulus at harvest.

Color and chlorophyll fluorescence are expressed as the hue angle (Alkarkhi et al., 2011) and *Fv*/*Fm* ratio, respectively. Chlorophyll fluorescence is an indirect measurement of the color in chlorophyll-