Postharvest Biology and Technology 85 (2013) 179–184

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Changes in the mesocarp of *Annona cherimola* Mill. 'Madeira' during postharvest ripening

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ARTICLE INFO

Article history:
Received 10 February 2013
Accepted 26 May 2013

Keywords:
Cherimoya
Chemical composition
Fruit ripening
Starch
Soluble sugars
Lipophilic extractives

ABSTRACT

Physicochemical changes during postharvest ripening of cherimoya (*Annona cherimola* Mill. 'Madeira'), were investigated to follow the principal modifications occurring during this process and to determine nutritional value. Fruit harvested at the mature green stage were analyzed during ripening using standard methods. Significant ($P < 0.05$) changes in chlorophyll, starch, titratable acidity, total free sugars and uronic acids were obtained, but no significant changes were found in ash, protein, lignin and lipid contents during ripening. The most obvious changes were chlorophyll degradation, an accentuated decrease of starch and an increase in total free sugars, with glucose the predominant sugar in the mesocarp, as revealed by GC analyses. Firmness loss was mainly attributed to depolymerization of pectin and lipid deterioration rather than hemicellulose degradation. Results also showed that the cherimoya variety evaluated in this study is a good source of minerals (mainly potassium), palmitic acid, linoleic acid, α-linolenic acid and sitosterol.

1. Introduction

The consumption of tropical and subtropical fruit is increasing worldwide (FAO, 2012), with consumer demand for new tastes. The cherimoya (*Annona cherimola* Mill.) is a soft subtropical fruit well adapted to the edaphoclimatic conditions of Madeira Island. The main cultivars of cherimoya produced in Madeira have been analyzed in a previous study (Caldeira et al., 1995), which conferred to 'Madeira' as superior in commercial and organoleptic characteristics, giving it a high potential for commercialization in national and international markets. Fruit are harvested when they have turned from pale green to yellow-green, the areas between the few small conical protuberances have filled out or when skin gives a little to touch. Thus, the tender skin and the short shelf-life, 5–7 days, makes the fruit vulnerable to physical injuries after harvesting, during handling, transport and marketing, restricting its commercialization. These facts imply that producers have to harvest before fruit ripening. To extend the postharvest life of this fruit, they can be stored either at low temperature, which usually leads to chilling injury below 10 °C (Alique et al., 1994), depending on the cultivar, or under controlled modified atmosphere (Alique and Oliveira, 1994). Although this is a common practice nowadays, it results in severe loss of fruit quality in relation to texture, taste and flavor (Pareek et al., 2011).

In general, fruit ripening is complex and the mechanisms by which fruit soften during this process are unclear and subject to speculation. During ripening some modifications in the chemical composition, either by enzymatic or non-enzymatic processes (Brummell, 2006), lead to remarkable changes in fragrance, flavor and a decrease in pulp firmness. Decrease in fruit firmness is due, at least in part, to the disassembly of the cell walls which are a complex intertwining network containing cellulose/hemicelluloses embedded in an amorphous gelatinous matrix formed mainly by pectins and stabilized by (glyco) proteins and phenolics. For many fruit species, postharvest ripening is accompanied by an increase in pectin solubility and loss of neutral sugars mainly galactose, arabinose and mannose which is related to depolymerization of pectins (Manrique and Lajolo, 2004). Although the modifications of cell wall polysaccharides seem to be widespread among several fruit species, variations in cell wall composition could lead to differences in the softening process depending on the species.

In cherimoya, some investigations have been undertaken on fruit quality parameters to ascertain the changes taking place during postharvest ripening (Martinez et al., 1993; Gutiérrez et al., 2005; Goñi et al., 2007). However, as the chemical composition depends on the cultivar, environmental conditions and also on the ripe stage of the fruit, the present study aimed to evaluate the main chemical changes that occurs on the mesocarp of cherimoya 'Madeira' which are essentially related to fruit quality. This

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http://dx.doi.org/10.1016/j.postharvbio.2013.05.014

knowledge is important to understand the nutritional value and commercialization potential of this fruit and may help to interfere with the process of ripening in order to extend postharvest life.

2. Materials and methods

2.1. Sample preparation and physical parameters

Cherimoya (*A. cherimola* Mill. 'Madeira') fruit were harvested (early January) from trees in a commercial orchard in Faial (Madeira Island, Portugal). Fruit at mature green stage with no evidence of physical or pathological injuries were selected. Each fruit was carefully washed with sodium hypochlorite (2%) to remove potential contaminants. Fruit were weighed at the beginning of the experiment and at the end of each storage period using a digital balance. The difference between initial and final fruit weight was considered as weight loss during that storage period and was expressed in percentage. The density of each cherimoya was determined by the ratio of weight and water volume displaced after fruit immersion into a measuring cylinder. Afterwards, lots of five fruit were randomly selected and stored at room temperature (20–22 °C) in a dimly-lit place. Every day after harvest, fruit were randomly chosen for physicochemical analyses. Fruit firmness was determined after removing the skin on two opposite sides at the middle of each fruit using a pressure-testing instrument (Model FT 327) fitted with an 11.3 mm cylindrical plunger. The force required to penetrate into the flesh was expressed in N. Penetration was carefully performed to avoid nearby seeds. Immediately, fruit were peeled (green peel was fully discarded), sliced, quick-frozen in liquid nitrogen and stored at −80 °C. From each sample a fresh slice was used to measure fruit water content using a humidity balance (Gibertini-Eurotherm) at 105 °C. Frozen mesocarp samples were lyophilized, milled and stored in the dark under vacuum for further analyses. In storage the average humidity was approximately 5%.

2.2. Chemical analyses

To determine chlorophyll content, 1 g of sample and 5 mL of acetone at 80% (v/v) were homogenized with a vortex and submitted to ultrasound extraction for 5 min at 25 °C. Samples were then centrifuged (Biofuge Stratos, Heraeus) at 1600 rpm for 5 min. The supernatant was placed in a quartz cell and its absorbance was measured by an ultraviolet-visible spectrometer (UV-2401, Shimadzu), at 663 nm (chlorophyll *a*) and at 646 nm (chlorophyll *b*) using 80% acetone as blank solution. Chlorophyll analysis was performed in duplicate in a dimly light room and its content was determined according to the equation of Lichtenthaler (1987). Unless otherwise stated, chemical analyses were performed according to AOAC (2000). Ash content was determined by complete incineration of 1 g of mesocarp sample in a Nabertherm furnace at 600 °C for 6 h. Crude protein content was calculated by converting the nitrogen content ($N \times 6.4$), determined by the Kjeldahl method in a Kjeldahl Selecta Alcodest still. The lignin content was determined using the Klason method (T 204 om-88). Titratable acidity was determined twice and the results expressed as citric acid equivalents. The content of uronic acids was obtained based on the method of *m*-phenylphenol with the galacturonic acid as standard. The iodine colorimetric method was used to measure starch content. Total free sugars (sucrose and reducing sugars) were determined according to Dubois et al. (1956), using glucose as the standard. The sugars monomers were determined by acid hydrolysis (Blakeney et al., 1983) followed by gas chromatography (GC), using a HP 5890 chromatograph equipped with a fused silica capillary column J&W DB-225 (30 m × 0.25 mm i.d.; 0.15 μm film thickness). Before sample injection, calibration curves for rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose were obtained using high purity commercial standards.

2.3. Lipophilic extractives

Milled mesocarp samples were extracted by Soxhlet with dichloromethane during 6 h. Dichloromethane was selected as a specific solvent for lipophilic extractives isolation for analytical purposes. The solvent was evaporated to dryness and the amount of extracts determined by gravimetry. The lipophilic extractives were identified and quantified by gas chromatography–mass spectrometry (GC–MS) as described by Oliveira et al. (2006). Briefly, 20 mg of each dried extract with a measured amount of internal standard was dissolved in 250 μL of pyridine. After the addition of 250 μL of bis(trimethylsilyl)trifluoroacetamide and 50 μL of trimethylchlorosilane, the mixture stayed at 70 °C for 30 min. GC–MS analyses were performed using the Agilent 6890N gas chromatography coupled to a 5975 Agilent mass selective detector, equipped with a DB-1 column (J&W: 30 m × 0.25 mm i.d.; thickness, 0.25 μm), using the conditions described previously (Oliveira et al., 2006). Components were identified based on the comparison of their spectra with two spectral libraries (NIST/EPA/NIH Mass Spectral Database, US), the retention times and, in some cases by comparing their fragmentation profiles with published data. For quantitative analysis, GC–MS was calibrated with pure reference compounds, representative of the major lipophilic extractives components (namely hexadecanoic acid, 1-eicosanol, 16-hydroxyhexadecanoic acid, ferulic acid and stigmasterol), relative to tetracosane used as internal standards. For each sample two injections were performed and results represent the average of six injections. Between injections the variation was less than 6%.

2.4. Statistical analysis

All chemical analysis and fractionation experiments were carried out at least twice and the results presented are the average of the values obtained for each fruit with a standard deviation lower than 6%. The experimental data were statistically analyzed by one factor analysis of variance (ANOVA) to determine its significance at $P < 0.05$, using the SPSS (*Statistical Package for Social Science*) version 15.0 for Windows.

3. Results and discussion

3.1. Physical parameters

The density of cherimoya fruit ranged from 0.88 g cm^{-3} to 1.11 g cm^{-3}, without any important variation during the ripening period. Weight loss increased steadily during storage (Fig. 1) and at the end of the experiment fruit had lost around 9% in weight. No statistical relationship was found between the density and the fruit weight. Fruit weight loss during ripening was also reported by others in cherimoya (Alique et al., 1994), custard apple (Prasanna et al., 2000) and soursop (Lima et al., 2006), being mainly attributed to water loss through respiration, transpiration and ripening. Water content in 'Madeira' cherimoyas ranged between 73 and 83% similarly to what has been reported for custard apple, cherimoya, soursop and sugar apple (Pinto et al., 2005).

Penetration force used to evaluate cherimoya softening was higher than 63.6 N on the first two days after fruit harvest and decreased sharply at day 4, reaching an average of 2.7 N at the end of storage (Fig. 1). At day 5 the fruit were over-ripe showing a softer texture that affects the quality for marketing. This enhanced rate of softening is in agreement with previous studies (Pareek et al.,