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Unilateral NMR, A New Approach to the Assessment of Rocha Pear Stored in Controlled Atmosphere during Long Periods

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Abstract

Rocha pear *Pyrus communis L.* can be stored for long periods in Controlled Atmospheres (CA) at very low oxygen partial pressure and temperature. Being this an important economical activity, it is of the utmost importance to develop efficient methods to follow the state of tons of pears stored in such extreme conditions. In an attempt to improve this situation, a project was set to make use of non invasive Nuclear Magnetic Resonance unilateral sensor (unilateral NMR) for studying Rocha pears populations evolution as a function of storage time in CA conditions. Pears originating from two different orchards were studied over a storage period of 7 months. Pear batches were removed from the controlled atmosphere chamber roughly every month and the evolution of their unilateral NMR and conventional physical and biochemical parameters were studied. First results for relaxation times, self-diffusion coefficients and conventional parameters data are presented and discussed.

Keywords: Unilateral NMR; Climateric; Rocha pear; Relaxometry; Postharvest; Soluble solid content

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Introduction

Rocha pear (*Pyrus communis L.*) cultivar is a Portuguese Protected Designation of Origin (PDO) recognised for its "firm body, juicy and sweet". It is one of the most important agricultural products for the economy of the west region of Portugal and represents an above 100 million Euros a year exportation trade. In addition to its organoleptic and nutritional properties [1], Rocha pear has the capacity to be stored for long periods (eight to nine months) in Controlled Atmosphere (CA) chambers in a very low oxygen partial pressure and temperature. A capacity that provides it with a huge commercial advantage regarding management of fruit flow. In this scenario it is of the utmost importance to access the Rocha pear quality stored for long periods where fruit may observe physical and/or chemical changes or even suffer physiological accidents [2] that diminish its nutritional and commercial value. Modern CA chambers have installed capacities to measure different gases, humidity, temperature and even fruit fluorescence (chlorophyll), and in Dynamic Control Atmospheres (DCA) equipments, the conditions inside the camera can be adjusted in face of the measured parameters.

It is well known that water activity [3] is an interesting parameter in food security and quality assessment; although it's molecular interpretation is still involved in some controversy. The concept is undoubtedly related with the water molecule "environment" in the complex matrix of food [4]. Still, while for some authors the major actor is the fraction of "free" water not "bound" around solutes, for others, the focus resides in the water structure built in a solution where solutes interfere with the hydrogen bonds supporting a water network in solution, some enhancing it and others destroying it. Others still, relate the water activity with the hydration number and stoichiometric of its clustering. Whatever the interpretation one assumes, water activity is related with "water mobility", and therefore it is but natural that Nuclear Magnetic Resonance (NMR) is used to throw some light into the issue. Standard NMR is thus widely used in food studies and also in fruit quality measurement, using either high resolution spectroscopy, imaging and/or relaxometry approaches

Figure 1: Pear in the NMR unilateral sensor.

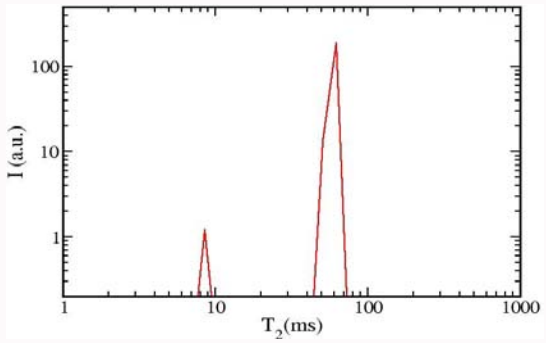

Figure 2: Example of a distribution of transverse relaxation times (T_2) for a pear at moment 0.

[5-7]. NMR is undoubtedly a powerful methodology in food science in general and in fruit or vegetables studies in particular.

In this work, we monitored Rocha pears from two orchards from the west region of Portugal during a period of eight months (September to April), by nuclear magnetic relaxometry and by diffusion studies, using a novel non-destructive and non-invasive NMR methodology, namely unilateral NMR [8,9]. Pears from two different origins were stored in the same CA environment, and were also subjected to a set of destructive conventional analysis procedures, for determination of firmness, soluble solid content, total titratable acids, acid ascorbic content and total phenolic compounds.

In unilateral or single-sided NMR, instead of placing the sample in a homogeneous magnetic field like in a NMR spectroscopy experiment or in a NMR imaging one, the magnetic field is created in a small region inside the sample. This means that instead of putting the sample inside the magnetic field, the field is colocated inside the sample. This implies that there are no restrictions to the sample volume and the measurement is completely non invasive and non destructive.

Unilateral NMR application to foodstuffs studies has been reported previously [10] but to best of our knowledge, this is the first time ever that a report on the use of unilateral NMR is presented regarding the systematic and exhaustive follow up of a fruit population stored for several months in CA conditions.

Longitudinal (T_1) and transverse (T_2) relaxation times, which give

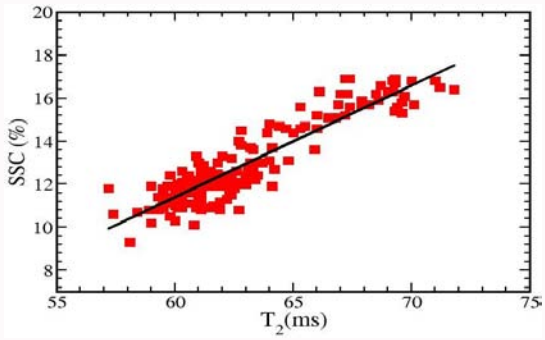

Figure 3: Soluble solid content versus transverse relaxation time for 177 pears from the two different orchards. The linear regression presents a R^2=0.9167424.

information about molecular mobility inside the fruit, were measured for each and every pear of the study. Taking advantage of the high magnetic field gradient of the unilateral NMR equipment, the measurement of self-diffusion coefficients inside the pears, is possible and was thus performed. Besides this, firmness, soluble solids content, total titratable acids, ascorbic acid and total phenolic compounds, widely used parameters for measurement of pears quality, were also determined.

In the present work, we present a general view and discuss data regarding a first set of results and conclusions. In future works, more detailed analysis on particular issues will be presented.

Materials and Methods

In this work, 720 pears were studied, half from an orchard here named Bombarral and the other half from an orchard here named Mafra. The Bombarral orchards being located roughly 60 km north from the Mafra orchard, both in the west region of Portugal. The pears were harvested in September and stored in a CA room at -0.5ºC with 90 to 95% of relative humidity, 0.5% O_2 and 0.5% CO_2 for several months.

Two samples composed of 45 pears from Bombarral and 45 pears from Mafra orchards, named delivery A, were measured at harvest. All the other pears were stored in CA. Afterwards similar deliveries (B, C, D, E, F, G and H) were obtained corresponding to different periods of storage in the CA conditions described above, namely: B 22 d, C 38 d, D 57 d, E 78 d, F 99 d, G 156 d and finally H 193 d.

For each delivery the two samples of 45 pears of each orchard were removed from the CA, and divided in three groups. The first group was measured after 24 h at room temperature, after removal from CA (moment 0). The 24 h period being set to assure the thermal equilibrium of the pears tissue. The second group was measured 7 d after (moment 7) and the third group was measured 14 d after being removed from CA conditions (moment 14). Groups corresponding to moments 7 and 14 were stored at 9ºC, in a standard house refrigerator.

The procedure in each of the moments 0, 7 and 14 was the following: the pears, once at thermal equilibrium after 24 h at 23ºC, were first investigated by unilateral NMR and afterwards transported to the Instituto Superior de Agronomia ULisboa in the day after, where the conventional measurements were performed. This procedure was set to assure the possibility to compare and correlate the conventional measurements with the ones obtained by unilateral NMR.

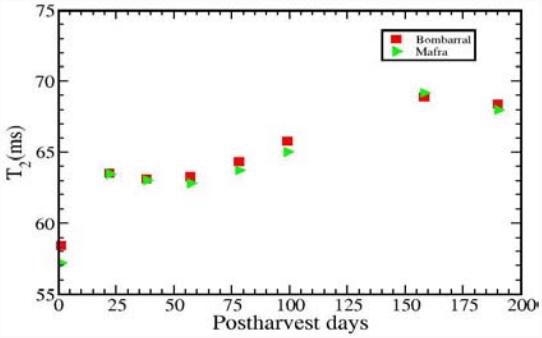

Figure 4: Transverse relaxation time evolution for pears of the two different orchards over a long storage period.

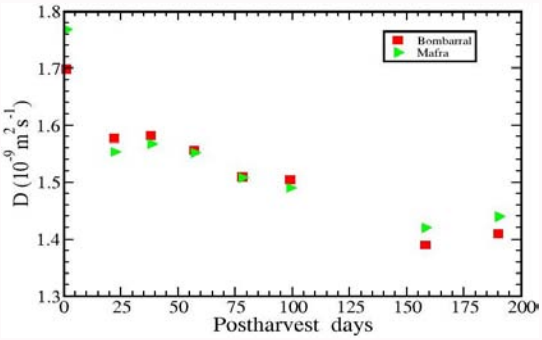

Figure 5: Self-diffusion coefficient evolution for pears of the two different orchards over a long storage period.

Unilateral NMR measurements

A Magritek Profile NMR Mouse PM25 System working at a 13.06 MHz frequency for proton, was used to obtain the relaxation times and the self-diffusion coefficients of pears in the long period of CA storage. The experimental apparatus can be observed in Figure 1.

Each pear, at thermal equilibrium at room temperature, was placed above the sensor and secured with plasticine, placed outside the sensor solid angle, in order to avoid displacements during the measurement.

The Magritek Profile NMR Mouse PM25 System allows moving the sensible slice from the surface to 0.0262 m within the interior of the sample. In this work all the measurements were made at 20500 μm from the pear surface.

The pears transverse relaxation time T_2 was measured using a CPMG pulse sequence having 1000 echos. The measured sensible slice being a parallelepiped with a 0.04×0.04 m^2 base and 50 μm thickness. The echo train was acquired 8 times with a repetition time of 1.8 s.

The self-diffusion coefficient was measured using a stimulated echo sequence with 1 ms for evolution period combined with a CPMG-train having 64 echos. The signal was collected 16 times with a repetition time of 1.2 s.

Conventional measurements

As referred, after the NMR measurements the pears were sent to the Instituto Superior de Agronomia ULisboa to be evaluated individually for a set of chemical and physical parameters, namely: firmness, soluble solids content, total titratable acidity, ascorbic acid content and total phenolic compounds. The firmness was assessed on two opposite sides of each fruit using a fruit pressure tester

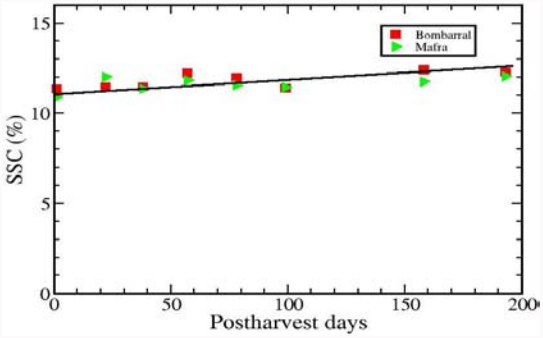

Figure 6: Soluble solid content for pears of the two different orchards over a long storage period.

(FT327) with an 8-mm probe, the subsequent analyses being carried out on clarified juices. Soluble solids contents were measured by refractometry using a Hanna HI-96801 refractometer, the titratable acidity was determined by potentiometric titration with NaOH 0.1 to pH 8.1 using a potentiometer Hanna pH 213 and the ascorbic acid by titration with DCPIP [11]. The total phenolic compounds were estimated by spectrophotometry, measuring the optical density at 280 nm, using gallic acid as standard [12]. These parameters are widely used for Rocha pear quality assessment [13].

Results and Discussion

In a previous work [14] cut samples of Rocha pears were studied using conventional relaxometry NMR measurements. In particular using a CPMG pulse sequence the magnetization evolution was interpreted as a continuous of exponentials decays with a continuous distribution of transverse relaxation times. The graphic representation of the T_2 ranges from 10ms to very high values presenting three maxima. Such a procedure allowed then, the identification of water in three sub-cellular structures. Those results were compared with results by Hills and Sanchez [5,15], and it was concluded that it was possible to identify water in the vacuole from the highest T_2 maxima, very similar to the T_2 of free water, water in the cell cytoplasm from the medium T_2 maxima, and a more bounded water, identified as cell wall water, by the shortest T_2.

In the present work, the fruit preserved intact were investigated using a completely non invasive NMR methodology, namely unilateral NMR. Due to the strong magnetic field gradient of the unilateral sensor the transverse relaxation time measured is an effective one [9]. Following the same procedure of spectral analysis (refereed in [5,14,15]) a continuous distribution of transverse relaxation times is considered for the unilateral NMR data. In Figure 2, a typical result is presented, it is possible to observe a pronounce maxima around 60 ms, and a small one around 8 ms. These can be interpreted as water in the vacuole and water in the cytoplasm respectively. The vacuole water transverse relaxation time presenting a value close to 52.8 ms, the value of transverse relaxation time measured for free water using the Magritek Profile NMR Mouse at 13.06 MHz.

The tiny peak reflects cytoplasm water; the bound water in the cell wall term is not present, because the strong magnetic field gradient of the unilateral sensor shortens the measured T_2 effective values, leading it to values smaller than the minimum that can be measurement with this equipment.

This result points a major difference between the two relaxometry methodologies. While the conventional NMR allows a better

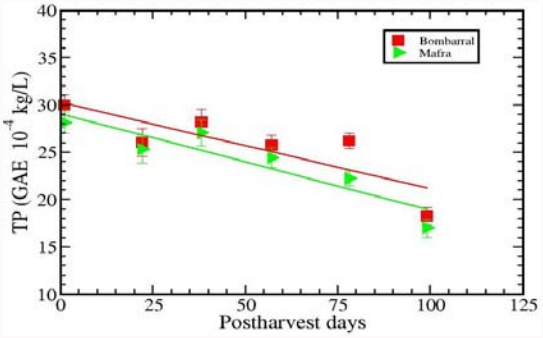

Figure 7: Total phenolic compounds for pears of the two different orchards over a long storage period. The error bars stand for the standard error of the mean.

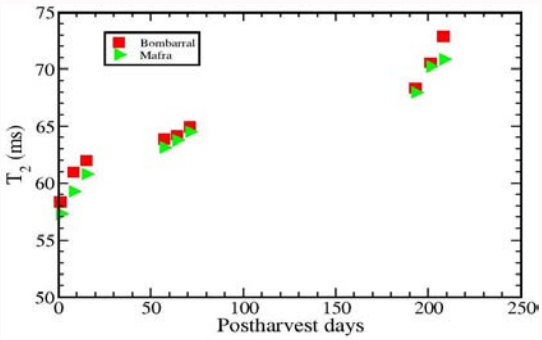

Figure 8: Evolution of T_2 in shelf time for deliveries A, D and H, for the two orchards.

discriminated T_2 spectral analysis, using the unilateral NMR sensor, the interpretation of the effective T_2 continuous distribution is limited. Still, on the other hand, the non destructive character of the unilateral NMR sensor and its important gradient field allows the access to other interesting information regarding the fruit state and evolution, while intact. For instance, it was possible to follow the fruit shelf life for different delivers corresponding to different storage periods in the CA.

Moment 0

In Figure 3, the good correlation between the measured soluble solids content for 177 pears and the respective transverse relaxation time, measured by unilateral NMR, is shown. Similar results for apples and strawberries based on NMR data measured using conventional NMR methodologies have been reported by Marigheto *et al.* [16]. It is important to note that, although the transverse relaxation time measured by a unilateral NMR sensor is an effective transverse relaxation time (T_2), because it is an inherent T_1 contribution [9], the correlation between the measured T_2 values and the soluble solid content values is still conserved.

The evolution of the transverse relaxation time with the CA storage period is represented in Figure 4 for the two orchards and for moment 0 for all the deliveries, with a standard error less than 1%. It can be observed that, at harvest day, the two orchards presented slightly different T_2 values, but the storage in CA renders them similar.

An abrupt transition in the T_2 value can be observed from harvest day to delivery B's moment zero (22 days in CA). From this day forward, the transverse relaxation time presents a slow growing tendency, with the exception of the last point. The fact that the pears,

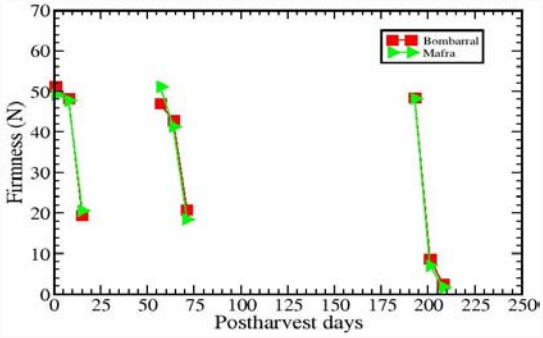

Figure 9: Evolution of firmness in shelf time for deliveries A, D and H, for the two orchards.

after 99 d of CA, were remove from the CA chamber where they were stored until then, and stored in another, although essentially in the same storage conditions, might eventually have influenced the T_2 evolution, something similar to the evolution in the beginning of the storage.

It is interesting to compare this result (T_2 increase with storage time) with the one obtained for other climateric fruit [17] in shelf evolution. The evolution is similar except that for fruit in control atmosphere the T_2 evolution is much slower.

Figure 5 presents the evolution of the pears' self-diffusion coefficient (D). The standard error being roughly 1%. It is interesting to observe that, at harvest, the two orchards presented different self-diffusion coefficients, and with the storage in CA the difference decreases. However for the two last deliveries (pears that were moved from one camera to another in similar conditions) the distinction in the self-diffusion coefficient values reappears, the Mafra pears presenting a higher self-diffusion coefficient than Bombarral pears. This detail in the behavior of the two orchards self-diffusion coefficient is not reflected in the T_2 evolution, showing that the relation between the two variables is not absolute. Also the self-diffusion coefficient of pears presents the same evolution that the one described for banana in shelf time reported by [17], and the D reduction can be attributed to the increase in concentration of soluble sugars (Figure 6).

Data for the longitudinal relaxation time for the long storage period for the two orchards measured at moment 0, shows a difference between the two orchards observed when the pears are harvested and preserved over the whole period of storage, the Bombarral pears always presenting a lower longitudinal relaxation time than the Mafra pears. The T_1 parameter seems therefore to reflect the origin of the fruit, a "label" that accompanies the pears throughout the storage period. An elemental analysis of the two soils revealed some important differences in elemental composition, and it is possible that the T_1 "label" relates to this.

Further details on T_1 data will be presented and discussed in a future publication. The firmness was measured for all the 30 pears of each delivery 24 h after the unilateral NMR measurement.

The results show that there is no evolution of the pears' firmness for the long storage period: their firmness was 48.51±0.98 N for the Bombarral pears and 49.98±0.98 N for the Mafra pears. There is also no distinction on the firmness of pears of the two orchards, at moment 0. These results were expected, as the harvest moment in each orchard is selected according to a certain firmness value previously decided and then the fruit physiological activity in the dynamic atmosphere is

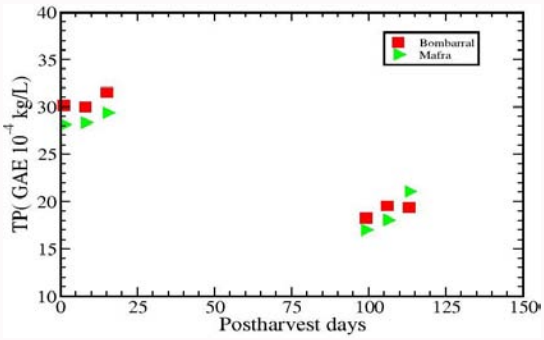

Figure 10: Evolution of total phenolic compounds in shelf time for deliveries A, F, for the two orchards.

Table 1: Titratable acids for the A delivery (from the tree) and H delivery (158 d in CA) for pears of the two orchards, Bombarral and Mafra. Values presented with the standard error of the mean.

Titratable acids (10^{-3} kg/L)		
Postharvest days	Bombarral	Mafra
0	1.13±0.05	0.99±0.06
158	0.55±0.08	0.61±0.07

strongly repressed in order to avoid degradation reactions that would naturally lead to a decrease in firmness.

In Figure 6 the soluble solids contents is represented for the two orchards for the long period of storage. The measurement was performed at moment 0. It is possible to observe a small increase, roughly 10 % in the soluble solids content over the 193 d. These results were also expected, although the metabolic activity is strongly suppressed, there is always some minimal physiological evolution that appears as the conversion of starch to monosaccharides that lead to soluble solids content small increase.

These 10% increase, are also seen in figure 4, apart from the discontinuity observed from delivery A to B.

In Table 1, the titratable acids for the two orchards are presented for two different instants, at harvest and 153 d after storage in CA. A decrease in the titratable acids can be noted which results from the consumption of acids in respiration process that occurs during storage.

The ascorbic acid was measured for pears at harvest, delivery A, and for all the following deliveries. The ascorbic acid showed no evolution in the studied period, and its value was the same for the two orchards, Bombarral and Mafra, 11.0±0.7 μkg/L, a magnitude often referred in literature for ascorbic acid in pears [13,18]. This absence of evolution in the ascorbic acid content during ripening in prolonged storage have also been reported [19].

In Figure 7, the evolution of the total phenolic compounds for several deliveries in the studied period of storage in CA is presented. A clear decrease in the total phenolic compounds can be noted for the two orchards in 100 days CA storage. This decrease is probably due to the activity of the enzyme polyphenoloxidase and is noteworthy, as the fruit loose their antioxidant ability and get much more susceptible to plagues and diseases when a low content of phenolic compounds is reached.

Shelf life

The pears were also studied regarding their evolution in shelf life. All the deliveries were measured at moment 0, moment 7 and 14.

In Figure 8, the evolution of the transverse relaxation time for the three moments of shelf life is presented for the deliveries A, D and H, corresponding to 0, 57 and 193 d in CA. It becomes clear that T_2 increases during the 14 d of shelf period, corresponding to fruit ripening. This increase has more expression in the A and H deliveries, and is less pronounced in delivery D, even though, deliveries A and D shelf were carried out in a house refrigerator at 9ºC, while delivery H shelf period was carried out at 20ºC.

The firmness clearly diminishes during a 14 d shelf period, more sharply in the 14th day for deliveries A and D. For delivery H (193 d in CA) the firmness diminished more sharply on the seventh day as shown in figure 9. Also, the value of the firmness in the 14th day for delivery H is very small, almost zero. These facts derive from the already mentioned difference in shelf storage of deliveries A and D, versus delivery H. While in storage at 9ºC the drop in firmness is more pronounced in the second week, in the case of storage at 20ºC, the full drop equivalent to that observed for deliveries A and D, takes place during the first week of storage, as expectable.

In Figure 10 it can be observed that the total phenolic compounds decrease with the storage time in CA, as previously noted in Figure 7. Still, in the 14 d shelf life considered, the total phenolic compounds show a small growth trend. This behaviour can be a defense reaction towards the sudden increase of temperature [20].

Conclusion

Rocha Pear samples, a Portuguese cultivar, from two orchards, were followed in long storage time in CA. The two populations were measured by non invasive unilateral NMR methodology, and by conventional physical and chemical quality parameters. To the best of our knowledge, this is the first time that such a detailed study following a long storage time in AC of Rocha Pear, using unilateral NMR data is reported.

It was observed that the unilateral NMR parameters provide information on the pears evolution, without the need to destroy the fruit, as it happens for the conventional measurements.

The transverse relaxation time clearly reveals the pear's soluble solid content without the need to use fruit juice, as it happens in the refractometry procedure, and therefore can be used in organoleptic test, with advantage, as the fruit can be opened up for tasting only at a selected instant of growth of T_2.

The transverse relaxation time showed to be sensible to the fruit storage conditions and the self-diffusion coefficient shows different reactions to these conditions for the two pear origins.

The total phenolic compounds showed a decrease with the storage time in CA as well as tritratable acids, while firmness and ascorbic acid showed no evolution in the long storage period in CA.

A non invasive methodology allows monitoring the evolution of fruit in shelf time.

We strongly believe that the unilateral NMR study opened a new observation window to fruit storage in CA conditions, with important scientific and commercial implications.

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