Instrumental acoustic-mechanical measures of texture in fresh-cut apples: influence of anti-browning dipping, modified atmosphere packaging and maturity degree, measured by time-resolved reflectance spectroscopy.

G. Cortellino[1], L. Piazza[2], L. Spinelli[3], A. Torricelli[4], A. Rizzolo[1]

[1] Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA), Unità di ricerca per i processi dell'industria agroalimentare (CREA-IAA), via Venezian 26, I-20133 Milan, Italy

[2] Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, via Celoria 2, I-20133 Milan, Italy

[3] Istituto di Fotonica e Nanotecnologie, CNR, piazza Leonardo da Vinci 32, I-20133 Milan, Italy

[4] Politecnico di Milano, Dipartimento di Fisica, piazza Leonardo da Vinci 32, I-20133 Milan, Italy

Corresponding author:

Giovanna Cortellino - Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA), Unità di ricerca per i processi dell'industria agroalimentare (CREA-IAA), via Venezian 26, I-20133 Milan, Italy

Phone: +39 -02-23 95 57 209

FAX: +39-02-23 65 377

E-mail: giovanna.cortellino@entecra.it

Abstract

Consumer acceptability of fresh-cut apples is affected by texture characteristics, mainly by crispness attribute, which can be dependent on fruit ripening. Reliable analytical tools to assess fruit crispness and maturity degree are the use of the combined instrumental acoustic-mechanical techniques and the measure of the absorption coefficient at 670 nm (μ_a670) by time-resolved reflectance spectroscopy (TRS), which has been shown to be an effective maturity index. The aim of this research was to evaluate the influence of maturity degree measured by TRS, modified atmosphere and anti-browning dipping on texture of fresh-cut apples by means of instrumental mechanical-acoustic test and rheological behavior of their cell wall material.

'Golden Delicious' apples were measured at harvest by TRS at 670 nm, ranked on the basis of decreasing μ_a670 (increasing maturity) and classified as less mature (LeM), medium mature and more mature (MoM). Then fruit were randomized in 20 batches of 12 fruit (LeM n=3; medium mature n=6; MoM n=3), each one used for one combination of packaging atmosphere, dipping treatment and storage time. LeM and MoM apples were peeled and cut into 8 wedges; half of them was dipped (2 min in 0.5 % ascorbic acid + 1 % citric acid solution) and the other was not dipped. All the samples were packed in polypropylene bowls using air or 80% Ar + 20% CO_2 (MA). Ethylene concentration and instrumental mechanical-acoustic characteristic were evaluated after 1, 4, 8, 11 and 15 days of refrigerated storage (4 °C). Rheological behavior of cell wall material was evaluated after 1 and 15 days. LeM and MoM samples showed a different ethylene evolution for fresh-cut apples packed in air, with MoM slices reaching the maximum ethylene production earlier than LeM ones. The mechanical-acoustic results allowed to evaluate that the maturity degree influenced distinctly the texture, being LeM apples firmer and stiffer producing also a higher number of acoustic events with more elevated maximum signal amplitude than MoM fruit, but did not affect their evolution during the 15 days storage at 4°C. Both dipping treatment and MA didn't affect texture in a well-delineated manner. The rheological behavior of cell wall material suggested an increase of elastic behavior with storage time, even if a clear influence of maturity degree, atmosphere and dipping was not found. In order to obtain a fresh-cut product with homogeneous texture characteristics, results suggested to process homogeneous maturity class of fruits, which could be efficiently selected by the absorption coefficient at 670 nm measured by TRS.

Key words: firmness, stiffness, number acoustic peaks, acoustic pressure peak, cell wall material.

1. Introduction

Consumers use textural properties as key indicators of food quality. One of the most studied textural property is crispness, a main attribute affecting consumer acceptability apart from taste, which is a desirable quality characteristic suggesting food freshness, the main evaluated and required property by the consumer at the time of purchase of fresh-cut products. Crispness has not only an hedonistic function, but it seems to have an important role for appetite psychology and satiety perception.

In the sixties for the first time Drake (1963) introduced the concept that auditory sensations are connected with texture perception. Texture is a quality attribute closely related to the structural properties of cellular tissue and the structure features have a large impact on the sounds produced when a food is bite. Products, such as apple, which contain a lot of fluid inside cells, are defined "wet crisp products", while products with a cellular structure containing only air are termed "dry crisp products". Wet cellular products are composed of turgid cells with elastic cell walls. The turgidity is created by the liquid inside the cell pressing on the cell wall, which opposes this force with strength and elasticity (Vickers and

Bourne, 1976). When the cells are broken, the contents expand rapidly when released, and a sound pressure wave is produced. This resulting sound is responsible for the perception of crispness. So a decrease in turgidity implies a decrease in crispness (Vickers and Bourne, 1976).

However crispness is not always connected to the turgidity pressure and force applied by wall cell and the loss of crispness during the ageing of an apple does not depend only on a significant humidity decline or to an evident histological modification. In fact the ageing process is combined with an increase of soluble pectin suggesting the progressive decay of substances which link together the cells. When a crisp apple is bitten, a sound is created by the break of middle lamella linking the cells together. When a not crisp and mealy apple is bitten, the weak sound derives from the cells separated each other. This phenomenon is due mainly to the pectin solubilization (Reeve, 1970) and to the resulting loss of force which connects the cells.

Drake (1963) firstly studied the food sound analyzing width and time of sounds produced during the chewing for a wide range of frequency. Vickers and Bourne (1976) were the first to postulate a psychoacoustical theory of crispness. The acoustic emission was described as an alternative analytical tool for the detection of apple crispness (Duizer, 2004). Sound measurement techniques, which are mainly destructive, consist in recording the sounds produced by instrumental compression probes or by the application of biting or chewing forces in the mouth.

Mealy and crisp “Cox’s Orange Pippin” apples could be distinguished by principal component analysis (PCA), applied to Fourier-transformed chewing sounds (De Belie et al., 2000). Other promising results were obtained by De Belie and Harker (2002) regarding the use of chewing sounds to assess small differences in crispness between ‘Royal Gala’ apples. Piazza and Giovenzana (2015) confirmed that a more reliable analytical way to assess fruit crispness is the use of the combined instrumental acoustic-mechanical techniques studying seven apples cultivars with different textural characteristics; they found that apples can be efficiently distinguished for crispness by means of coupled acoustic and mechanical texture analysis. Merging distinctive parameters taken from the mechanical signals and simultaneously recorded acoustic traces allowed apples to be clustered based on their crispness attributes using PCA (Piazza and Giovenzana, 2015). Time-resolved reflectance spectroscopy (TRS) is a non-destructive method for optical characterization of fruit. In TRS a short pulse of monochromatic light is injected within diffusive medium. Among its advantages compared with more traditional spectroscopic techniques, there is the feasibility to derive simultaneously two independent optical parameters, both being dependent on wavelength: the absorption of the light inside the irradiated body (μ_a, absorption coefficient), and the scattering of the photons across the tissue (μ'_s, transport scattering coefficient) (Torricelli et al., 2008). Light penetration achieved by TRS in most fruit and vegetables can be as great as 1–2 cm, depending on the optical properties (Cubeddu et al., 2001). Hence, TRS provides information on the internal properties of the medium and is not significantly affected by surface features (Saeys et al., 2008).

Chlorophyll absorbs light with a peak at 672 nm. It has been proved that the absorption coefficient measured at wavelengths near the chlorophyll peak (between 630 and 690 nm) is an effective maturity index for different fruit species, and it was used to group fruit in a batch into TRS maturity classes, ranging from less mature class (LeM) with higher μ_a values, to more mature class (MoM) having lower μ_a values. In 'Jonagored' apples harvested at two dates and classified at harvest by μ_a at 630 nm (μ_a630), the absorption coefficient μ_a630 was significantly higher in first harvest apples, indicating less mature fruit. Apples with higher μ_a630 had lower fruit mass and lower per cent blush. Fruits classified as more mature by TRS had less titratable acidity at harvest and more soluble solids after storage; at sensory analyses these fruits were significantly sweeter, more aromatic and pleasant (Vanoli et al., 2005). Furthermore, high μ_a630 fruit (less mature) had at harvest a less advanced breakdown of insoluble protopectins to soluble pectins, compared to the low μ_a630 ones (more mature) (Vanoli et al., 2009). In a study on 'Braeburn' and 'Cripps Pink' apples classified according to μ_a670 measured after six months of storage, a different evolution of the pulp mechanical properties with shelf life as a function of harvest date and TRS maturity class was reported (Vanoli et al., 2013). Less mature 'Braeburn' apples showed the highest values of firmness, stiffness and energy-to-rupture and softened during the shelf life for all the harvests, whereas more mature 'Braeburn' apples, characterized by lower values of pulp mechanical properties, softened during shelf life only in fruit of early harvest. In contrast, the classification of 'Cripps Pink' apples was not so effective and firmness decreased with shelf life only in less mature apples from early harvest (Vanoli et al., 2013). Moreover Zanella et al. (2013) reported that the relationship between μ_a and firmness is cultivar-specific, as they found a good correlation for 'Braeburn' apple, even if not sufficient for a reliable nondestructive firmness estimation, and no correlation for 'Cripps Pink' cultivar.

Rizzolo et al. (2011) firstly applied TRS to apples that are to be processed. This methodology might be used as a management tool in selecting apple fruit in order to produce rings with constant sensory characteristics. The classification of apples at harvest based on μ_a670 was able to segregate fruit generating air-dried rings of different quality. The differences found in raw material, influenced by TRS maturity class, affected the changes occurring in apple rings with air-drying, mainly influencing weight loss, area shrinkage and how much ring colour changed due to browning phenomena. For 'Golden Delicious' and 'Pink Lady®' cultivars, by processing the more mature fruits, i.e. either after long cold storage or by using apples having lower μ_a670 at harvest, air-dried rings with low shrinkage and low color changes (i.e. showing less browning) with lower ring hardness and crispness index were obtained (Rizzolo et al., 2012).

Recently Rizzolo et al. (2014) carried out an interesting approach combining the TRS technique with the coupled acoustic and mechanical texture analysis. The results indicated that using less mature apples based on μ_a670 measured at harvest by TRS, air-dried ring with higher porosity and higher average sound pressure level of peaks lower than 60 dB ($SPL_{av<60}$) could be produced, as well as osmo-air dried ring having a more connected solid structure, with lower tissue and pore degree of anisotropy, and defined less crispy by acoustic parameters (lower $SPL_{av>60}$ and lower average SPL of total sound peaks) than osmo-air-dried rings produced from more mature fruit.

The stress caused by technological steps involved in fresh-cut products processing, such as peeling and cutting, produces a physiological response with increased ethylene production and respiratory activity, with effects being observed very rapidly (Toivonen and Brummel, 2008). In climacteric fruit, wound-induced as well as exogenous ethylene may cause a hastening of ripening and softening (Toivonen and Brummel, 2008). The general effects of ethylene are usually detrimental to fruit quality (Saltveit, 1999); therefore, its concentration or activity should be minimized to lengthen product shelf life. As apple is a climacteric-type fruit, it results very sensitive to ethylene. It has been reported that low O_2 atmospheres and elevated CO_2 levels synergically act to reduce ethylene production and respiration rates (Soliva-Fortuny and Martín-Belloso, 2003a). There are very few studies on the effect of alternative modified atmosphere on the evolution of ethylene during cold storage; Cortellino *et al*. (2015) demonstrated that an Ar and CO_2 mixture (80**%** Ar + 20% CO_2) was able to completely inhibit ethylene accumulation for the whole cold storage period.

The present study aimed at evaluating the influence of maturity degree, modified atmosphere and anti-browning dipping on texture ageing kinetic of fresh-cut apples by means of instrumental mechanical-acoustic test. Moreover the rheological behavior of cell wall material isolated from the fruit was assessed in order to better understand the relationship between measured texture characteristics and the structure-forming properties of the cell wall materials (CWM) isolated from fruit.

2. Materials and methods

2.1. *Fruit and experimental plan*

The experiment was carried out on ’Golden Delicious‘ apples (*Malus x domestica,* Borskh.) picked at commercial ripening degree by the Società Cooperativa Agricola Melavì (Ponte di Valtellina, Italy).

At harvest, the absorption coefficient at 670 nm (μ_a) was measured by TRS on two opposite sides of each fruit (n=240). The average μ_a per fruit was used for apple ranking from less mature to more mature. The 240 ranked apples were grouped by 12, corresponding to 12 μ_a 670 levels from less mature to more mature, with a total of 20 groups. Fruits from each group were randomized in 20 samples of 12 fruit each one in order to have fruit from the whole range of μ_a 670 for every sample. In each sample only 6 fruit, characterized by the highest (3 fruits) and the lowest (3 fruits) μ_a 670, respectively the less mature (LeM maturity class) and the more mature (MoM maturity class), were processed in order to have two sets of apples with a well-defined and different maturity degree. Each 3-apple set (n=40) was used for one combination of maturity degree, packaging atmosphere, dipping treatment and storage time according to the experimental plan shown in Figure 1.

Apples were washed in tap water and dried with absorbant paper. Then they were manually peeled, cored and cut into 8 slices. This wedge cut allowed maximum use of the apple. Slices were immediately dipped in an antioxidant solution (fruit to antioxidant solution ratio 1:10) of ascorbic acid 0.5 % (w/w) and citric acid 1 % (w/w) for 2 min (Cortellino et al., 2015). The slices were drained and the excess water removed with absorbant paper. Half of the fruits were not dipped. All the samples (one apple/one bowl), dipped (dip) and undipped (nodip), were packed in polypropylene bowls (O_2TR: 0.31 $cm^3 pack^{-1} d^{-1} bar^{-1}$; CO_2TR: 4.9 $cm^3 pack^{-1} d^{-1} bar^{-1}$) and hermetically sealed with a film (O_2TR: 47.6 $cm^3 m^{-2} d^{-1} bar^{-1}$; CO_2TR: 217 $cm^3 m^{-2} d^{-1} bar^{-1}$) using a packaging machine Mod. TSM 95 (MINIPACK-TORRE, Dalmine, Bergamo, Italy) and stored at 4 ± 1 °C up to 15 days. Bowls, sanitized by UV radiation before packaging, were filled with air or 80 % Ar + 20 % CO_2 (MA), a commercial mixture purchased from Sapio S.r.l. (Monza, Italy). The ratio between the amount of product and the injected gas mixture was 1:2. At each storage time (1, 4, 8, 11 and 15 days), samples were analysed for ethylene headspace concentration and instrumental acoustic-mechanical measures, while after 1 and 15 days of storage CWM was prepared and analysed for rheological properties.

Hearafter slices of each atmosphere of the less mature TRS maturity class are referred to as Air_LeM and MA_LeM and those of the more mature TRS maturity class as Air_MoM and MA_MoM.

2.2. *TRS measurement*

For TRS measurement, a compact system was used, working at 670 nm, based on a pulsed laser diode (mod. PDL800, PicoQuant GmbH, Germany), with 80 MHz repetition frequency, 100 ps duration, and 1 mW average power, a compact photomultiplier (mod. R5900U-L16, Hamamatsu Photonics, Japan) and an integrated PC board (mod. SPC130, Becker&Hickl GmbH, Germany). Typical acquisition time for time-correlated single photon counting is 1 s per point. A couple of 1 mm plastic fibers (Mod. ESKA GK4001, Mitsubishi, Japan) delivers light into the sample and collects the emitted photons at a distance of 1.5 cm. A band pass filter turned at 670 nm was used to cut off the fluorescence signal due to chlorophyll. Overall, the instrumental response function duration was < 160 ps. The absorption coefficient (μ_a) was obtained by fitting the experimental TRS data with a standard solution of the diffusion approximation to the transport equation for a semi-infinite homogenous medium. The extrapolated boundary condition was used (Contini et al., 1997) to take into account the refractive index mismatch at the surface.

2.3. *Ethylene*

1 mL of the head space gas for each apple/bowl was sampled and analyzed for the ethylene content following the conditions reported by Rizzolo et al. (2005) using a deactivated aluminum oxide F1 (89-100 mesh) column ($^{1}/_{8}$ in. x 200 cm); column temperature 100°C, injector temperature 100°C and FID temperature 225°C. Quantitative data were obtained by relating ethylene peak area to that of a 10 μL L^{-1} standard and were expressed as μmoles per kilogram in standard condition; GC data were corrected for fruit mass and empty volume of the bowl.

2.4. *Instrumental mechanic-acoustic test*

Recording sounds and/or a stress/strain pattern produced during the application of a force to a noisy product is an experimental way to obtain quantitative information regarding crisp sounds and to predict the sensory sensation of crispness. In order to obtain instrumental texture parameters, a mechanical test was performed with the TA.XT. plus Texture Analyser (Stable Micro Systems, Godalming, UK), and the simultaneous acoustic emission was measured with the Acoustic Envelope Detector (AED) (Stable Micro Systems, Godalming, UK), which is combined with the dynamometer.

Regarding the mechanical puncture test, cylinders of apple flesh (17 mm diameter and 10 mm height) were cut with a metal borer. Two cylinders obtained from each slice were tested, so 16 data/apple were acquired. Puncture measurements were carried out using a 4 mm diameter probe, at the cross–head speed of 100 mm min^{-1} up to 90% penetration depth in the flesh. A load cell of 50 kg was used. The raw mechanical data were expressed as force (N) vs. strain (%). The cross-head speed is kept constant, and hence, it is possible to establish a relationship between strain (relative height variation of the sample due to compression) and testing time. From the recorded curves, mechanical and acoustic discrete parameters were extracted by means of the software Texture Exponent Exceed TEE32 (Stable Micro System, Godalming, UK). Force/displacement and sound/displacement curves were simultaneously plotted. From the force curve the following parameters were extracted: firmness corresponding to the maximum force (*firmness*, N) and stiffness corresponding to Young's modulus (*stiffness*, N mm^{-1}).

The sounds emitted during instrumental deformation of the samples were measured and the energy of the sound was calculated as the acoustic component. The acoustic profile is presented as a plot of sound pressure level (SPL) (dB) vs. strain (%), where the sound pressure level is defined as the ratio between the measured acoustic pressure level and the reference acoustic pressure level (corresponding to zero acoustic emission), on a logarithmic scale. The Acoustic Envelope Detector (AED) is an acoustic emission monitoring system consisting of an electro-acoustic transducer, a pre-amplifier, a signal conditioning system, and a data acquisition system. The AED operates in the frequency range of 3.125-12 KHz. Piazza and Giovenzana (2015) identify 8 KHz as the optimum frequency for the current method. The microphone (ECM-2 005, Monacor) was placed 20 mm far from the axis of the puncture probe and was positioned at mid-height of the cylindrical apple sample, in order to get a standardized acquisition of the acoustic signal. All tests were performed at room temperature in a laboratory with no special soundproof facilities. The acoustic profile was affected by ambient ground noise. The level sound (dB) able to distinguish the ground noise from the product sound was identified as 1.3 dB. From the sound trace the following discrete acoustic parameters were extracted: total number of the acoustic peaks (N_{sounds}, adimensional) and the maximum value among the acoustic pressure peaks (dB_{max}, dB).

2.5. *Preparation of cell wall material*

Cell wall material (CWM) was extracted according to Roversi and Piazza (2015). Ethanol, methanol, chloroform and pure acetone were of analytical grade and purchased from Sigma Aldrich. Twenty grams of apple flesh taken from the sealed packages, both at the beginning and at the end of storage (1 and 15 days), were boiled in a solution of 95 % (v/v) ethanol (150 mL) and distilled water (10 mL) for 20 min to inactivate potential wall-modifying enzymes. Then they were homogenized at top speed in a polytron (Brinkman Instruments) for 3 min and filtered under pressure by using glass microfiber filters (Whatman), pore size 125 μm mm. A solution of methanol-chloroform 1:1 was used to wash the resulting pellet until complete discoloration. The pellet was washed in acetone to remove water from cell wall material and finally dried at room temperature. In order to perform the rheological measurements, dried samples were rehydrated with deionized water at 20°C for 3 h (final concentration 2% w/w).

2.6 *Rheological measurements of CWM*

The linear viscoelastic properties of CWM were evaluated using a CMT (combined motor and transducer) rheometer (DHR-2, TA instruments). All measurements were performed using parallel geometry (40 mm diameter) at 23°C with a gap of 2 mm. A solvent trap provided by manufacture was used to prevent loss of solvent. First, oscillatory strain sweeps were performed to determine the linear viscoelastic region for each sample. Then, the dynamic moduli *G*' and *G*'' (Pa) were measured by means of an oscillatory sweep test at a strain of 0.1% (in linear viscoelastic region) in the frequency region of 0.01 – 200 rad s^{-1}. All measurements were performed in triplicate (n=3) to ensure data reproducibility. The results were expressed as tan δ, the ratio between *G*'' (loss modulus) and *G*' (elastic modulus) at the frequency of 100 rad s^{-1}.

2.7 *Statistical analysis*

Data of ethylene concentration and mechanical-acoustic parameters were submitted to multifactor analysis of variance considering packaging atmosphere, dipping treatment, storage time, TRS maturity class and their interactions as source of variation, and means were compared by Tukey's multiple range test to determine statistically significant differences ($P \leq 0.05$). The software used was Statgraphics version 7 (Manugistic Inc., Rockville MD, USA).

3. Results

3.1 *Absorption coefficient at harvest*

The absorption coefficient at 670 nm ranged from 0.302 cm^{-1} for the least mature fruit to 0.054 cm^{-1} for the most mature apple; the optical properties at harvest of LeM and MoM apples selected for this study were (average ± standard error): LeM maturity class: 0.162 ± 0.0042 cm^{-1} ; and MoM maturity class: 0.079 ± 0.0011 cm^{-1} .

3.2. *Multifactor analysis of variance*

To investigate the effect of packaging atmosphere (A), dipping antibrowning treatment (D), storage time (S) and TRS maturity degree (M) and their interaction on the data set of ethylene and mechanical-acoustic parameters, a multifactor analysis of variance was performed and the results are reported in Table 1. Except for the interaction AxDxM, which had no significant effect, all main factors and their interactions had a very strong significance (99.9%) on both mechanical parameters. Regarding the acoustic parameters, N_{sounds} was strongly influenced by all main factors and their interactions, but in a slightly lower way by the interaction AxDxS and DxSxM. Unlike, the other acoustic parameter dB_{max} was significantly affected by the four main factors, and by all the interactions, apart from AxDxS and AxSxM which had no significant effect, even if with different significance levels. On the other hand the multifactor analysis of variance showed that the ethylene headspace concentration was strongly affected only by the factors atmosphere and storage time and their interaction (AxS). Both dipping and maturity factors, as well as most of the interactions had no influence on this parameter. The overall multifactor analysis of variance data highlighted a wide and complex framework, not only due to the strong significant effect of the four single factors but also to the very high number of significant interactions.

3.2 *Ethylene*

The multifactor ANOVA pointed out that the dipping treatment main factor and its interactions with the other three factors had not significant influence on ethylene production (Table 1), so it was not considered in the data presented in Table 2. Ethylene headspace concentration was noticeably higher in apples packaged under air atmosphere. In contrast, the Ar+CO_2 mixture was able to completely inhibit ethylene accumulation for the whole storage period. No difference between TRS maturity classes were highlighted for MA samples. In contrast, both TRS maturity classes packaged under air showed a very similar trend till the fourth day of storage; then, during the following storage time, the behaviour of the two TRS maturity classes significantly differed: the more mature apples showed a maximum peak of ethylene concentration at 8 days of storage, whereas the less mature samples showed their highest level after 11 days. At the end of storage they had similar ethylene concentrations.

3.3 *Mechanical and acoustic parameters*

The complex data (Fig. 2) highlighted a negative trend linked to storage time for all mechanical and acoustic parameters. The results were characterized by very small values of standard error of the mean.

The firmness value ranged from 11.25 ± 0.009 N of the firmest sample (nodip Air_LeM after just one day) to 6.69 ± 0.001 N of the least firm one (nodip MA_MoM at the end of storage). As it was predictable, firmness values showed a decreasing trend throughout the storage time with a medium difference of 2.83 ± 0.77 N between the first and the fifteenth day. Both dip and nodip MA_MoM samples showed the highest decrease in firmness, while dip Air_LeM and nodip MA_LeM showed the lowest difference. The maturity degree remarkably influenced firmness of apple wedges: in fact most of LeM samples resulted firmer than the respective MoM samples. In detail, dip and nodip Air_LeM slices were firmer than dip and nodip Air_MoM ones just after processing (1 day) and showed a same decreasing trend with storage, while dip and nodip MA_LeM slices were as firm as dip and nodip MA_MoM ones until the fourth day of storage, but, prolonging the storage time, dip and nodip MA_LeM slices preserved better this texture parameter. The dip MA_LeM and MA_MoM and the nodip MA_MoM samples showed higher firmness than the respective packed in air just after processing, but successively the MA samples highlighted a stronger decreasing trend than air samples did, and consequently they reached the same or lower final firmness of air samples. Differently, the nodip Air_LeM was firmer than nodip MA_LeM at the beginning of storage, even if this difference was reduced throughout storage. Concerning the influence of antibrowning treatment, the samples packed in air and in modified atmosphere showed a different behaviour. Air samples not subjected to dipping step were firmer than the respective dipped ones both at the beginning and throughout storage, whereas the dipped samples packed in modified atmosphere highlighted a similar or higher firmness than the respective no-dipped ones.

The stiffness value ranged from 11.43 ± 0,015 N mm^{-1} of the stiffest sample (dip MA_MoM apple slices after just one day) to 6.36 ± 0.004 N mm^{-1} of the least stiff one (nodip MA_MoM at the end of storage). All samples showed a decreasing trend throughout storage with a medium reduction of 2.56 ± 0,77 N mm^{-1}. The two samples dip and nodip

MA_MoM characterized by the highest reduction of firmness presented also the highest decrease in stiffness, while the lowest reduction was observed for nodip MA_LeM. As it was described for firmness property, the maturity degree remarkably influenced also stiffness since LeM samples resulted characterized by higher values of this texture parameter than the respective MoM samples, except for MA samples at the first day of storage, for which LeM apple slices were equally or less stiff than the respective MoM ones. Nearly all samples packed under modified atmosphere showed higher values of stiffness than the respective air samples in the first part of storage, but successively they presented similar or even lower values than air samples, with the exception of nodip Air_LeM, which showed a stiffness value higher than the nodip MA_LeM apple slices. It was not possible to highlight a well-defined influence of the dipping step on stiffness property as some dip samples were stiffer, while others were less or equal stiff than the respective nodip ones.

The N_{sounds} value ranged from 29.67 ± 0.12 of the dip MA_LeM apple slices after just one day to 14.13 ± 0,04 of the dip MA_MoM at the end of storage. All samples showed a decreasing trend with storage time with a medium reduction of 7.72 ± 2.57. The highest decrease was presented by dip MA_MoM sample and the lowest by dip Air_LeM ones. The influence of maturity degree was noticeable also for N_{sounds} parameter. The most part of LeM samples produced a higher number of sound peaks than the respective MoM apple slices, apart from dip Air_MoM ones, for which the number of sound events was more elevated than that produced by dip Air_LeM sample till the fourth day of storage. Modified atmosphere had a negative influence on this acoustic parameter, as most MA samples emitted lower number of sound peaks than the respective air samples, except for dip Air_LeM and dip MA_LeM, which presented opposite results. The antibrowning dipping had a conflicting effect on the number of sound peaks: positively for Air_MoM, MA_LeM and MA_MoM slices only till the fourth day, but negatively for Air_LeM in the first part and MA_MoM in the second part of storage.

The maximum value of acoustic pressure level (dB_{max}) ranged from 66.06 ± 0.007 dB of dip MA_LeM apple slices after just one day to 59.83 ± 0,049 dB of nodip MA_MoM at the end of storage. All samples showed a decreasing trend throughout storage with a medium reduction of 3.47 ± 1.09 dB. The highest decrease was presented by nodip MA_MoM sample and the lowest by dip Air_LeM sample. The less mature apples produced peaks whose maximum level was mainly higher than those emitted by the more mature fruits, even though this happened only after the eight day of shelf life for dip Air_MoM and nodip MA_LeM samples. This parameter resulted affected by modified atmosphere, both positively in case of dip MA_LeM and dip MA_MoM samples and negatively for nodip Air_LeM and nodip Air_MoM ones. The dip samples had higher dB_{max} values than the respective nodip ones in most cases with the exception of nodip Air_LeM ones.

3.4 *CWM rheological properties*

Firstly *G*' (elastic modulus) resulted higher than *G*'' (loss modulus) in any case (Fig.3), as the aqueous suspension of cell wall material behaved as gel. The effect of maturity degree was controversial as, if dipping was applied, MoM apples after 1 day presented higher tanδ values than LeM ones, but if samples were not submitted to antibrowning treatment the results were opposite. At the beginning of the storage it was noticeable that packaging in modified atmosphere had a positive influence, forasmuch as all MA samples showed higher tanδ values than the respective air samples; this difference, even though slight, was maintained till the end of storage. Dipping had a positive effect in case of MoM apples but had no influence on LeM slices. For all samples the remarkably decrease of tanδ with the ageing of slices revealed an increase of the elastic performance. At the end of storage nodip Air_LeM and nodip Air_MoM samples highlighted a particular strong decrease of this rheological parameter.

4. Discussion

Most of the ethylene synthesis may come from tissue wounding due to technological steps as peeling and cutting. The Ar+CO_2 mixture was able to inhibit completely the ethylene release for the whole storage period as already reported by Cortellino et al. (2015). To our knowledge there are no other studies on the effect of alternative modified atmosphere on the evolution of ethylene during cold storage. These results confirm the inhibition of ethylene production under anaerobic or low O_2 conditions as observed by many authors (Soliva-Fortuny et al., 2002; Gil et al., 1998; Anese et al., 1997; Soliva-Fortuny et al., 2005; Rojas-Graü et al., 2007). It was also suggested that oxygen is involved in the conversion of 1-amino-cyclopropane-1-carboxylic acid (ACC) to ethylene (Yang, 1981). On the other hand the action of CO_2 is complex, since at low concentrations, carbon dioxide activates the biosynthesis of ethylene via the latter's role as co-factor for 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase (Smith and John, 1993), but at high concentrations, carbon dioxide may induce the activity of ACC oxidase while inhibiting its synthesis (Cheverry et al., 1988). The statistical approach pointed out that the dipping step in antibrowning agents, applied to preserve color, had not significant influence on ethylene production. This results was in accordance with Soliva-Fortuny *et al*. (2002) and Rojas-Graü *et al*. (2007) findings, demonstrating that dipping in ascorbic acid solution does not lead to evident changes in ethylene emission. By contrast Gil *et al*. (1998) found that this type of dipping reduced ethylene production only when air atmosphere packaging was used. Furthermore the maturity degree did not significantly influence the average amount of ethylene production, even though the LeM and MoM samples showed a different evolution of this compound in the headspace atmosphere of fresh-cut apples packed in air, with

MoM slices reaching the maximum ethylene production earlier than LeM ones, in agreement with Rizzolo et al. (2006) findings for ‘Golden Delicious’ apples during post-harvest shelf life, with fruit harvested later reaching the maximum rate of ethylene production sooner than fruit harvested one and two weeks earlier. However, our results on ethylene development may appear in contrast with previous observations by Cortellino et al. (2014) and Soliva-Fortuny et al (2002), who reported that fresh-cut apples, characterized by a different stage of ripeness, showed a diverse ethylene physiology with higher production in less ripe apples. The reason of this disagreement may be due to the fact that in Cortellino et al. (2014) and Soliva-Fortuny et al. (2002) works the less mature fruit corresponded to fruit processed at harvest, which is known producing higher ethylene than fruit after storage, and the more mature ones to those processed after storage, whereas in this research the LeM and MoM TRS maturity classes indicates a different biological age of fruit on the tree for fruit belonging to the same harvest time, which influenced ethylene production in the same way as different harvest times do.

For all the samples the mechanical and acoustical parameters decreased with the ageing time, as it was expected for apples that macroscopically become soft and mealy fruits. Firmness is determined largely by the physical anatomy of the tissue (cell size, shape and packing, cell wall thickness and strength), and the extent of cell-to-cell adhesion, together with turgor status (Toivonen and Brummel, 2008). The severity of wounding (peeling, coring and slicing) can be greater for climacteric fruit for which wound-induced ethylene promotes ripening and softening. Another factor involved in the decline of desirable texture is water loss, which leads to a decrease in turgor of the tissue cells. Previous results by Roversi and Piazza (2015) showed that water content of ‘Fuji’ apple slices decreased from 86.5% to 84% in 30 days of storage at 4°C. This is a non-physiological process associated with the postharvest dehydration of the fruit, and was enhanced with the increasing of storage temperature (Roversi and Piazza, 2015). The dehydration process is rapid in fresh-cut products due to the absence of cuticle and sub-epidermal layers and, hence, to the exposure of internal tissue. It causes a decrease in cell-to-cell adhesion; in wet conditions, the tissue is able to “take up” water and to maintain a turgid pressure inside the cells, which results in high tension of the cell wall and justify the higher elasticity of fresh apples (rigidity or stiffness, *d*F/*d*S) (Roversi and Piazza, 2015). As the turgor pressure declines, due to a decrease in available overall moisture, the plant tissue softens. Lin and Pitt (1986) and Niklas (1992) observed that cell tissue under high turgor fails by cell rupture, whereas tissue under low turgor fails by cell separation.

When the apple tissue deforms due to an instrumental test or a human biting, a sudden emission of acoustic energy happened. The consumer, hearing many of such small emission, interprets them as a crispy sound (Do Trong at al., 2014). The mechanical fracture and crack propagation properties of the flesh tissue, linked to the characteristics of the cellular structure in conjuction with turgor pressure, were responsible of the sensory perceived texture of the fruit. The recent technique to acquire quantitative information, in order to predict the sensory sensation of crispness is recording sounds and/or a stress/strain pattern produced during the application of a force (Herremans et al., 2014). Two main phenomena are possible to be highlighted by acoustic analysis: firstly the propagation of the sound and secondly cracking events generating the sound. As already reported by Zdunek et al (2010), the former is well connected to the signal amplitude (dB_{max}), whereas the latter is highly linked with the number of acoustic events (N_{sounds}). In the Roversi and Piazza (2015) experiment, dB_{max} data, after normalization for the experimental values of water content, indicated that this parameter is sensitive only to changes in the turgor pressure inside cells. Zdunek et al. (2010) previously identified the decrease in the turgor pressure as the major factor contributing to the attenuation of the sound elastic waves, thus weakening crack propagation in fruits under compression. The

same author suggested that N_{sounds} is sensitive not only to the turgor pressure changes but also to the structural modification occurring inside the cell wall.
The scenario in this work was very complex as suggested by the high significance of all factors and their interactions. The overall results revealed a significant influence of TRS maturity degree as LeM apples resulted firmer and stiffer producing also a higher number of acoustic events with more elevated maximum signal amplitude than MoM ones. The higher values of these parameters in LeM apples were observed just after 1 days of storage, but it was not possible to identify a clear difference in the decreasing trend throughout storage between the two TRS maturity classes. This fact suggests that the classification of fruit at harvest by TRS influenced the texture characteristics of raw fruits, but did not affect their evolution during the 15 days storage at 4°C, even if it was found that TRS maturity class influenced firmness and stiffness trends for apple fruit stored for much longer periods (till 5 months) (Vanoli et al., 2005, 2009).
The results of this study suggest that modified atmosphere packaging was not able to preserve the texture of fresh cut apples better than air packaging, as MA samples were firmer and stiffer at the beginning of storage, but less firm and stiff than air samples at the end of storage. This was confirmed also by acoustical results, as Ar+CO_2 mixture packaging influenced negatively not only the number of sound emission of all samples for the whole storage time by reduction of the number of sound events, but also the maximum value of peaks in the samples not submitted to the antibrowning treatment, by lowering the dB_{max} value. This result was not in accordance with previous findings (Cortellino et al., 2015), showing that Ar+CO_2 combination positively and significantly influenced the firmness parameter during the whole storage time, even if the beneficial effect of MA packaging was partially nullified by the dipping treatment. Indeed, the adverse influence of MA packaging on apple slice firmness was not expected as many studies have demonstrated that the decrease of apple firmness during storage time is strongly

dependent on the availability of oxygen given mainly by packaging atmosphere. Soliva-Fortuny tested the packaging in 100% N_2 combined with bags of low oxygen permeability, with effective results in preserving apple softening till 21 (Soliva-Fortuny *et al*., 2005) and 60 (Soliva-Fortuny *et al*., 2002; Soliva-Fortuny et al., 2003b) days of storage. Cortellino et al. (2015) also proved that conventional gas mixture without or with low oxygen level (99% N_2 + 1% O_2 and 90% N_2 + 5% CO_2 + 5% O_2) preserved better this important quality parameter than the traditional air, even though the difference was not always statistically significant, provided that dipping was not applied. The outcome of the present study did not confirm the relationship between ethylene production and retention of firmness during the shelf life. The incongruity among our results and those by others research groups could be reasonably due to the different length of storage (15 days *vs* 60 days).

In disagreement with the expectations, the dipping step did not negatively influence the texture properties of apple slices; in fact, stiffness and N_{sounds} did not seem to be affected by dipping, meanwhile dB_{max} of dipped samples resulted higher than the respective no dipped ones and the firmness of dipped samples packed in air resulted higher than the respective no dipped. In point of fact various authors found the neutralizing effect on the apple tissue preservation from softening by MA packaging of the dipping treatment in ascorbic and citric acids solution, causing a structural breakdown of fruit tissue (Cortellino et al., 2015; Cocci et al.,2006; Rojas-Graü et al., 2007; Ponting et al., 1972).

Physical and chemical changes may affect textural integrity, but also the enzymatic hydrolysis of cell wall pectic substances, resulting in a loss of firmness (Varoquaux et al., 1990). It has been reported that varoius factors may also affected tissue softening, such as hydrolysis of protopectins to water soluble pectins, decrease in cellulose crystallinity, thinning of cell walls, diffusion of sugar to the intercellular spaces and ion movement from the cell wall (Toivonen and Brummel, 2008). From a biochemical point of view, texture changes are supposed to mainly be dependent on the action of pectic enzymes, especially polygalacturonases (Knee, 1973), which degrades polygalacturonan, present in the cell walls of fruits, by hydrolysis of the glycosidic bonds linking galacturonic acid residues. The decrease trend of Tan δ values was presumably due to the degradation of protopectins in pectic acid by pectinesterase enzyme. Successively the pectic acid, as polymer of galacturonic acid, was hydrolyzed and made soluble by polygalacturonases, with a consequent increase of the hydration property of CWM network. The decrease of Tan δ values by the ageing time, suggesting an increase of elastic behaviour, was in accordance with the increase of stiffness parameter. Furthermore also TRS maturity class influenced the polyuronide pattern (Vanoli et al., 2006; Vanoli et al., 2009). 'Jonagored' apple fruit of different TRS maturity class had a different polyuronide content, even if their firmness was not different, with LeM fruit showing at harvest higher total galacturonic acid content, residue insoluble pectin and protopectin index, and lower galacturonic acid content in oxalate-soluble pectin fraction than MoM fruit, indicating a less advanced breakdown of insoluble protopectins to soluble pectins.

5. Conclusion

The acoustical approach revealed a useful technique to complete mechanical results in order to monitor the texture, specifically crispness, of apple slices during shelf life. Consequently the comprehensive results allowed to evaluate better the influence of each factor involved in the processing and storage, such as maturity degree, dipping and modified atmosphere, on the texture parameters of fresh-cut apples. Overall data revealed that only the maturity degree influenced distinctly the texture, meanwhile both dipping and modified atmosphere didn't affect it in a well-delineated manner. The 15 days storage time at 4°C used in this work, even though being the actual maximum storage time applied for fresh-cut apples in the market, was definitely shorter than those considered in other studies (till 60 days), and it probably did not allow to highlight the influence of modified atmosphere, by stopping ethylene production, on texture quality. The results suggested that it would be desirable processing raw materials previously selected according to maturity class in order to obtain a fresh-cut product with homogeneous texture characteristics. So the methodology based on the absorption coefficient at 670 nm measured by TRS at harvest might be used as a management tool in selecting raw apple fruit intended to be processed as fresh-cut product.

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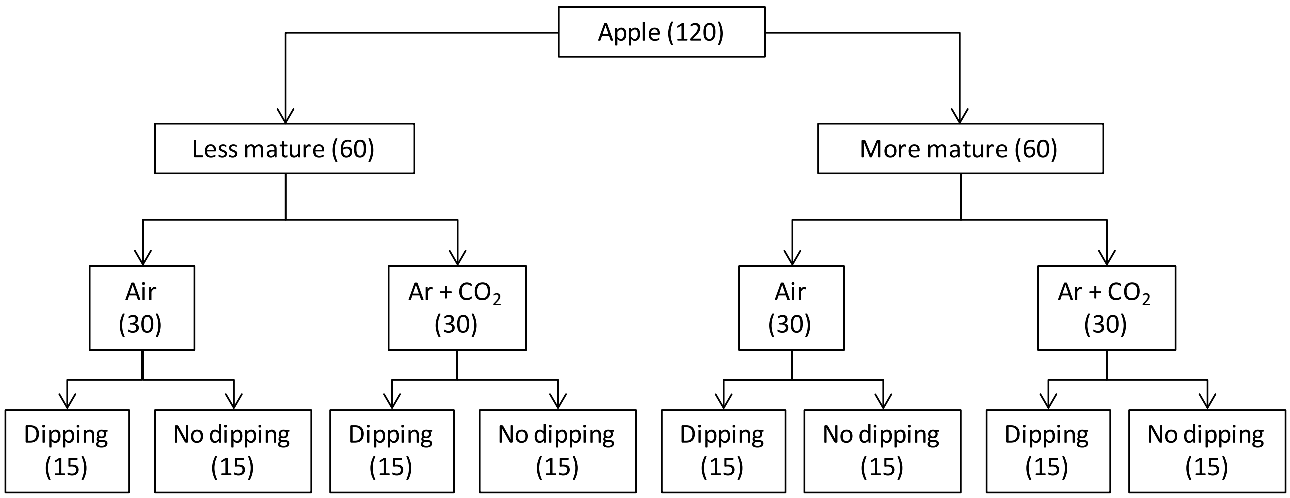
List of Figures

Figure 1. Experimental plan. In brackets the number of processed fruits.

Figure 2. Firmness (N), stiffness (N mm^{-1}), total number of the acoustic peaks (N_{sounds}, adimensional) and the maximum value among the acoustic pressure peaks (dB_{max}, dB) (mean ± standard error) of apple slices processed at two different maturity degree (LeM and MoM), submitted or not to dipping treatment (dip and nodip) and packed in air and in modified atmosphere (MA).

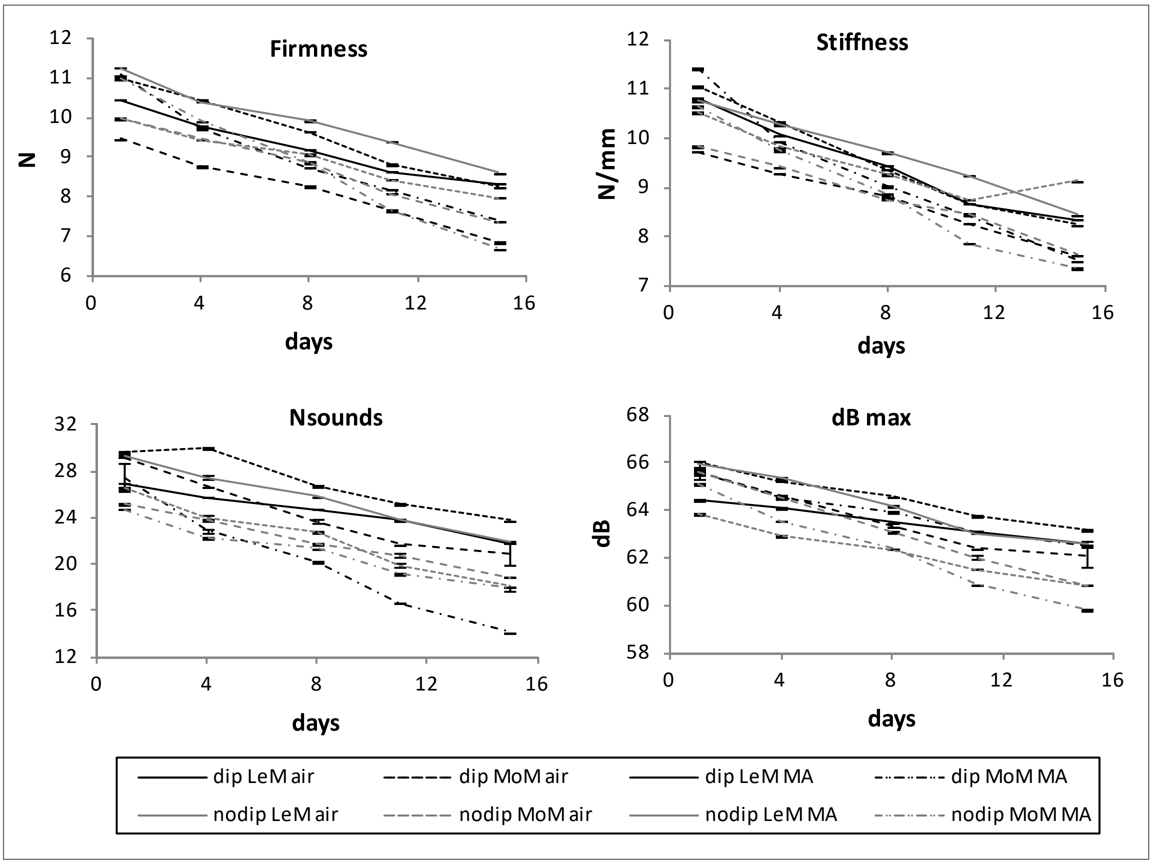
Figure 3. Tan δ values at frequency of 100 rad/s of CWM isolated from apple slices coming from fruits processed at two different maturity degree (LeM-left and MoM-right), submitted or not to dipping treatment, packed in air or in modified atmosphere (MA) after 1 and 15 days of storage. Mean ± standard deviation.

Fig 1



Apple (120)
Less mature (60)
More mature (60)
Air
(30)
Ar + CO_2
(30)
Air
(30)
Ar + CO_2
(30)
Dipping
(15)
No dipping
(15)
Dipping
(15)
No dipping
(15)
Dipping
(15)
No dipping
(15)
Dipping
(15)
No dipping
(15)

Fig 2



Firmness
N
days
Stiffness
N/mm
Nsounds
dB max
dB
dip LeM air
dip MoM air
dip LeM MA
dip MoM MA
nodip LeM air
nodip MoM air
nodip LeM MA
nodip MoM MA

Fig 3

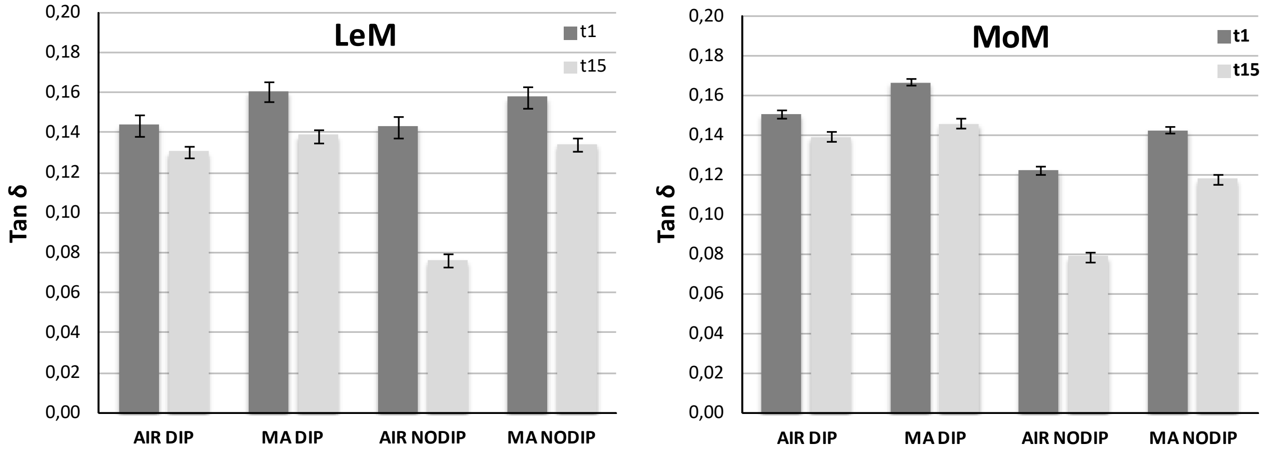

LeM
t1
t15
Tan δ
0,00
0,02
0,04
0,06
0,08
0,10
0,12
0,14
0,16
0,18
0,20
AIR DIP
MA DIP
AIR NODIP
MA NODIP
MoM
t1
t15
Tan δ
AIR DIP
MA DIP
AIR NODIP
MA NODIP

Table 1 Significative influence (* P<0.05; ** P<0.01; *** P<0.001) of the main factors TRS maturity class, dipping treatment, atmosphere and storage time and their interactions on ethylene concentration and mechanical-acoustic parameters of apple slices.

	Mechanical parameters		Acoustic parameters		
	Firmness	Stiffness	N_{sounds}	dB max	Ethylene
Main effects					
Atmosphere	***	***	***	***	***
Dipping	***	***	***	***	ns
Storage	***	***	***	***	***
Maturity	***	***	***	***	ns
Interaction					
AxD	***	***	***	***	ns
AxS	***	***	***	**	***
AxM	***	***	***	**	ns
DxS	***	***	***	***	ns
DxM	***	***	***	**	ns
SxM	***	***	***	***	*
AxDxS	***	***	**	ns	ns
AxDxM	***	ns	***	***	ns
AxSxM	***	***	***	ns	*
DxSxM	***	***	**	***	ns
AxDxSxM	***	***	***	***	ns

Table 2. Ethylene concentration (μmol kg^{-1}) in packages of apple slices processed at two different maturity degree (LeM and MoM) and packed in air and in modified atmosphere (Ar + CO_2). Different letters correspond to a significant difference ($P \leq 0.05$).

	air		$Ar+CO_2$	
Storage	LeM	MoM	LeM	MoM
1	25.9 ± 1.6^{d}	27.9 ± 1.0^{d}	11.4 ± 2.3^{d}	9.6 ± 1.6^{d}
4	72.0 ± 3.4^{bc}	68.4 ± 3.0^{c}	7.8 ± 1.0^{d}	9.2 ± 0.7^{d}
8	97.7 ± 12.8^{abc}	126.0 ± 9.3^{a}	8.3 ± 1.2^{d}	8.9 ± 1.1^{d}
11	120.2 ± 2.7^{a}	99.5 ± 4.8^{ab}	8.5 ± 0.4^{d}	10.2 ± 2.0^{d}
15	111.3 ± 10.9^{a}	120.8 ± 14.5^{a}	6.9 ± 1.1^{d}	8.2 ± 0.6^{d}