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# Fresh-cut melon quality during storage: An NMR study of water transverse relaxation time

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ABSTRACT

Molecular mobility is a fundamental parameter which reflects the dynamic properties of food components and contributes to food degradation reactions comprehension. Fresh-cut fruits have become an important food market segment. However, processing of fruits promotes faster its physiological deterioration, biochemical changes and microbial degradation. The purpose of this work was to use NMR methodology as a tool to evaluate fresh-cut fruit quality, during storage at refrigerated conditions. The fresh-cut melon transverse relaxation time ($T_2$) was measured for a period of 7 days of storage at 5 °C. The relationship between the obtained values, microstructure and quality parameters was investigated. In general, results show the existence of one class of water fluidity in the system, the one present in cells after processing. $T_2$, a measure of this fluidity, is affected by the processing and storage time. Also, it is possible to find a close relationships between $T_2$ and quality parameters of total colour difference (TCD), firmness and $a_w$. As $T_2$ increases TCD also increases, while firmness and $a_w$ decrease. These results highlight the usefulness of NMR methodology application in food science.



## 1. Introduction

Stability of biological systems, including foods, depends strongly on molecular mobility (Roudaut et al., 2004) and water "availability". This availability is a manifestation of how freely water molecules can participate in reactions, namely degradation reactions (Ruan and Chen, 1998). Understanding, the integral concept of changes in location and mobility of water is particularly important considering that water molecular mobility profoundly influences the chemical, physical, and microbiological quality of foods (Vittadini et al., 2003).

Water activity has been recognised, for a long time, as a primary guideline for safety and quality control of foods (Labuza, 1977). However, the limitation of this measurement has been expressed (Hills et al., 1996; Mathlouthi, 2001; Slade and Levine, 1991), since it is based on the assumption that foods are in its equilibrium state, being indifferent to the solute–solute and solute–water interactions, factors that have deep impact on food system's reaction kinetics (Mathlouthi, 2001; Ruan and Chen, 1998).

In recent years, nuclear magnetic resonance spectroscopy has evolved to become a powerful tool to probe the structure and dynamics of food constituents in solid state. Specifically, $^1$H NMR has been used to investigate water dynamics and physical structure of foods through analysis of nuclear magnetisation relaxation times (Fundo et al., 2014; Li et al., 2000). In these measurements, the samples are submitted to a static magnetic field and the protons are excited by means of a radiofrequency pulse. The analysis of the signal emitted while the samples return to equilibrium (FID) allows the determination of the spin–lattice or longitudinal ($T_1$), and spin–spin or transverse ($T_2$) relaxation times. The latter is related with the mobility of the protons in the sample matrix (Fundo et al., 2014). This methodology has been applied in complex food systems such as crackers (Yan et al., 1996), wheat starch (Choi and Kerr, 2003), chicken meat (Li et al., 2000), carrots (Rutledge, 2001), kiwi fruit (Tylewicz et al., 2011) and even model bread crust (Chen et al., 1997).

Foods and biological materials consist largely of water and macromolecules rich in protons and, since water protons are major contributors to the proton relaxation, the interactions between water and macromolecules represent the most important factors affecting the proton relaxation process (Ludescher et al., 2001).

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Thus, the application of NMR technique may be very useful in predicting physicochemical changes and understanding structures and dynamics of complex macromolecular systems (as foods) (Domjan et al., 2009) in solutions and/or in solid state. Moreover, this could be an interesting technique to evaluate food quality during storage period, since degradation reactions, water interactions, structure and chemical compounds changes result in altered NMR properties (Ludescher et al., 2001).

Minimally processed fruit has become an important market segment due to the increasing demand for fresh, healthy and convenient foods (Rico et al., 2007). However, it is well known that processing fruits enhances physiological and biochemical changes, and microbial degradation, which result in degradation of fruit colour and texture. Wounding damage is sensed by sensors/receptors that recognise increased concentrations of endogenous molecules or their fragments, and tissue responds producing ethylene (Amaro, 2012; Heil, 2012), increasing respiration rate and membrane deterioration (Toivonen and Brummel, 2008; Watada and Qi, 1999).

Melon is one of the most important fruits in the world of fresh-cut fruit market (Aguayo et al., 2004). Fresh-cut melon degradation during storage may be characterised by many physical and chemical parameters, such as changes in colour, firmness, aroma (Oms-Oliu et al., 2008) and water activity. Due to processing operations, a great number of cells are disrupted, which induces the release of enzymes and their substrates and promotes oxidative enzyme-catalysed processes, resulting in accelerated quality losses.

The purpose of this work was to utilise NMR parameters to characterise fresh-cut melon along refrigerated storage and relate NMR measurements with some of its most important quality parameters, i.e. colour and softening rate.

## 2. Materials and methods

### 2.1. Fruit material, processing, packaging and storage conditions

*'Piel de Sapo'* melons were obtained at a local supermarket, at commercial maturity stage. In order to characterise the fruit initial maturity state, soluble solids content was determined using a refractometer method and ranged between 8.6 and 10.6°Brix (Amaro, 2012; Simandjuntak et al., 1996). Fruits were carefully inspected for bruising and compression damage and only those without visual defects and uniform in shape and size were selected for processing and analysis.

Melons were washed in running cold water, dipped in 100 μg $L^{-1}$ hypochlorite solution for 2 min, rinsed with deionised water and allowed to drain. The rind was removed with a sharp stainless steel knife, the blossom and stem ends were discarded, placental tissue and seeds were removed, and the mesocarp was prepared in cubes of ~2.5 $cm^3$. All cutting tools and containers were sanitised with 70% ethanol and allowed to dry before usage. Fresh-cut melon cubes were randomly placed in vented polypropylene clamshells (~175 g) and stored at 5 °C for maximum 7 days. To avoid the accumulation of ethylene and carbon dioxide inside the packages (Vilas-Boas and Kader, 2007), clamshells were perforated with single 6 mm vents. Samples were analysed at days 0, 1, 3, 4, 5 and 7 after cutting preparation.

### 2.2. Transverse relaxation times measurement

A Bruker AVANCE III solid state NMR spectrometer (300 MHz for proton) was used to determine the samples transverse or spin–spin relaxation times, $T_2$. The transverse relaxation time was obtained with a Carr–Prucell–Meiboom–Gill (CPMG) pulse sequence with a 90–180° pulse spacing of 500 ms and a repetition time of 15 s. The magnetisation was recorded after 18 echos arrays, with the precaution that the number of echos always permits to define an exponential decay for the magnetisation. The samples were cut in small cylinders, 1.5 cm high, and placed in a 5 mm standard NMR tube, for the $T_2$ measurement. It is clear that the relaxation time measurements are not spatial resolved, but subcellular water compartmentation can be monitored (Hills and Remigereau, 1997).

For each day 0, 1, 3, 4, 5 and 7, after cutting, three samples were studied for their transverse relaxation time evaluation.

### 2.3. Microscope techniques

Optical and scanning electron microscopes were used to observe any microstructural changes that occur in melon during storage. Hence, at each storage time (0, 2, 4 and 6 days), a thin surface layer of the fruits tissue was removed, at 3 different sections and in duplicate, for both type of microscopy techniques.

For the optical microscopy, the fruit sample was emerged to staining, in a solution of toluidine blue O at 0.5% (w/v), during 2 h. The stain solution was disposed, and the fruits stained portions were washed in ethanol at 97%, during 10 s, and then washed with water during 30 s. Then the stained tissues were dried in microscope slides in a desiccator, during 24 h. After this time, visualisation was made using a final magnification of 400 times.

Typical fixation of the material for SEM investigations involves dehydration, which can remove or alter lipids that form the wax coating on the fruit surface, and a critical point of drying is that it can shrink and destroy tissues. Therefore, a modified and simplified methodology was used in order to prevent destruction of the epicuticular wax. The cut samples were wiped with a paper towel, carefully mounted onto stubs and examined under a JEOL-5600 Lv microscope (Tokyo, Japan), operated under low vacuum mode, using a spot size of 30 and a potential of 10–15 kV. All analyses were performed at room temperature (20 °C). The visualisation was made using a magnification of 200 times.

For both techniques, photos were obtained of three sections cut from each fruit.

### 2.4. Measuring quality parameters

The fruit quality parameters evaluated were total colour difference (TCD), softening rate and water activity.

Colour of the fresh-cut melon surface was measured in the CIE $L^*a^*b^*$ colour space with a Konica-Minolta CR-400 chromameter (Osaka, Japan) equipped with a $D_{65}$ illuminant and the 2° observer for colour interpretation. $L^*$ ranges from 0 (black) to 100 (white), $a^*$ indicates the degree of greenness (for negative values) to redness (for positive values), and axis $b^*$ also ranges from negative to positive values indicating, respectively, degree of blueness to yellowness. $L_0^*$, $a_0^*$ and $b_0^*$ were evaluated from freshly cut fruit (time 0). Colour changes were assessed using TCD, calculated through the formula $TCD = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$.

Measurements (five cubes from each three replicate clamshells) were carried out. One measurement was made in each five cubes from three duplicated clamshells per replicate.

Firmness was measured using a TA-XT2 Plus texture analyser (Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell. The force to drive a cylindrical probe with 5 mm diameter to perforate 5 mm into the tissue at a speed of 1.5 mm $s^{-1}$ was recorded (Amaro et al., 2013). One measurement was taken on the lateral surfaces of each five cubes from three clamshells, respecting to one replicate, from a total of two true replicates, for each sampling day.

Water activity was measured using a dew point hygrometer (Aqualab Series 3, Decagon Devices Inc., Pullman, WA, USA).

Three measurements were done for each true replicate and for each sampling day.

The quality data was subjected to statistical analysis performed using the software packages STASTICS© 6.0 (StatSoft, Tulsa, OK). An individual package constituted an experimental unit which was used as one replicate on each sampling day. Three replicated packages were analysed. The experiment trial was carried out twice.

## 3. Results and discussion

Maturity stage is an important factor that may affect the intensity of wound response in fresh-cut tissues (Beaulieu and Lea, 2007; Watada and Qi, 1999). These variances in samples maturity stage contribute, along with the natural variability between complex biological systems, for the differences obtained between the three replicates. To characterise the melon maturity stage, the soluble solids content (SSC) was measured. The initial SSC ranged between 8.6 and 10.6°Brix, with no significant differences observed throughout storage. Melon SSC undergoes minor changes during postharvest storage of whole or fresh-cut fruit (Portela and Cantwell, 1998).

### 3.1. Transverse relaxation times

Spin–spin relaxation time ($T_2$) of the samples was obtained with the purpose of evaluating water molecules dynamics and environment during the fruit storage degradation process. The CPMG data were analysed as a continuous distribution of exponential relaxation times with CONTIN program (Provencher, 1982), and the result is presented in Fig. 1.

It is clear from Fig. 1 that all values of $T_2$ from about 50 ms to very high values (<10 s) are presented in the samples, with only one pronounced maximum. In the first 24 h after processing, the maximum amplitude $T_2$ value evolves from 329 to 285 ms. At the third day of storage, the maximum amplitude $T_2$ value is 382 ms and after that remains unchanged at 442 ms until day 7.

Comparing Fig. 1 with the results on whole apples, kiwifruits and pears, reported elsewhere (Hernández-Sánchez et al., 2007; Hills and Remigereau, 1997; Tylewicz et al., 2011), where pools of water are attributed to vacuole, cytoplasm and cellular wall, in our experiment only one peak for water relaxation times was detected, probably due to loss of cellular compartmentation, as a consequence of wounding.

Regarding the maximum amplitude, $T_2$ value shifts to shorter values in the first 24 h after processing. Literature reports that after wounding there is major tissue disruption, whereby enzymes and substrates sequestered in different organelles come into contact (Beaulieu and Gorny, 2001) and signalling-induced wound responses are initiated with microbiological, enzymatic, and physicochemical reactions simultaneously taking place, decreasing water availability (Artés et al., 2007) possibility of an increase in 'water binding' (Chen et al., 1997), as the subcellular structures are disrupted and the release of solutes that were retained in the organelles occurs, with the association of these solutes with water through hydrogen bonds. After this 24 h period, where metabolic rate is elevated, water is continuously released from the physical barriers in the system and the observed $T_2$ is more often at values closer to the free water $T_2$. By day 4 of storage, $T_2$ maximum amplitude occurs for the highest $T_2$ value and remained constant until the end of storage. This may indicate that by day 4 of storage, cells reach a threshold where metabolic rate is decreased, wounding reactions are diminished along with increased membrane degradation and turgor loss. These alterations lead to a higher water transverse relaxation time expression in cells. This interpretation is according with quality changes data obtained and discussed in point 3.3.

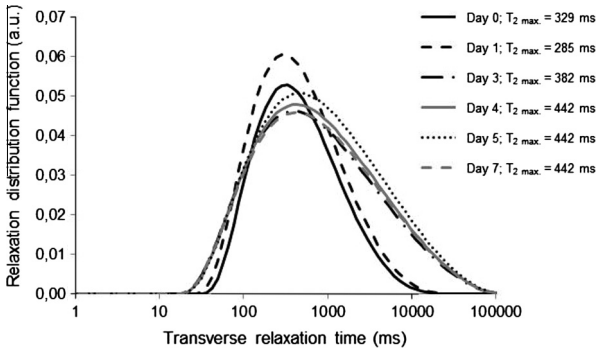


**Fig. 1.** Distribution of transverse water proton relaxation times ($T_2$) in fresh-cut melon measured at 300 MHz and room temperature.

The maximum amplitude $T_2$ value evolving to higher $T_2$ values with storage time can be interpreted as the enhanced range of water relaxation time detected and attributed to cell structure disorder due to the occurrence of membrane rupture and plasmolise (Toivonen and Brummel, 2008).

Once this water proton behaviour indicates an alteration in cellular structure and also in water solute bonds, which can be associated with fresh-cut fruits quality loss during the storage period. This relation will be explored in the following sections.

### 3.2. Microstructure analysis

The use of two different techniques allows obtaining complementary results: with light microscope (LM) it is possible to get a qualitative description of the samples structure, while scanning electron microscope (SEM) is used to examine surfaces, with an improved resolution (Kaláb et al., 1995).

Fig. 2 presents the Light microscopy (LM) photos of the transversal cuts of fresh-cut melon. Toluidine Blue, used as dye, is especially useful the for examination of fruit tissues, more specifically the fruit parenchyma cells, which constitute the fruit mesocarp (Kaláb et al., 1995). At day 0 of storage, intercellular spaces and vesicles are visible in fresh-cut melon mesocarp. All spaces presented are round and turgid with a visible cellular wall structure. This visual definition is mainly attributed to the water inside the cells. By day 4 of storage, it is already possible to observe a decrease in cell wall strength, which could be related with its pectin solubilising (Fernandes et al., 2008). The observed changes correlate with the $T_2$ distribution function, discussed in the previous section, and are also supported by the changes in the quality parameters measurements discussed below (see Section 3.3).

As for the images obtained with Scanning Electron Microscope (SEM), Fig. 2 shows, for day 0, closely bonded cells and defined cellular walls, reinforcing the results obtained with light microscope (LM). In fact it is actually possible to observe chloroplasts. Chloroplasts are an important cellular organelle, as they contain chlorophylls and carotenoids, that are pigments responsible for melon colour, as discussed in Section 3.3. After 4 days of storage, image shows a great number of cell walls broken down and the few remaining cells with severely distorted walls. This phenomenon stimulates the cellular disorganisation and cell size and shape variations. The cell plasmolise is also seen superficially by SEM, and confirms the observations of optical microscopy. Also after day 4, the observation of chloroplasts becomes more difficult.

### 3.3. Quality parameters

As discussed in the introduction section, colour, firmness and water activity are considered important parameters in fresh-cut

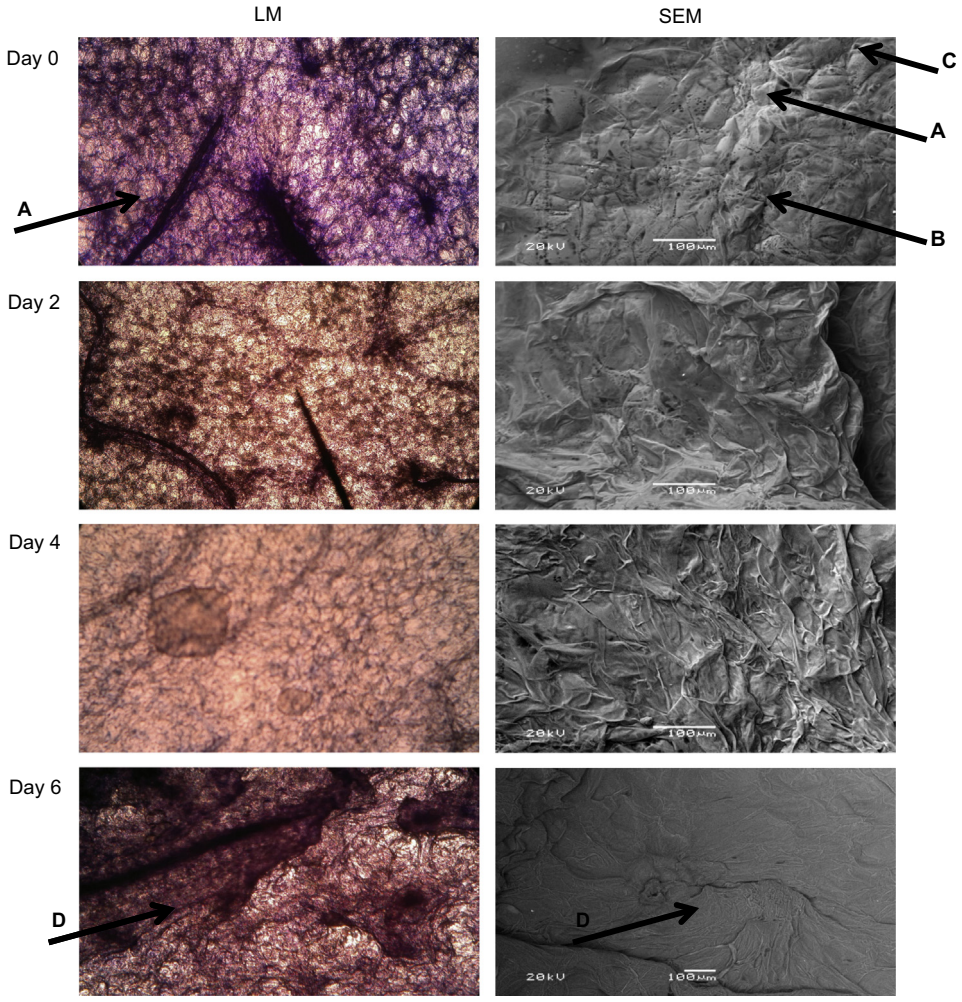


**Fig. 2.** Light and scanning electron microscope images of fresh-cut melon, at different days of storage. (A – cellular wall; B – cellular organelles; C – chloroplasts; D – plasmalemma).

fruit quality assessment (Fig. 3). Differences found between measurements are explained by the fruits initial maturity and the natural variability of the complex biological systems, as explained above.

Fig. 3(a) presents melon samples total colour differences (TCD) tendency, along storage. As expected, and in accordance with literature (Toivonen and Brummel, 2008), TCD increases with storage time. In the specific case of fresh-cut melon, changes in colour are attributed to different biochemical processes, mainly chlorophyll and carotenoids degradation, since melon is not very susceptible to surface browning (Munira et al., 2013; Toivonen and Brummel, 2008). The increase in colour changes observed during fresh-cut melon storage is generally attributed to translucency or water-soaking symptoms (Munira et al., 2013; Portela and Cantwell, 1998). Particularly, literature reported (Portela and Cantwell, 1998), also for a non-climacteric melon, that colour is attributed to the combination of low concentration of carotenoids and chlorophylls in plastids, that are inside the chloroplasts. As the storage time increases and the plastids degradation occurs, the pigment concentrations in melon changes and consequently so does colour.

Fresh-cut melon firmness during the storage period is shown in Fig. 3(b). Results demonstrate a rapid increase in firmness loss with storage time, particularly until day 4 of storage. At the end of

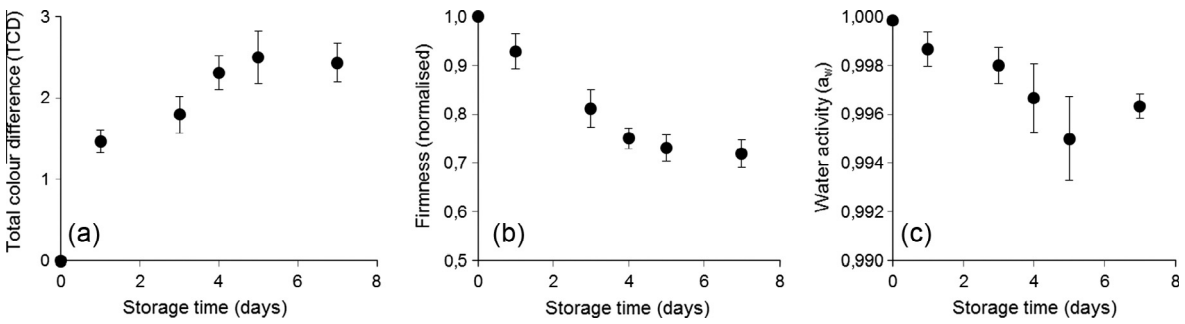


**Fig. 3.** Fresh-melon quality parameters: (a) TCD, (b) firmness, and (c) water activity, during 7 days of storage. Vertical bars present the mean standard error.

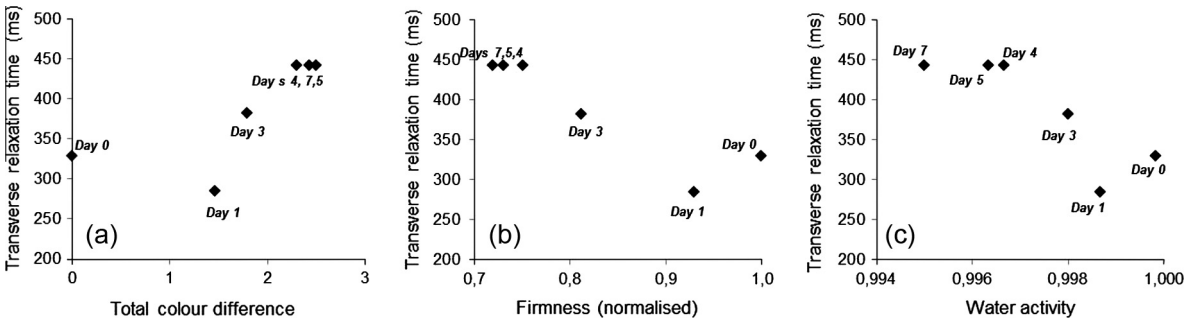


**Fig. 4.** Transverse relaxation time ($T_2$) as function of (a) TCD, (b) firmness, and (c) water activity.

storage, samples showed a degree of firmness around 30% (expressed as percentage of loss compared with the firmness measured at day 0). These changes in melon cubes firmness during storage were already reported for other melon cultivars and also for 'Piel de Sapo' (Aguayo et al., 2004). Fresh-cut melon is very susceptible to softening during storage, even under low temperatures, due to enzymatic degradation of the cell wall, specifically the middle lamella, and to loss of cell adhesion (Toivonen and Brummel, 2008). The enhanced activity of melon cell wall hydrolases in the first hours after processing, along with the transformation of protopectin to water-soluble pectin, lead to later alterations in structural features, namely thickness of the cell wall size and shape of cells, and volume of intercellular spaces (Rojas et al., 2001; Toivonen and Brummel, 2008) These modifications are according to the observed light and scanning microscope images, presented in Fig. 2.

Fig. 3(c) shows fresh-cut melon samples water activity decreasing until day 5 and then remained almost constant till the end of storage period. Literature reports water activity as a parameter for food stability control, namely chemical reactions in foods (Labuza, 1977). As discussed in Section 3.1. the decrease in this parameter may be due to the fact that water is being used for the physic and biochemical degradation reactions and/or microbial growth, occurring during the storage period.

### 3.4. Relaxation time vs quality parameters

In Fig. 4 it is possible to observe the behaviour tendency between maximum distribution $T_2$ value and fresh-cut melon quality parameters, TCD, softening rate and water activity. Fig. 4(a) shows the maximum distribution $T_2$ value against the total colour difference. Although a weak tendency was noticed, it is possible to observe $T_2$ increasing with TCD. This tendency may result from translucency or water-soaking symptoms derived from disruption of cellular structures. As discussed above, alterations in fresh-cut melon colour are mainly attributed to altered combination of low carotenoids and chlorophylls in plastids (Portela and Cantwell, 1998), and not so related with water system dynamics.

Fig. 4(b) demonstrates the relationship between maximum distribution $T_2$ value and firmness loss/softening. As expected, $T_2$ maximum value increases with the melon softening (lower firmness) At day 4 maximum distribution $T_2$ value reaches the highest value, while softening of fresh-cut melon tissue stabilises from this day on.

The softening together with cell wall degradation and loss of physical barriers, possible to observe by microscope images in Fig. 2, allows the leakage of cellular osmotic solutes into the apoplastic space, which then results in altered water mobility/availability (Toivonen and Brummel, 2008).

Water activity relationship with water relaxation time is presented in Fig. 4(c). It is possible to observe a tendency between these two parameters, i.e., cell water maximum distribution $T_2$ value decreases with increasing water activity. Despite of water activity being considered as a critical parameter of food systems stability (Labuza, 1977), the usual measuring methods do not consider microstructure nor the possibility that there may be local regions differing in water content, and presumably, water availability (Hills et al., 1996; Mathlouthi, 2001). These results demonstrate that, considering the lowest water activity values, the increase in this parameter does not reflect on water mobility. Although it is possible to observe a relationship between these two parameters, water activity measurements may not provide, for example, the relationship of the evolution of the structural changes of the food material with the changes of the water–macromolecules and water–water interactions that occur during food shelf-life (Wang and Liapis, 2012), and studies have stressed that under many common circumstances the thermodynamics activity of water is far less relevant to processing and storage than structure-related properties, which can restrict the mobility and diffusion of the reactants (Anese et al., 1996; Slade and Levine, 1991).

## 4. Conclusions

Fresh-cut melon samples were analysed in terms of water molecular dynamics (through the determination of $T_2$), microstructure and quality parameters, during maximum 7 days of refrigerated storage period.

The effect of wounding was observed by the $T_2$. distribution function analysis, that presents only one peak corresponding to cells total water. NMR experiments also allowed to evaluate the storage effect. The peak position ($T_2$) decreased in the first day of storage, increased from day 1 to 4, and remained constant until the end of storage. This indicates an increase in biochemical reactions and water–solutes bonds in the first 24 h after processing, followed by cellular structure degradation, where water became free from physical barriers. These results are supported by light and scanning electron microscope images.

The analysed quality parameters demonstrate a close relationship with the value of $T_2$, where the distribution function is maxima. These relationships are explained by several phenomena, such as loss of membrane integrity, cellular structures disruption and leakage of cellular osmotic solutes into the apoplastic space, which are alterations enhanced by processing-related wounding.

The results clearly established the potential of relaxometry techniques in monitoring the effect of physiological changes on the dynamic state of water and solutes. All these studies show the usefulness of gathering the NMR concept and methodologies with food science, and demonstrate the great value of these studies

on degradation reactions and stability in more complex food systems.

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