Development of a dielectric spectroscopy technique for the determination of apple (Granny Smith) maturity

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1. Introduction

Apple industry has progressed recently because of its advances in production, storage, product development and marketing efficiencies (Sinha, 2006). To further improve of apple industry competitiveness, objective techniques for determining fruit maturity are needed in order to decide the best uses and storage time of the fruit. Immature fruits are more subject to shriveling and mechanical damage; and overripe fruits become soft and mealy with insipid flavor soon after harvest (Kader & Barret, 1996).

Fig. 1 presents an overview of the most relevant metabolic processes and changes during the growing, ripening and senescence in climacteric fruits. Apple is a climacteric fruit and is characterized by the starch accumulation during fruit growth, which is hydrolysed to monosaccharides, mainly glucose and fructose, during the ripening process. Starch hydrolysis is a process that requires high energy consumption and it is related to an increase of fruit respiration rate (climacteric crisis), until reaching its maximum at the end of ripening (climacteric peak). Finally, respiration rate decreases during senescence (Lal Kaushal & Sharma, 1995). The main biochemical changes of fruit maturity are produced during the climacteric crisis.

Ethylene effect on fruit maturity is also important in climacteric fruits. A climacteric rise in ethylene production precedes the increase in respiration rate (Fig. 1), suggesting that ethylene is the hormone that triggers the ripening process (Lanahan, Black, & Ecker, 1996). Organic acids are also strongly related to maturity process of climacteric fruits, in which ethylene is produced from l-amine-1-carboxyl ciclopropane acid (Mansour, Latche, Vaillant, Pech, & Reid, 1986); moreover, the intense respiratory activity consumes malic acid in an oxidative decarboxylation (Hulme, Dones, & Wooltorton, 1963). Malic acid can also be used in gluconeogenesis (Ribereau-Gayon & Peynaud, 1971).

The relationship between soluble solids content and organic acid concentration is called Maturity Index and is usually used in industry as a reference parameter of fruit state (Harker, Marsh, Young, Murray, Gunson, & Walker, 2002). Thiault Index (TI) is one of the maturity indexes frequently employed in apple fruit; it is related to soluble solids content and malic acid concentration (Varela, Salvador, & Fiszman, 2005). Therefore, there exist an increasing interest in determining organic acid and sugar composition of apple because of the possibility of employing these determinations for predicting fruit maturity index. There exist numerous instrumental techniques to carry out these determinations but require samples from fruit internal
tissues and, therefore, are destructive tests. On the other hand, recent researches are developing nondestructive techniques to measure food quality attributes; these techniques are mainly based in electrical properties of foods, known as dielectric properties. They were recently used to predict soluble solids content in honeydew melons (Nelson, Trabelsi, & Kays, 2006) and watermelons (Nelson, Guo, Trabelsi, & Kays, 2007) with promising results. On the other hand, no good correlations were obtained in apple during storage at frequencies between 10–1800 MHz (Guo, Nelson, Trabelsi, & Kays, 2007). More studies are needed for determining the real possibility of sensing apples quality from their dielectric properties.

Dielectric spectroscopy determines the dielectric properties of a medium as a function of frequency. It is based on the interaction of an electric external field with the sample (Metaxas & Meredith, 1993; Nelson & Datta, 2001); complex permittivity ($\varepsilon'$) (Eq. (1)) is the dielectric property that describes this interaction (Metaxas & Meredith, 1993; Nelson & Datta, 2001). The real part of complex permittivity is called dielectric constant ($\varepsilon''$) and the imaginary part is called loss factor ($\varepsilon''$). The dielectric constant is related with the capacitance of the material and its ability to store energy, and the dielectric loss factor is related to the absorption and dissipation of the electric field energy in other kinds of energy such as the termic one.

$$\varepsilon' = \varepsilon'' + i\varepsilon'' \quad (1)$$

There are different mechanisms affecting the dielectric behaviour of biological tissue. Relaxation phenomena are produced at microwave and radio frequencies and characterize, with the conductivity, the dielectric behaviour of practically all tissues at these frequencies. Permittivity decreases with the frequency increase in different steps called dispersions. It is important to highlight that these dispersions are not produced instantaneously and are characterized by the correspondent relaxation phenomena (Schwan, 1988). In biological systems, there are four main relaxation regions: $\alpha$, $\beta$, $\delta$ and $\gamma$ (Gabriel, 2006). Each of these steps characterizes a type of relaxation that occurs in a specific frequency range and which allows to identifying different phenomena. At the frequencies employed in present work, the $\gamma$ is the main dispersion (Fig. 2). The $\gamma$-dispersion, also called orientation polarization, is located at GHz region, and it is due to the orientation of dipoles, fundamentally free water molecules (Feldman, Ermolina, & Hayashi, 2003). Relaxation frequency is that in which the loss factor reaches its maximum value in $\gamma$-dispersion (Fig. 2).

In some cases, to analyze the energy dissipation of these relaxation phenomena in terms of loss factor spectra could be useful. Loss factor can be expressed by the Eq. (2), which reflects the different contribution phenomena to the loss factor spectrum in the frequency range of the present study.

$$\varepsilon'' = \varepsilon''_d + \varepsilon''_o$$  \quad (2)

where:

$\varepsilon''_d$ represents the loss factor caused by the dipolar orientation or dipolar relaxation.

$\varepsilon''_o/\varepsilon''_d\omega$ represents the loss factor due to effect of ionic conductivity, where $\sigma$, $\varepsilon_0$, and $\omega$ are the conductivity of the material, the dielectric constant in vacuum and the angular frequency, respectively.

In previous work, the high correlation of dielectric properties with soluble solids and malic acid content was demonstrated in standard solutions which simulate the apple liquid phase (Castro-Girález, Fito, Chenoll, & Fito, 2010). The objective of the present study was a further step in the use of dielectric spectroscopy to determine the harvested apples maturity in a rapid and nondestructive way.

Fig. 1. a) Qualitative evolution of respiration rate, fruit growing, ethylene levels and commercial life of Granny Smith apple; b) Qualitative evolution of starch, soluble solids and organic acids content during the ripening and senescence (Elaborated by the authors from data of different sources: Hulme, 1958; Pearson & Roberson, 1954; Taiz & Zeiger, 2002).

Fig. 2. Ideal representation of dielectric constant and loss factor spectra in biological tissue, where $f_R$ is the relaxation frequency; $\omega$ represents the loss factor caused by the dipolar relaxation; $\sigma/\varepsilon_0\omega$ represents the loss factor due to effect of ionic conductivity, where: $\sigma$, $\varepsilon_0$, and $\omega$ are the conductivity of the material, the dielectric constant in vacuum and the angular frequency, respectively.
2. Materials and methods

2.1. Raw material

20 Granny Smith apples were used, trying to get homogeneity in size. Pieces with superficial defects were refused. Apples were acquired one day before starting the experimental and were stored at 6 °C until the measurements. At each storage duration interval, four apples were taken and allowed to warm to 30 °C overnight in a controlled temperature chamber. Measurements were taken at one week intervals during five weeks storage period.

2.2. Experimental procedure

Apples were removed from 6 °C storage the night before the experimental and allowed to equilibrate to 30 °C. Apples were cut into two halves. One of them was used to structured tissue measurements which consisted in water activity, moisture and dielectric spectra. The other half was liquidized and paper filtered, and the clarified juice obtained was used to the measurements of water activity, sugar concentration (°Brix), estimated acidity and dielectric spectra.

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 software of the network analyzer calculates the dielectric constant and loss factor as a reflected signal function. For these measurements the probe was fixed to a stainless steel support, and an elevation platform brings the sample near the probe to avoid possible phase changes due to cable movements after calibration.

The system was calibrated by using three different types of loads: air, short-circuit and 25 °C Milli-Q water. Once the calibration was made, 25 °C Milli-Q water was measured again to check calibration suitability. The dielectric properties were measured by contacting the sample surface with the probe and by introducing the open-ended probe at least 5 mm deep in the clarified apple juice. The Mean values of ten replicates are reported in this article. All determinations were made at 30 °C from 500 MHz to 20 GHz.

2.4. Physical-chemical analysis

The water activity was determined by using a dew point hygrometer Aqualab® series 3 TE (Decagon Devices, Inc., Washington, USA). Water activity was measured in clarified liquid and in structured apple tissue. Sugar content was determined by a refractometer (ABBE, ATAGO Model 3-T, Japan). Moisture content and titrable acidity (expressed as malic acid) were determined according to the AOAC (1984) methods 22.013, 22.008, respectively. Analytical determinations described above were obtained by triplicate.

2.5. Maturity index

Thiault Index (TI) was used as an indicator of apple maturity. The Thiault Index is defined by Eq. (3) (Thiault, 1970).

\[
TI = c_s + 10 \times Ac
\]

where, \( c_s \) represents the sugar concentration (g/L) measured by refractometry, \( Ac \) represents the NaOH estimated acidity (expressed as g/L). The Lewis expression of sugar solutions density (Lewis, 1987) was used to transform the sugar mass fraction (g/g) to sugar concentration (g/L) (Eq. (4)).

\[
zs = \rhoss (g_sugar / g_juice) = \frac{z_s}{\rho_s}
\]

In apple, if TI is equal to 170, is the minimum to an acceptable fruit quality; if TI is equal to 180, is recommended to harvest the fruit; if TI is more than 180, the fruit quality is excellent (Porro, Datlaserra, Dorigatti, & Zatelli, 2002; Varela et al., 2005).

3. Results and discussion

In Fig. 3, some dielectric spectra of clarified apple juices can be observed. The figure shows that the ionic losses are the main contribution to loss factor at low frequencies. Ionic losses are directly
related to malic acid content (Castro-Giráldez et al., 2010). At the higher frequencies of the spectra, a slightly decrease in dipolar relaxation frequency can be observed; it can be attributed to differences in sugar content (Castro-Giráldez et al., 2010). In Fig. 4 the relation between malic acid concentration and the loss factor at 0.5 GHz can be observed. In the same figure, the relation between soluble solids content and the loss factor at relaxation frequency is also shown.

Fig. 4 shows that the increase in malic acid produces an increase in the loss factor value at 0.5 GHz due to the increase in ionic losses. It can be affirm that malic acid is in malate form in apple juice, and the strong negative charges of the molecule have an important effect on loss factor at low frequencies of the spectrum. The negative relation of soluble solids content with loss factor at dipolar relaxation frequency can also be observed. The increase of viscosity caused by the presence of sugar molecules reduces water mobility and displaces dipolar relaxation frequency to lower frequencies of the spectrum (Fig. 5), decreasing also the loss factor value at the relaxation frequency (Fig. 4).

In Fig. 5, dielectric spectra of apple tissue are shown. If compared apple juice spectra to apple tissue spectra (Figs. 3 and 5, respectively), it can be observed that apple juices spectra take higher values than apple tissue spectra; these differences can be due to the compartmentation of apple tissue and the effect of the matrix on the dielectric spectra. At these frequencies, insoluble matrix does not interact with radiation producing a concrete polarization, but its presence, taking part of a highly ordered structure, has the effect of limiting the mobility of water and solutes molecules. In the same figure, the relation between sugars concentration and the relaxation frequency can be observed. The increase in sugars content decreases relaxation frequency as can be observed in this figure.

3.1. Maturity index

As it can be observed in Eq. (3), Thiault Index is an indicator of soluble solids content and malic acid concentration. Both soluble solids content and acidity are quantified in the same order, for this reason the acidity is multiplied by 10.

Due to the fact that malic acid concentration is positively related to loss factor at 0.5 GHz, and the concentration of soluble solids is
negatively related to loss factor at the relaxation frequency, a new Maturity Index based on dielectric properties ($M_{\text{dielectric}}$) can be defined (Eq. (5)).

$$M_{\text{dielectric}} = e'(f_{\text{relaxation}}) - e'(f_{0.5GHz})$$ (5)

It is important to highlight that an increase in malic acid produces an increase in loss factor at 0.5 GHz; on the contrary, an increase in sugar content produces a decrease in the loss factor at relaxation frequency. As it was explained above, Thiault Index quantify both soluble solids content and malic acid concentration; for this reason, to define a Dielectric Maturity Index it is necessary to subtract the loss factor at relaxation frequency from the loss factor at 0.5 GHz. It could be interesting to remark that loss factor at relaxation frequency is higher than loss factor at 0.5 GHz, for this reason and to avoid negative signs, the subtraction was define as specified in Eq. (5). Moreover, it is also important to denote that an increase in Thiault Index will produce a decrease in Dielectric Maturity Index.

In Fig. 6, the Dielectric Maturity Index was represented as a function of the Thiault Index. It can be appreciated that there exist a linear correlation between both Indexes.

In Fig. 7, the Dielectric Maturity Index was represented as a function of the Thiault Index for standard solutions (Castro-Giráldez et al., 2010), apple juice and Granny Smith apple tissue. It is affirmed that the fruit maturity can be good predicted with the Maturity Index calculated from dielectric properties of apple tissue. The on the other hand worse correlation was found between Thiault Index and the Maturity Index calculated from dielectric properties of apple juice. It might be due to the fact that the liquidized operation produces a destructuration of tissue and thus compositional changes and biochemical reactions can occur, mainly oxidation reactions.

**Fig. 6.** Dielectric Maturity Index ($M_{\text{dielectric}}$) as a function of the Thiault Index ($TI$) for Granny Smith apple tissue.

**Fig. 7.** Dielectric Maturity Index ($M_{\text{dielectric}}$) as a function of the Thiault Index ($TI$) for standard solutions (•) (Castro-Giráldez et al., 2010), apple juice (♦) and Granny Smith apple tissue (♦).

