Postharvest Biology and Technology 145 (2018) 117–124

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Ethanol vapor is efficient for reduction of astringency compounds in cashew apple

Jaqueline Visioni Tezotto-Uliana*, Juliana Tauffer de Paula, Magda Andreia Tessmer, Ricardo Alfredo Kluge

São Paulo University, "Luiz de Queiroz" College of Agriculture, 13418-900, Piracacaba, SP, Brazil

ARTICLE INFO

Keywords:
Anacardium occidentale L.
Deastringency
Proantocyanidins
Postharvest
Pseudofruit

ABSTRACT

Cashew nut is known and appreciated worldwide, but the cashew apple has a low consumption, which is related to its high astringency. Condensed tannins or proanthocyanidins are the polyphenols responsible for this gustatory sensation. The use of ethanol as a deastringency agent is not the most used technique for astringency removal, but it has low cost, simplified application and high efficiency. This study aimed to verify whether ethanol vapor is able to penetrate cashew apples and reduce their astringency without causing fermentation, as well as to determine the best dose and exposure time of cashews to ethanol. Two experiments were conducted: in the first, the exposure time of the cashews to ethanol was 12 h and, in the second, it was 24 h. In both experiments, cashew apples early dwarf clone CCP-76 was used, the tested doses were 0.0 (control), 1.75, 3.50, 7.00, and 14.00 mL kg^{-1} of fruit, and the storage period was 16 d, in a cold chamber at 5 °C and 90% RH. Anatomical analyses showed the presence of secretory canals distributed in the parenchyma and stomata in the epidermis, indicating that the ethanol vapor was able to penetrate the cashew apples. Chromatographic analyses performed during treatment application showed that the ethanol vapor was absorbed by the pseudofruit and that the required doses were correct. The contents of ethanol, acetaldehyde, proanthocyanidins and total polyphenols of the cashew apple pulp were influenced by the dose, but not by the exposure time to ethanol, with 3.50 mL kg^{-1} being enough to reduce the tannins to the lowest levels. The two highest doses of ethanol resulted in cashew apple fermentation, skin depigmentation, which altered its color, and increased weight loss. Firmness and decay incidence were not altered. Thus, ethanol application at a dose of 3.5 mL kg^{-1} of fruit for 12 h of exposure time reduced the astringency compounds of the cashew apples without impairing their postharvest quality.

1. Introduction

Cashew (*Anacardium occidentale* L.) belongs to the Anacardiaceae family, is native to the Amazon region, and has Brazil as the main producer and consumer, of both the nut, which is the true fruit, and the peduncle, which is a pseudofruit adhered to the nut (FAO, 2016; Ramos et al., 1996). The cashew apple is considered a tropical pseudofruit, which is non-climacteric, succulent, aromatic and extremely perishable. Furthermore, it is a rich source of carbohydrates, minerals, vitamins, amino acids, phenolic compounds, organic acids and antioxidants (Assunção and Mercadante, 2003; Menezes and Alves, 1995; Talasila et al., 2012).

Although cashew nut is known and appreciated all over the world, the *in natura* cashew apple has a low consumption, which is related to its elevated astringency (Assunção and Mercadante, 2003). Astringency is a tactile sensation in which the normal lubrication of the mouth surface is impaired because of precipitation of the salivary proteins (Lyman and Green, 1990). In astringent fruit, this precipitation occurs due to the establishment of insoluble complexes between the proteins and the condensed tannins, present in the vacuoles of specialized cells in the pulp.

Condensed tannins or proanthocyanidins are polyphenols resulting from the polymerization of flavan-3-ols (catechins) and flavon-2,4-diol (leucoanthocyanidins) units (Dixon and Paiva, 1995). These compounds were identified in the pulp and skin of cashew apples, even when the pseudofruit was harvested completely ripe (Michodjehoun-Mestres et al., 2009; Queiroz et al., 2011a,b). The amount of condensed tannins reduces with the development of the fruit and differs among cultivars and clones. The clone CCP-76, when completely ripe, contains 0.1 and 0.5 g kg^{-1} in pulp and skin, respectively (Michodjehoun-

* Corresponding author.
E-mail address: jaqueline.tezotto@usp.br (J.V. Tezotto-Uliana).

https://doi.org/10.1016/j.postharvbio.2018.07.002
Received 14 January 2018; Received in revised form 22 June 2018; Accepted 5 July 2018

Mestres et al., 2009; Queiroz et al., 2011a,b), indicating the need for studies regarding artificial removal of cashew apple astringency.

Artificial deastringency is performed in the postharvest, forcing condensation or polymerization of the tannins with acetaldehyde molecules, as polymerized tannins are insoluble and unable to react with the proteins of the mouth saliva (Matsuo and Itoo, 1982). One of the ways of deastringency consists in the application of ethanol vapor, which must be absorbed by the fruit surface and, inside the cell, transformed into acetaldehyde by the enzyme alcohol dehydrogenase, naturally present in the cell. This enzyme also acts in the reverse reaction, transforming ethanol into acetaldehyde, once its function is to balance the concentration of these compounds (Edagi and Kluge, 2009; Oshida et al., 1996). Although the use of ethanol as a deastringency agent is not the most used technique worldwide, it is the most common in Brazil, given its low cost, simplicity of application and high efficiency (Antoniolli et al., 2000; Biasi and Gerhardt, 1992).

Several factors may interfere in the deastringency from ethanol, such as maturation stage, harvest season, dose, time and temperature of exposure to this deastringency agent (Taira et al., 1990; Vitti, 2009). Studies with other astringent fruit, especially persimmon, indicate that, when these factors are not employed correctly, there may be undesirable consequences, such as firmness loss, emergence of spots in the skin and alterations in flavor and odor (Edagi et al., 2009; Edagi and Kluge, 2009; Ittah, 1993; Pesis, 2005). Reports are lacking on deastringency resulting from ethanol application in cashew apples; nevertheless, preliminary studies have indicated a difficulty in ethanol absorption, occurrence of fermentation and skin discoloration of pseudofruit, which did not lose astringency (data not published).

Considering that, for astringency reduction, it is necessary to explore efficient and low-cost techniques, which may improve cashew apple shelf-life, this study had two goals: (a) to verify whether ethanol vapor can penetrate cashew apples and reduce their astringency without resulting in fermentation and (b) to determine the best dose and exposure time of the pseudofruit to ethanol. To address these issues, it was necessary to study the anatomical, physicochemical and biochemical aspects of cashews.

2. Material and methods

2.1. Plant material

The pseudofruit was obtained from a commercial property located in Arthur Nogueira, SP (22°34′23″ South, 47°10′21″ West and 588 m of altitude). Cashew apples early dwarf clone CCP-76 were harvested when their nuts were completely ripe and their pseudofruit presented 70–75 % of the skin with an orange-reddish color and the apical end of the peduncle was smaller than the basal end. This is a maturity point use for some growers. Selective harvest was performed manually and in the morning; he cashew apples were placed in cardboard boxes, without overlapping, and transported in a refrigerated vehicle to Piracicaba, SP. In the laboratory, the pseudofruit was subjected to selection and homogenization regarding color, size, absence of mechanical damage and visible pathogens. The selected cashew apples were sanitized with sodium hypochlorite (5 g L^{-1} of StartClor®, for 10 min), placed to dry on an absorbent paper, at room temperature, for 2 h, and then divided in groups of 60 cashew apples each (approximately 4.2 kg).

2.2. Treatments

For ethanol application, each group of cashew apples was packaged in a dark and hermetic 54.5 L plastic box of, using approximately one third of its volume. Two open Petri dishes, containing specific amounts of ethanol (99.9%), were placed in the bottom of each plastic box. A fan was fixed in the box lids, in order to force ethanol evaporation, as well as ventilation and homogenization of the air. Two experiments were conducted: in the first, exposure time of the cashew apples to ethanol was 12 h and in the second, 24 h. In both, the tested doses of ethanol were: 0.0 (control), 1.75, 3.50, 7.00 and 14.00 mL kg^{-1} of cashew apple, which corresponds to the following concentrations respectively: 0.00, 0.03, 0.06, 0.12 and 0.25 mL L^{-1}. During treatment applications, the boxes stayed at room condition (25 °C and 60% RH) and immediately after their opening, the cashew apples were stored at 5 °C and 90% RH, for 16 d. One hour after closing and one hour before opening of the boxes, gas samples were collected and injected in a gas chromatograph, in order to verify the ethanol concentration inside them. Analyses of cashew occurred on the day of harvest, before treatment application, and after it, every 4 d, with 12 h of simulated marketing at 15 °C and 70% RH. Each experimental design was completely randomized, in a 5 × 5 (doses x days of analyses) factorial design. In both experiments, four repetitions were used, composed of three cashew apples each. Spectrophotometric analyses were performed in duplicates.

2.3. Determinations

Anatomical analysis at the light microscope (LM) was performed in cross sections of the equatorial regions of the pseudofruit. The samples were fixed in the Karnovsky' solution (Karnovsky, 1965), subjected to a vacuum pump to remove air from the intercellular spaces and dehydrated in a growing ethyl series to 100% ethanol. The samples were infiltrated in Leica Historesin® (Heraeus Kulzer, Hanau, Germany) for the preparation of the blocks and sectioned in a rotating microtome (Leica RM 2045) at a 5–8 µm thickness. The sections arranged in histological slides were stained with 0.05% toluidine blue in phosphate and citric acid buffer at pH 4.5 (Sakai, 1973) for the usual histological analyses. Paradermal sections were also performed in fresh material and further test with Sudan IV was conducted to identify cuticle lipid (Pearse, 1968). The photomicrographs were captured in a Leica DM LB trinocular microscope (Leica Wetzlar, Germany) linked to the Leica DC 300 F video camera with LAS 4.0 software used for image analysis.

Fresh weight loss (WL) was determined by the difference, in %, between the initial weight of the repetition and the weight verified on each day of analysis, using a precision balance (Gehaka 8000). Decay incidence (DI) was performed visually, by scores, in relation to the area of the skin affected by a visible mycelium of the pathogen, with scores 0 = 0, 1 = 1–5, 2 = 6–25, 3 = 26–50 and 4 = > 50% of the skin infected. Skin color index (CI) was determined with a colorimeter (Konica Minolta, model CR-400), using L*a*b* system and calibration at D65. Eight reads were performed around each pseudofruit and an index was calculated from the following formula: CI = (100 x a)/(L x b), with values varying from 0.0 to 2.0, the lowest values corresponding to the greenest pseudofruit and the highest values corresponding to the reddest pseudofruit. This index may be successfully used for cashew apples since they present different colors (green, orange and red) throughout their length. Pulp firmness was determined using a benchtop digital penetrometer (Turoni Italy, model 53,205) with an 8 mm diameter tip, consisting of two reads in opposite sides in the equatorial region of the pseudofruit, after skin removal. The values were expressed in N.

Acetaldehyde and ethanol production was performed according to (Davis and Chace Jr., 1969) with the following modifications: 1 g of crushed pulp was sealed in a 12 mL glass bottle and frozen at -26 °C. At the moment of analysis, the bottles were placed in a water bath (Tecnal, TE-0541) at 50 °C, for 30 min and after this period, 0.5 mL of gas from the bottle's headspace was collected and injected in a gas chromatograph (Thermo, Trace GC Ultra model, with a flame ionization detector - FID - and Porapak N column), with the results expressed in g kg^{-1} of pulp.

Proanthocyanidin content of the pulp and skin (condensed tannins) was determined by the Vanillin-HCl method, according to (Nakamura et al., 2003). In test tubes, 1 mL of sample (extracted with methanol), 2.5 mL of a 1% vanillin solution in methanol and 2.5 mL of 9 N HCl in methanol were added. The mixture was incubated for 20 min at 30 °C