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Study of pomegranate ripening by dielectric spectroscopy

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ABSTRACT

Pomegranate (*Punica granatum* L.) is one of the fruits most recently studied for its many health benefits and its high antioxidant capacity and total phenolic content. Currently, the industry uses destructive methods to ensure the quality standards demanded by consumers. In this context, dielectric spectroscopy is presented as an interesting technique to monitor, on-line, fruit quality standards and ripening changes. The aim of this study is to analyze the effect of the major components of pomegranate and its structure on the dielectric spectrum between 500 MHz and 20 GHz. Some physical, chemical and dielectric measurements were carried out in the arils, spongy white tissues and peel. A maturity index was defined based on dielectric properties of fruit at two different frequencies, 2.4 and 1.2 GHz. The results demonstrated the utility of this index for pomegranate.

1. Introduction

Pomegranate (*Punica granatum* L.) is an ancient fruit that has been recently studied for its numerous health benefits such as the reduction of blood pressure, the anti-atherosclerotic effect and the reduction of LDL oxidation (Aviram et al., 2000, 2004, 2008; Aviram and Dornfeld, 2001; Kaplan et al., 2001); moreover, this fruit presents some components with anticarcinogenic action (Kim et al., 2002; Lansky et al., 2005; Malik et al., 2005; Malik and Mukhtar, 2006; Shishodia et al., 2006) and that inhibit tumour initiation and development (Adams et al., 2006; Khan et al., 2007). Some studies demonstrated that pomegranate fruit also confer an anti-inflammatory activity (Lansky and Newman, 2007; Larrosa et al., 2010; Lee et al., 2010; Shukla et al., 2008), antidiabetic properties (Bagri et al., 2009; Katz et al., 2007; Li et al., 2008; Parmar and Kar, 2007) and antimicrobial properties (Al-Zoreky, 2009; Choi et al., 2009; Gould et al., 2009; McCarrel et al., 2008; Reddy et al., 2007). These beneficial effects of pomegranate have been directly related to its high antioxidant capacity and total phenolic content (Aviram et al., 2000; Gil et al., 2000; Seeram et al., 2005; Basu and Penugonda, 2009; Viuda-Martos et al., 2010).

The edible part of pomegranate fruit is the pulp (arils), which surrounds the seeds. The fruit also contains membranous walls and spongy white tissues that are called locula septa. Both locula septa and the pulp form the whole pericarp. Moreover, the fruit is surrounded by a thin peel (Kader, 2006). Some studies have shown that the functional components of pomegranate fruit are located in the different parts of the fruit, not only in the arils (Negi et al., 2003). Besides the polyphenols and the antioxidant components, the edible part of pomegranate is also rich in sugars, mainly glucose and fructose, vitamins, polysaccharides, minerals and organic acids, fundamentally, citric acid (Melgarejo et al., 2000).

Pomegranate is a non-climacteric fruit and therefore a minimum maturity state is necessary at harvest (Elyatem and Kader, 1984). Kader (1999) defined the maturity state of fruits using the relationship between sugar content and acidity in liquid phase (maturity index), distinguishing between fruit that are not able to continue their ripening process once removed from the plant, and fruit that can continue the process once collected. Pomegranate fruit belong to the first group and have a low respiration rate and a non-climacteric respiration pattern, which has not received much study.

Objective techniques for determining the maturation state after harvesting are needed in order to decide the best uses and storage time of this kind of fruit. There exist numerous instrumental techniques to carry out these determinations but these techniques require samples from fruit internal tissues and, therefore, are destructive tests. On the other hand, recent studies developed non-destructive techniques to predict soluble solids content in honeydew melons (Nelson et al., 2006) and watermelons (Nelson et al., 2007) with promising results. Moreover, a maturity index based on dielectric properties in the microwave frequency range was developed for predicting maturity of climacteric fruits (Castro-Giráldez et al., 2010a,b).

The aim of this paper was to study the changes in terms of respiration rate, chemical composition and dielectric properties of pomegranates during storage at 5 °C for a period of 79 d, trying to evaluate the maturity index of the fruit by a non-destructive technique.

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2. Materials and methods

2.1. Raw material

Pomegranates (cv Mollar Valenciana) were harvested at the end of September from a plantation located in Valencia, Spain. The fruit were selected for homogeneity in size. Pieces with superficial defects were refused. All the fruit were at the commercial maturity state and were stored at 5 °C.

2.2. Experimental procedure

Prior to the studies of pomegranate fruit, different standard solutions simulating the liquid phase of pomegranate fruit were prepared. The chemical composition of the standard solutions was based on bibliographic sources (Al-Maiman and Ahmad, 2002). The dielectric properties of these solutions were measured at 5 °C in order to describe the different dispersions that occur in the microwaves range.

The additive used for preparing standard solutions was: citric acid 1-hydrate (E-330, F.C.C., Panreac Quimica S.A.U., Barcelona, Spain). Standard solutions were prepared with Milli®-Q water. Citric acid concentrations of 0, 1, 1.5, 2, and 2.5 g L^{-1} were assayed in solutions of 0 and 15% mass fraction of sugar. The sugar composition was obtained by mixing glucose and fructose in the equal proportions simulating the composition of the pomegranate fruit (Melgarejo et al., 2000).

The measurements of pomegranate fruit were made at 2, 9, 23, 37, 51, 65 and 79 d after the fruit harvest date. At each measurement date, three pomegranates were used to measure the respiration rate before the destructive measurements. After the respiration measurements were made, the pomegranates were divided in two halves. One of them was used for dielectric spectra measurements and afterwards for polyphenol content determination. The other half was used to measure water activity, sugar mass fraction in liquid phase (weight of sugar by weight of liquid phase), titratable acidity, and pH.

In parallel, at each measurement date, other pomegranate fruit were analyzed as respiration control fruit (these fruit were kept intact till the end of the experiments).

2.3. Dielectric properties measurement

The system used to measure dielectric properties consists of an *Agilent 85070E* open-ended coaxial probe connected to an *Agilent E8362B* vector network analyzer.

The dielectric properties were measured by contacting the coaxial probe with the pomegranate surface. The dielectric properties of the standard solutions were measured by inserting the coaxial probe into the liquid.

The dielectric properties of pomegranate fruit were measured in the arils, in the spongy white tissues of the locula septa, and in the peel. The mean values of five replicates are reported in this article. All determinations were made at 5 °C from 500 MHz to 20 GHz.

2.4. Physical–chemical analysis

Water activity was determined by using a dew point hygrometer, Aqualab® series 3 TE (Decagon Devices, Inc., Washington, USA). Water activity was measured in the arils and also in the spongy white tissues.

Sugar content was determined by a refractometer (ABBE, ATAGO Model 3-T, Japan).

Titratable acidity (expressed as citric acid; g L^{-1}) was determined according to the AOAC (1984) method 22.008 (AOAC, 1984).

Analytical determinations described above were obtained by triplicate.

The total phenolic content (TPC) was determined using the Folin–Ciocalteu's reagent (Chang et al., 2006). Pomegranate arils (0.5 g) were extracted with 5 mL methanol for 1 h, then the methanolic extract (ME) was diluted 1:1 (L L^{-1}) with distilled water. 125 μL of the diluted extract was mixed with 0.5 mL of distilled water in a test tube followed by addition of 125 μL of Folin–Ciocalteu reagent (FCR) and allowed to stand for 6 min. Then, 1.25 mL of 7% sodium carbonate mass fraction in water solution and 1 mL of distilled water were added. Each sample was allowed to stand for 90 min at 25 °C. Absorption at 760 nm was measured with a UV–vis spectrophotometer (JASCO V-630) and compared to a gallic acid calibration curve. The results were expressed as gallic acid equivalents (GAE) by sample mass (mg kg^{-1}). Each assay was carried out in triplicate.

Optical measurements were made by an Apochromatic Stereomicroscope Leica MZ (Leica Microsystems Ltd., Heerbrugg, Switzerland).

2.5. Low temperature scanning electron microscopy (cryo-SEM)

A Cryostage CT-1500C unit (Oxford Instruments, Witney, UK), coupled to a Jeol JSM-5410 scanning electron microscope (Jeol, Tokyo, Japan), was used. The sample was immersed in slush N_2 (−210 °C) and then quickly transferred to the Cryostage at 1 kPa, where sample fracture took place. Sublimation (etching) was carried out at −95 °C; the final point was determined by direct observation in the microscope, working at 5 kV. Then, once again in the Cryostage unit, the sample was coated with gold in vacuum (200 Pa), applied for 3 min, with an ionization current of 2 mA. The observation in the scanning electron microscope was carried out at 15 kV, at a working distance of 15 mm and a temperature ≤−130 °C.

2.6. Respiration analysis

A closed or static system was chosen to measure the respiration rate. Fruit were placed in 2 L hermetic glass containers provided with a rubber septum and were stored at 5 °C in a temperature-controlled chamber (J.P. Selecta S.A., Hot-Cold M, Barcelona, Spain). The volume of air from the headspace was withdrawn at different times with a needle connected to a gas analyzer. A head-space-gas analyzer (PBI Dansensor A/S, CheckMate 9900, Ringsted, Denmark) was used to determine the oxygen and carbon dioxide contents (L L^{-1}). Gas sampling was carried out every 60 min for 8 h. The relative humidity was analyzed before and after the respiration analysis by a hygrometer (φ). The respiration rate, expressed as oxygen consumption rate and carbon dioxide production rate, was calculated by Eq. (1):

$$RR_i(\mu g_i\, kg_F^{-1}\, s^{-1}) = \pm \frac{dx_i}{dt} \cdot \frac{V_{HS} \cdot MW_{air}}{M_{Fruit}} \quad (1)$$

In this equation, the subindex '*i*' represents oxygen or carbon dioxide, the symbol '+' is used to indicate the carbon dioxide production and the symbol '−' for oxygen consumption. The mass fraction by time represents the slope of the fitting data of '*i*' fraction measured by time; the head space volume (V_{HS}) was calculated from the volume of the glass and the volume of fruit obtained from its mass and density; M_{Fruit} represents the mass of the sample and MW_{air} represents the molecular weight of air estimated with Eq. (2):

$$MW_{air} = x_{O_2} \cdot MW_{O_2} + x_{CO_2} \cdot MW_{CO_2} + \frac{p_s^{5\,°C} \cdot \varphi}{P} \cdot MW_{H_2O} + \left(1 - \left[x_{O_2} + x_{CO_2} + \frac{p_s^{5\,°C} \cdot \varphi}{P}\right]\right) \cdot MW_{N_2-Ar} \quad (2)$$