ASSESSMENT OF AVOCADO TEXTURAL CHANGES DURING RIPENING BY USING CONTACTLESS AIR-COUPLED ULTRASOUND

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ABSTRACT

In the present study, the use of the air-coupled ultrasonic technique has been analysed as a new tool for the contactless assessment of the avocado post-harvest textural modifications during ripening. Thus, ultrasonic parameters, such as maximum wave amplitude and ultrasound velocity, and textural ones, such as hardness, elastic modulus and relaxation capacity, were measured on avocado slices. During ripening, avocado reduced its elastic modulus (from 2.29±0.75 to 0.16±0.08 MPa), became softer and became more viscoelastic, which was well described from zero and first-order kinetic models. These changes increased ultrasound attenuation, decreasing the maximum amplitude of the ultrasonic signal (from 336.6 to 55.4 V/m), while the ultrasonic velocity remained constant, between 320.1±6.9 and 316.4±82.6 m/s. Thereby, the maximum ultrasonic amplitude, which adequately correlated with textural parameters ($r_{avg.}$=0.85), could be used to assess the post-harvest ripening on avocado slices.

Keywords: avocado, air-coupled ultrasound, texture, ripening.

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1. **INTRODUCTION**

Avocado (*Persea Americana Mill.*) is an oleaginous climacteric fruit, appreciated worldwide due to its high quality nutritional value. Specifically, in mature avocado fruit, the oil fraction is composed of 75% of monounsaturated fatty acids (mainly oleic acid), 10% to 15% of polyunsaturated fatty acids, such as linoleic, and is considered an excellent source of antioxidants, such as vitamins E and C (Alnasan and Yamanishi, 2018). This composition is related to health benefits, including the prevention of cardiovascular diseases, anti-cancer activity and diabetes (Ding *et al.*, 2007; Magwaza and Tesfay, 2015). In recent years, avocado production has increased considerably, with Mexico (31%), The Dominican Republic (8%), Colombia (6%), Peru (6%) and Indonesia (6%) being the world's leading producers (Hurtado-Fernández, Fernández-Gutiérrez and Carrasco-Pancorbo, 2018). The main importers of this tropical fruit are The United States (45%), The Netherlands (11%), France (8%) and Japan (5%) (FAO, 2013). Consequently, long transportation times are frequent, which are relevant in terms of ripening and fruit quality (Villa-Rodríguez *et al.*, 2011; Hernández *et al.*, 2016).

The avocado ripening process is triggered once the fruit is harvested from the tree and leads to physicochemical modifications, such as colour, texture and flavour (Prasanna, Prabha and Tharanathan, 2007). During ripening, it has been reported that the pulp, or mesocarp, composition changes gradually, increasing the oil content and decreasing the sugar, starch and moisture content (Alnasan and Yamanishi, 2018). Conversely, other studies have reported that the total oil and solid content do not change but a migration of both components to the extracellular spaces occurs as a consequence of the cell wall degradation. Differences in the literature may be explained by the highly variable nature of this fruit in terms of oil, solid and water content (Mizrach and Flitsanov, 1999), which hinders the monitoring of the evolution of these components. Thus, the ripening characterization by using chemical parameters, such as moisture content, dry matter or oil content (Blakey et al., 2012; Magwaza & Tesfay, 2015), has to be complemented with more appropriate tools that shed light on the phenomena occurring. In this regard, during avocado ripening, the firmness of the fruit decreases and the rough external peel or exocarp detaches from the pulp, due to the swelling and weakening of the cell wall (Carrington, 2011; Ortiz-Viedma *et al.*, 2018). These modifications are related to changes in sensory attributes which can be assessed by the use of instrumental texture by using compression, puncture or shear tests (Blanpied et al., 1978; Bourne, 1979). It has to be remarked that its use for viscoelastic and highly inhomogeneous materials, such as fruits and vegetables, can lead to inaccurate assessment in some cases (Bourne, 1982). These methods are destructive and time consuming, and as a consequence they can only be applied to a limited number of samples of a batch. Hence, there is a need to develop fast, economic and non-destructive techniques for instantaneous inspection, which may enable real-time decision making (Ibba et al., 2020; Islam et al., 2018; Peleg et al., 1990). These strategies are perfectly aligned with the concept of Industry 4.0 and Smart Manufacturing.

Since the last century, ultrasound techniques have been used for non-destructive testing of food materials. Ultrasound offers advantages over other non-destructive technologies based on electromagnetic energy, such as Magnetic Resonance Imaging or X-Ray, since they do not comprise any ionizing radiation; thus, the equipment and personal manipulation are simpler (Chen *et al.*, 1993; Harker *et al.*, 2010). Conventional ultrasonic systems with direct contact between the transducer and the fruit have been tested. Thus, Mizrach et al. (1989; 1999) designed a high power, low frequency ultrasound (50 kHz) contact system to study the tissue of different agricultural specimens. Meanwhile, Self et al. (1994) used commercial ultrasonic devices for non-destructive testing: this work in direct contact with the peel to evaluate avocado flesh while ripening. Afterwards, attenuation and ultrasonic velocity have been measured during ripening in the whole avocado fruit and correlated to its composition (Mizrach *et al.*, 1999; Mousavi *et al.*, 2005) and textural parameters (Mizrach and Flitsanov, 1999; Flitsanov *et al.*, 2000). As regards the avocado ripening process, the main results found in the literature showed a direct relationship between the ripening time and ultrasonic attenuation, whereas the relationship of the ultrasonic velocity with the ripening state was unclear (Mizrach, 2008). Firmness declines as the fruit matures and it was correlated satisfactorily with the increase in attenuation (Mizrach *et al.*, 1999; Magwaza and Tesfay, 2015). These previous experiments were carried out by attaching the ultrasonic transducers to the avocado peel surface in order to prevent the high attenuation of the flesh. The contact force between those transducers and the surface of the avocado is extremely important, since small variations might greatly alter the measurements. Other factors that might negatively impact the measurements are the presence of edible coatings, such as wax in the peel, and the surface roughness (Carrington, 2011; Magwaza and Tesfay, 2015). Moreover, these techniques require the use of coupling materials (water, glycerine or oil), which may not be allowed in some food products (Gan, Pallav and Hutchins, 2006; Mohd Khairi *et al.*, 2016) and its use dramatically slows down the measurements and increases the risk of cross-contamination.

Alternatively to traditional ultrasonic measurements, contactless ultrasound techniques emerge as a powerful tool in the food industry and are considered the most suitable for the purposes of physicochemical analysis without product modifications (Awad *et al.*, 2012; Chandrapala, 2015). The main advantage is that there is no direct contact with the sample under study, namely, the transducer is separated from the product surface and the airborne measurement is feasible. Thereby, the measurement is absolutely non-invasive and it is better adapted to the required fast inspection of food processing lines. The first studies to apply the non-contact ultrasonic technique in foods used commercial piezoelectric transducers whose central frequency was 1 MHz (Saggin and Coupland, 2001) and studied some mechanical properties (Cho and Irudayaraj, 2003a) and quality parameters (Cho and Irudayaraj, 2003b; Gan, Pallav and Hutchins, 2006) of different products, such as cheese and chocolate (Watson *et al.*, 2014). The low efficiency of commercial airborne transducers involved low signal-to-noise ratios, which in the past decades has been an enormous obstacle to the development of air-coupling ultrasonic technology towards the attainment of ambitious goals. Highly-efficient piezoelectric transducers in the frequency range [0.15 – 0.35] MHz have been developed by coupling multi-layer matching materials to the active piezoelectric (Gómez Álvarez-Arenas, 2004). Thus, a better matching with the air is achieved and higher energy levels are transmitted through the analysed material. Recently, these transducers have been adapted for their use in vegetal tissues as plant leaves (Sancho-Knapik, Peguero-Pina, Fariñas, *et al.*, 2013; Fariñas and Gómez Álvarez-Arenas, 2014; Gómez Álvarez-Arenas *et al.*, 2016) and exploratory tests have also been carried out on foodstuffs (Corona *et al.*, 2013; Ginel and Gómez Álvarez-Arenas, 2019). However, no study has been found covering the use of these novel air-coupled transducers to assess textural changes in highly attenuative fruits, such as avocado. Therefore, the aim of this study is to test the feasibility of using non-contact ultrasound as a rapid, non-destructive and non-invasive method to assess the textural modifications in avocado slices during storage and ripening.

2. MATERIALS AND METHODS

2.1. Fruit and ripening conditions

The avocados (*Persea americana var*. Hass) used in this study are classified as Category I and calibre 10 and were purchased in a local market (Valencia, Spain). The avocados were imported from La Libertad (Peru) and were transported by ship freshly-harvested under controlled temperature and humidity conditions (4ºC, 80%RH), which guaranteed the fruit was received in an unripe state. Ripening was carried out in a temperature-controlled chamber at 20±1ºC, without the addition of ethylene, the aim of which was to simulate a domestic storage environment. To evaluate the ripening state, samples were analysed after 0, 1, 2 and 5 days of storage in order to obtain a wide range of textures, covering from unripe to very ripe fruits.

2.2. Sample preparation

Prior to performing ultrasonic and textural tests on each piece of avocado, the mesocarp was cut into slices along the equatorial plane; then the slices were wrapped in plastic film and kept at 4ºC. Slices of 2 and 5 mm thickness were cut for the ultrasonic and the textural analysis (Fig. 1A), respectively. The analysis focused on the mesocarp, hence the slices containing endocarp or exocarp were discarded (Fig. 1B). Following this procedure, 10 avocado fruits were used per storage day, 6 slices (2 mm) from each fruit were used for the ultrasonic measurements and 3 points were measured in each slice (18 measurements per fruit). In the case of the textural tests, however, 2 slices were used per fruit and 3 tests were carried out per slice, which leads to 6 textural measurements per avocado. Both ultrasonic and textural measurements were carried out in the centre of the slices, which matches with the equatorial zone of the fruit where the seed is located, following a triangular pattern (side 1 cm).

2.3. Non-contact ultrasonic measurement

The non-contact ultrasonic technique consisted of a pair of piezoelectric transducers specifically designed to optimize their performance on air (US-BioMat lab; ITEFI-CSIC, Madrid). An optimized impedance matching with the air is achieved by the coupling of active and passive multilayer materials to the piezoelectric ceramic in order to create a gradual decrease in the acoustic impedance and, thus, improve energy transfer in the air-transducer interface (Gómez Álvarez-Arenas, 2004). The central frequency of the transducer was 250 kHz, the frequency band 150 –350 kHz, the peak sensitivity -25 dB, the electrical impedance 100 Ω and diameter 20 mm (Álvarez-Arenas, 2013). In order to drive the 200 V-amplitude semi-cycle of square wave tuned to the transducer center frequency, a commercial pulser/receiver was used (5077PR, Olympus, Houston, TX, USA). The electric signal received was filtered using the built-in Low Pass Filter with a 10 MHz cut-off frequency. The signal was amplified 59 dB before sending it to a digital oscilloscope (MDO3024, Tektronix, WA, USA) with the impedance set at 1MΩ, the bandwidth at 20 MHz and averaged 128 samples. Then, the signal was digitized at 10 MS/s and 8 bit (vertical resolution). The result was transferred and stored in a PC using Labview® (National Instruments, Austin, TX).

In order to maximize the energy received, a through-transmission configuration was used (Fig. 2A). The avocado slices were placed normal to the wave propagation direction in a sample holder at a distance of 9 mm from each transducer. The sample holder contained a cylindrical hole whose diameter was two millimeters bigger than the transducer but smaller than the sample to ensure that the ultrasonic wave propagated through the material under study (Fig. 2A).

The ultrasonic signal excited by the transmitter's transducer propagates first through the air, then through the sample and finally, through the air again until it reaches the receiver's transducer (Fig. 2B). In order to obtain the ultrasonic velocity, the time of flight of the ultrasonic wave through the avocado slice was calculated. Firstly, the cross-correlation between the measured ultrasonic signal received before placing the sample in the holder and the signal measured through the sample was computed. Then, the Hilbert transform was applied to compute the envelope of the correlation, in order to obtain the time of flight from the location of its maximum (Burrascano *et al.*, 2015). Thicknesses of every sample were measured using a caliper (192-633 Serie, Mitutoyo, Japan). Finally, the ultrasonic velocity through the sample can be calculated using equation 1:

$$v = \frac{t}{\frac{t}{v_o} - \Delta d} \qquad \text{Eq. 1}$$

where v is the ultrasonic velocity through the avocado slice (m/s), t is the thickness (m), v_o is the ultrasonic velocity in the air (340 m/s) and Δd is the time of flight difference between the reference signal that propagated through the air and the one measured with the sample, obtained as explained above (s).

The second ultrasonic parameter considered in this study was the maximum amplitude of the ultrasonic signal divided by the avocado thickness (A_t), which was computed from the following equation:

$$A_t = \frac{\max(V)+|\min(V)|}{t} \qquad \text{Eq. 2}$$

where *max(V)* and min(V) are the maximum and the minimum peak amplitudes of the ultrasonic signal (V) and *t* the thickness of the sample (m).

Python software (Numpy, Matplotlib and Scipy packages) was used to perform the analysis of all signals acquired to obtain the ultrasonic parameters defined above.

2.4. Textural analysis

The textural analysis applied in this study was the stress-relaxation test, as defined by Landahl et al. (2009). The analysis was carried out with a texturometer (TA.XT2i, Stable Micro Systems, Surrey, UK) set with a cylindrical probe of 6 mm diameter (SMS P/6, ANAME, Madrid, Spain). The textural tests were carried out in a temperature controlled chamber (at 4±1ºC) and at a compression rate of 10 mm/min, 0.6 mm of deformation and a relaxation time of 60 seconds. The avocado slices (5 mm thick) were prepared as indicated in 2.1 and as already mentioned, the measurements were taken in the equatorial zone of every avocado slice sample in triplicate.

Fig. 3A shows a typical curve obtained in a stress-relaxation test while Fig. 3B includes some experimental tests performed on avocado pulp slices on different storage days. Two parts can be distinguished in the stress-relaxation curves. Firstly, the sample is compressed at a constant rate until it reaches the established deformation target. Secondly, the probe is stopped and the force recorded for 60 s in order to analyse the relaxation behaviour. The stress-relaxation test is used to quantify the viscoelastic behaviour of cellular solids and other viscoelastic materials (Peleg and Calzada, 1976). The textural parameters analysed from the stress-relaxation tests were the hardness, which is computed as the maximum compression force (F_{max}), and the residual force (F_{60s}), which corresponds to the load obtained 60 seconds after the maximum compression force was reached (Fig. 3A). Additionally, the elastic modulus (E) was computed from the slope of the curve (Fig. 3A) in the compression stage using equation 3.

$$E = \frac{F_{max} - F_{2s}}{S \cdot \varepsilon} \qquad \text{Eq. 3}$$

where F_{2s} is the force after 2 seconds of compression loading, *S* the surface of the texture probe and ε the sample strain reached. The initial part of the curve (compression time < 2 s) was not considered in the assessment of the elastic modulus since it matches the coupling distance of the flat probe to the avocado slice surface.

Additionally, the Total Relaxation Capacity (TRC) was assessed, which provides information on the level of cellular structure loss as a decrease in the relaxation capacity (Jaya and Durance, 2005). TRC was calculated as indicated in equation 4.

$$TRC = \frac{F_{max} - F_{60s}}{F_{max}} \qquad \text{Eq. 4}$$

The evolution of the textural parameters (TP) during storage was mathematically described using kinetic models of *n*-order (Eq. 5), which are widely employed to model the degradation kinetics of foods (Sila *et al.*, 2006; Chen and Opara, 2013). Thereby, zero ($n=0$), first ($n=1$) and second ($n=2$) models were tested.

$$\frac{dTP}{dt} = k \cdot TP^n \qquad \text{Eq. 5}$$

where *t* is the time (days), *k* the kinetic constant ($days^{-1}$) and *n* the order (dimensionless).

2.5. Statistical analysis

The influence of the ripening time on the textural and ultrasonic parameters was analysed using a simple analysis of variance (ANOVA). The comparison of the means was performed using the Fisher Least Significant Differences (LSD) test with 95% confidence interval. The statistical analysis was carried out using Statgraphics Centurion XVII (Statgraphics Technologies Inc., VA, USA).

3. RESULTS AND DISCUSSION

3.1. Textural modifications during storage

The evolution of the textural parameters during storage reports information about the progress of ripening in avocado fruit, since it undergoes physical and chemical modifications which alter its cell structure. The evolution of the textural parameters analysed throughout the storage is depicted in Fig.4. On the one hand, the avocado was observed to become less hard (Fig. 4A). Thus, the hardness, defined as the F_{max}, was reduced from 14.0 N on day 0 to 1.1 N on day 5, which points to the fact that ripening takes place relatively quickly if the fruit is stored under conditions (20ºC) close to standard room temperature. The change in hardness was not significant ($p>0.05$) during the first day of storage at 20ºC, and the most relevant decrease was found between days 1 and 2, where the F_{max} decreased from 12.5 to 5.6 N. The decrease in hardness between days 2 and 5 was slightly more moderate (from 5.6 to 1.1 N). Thus, three homogeneous groups of hardness were distinguished according to storage time (Fig. 4A). The reduction in hardness is promoted by the rise in cellulase and pectinase activity on the first days of storage, which induces the cell wall softening (Landahl, Meyer and Terry, 2009) and has been described in the literature. In this regard, Ortiz-Viedma *et al.*, (2018) reported a change in the maximum fracture stress, a parameter directly related to hardness, from approximately 250 to 75 kPa in avocado Hass mesocarp stored for 40 days at 10ºC. On the other hand, a high degree of experimental variability was found in every trial, but especially between days 0 and 2. This fact confirms that, although the pieces belong to the same batch, the great heterogeneity among the avocado fruits purchased leads to different patterns in the textural changes. The highly heterogeneous nature of the avocado hardness represents a relevant handicap for the fruit logistics and retail companies, leading to unreliability in terms of the ripeness of the fruit delivered to the consumers , given the high product demand in the ready-ripe stage (Blakey, Bower and Bertling, 2009; Fuentealba *et al.*, 2016).

The existence of an initial linear stage (after the probe coupling) in the stress-strain curves allowed the elastic modulus (E) to be computed. The calculated values for the elastic modulus ranged from 2.29 to 0.16 MPa and agree closely with others previously reported in the literature. Thus, Baryeh (2000) reported an elastic modulus for avocado of 0.46 MPa, while, banana, another climacteric fruit, presents elastic modulus values of between 0.8 and 3 MPa (Lewis, 1996). As observed in Fig. 4B, the change in the elastic modulus followed the same pattern as the one found for hardness.

Fig. 4C. shows the evolution of the residual relaxation force during storage, defined as F_{60s}. The evolution of F_{60s} also follows an identical pattern to the one found in the F_{max}. Thereby, an overall decrease in F_{60s} from day 0 (8.8 N) to day 5 of storage (0.42 N) was found. From day 0 to day 1, there are no statistically significant ($p>0.05$) differences observed between the two groups of avocados. The highest reduction in F_{60s} was computed between days 1 and 2, when experimental values dropped from 7.7 to 3.1 N. Additionally, the high degree of experimental variability between fruits is also reflected in this parameter. The reduction in the residual relaxation force reflects the fact that maturation results in a modification of avocado viscoelastic behaviour; thus, there is a transformation to a more viscous material. This fact is better analysed by computing the total relaxation capacity parameter (TRC), which showed a significant ($p<0.05$) increase during storage time after day 1 (Fig. 4D). The increase in the average TRC is inversely related to the capacity of the avocado tissue to recover its structure after being deformed;

as a consequence, its increase reflects more viscoelastic behaviour. This result was already reported by Ortiz-Viedma et al. (2018), who found that avocado fruit increased its viscous character, to the detriment of the elastic component, as maturation progresses. The loss of cell wall structure is due to the degradation of cross-links between cellulose microfibrils that produces a reduction in the elastic properties of the cell wall (Sakurai and Nevins, 1997; Defilippi *et al.*, 2018)) through the release of cellular materials and pectin (Ortiz-Viedma *et al.*, 2018).

The evolution of the texural parameters during storage was accurately modelled using *n*-order kinetic equations (Table 1). While the change of the hardness, elastic modulus and residual relaxation force was better described using firstorder kinetics, the evolution of TRC was closer to the characteristic pattern of zero order kinetics. The fit of the second-order model provided slightly lower regression coefficients (Table 1) for every textural parameter.

Textural changes are characteristic throughout fruit ripening as a consequence of structural modifications. Specifically, previous studies have concluded that there is a strong relationship in avocado between a reduction in the concentration of mesocarp carbohydrates and a reduction in hardness (Mizrach, 2008; Awad *et al.*, 2012; Blakey *et al.*, 2012). The fall in the concentration of carbohydrates is a natural postharvest process in fruit , which results in changes in the fruit composition and structure. These are due to modifications in cellulose, glucose and pectin concentration due to the cellulase and pectinase activity (Blakey *et al.*, 2012). On the one hand, experimental results highlighted the highly variable nature of the avocado fruit, considering the initial textural parameters. On the other hand, textural modifications are temperature-dependent and are affected by initial textural properties, which makes the prediction of textural changes during ripening-storage time highly complex. Therefore, in this context, the search for novel methods as a means of characterising initial texture as well as textural modifications during ripening in order to predict the optimum ripening time is of great relevance.

3.2 Ultrasonic measurements

The ultrasonic parameters analysed were the maximum amplitude (A_t) and the ultrasonic wave velocity (v) of the signal transmitted through the avocado mesocarp slices. Fig. 5A. shows the evolution of A_t during storage; what must first be mentioned again is the high degree of experimental variability observed, which was similar in relative magnitude to the one found for the textural parameters. This is explained by the highly heterogeneous nature of avocado fruit, related not only to their textural properties but also to their composition. In this sense, Mizrach and Flitsanov, (1999) mentioned that the oil content of avocado at harvest could range from the minimum 9% up to 25%. A statistically significant reduction ($p<0.05$) in A_t was found over storage time, following a similar pattern to that given in the textural parameters (Fig 4). On day 0, A_t was 336.6 V/m, reaching its minimum value on day 5 of storage (55.4 V/m). As observed in Fig. 5A., the largest modification of A_t was found after the first day of storage, where values dropped from 413.0 to 182.5 V/m on day 2. This decay in signal amplitude matches results from previous studies, such as Mizrach and Flitsanov (1999), who reported an exponential increase in signal attenuation during ripening. The measurement of attenuation using the direct-contact ultrasonic technique is complicated in solid materials, due to a lack of accuracy linked to the energy loss in the transducer/sample coupling interface. For that reason, most applications of contact ultrasound use velocity to estimate food properties, despite the fact that attenuation could provide better information. Air-coupled ultrasound avoids that problem, being very useful when attenuation measurements are of interest. The attenuation of ultrasound in a solid material is mainly due to scattering and absorption (You *et al.*, 1991). During ripening, there exists a degradation of the parenchyma and oil cell walls (Davenport and Ellis, 1959), which results in an increase in both the extracellular oil and solid contents (Ortiz-Viedma *et al.*, 2018). This could have an impact on the scattering phenomenon related to attenuation, but the loss in ultrasonic energy during wave propagation in the ripe avocado is mainly related to the damping phenomenon that takes place in viscoelastic materials (You *et al.*, 1991). The absorption of ultrasonic energy in elastic materials gradually increases, as the material behaves as a more viscoelastic medium, due to the viscous attenuation. Viscous attenuation is based on the relative movement of the particle and the surrounding medium which causes dissipation of the acoustic energy due to shear friction (Geier *et al.*, 2014). The use of efficient airborne transducers with multilayer matching materials (Gómez Álvarez-Arenas, 2004; Ginel and Gómez Álvarez-Arenas, 2019), like the ones used in the present study, makes the ultrasonic measurement in highly attenuating materials, such as avocado slices, feasible and may provide a more accurate assessment of the attenuation than conventional direct-contact ultrasonic techniques. Future research should deal with the optimization of the airborne ultrasound transducers in order to improve its energy performance, which is absolutely necessary to address the challenge of the inspection of the whole fruit.

Fig. 5B shows that the ultrasonic velocity of avocado mesocarp remained constant during storage. Thereby, velocity ranged between 320.1 and 321.4 m/s for days 0 and 2, and only a small reduction was found on day 5 (316.4 m/s). Since ultrasound is a mechanical wave, its velocity depends on the elastic properties of the material under study. In the case of avocado mesocarp, the textural post-harvest modifications are a consequence of changes not only in the cell wall structure but also in the water-oil ratio (Márquez *et al.*, 2014). According to the literature, the reduction in the elastic properties should imply a more significant reduction in the ultrasonic velocity in vegetal tissues (Sancho-Knapik, Peguero-Pina, Medrano, *et al.*, 2013), but the experimental results do not reflect the expected changes. Previous literature has also shown a rather ambiguous relationship between the ultrasonic velocity and the storage time for avocado flesh (Self *et al.*, 1994). In this context, Mizrach (2000) and Mizrach and Flitsanov (1999) also demonstrated an unclear evolution of the ultrasonic velocity during ripening, which was mathematically modelled by considering second and third order polynomial coefficients to explain a slight increase in the velocity at the end of storage. This tendency in the velocity over storage time modelled by Mizrach and Flitsanov (1999) seems to be counterintuitive from a physicomechanical point of view, since a reduction in the velocity should be expected during ripening, as a result of the reduction in the elastic modulus, such as those shown in Fig. 4B. It could be expected that the effect of the change in the elastic properties on the ultrasonic velocity during ripening could be counteracted by compositional modifications that could increase the ultrasonic velocity due to density changes. However, literature reflects that the amount of oil and solids does not change during ripening (Landahl, Meyer and Terry, 2009). On the contrary, it has been reported that the water content could be reduced by slight dehydration, which can be partially minimized by a waxy coating (Self et al., 1994). In addition, Self *et al.* (1994) also reported that the air fraction could be reduced by a factor of up to 4 during ripening, as previously described by Nobel (1991). Therefore, further research should elucidate the phenomena compensating the expected reduction in the ultrasonic velocity during ripening.

Ultrasonic velocity values reported in the present study are similar to the ones already published using conventional contact ultrasonic technologies. Thus Mizrach and Flitsanov (1999), assessed the ultrasonic velocity in avocado from measurements on the fruit's surface and found that values lay between 361 and 330 m/s. The direct comparison between ultrasonic values shown in previous studies is complex, not only because of the aforementioned problem with intra and inter fruit heterogenity, but also due to the different ultrasonic techniques used.

3.3 Relationship between ultrasonic and textural parameters

Figs. 6 and 7 show the relationship between the textural (hardness, elastic modulus residual relaxation force and total relaxation capacity) and the ultrasonic maximum amplitude (A_t). In both Figs. 6 and 7, the average values for the textural and ultrasonic parameters of each avocado fruit tested are plotted. In addition, the average figure for each storage day has also been included. First of all, it has to be highlighted that the experimental design provided a homogenous distribution of samples in the range of each textural parameter analysed.

For every textural parameter, significant ($p<0.05$) linear regressions were established (Figs. 6 and 7). In this sense, linear models have also been used by Mizrach, (2000) to relate the firmness and the attenuation (dB/mm), whereas While, Mizrach and Flitsanov (1999) presented a second degree polynomial model to relate these variables. The loss in elastic properties and the transformation of the avocado into a more viscoelastic material during ripening is reflected in the decrease in E and the increase in TRC, which indirectly also affects F_{max} and F_{60s}, as indicated in section 3.1. Both facts lead to an increase in viscous attenuation and a decrease in A_t, which pointed to a positive relationship with E, F_{max} and F_{60s} but a negative one with TRC. The best correlations were found between E and A_t (r=0.87) and F_{max} and A_t (r=0.86). Better correlations using a lower number of experimental data were computed by Mizrach and Flitsanov (1999) and Mizrach (2000). However, in the present study, the use of non-contact ultrasound is a great improvement on the industrial technology proposed. Parameters related to relaxation and the viscoelastic behaviour of the material (F_{60s} and TRC) obtained slightly lower correlation figures: r=0.85 between F_{60s} and A_t, while r=-0.83 between TRC and A_t. The experimental variance not explained by the linear models identified in this study can be linked to the effect of the heterogeneous composition of avocado not only in terms of the oil and water content, but also its air fraction, which also has a big impact on the attenuation. Therefore, further attempts have to be made to consider the interaction of composition in the prediction of avocado texture based on ultrasonic parameters.

4. CONCLUSIONS

Efficient piezoelectric transducers tuned with multilayer matching materials made feasible the airborne ultrasonic inspection in a highly attenuating product, such as avocado. Thereby, the air-coupled ultrasonic technique enabled the rapid and contactless determination of ultrasonic parameters, such as ultrasonic velocity and maximum amplitude (A_t), in avocado pulp slices, which has been used to characterise the textural modifications of this fruit during ripening at 20ºC. Ripening caused a change in the A_t, which reduced as the fruit progressively ripened, while the ultrasonic velocity remained fairly constant. The change in the A_t during ripening followed a similar pattern to the one observed in the textural parameters, such as hardness and elastic modulus, as well as to those related to the viscoelastic behaviour of the material. Thus, significant linear models were established between A_t and the textural parameters (regression coefficients from 0.83 and 0.87) , which can be used for computing the textural modifications undergone by the product during ripening.

Further work has to be undertaken in order to improve the use of this novel technique for avocado evaluation. Firstly, considering the high degree of variability of avocado in terms of composition, the characterisation of the textural changes has to be analysed, taking into account the initial composition and its changes during ripening. Secondly, the ultrasonic analysis could be shifted to the frequency domain in order to better compute the ultrasonic velocity and attenuation; in this study this has been prevented by the low signal-to-noise ratio, which became more marked as ripening progressed. Thirdly, an engineering solution is required for non-invasive inspection, not only for the purposes of increasing the signal-to-noise ratio for a more acute analysis, but also for addressing the characterisation of whole avocado pieces using the air-coupled ultrasound technique and transducers used in this study. This would contribute to the in-line implementation of this technology for non-invasive and real time whole avocado fruit sorting at industrial scale.

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FIGURE CAPTIONS

Fig. 1. A) Left: 2 mm thick avocado slices for ultrasonic analysis; Centre: picture of the surface of one slice; Right: 5 mm thick avocado slice for textural analysis. B) Schematic view of the spatial distribution of slices along the equatorial plane of each sample. Those coloured red were discarded.

Fig. 2. A) Diagram of the ultrasonic experimental set-up. B) Ultrasonic signals acquired on different storage days of avocado slices.

Fig. 3. A) Schematic representation of the stress-relaxation test and the parameters comprised. B) Stress-relaxation experimental curves of avocado mesocarp slices on different ripening days.

Fig. 4. Evolution of textural parameters during storage: A) Hardness, defined as the maximum compression force (F_{max}, N); B) Elastic Modulus (E, MPa); C) Residual force at 60s (F_{60s}, N); D) Total Relaxation Capacity (TRC, dimensionless). Average values and standard deviation are plotted ($n = 60$). Different letters represent homogeneous groups established from LSD intervals with 95% confidence.

Fig. 5. Evolution of ultrasonic parameters during storage time. A) Maximum amplitude to thickness ratio (A_t, V/m); b) Ultrasound velocity (v, m/s). Average values and standard deviation are plotted ($n = 180$). Different letters represent homogeneous groups established from LSD intervals with 95% confidence.

Fig. 6. Correlation between ultrasonic and textural parameters over storage time: A_t (V/m) with F_{max} (N) (grey circles); and with F_{60s} (N) (black triangles). Solid markers represent mean values per storage day. Empty markers represent mean values per piece of avocado. Solid lines correspond to linear regressions considering mean values per avocado fruit.

Fig. 7. Correlation between ultrasonic and textural parameters over storage time: A_t (V/m) with E (MPa) (grey circles); and with TRC (black triangles). Solid markers represent mean values per storage day. Empty markers represent mean values per piece of avocado. Solid lines correspond to linear regressions considering mean values per avocado fruit.

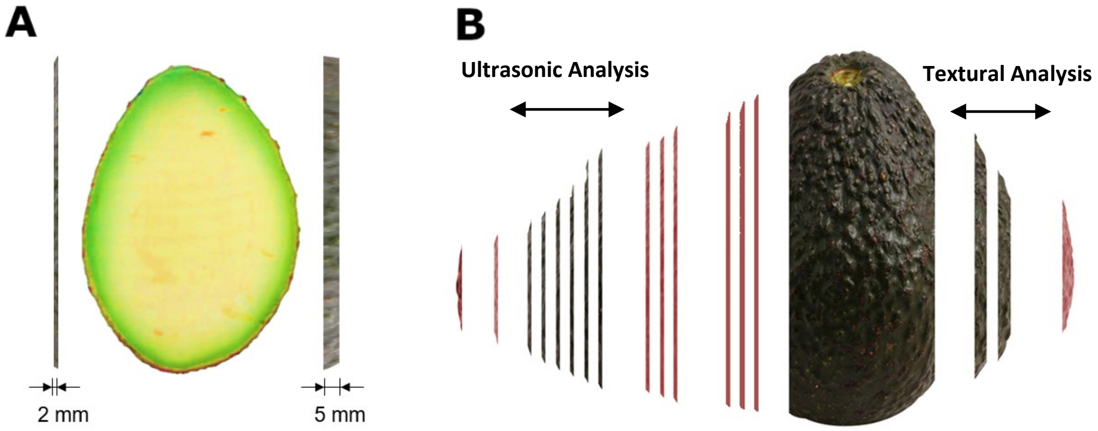
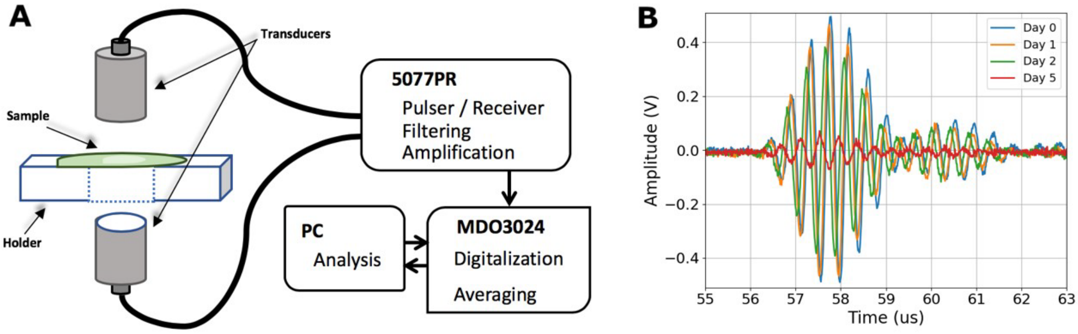
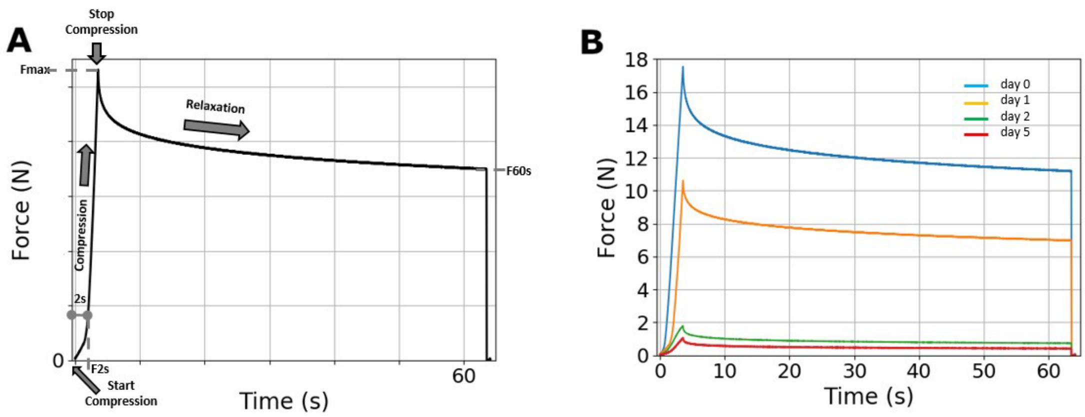


Figure 1



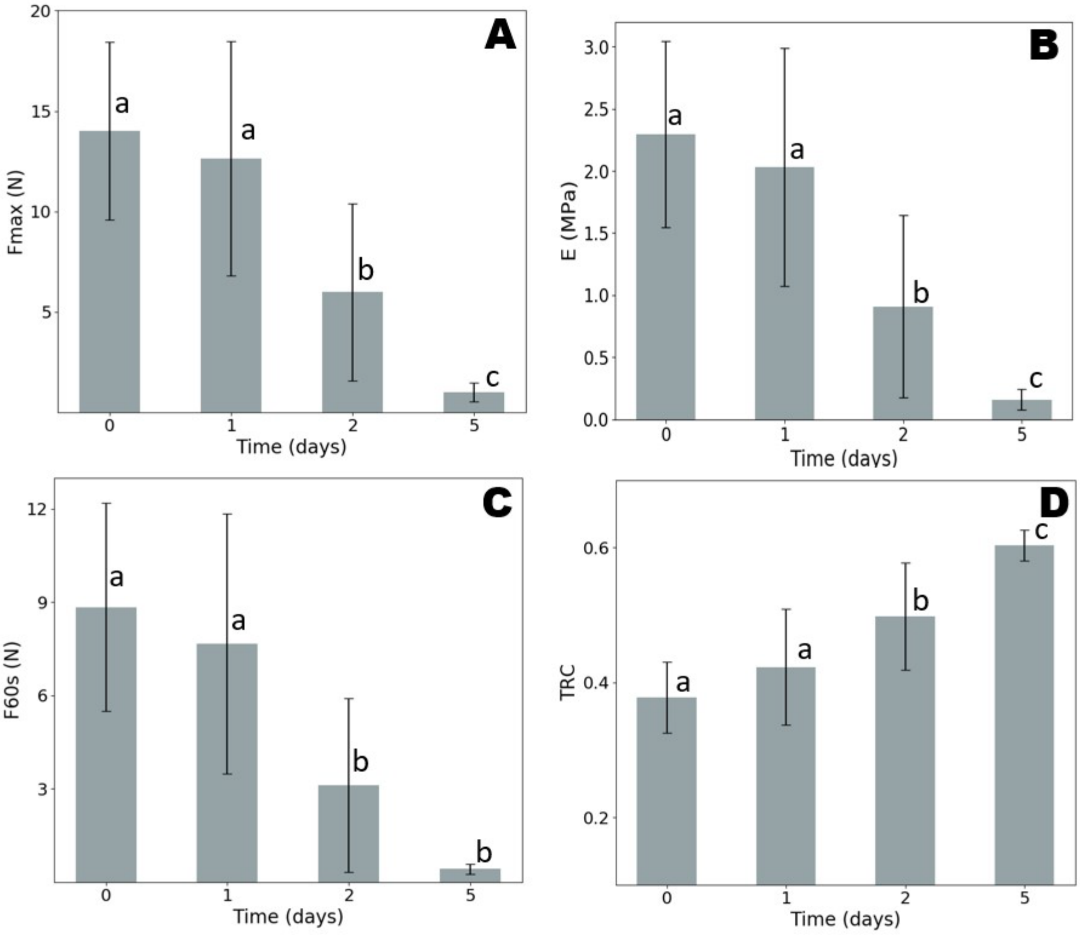
A
Transducers
Sample
Holder
5077PR
Pulser / Receiver
Filtering
Amplification
PC
Analysis
MDO3024
Digitalization
Averaging
B
Day 0
Day 1
Day 2
Day 5
Amplitude (V)
Time (us)

Figure 2



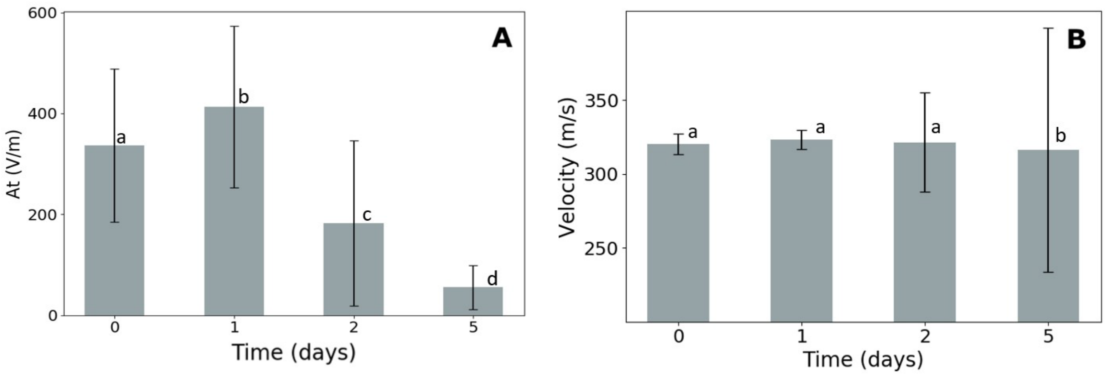
A
Stop
Compression
Fmax
Relaxation
Force (N)
Compression
F60s
2s
0
F2s
60
Start
Compression
Time (s)
B
18
16
14
12
10
8
6
4
2
0
day 0
day 1
day 2
day 5
Force (N)
0
10
20
30
40
50
60
Time (s)

Figure 3



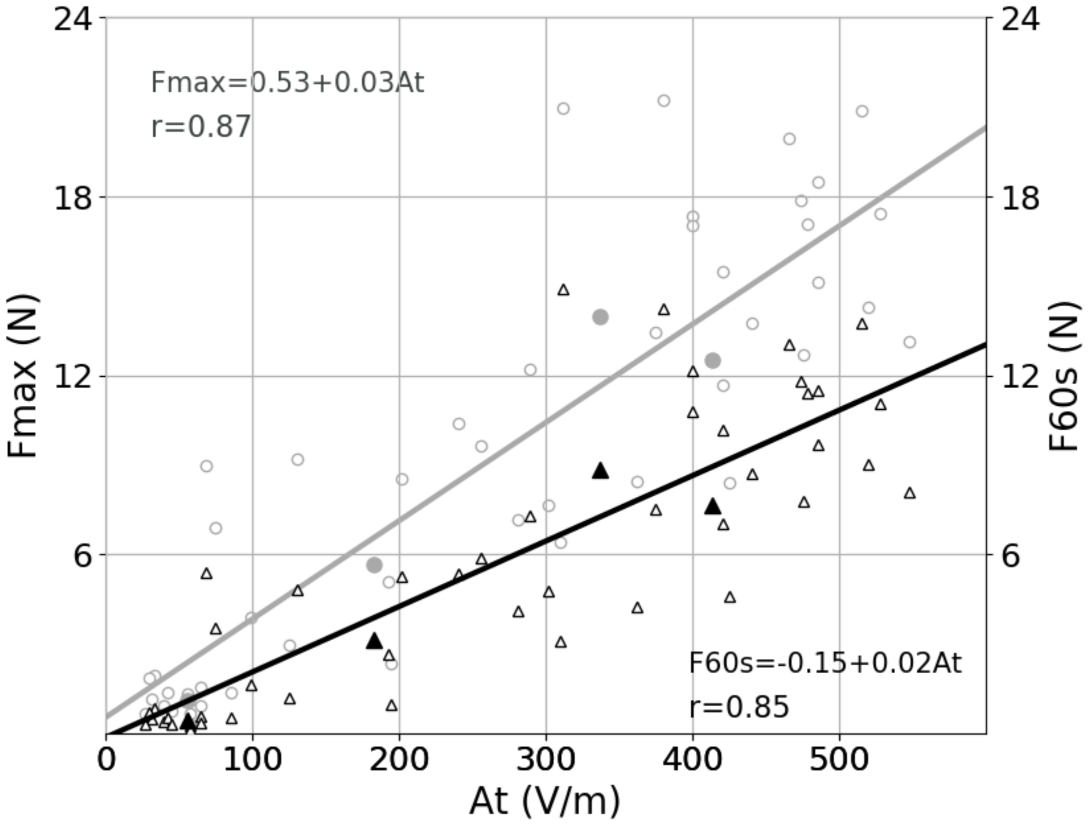
A
Fmax (N)
20
15
10
5
a
a
b
c
0
1
2
5
Time (days)
B
E (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
a
b
c
0
1
2
5
Time (days)
C
F60s (N)
12
9
6
3
a
a
b
b
0
1
2
5
Time (days)
D
TRC
0.6
0.4
0.2
a
a
b
c
0
1
2
5
Time (days)

Figure 4



A
600
400
200
0
At (V/m)
a
b
c
d
0
1
2
5
Time (days)
B
350
300
250
Velocity (m/s)
a
a
a
b
0
1
2
5
Time (days)

Figure 5



Fmax=0.53+0.03At
r=0.87
F60s=-0.15+0.02At
r=0.85
Fmax (N)
F60s (N)
At (V/m)
24
18
12
6
0
100
200
300
400
500

Figure 6

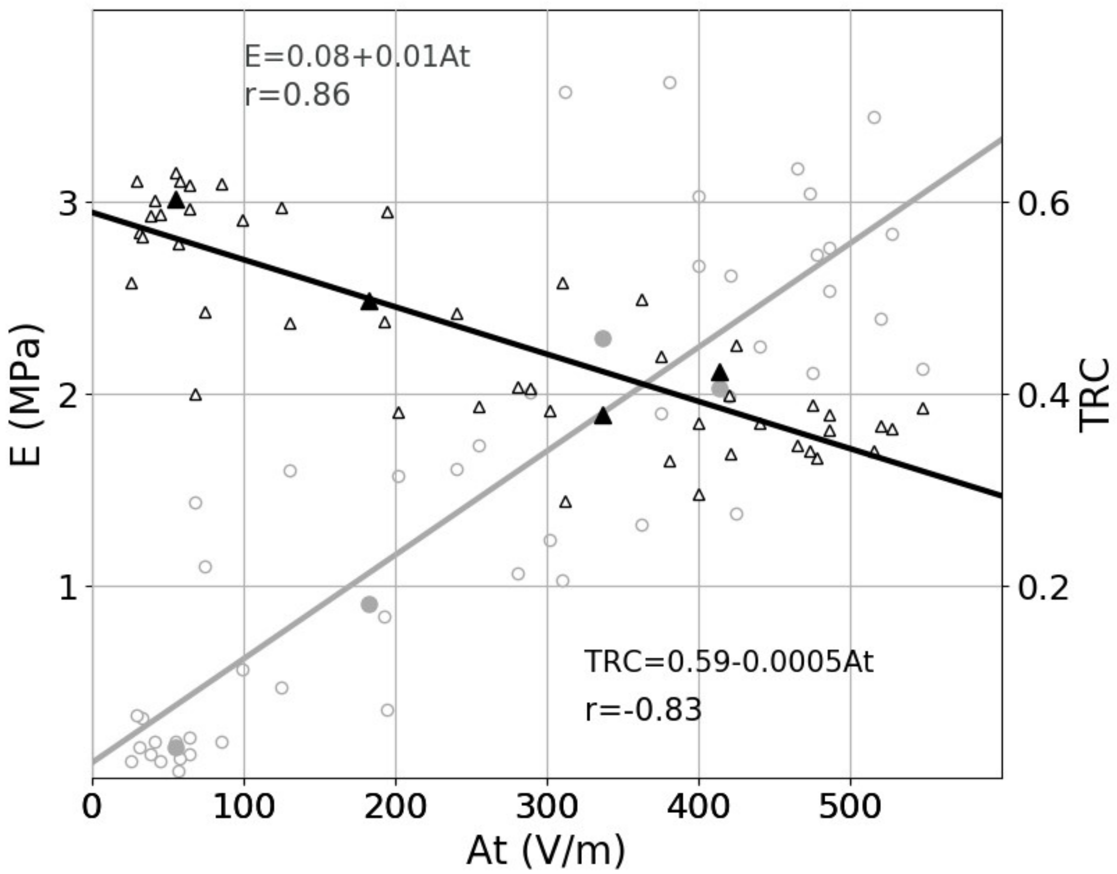

E=0.08+0.01At
r=0.86
TRC=0.59-0.0005At
r=-0.83
E (MPa)
TRC
At (V/m)
0
100
200
300
400
500
1
2
3
0.2
0.4
0.6

Figure 7