

Investigation on Shelf-Life Of Roasted Chestnuts with Different Packaging Systems through Low-Field NMR Analysis

Noemi Proietti^a, Loredana Liguori^b, Valeria Di Tullio^a, Giuseppina Adiletta^b, Paola Russo^{c*}

^aLaboratorio di Risonanza Magnetica "Capitani - Segre", Istituto per i Sistemi Biologici (ISB), CNR, Monterotondo, 00015 Rome, Italy

^bDepartment of Industrial Engineering, University of Salerno, Via Giovanni Paolo II 132, 84084, Fisciano, Salerno, Italy

^cDepartment Chemical Engineering Materials Environment, Sapienza University of Roma, Via Eudossiana 18, 00184 Roma, Italy

paola.russo@uniroma1.it

Raw chestnut has a limited shelf life due to the high moisture content. Diverse processing methods have been employed to make chestnut palatable and safe for consumption. Roasting is considered a suitable process for maintaining the nutritional profile and minimising the loss of nutrients of chestnut, but the residual moisture content does not assure a prolonged shelf life. To this aim, two different packaging methods are compared in this work, specifically a modified atmosphere package and a semipermeable film. The weight loss and the O₂ and CO₂ concentrations were measured during time. Moreover, low-field 1 H NMR single-sided relaxometry was used to have information on water compartments, diffusion and movement obtained by detecting proton signals prevalently ascribable to H₂O contained in chestnuts. The main advantage of this technique is that it does not require any pre-treatment of the sample, it is non-invasive and hence chestnut sample is here analysed within its packaging system during shelf life. Results showed that modified atmosphere packaging allowed the roasted chestnut to be marketable up to 90 days, while the semipermeable film up to 30 days.

1. Introduction

The chestnut (*Castanea sativa* Miller) is an important traditional and seasonal nut in Mediterranean countries (Moreira 2007). It is widely consumed throughout America, Asia, and Europe (Liu et al., 2015), with a total production of 2.35 million tonnes worldwide in 2018 (FAOSTAT, 2020). It is a highly appreciated food all over the world especially for its unique sweet taste and flavor as well as its high nutritional value (Kan et al., 2016).

The primary components (on a wet basis) of fresh chestnuts are water (49%), carbohydrates (46%; mainly starch and sugars such as sucrose and then glucose and fructose), proteins (3%), cellulose (1%) and minerals (1%; K, P and Mg) (Yang et al., 2010). Due to the high-water content, chestnuts are perishable seasonal fruits, so post-harvest treatments are required to extend the shelf-life of this nut for a long time (Moreira, 2007).

After harvesting, chestnuts are subject to significant water losses, which can cause serious damage to industry and trade. A significant number of raw chestnuts, not consumed at the time of harvest, is processed with different techniques such as blanching before freezing, boiling, roasting for long-term storage (Jermini et al., 2006). In addition, boiled and roasted chestnuts are favourite preparations of most consumers.

Understanding the effect of different processing operations on chestnut quality is fundamental for a better use of this crop (Zhu, 2016). Indeed, the impact of processing on the chemical composition depends on the variety, processing conditions, and their interactions.

Roasted chestnuts have lower mass, due to lower moisture content, than boiled or raw fruit. The average moisture content increased by 4% with boiling but decreased by 20% with roasting compared to raw chestnuts (Silva et al., 2011). After treatment by home cooking or industrial processing, it has been observed that the nutrition, taste, and shelf-life of chestnut fruit change. De Vasconcelos et al. (2010) studied the effects of

different cooking treatments on the nutritional composition of chestnut fruits. This study revealed that chestnut fruits lost about 25% of their energy value and gained moisture after boiling, while the energy level increased dramatically with roasting (200°C, 40 min) and sugars available increased by 25%. Gonçalves et al. (2010) showed that the content of protein, insoluble and total dietary fiber increases after roasting, while the fat content decreases and the citric acid, gallic acid and the total phenolic content increase while the malic acid decreases. As confirmed by Kursh et al. (2001) roasting had little effect on the chemical composition, as the amount of starch, sucrose and fatty acids remained the same. In this regard, roasting chestnuts at a temperature below 210 °C can be considered a mild treatment. However, the residual moisture content of roasted chestnuts does not guarantee an extended shelf life. Existing data suggest that further research should be conducted to confirm the published results, particularly on the shelf life of roasted chestnuts. In this context, the aim of this study was to evaluate changes in physico-chemical and microbial traits of roasted chestnut packaged in two different packaging systems: modified atmosphere packaging (MAP) and passive modified atmosphere with semipermeable film (SP) during cold storage.

In order to monitor fruit samples directly in the package during storage, a single-sided NMR technique was used. The main advantages of this technique are that it does not require any sample pre-treatment, and once developed, standard protocols based on fast measurements can be easily transferred to quality control applications. Unlike conventional NMR instruments, the open geometry allows non-invasive measurements even on packaged products; the packaging material does not significantly affect the measurement as it is relatively thin (Capitani et al., 2017). The ¹H pulsed low field NMR allows to obtain information on compartmentalization and diffusion of water by detecting the proton signal due to the H₂O contained in plant tissue. In literature, single-sided NMR has been used to monitor, in a completely non-invasive way, the pear during drying (Adiletta et al., 2015; Proietti et al., 2018), the growth of kiwifruit (Capitani et al., 2013), to investigate the water status of fresh and withered blueberries (Capitani et al., 2014), to detect adulteration of virgin olive oil in sealed bottles (Xu et al., 2014), deterioration of tomato paste (Pinter et al., 2014) and assessing the fat content in live fish (Veliyulin et al., 2005).

In this work, the low-field single-sided ¹H NMR was used to obtain information on the water compartments and on the movement obtained by detecting proton signals mainly ascribable to the H₂O contained in chestnuts.

2. Materials and methods

2.1 Chestnuts

Chestnuts (*Castanea sativa* Mill.) var. *Judia* were harvested in the growing season 2018-2019 in a commercial chestnut grove in the Italian region of Campania. Upon arrival at a local factory, the nuts were manually selected to remove the kernels that showed insect damage and/or fungal infections. The nuts were processed and subjected to seasoning in water and subsequently drying at room temperature. Before hot processing, the whole chestnuts are cut in a rotatory machine, arranged in a single layer, and placed in rotating flame oven. Roasting was carried out at 140°C for 30 min. The samples were left to cool to room temperature and once delivered to the Food Technology Laboratory at University of Salerno, the fruits were peeled manually before packaging.

2.2 Packaging and storage

Samples of roasted chestnuts (250 g) were packaged in bags of (18 cm x 21 cm) with different films: semipermeable (SP) and barrier with modified atmosphere (MAP). The main characteristics of films have been reported in Table 1. The modified atmosphere used was constituted by N₂ 70% vol. and CO₂ 30% vol. The packages were stored at 5°C, 90 ± 2% RH. Measurements of the different parameters (e.g., moisture, water activity, weight loss) on three replicates were performed at each storage time (15, 30, 45, 60 and 90 days). All results were expressed as mean ± SD.

Table 1. Main Characteristics of the films used in the experimental tests of roasted chestnuts.

	MAP film	SP film ®
Manufacturer	Orved	Cryovac
Material	PA/PE	Polyester
Thickness (µm)	140	25
O ₂ TR (cm ³ /m ² /24h) (23 °C, 0% RH)	40	72
CO ₂ TR (cm ³ /m ² /24h) (23 °C, 0% RH)	<130	800
MVTR (g/m ² /24 h) (38 °C, 100% RH)	<3	47

2.3 Single sided NMR measurements

Measurements were made at 13.55 MHz with an NMR instrument from Bruker Biospin interfaced with a single-sided sensor by RWTH Aachen University, Germany. This sensor generates a magnetic field with an extremely uniform gradient to resolve the near surface of arbitrary large samples. Longitudinal relaxation times (T1) were measured with the Aperiodic Saturation Recovery sequence, using a logarithmic increment, 64 blocks were collected in each experiment. Effective transverse relaxation times (T2) were measured using the CPMG pulse sequence with a 2τ echo time of 104 ms, 4096 echoes. Measurements were carried out at 1mm and 5mm of depth within the sample. Four roasted chestnut samples for each package were analyzed during storage (15, 30, 45, 60 and 90 days). After each analysis the samples were stored at 5°C.

2.4 Headspace gas composition

The gas composition inside the packages was measured by O₂/CO₂ gas analyser (PBI Dansensor, Checkmate3, Ringsted-Denmark) with the accuracy of 0.1%. The gas analyser needle was inserted through an impermeable rubber seal attached on the film.

2.5 Microbial count

Approximately 10 g of product from each package were homogenised with 90 mL of sterile saline in a stomacher (International PBI, Milan, Italy) for 2 min at room temperature. Subsequently, serial dilutions (1:10) of homogenates and microbiological counts of total mesophilic bacteria (TBC) and moulds and yeasts were performed respectively by Plate Count Agar (PCA, Oxoid, Milan, Italy) at 30°C for two days (ICMSF, 1978) and Oxytetracycline-Glucose-Yeast Extract Agar (PCA, Oxoid, Milan, Italy) at 22°C for 5 days (Di Matteo et al., 2012).

2.6 Statistical analysis

Mean data values and their standard deviations were calculated from three replicates. One-way analysis of variance (ANOVA) using Tukey's test ($p < 0.05$) was conducted to compare mean values.

3. Results

The water self-diffusion process is of primary importance for evaluating enzymatic activity, sensory perception and food spoilage. In particular, the water present in chestnut structure is classified into "bound water", "immobilized water" and "free water", respectively located in the starch granules, between the starch granules and outside the starch granules. The differences in the longitudinal relaxation times (T1) are generally not sufficient to identify the water in the different compartments while the differences in the transverse times (T2) are more pronounced as the exchange of water between the different domains results in a partial average which depends on the dimensions of these and the water permeability of the membrane (Proietti et al., 2018). Hence, T2 measurements have more detailed information on the breakdown of water in cell tissue than T1.

After roasting, the chestnut has already undergone a partial loss of water which occurred mainly outside the starch granules, but the reduction of the moisture content continues inside the packages, with different kinetics in the two cases under examination. The relaxation time T1, which has a monoexponential behavior, provides information about the water present in the larger clusters, mainly free water. In the case of MAP, the net change in T1 is not significant; as shown in figure 1a, it changes from 29 ms at time zero to 26 ms at 90 days. This trend is due to the high humidity values that can be reached in the package due to the poor permeability of the polymeric films to water vapor. Consequently, the reduction of water content is slowed down and hindered, which is instead clearly visible in the case of the semipermeable film, where the decreasing trend can be approximated by means of a second-degree polynomial, as illustrated in Figure 1b.

The effective transverse relaxation time T2 is influenced by the diffusion of water, mainly "bound water", "immobilized water", in the granules and between the starch granules which exhibit multi-exponential decays as is also observed in tissue membranes and walls plant cells (Stilbs, 1987). In this case, only two components T2a and T2b are used, whose weight is indicated by the values of Wa and Wb. The lower values (T2a), in the range 4-8 ms, have been attributed to component A and indicate the water more linked to the cellular structure, responsible for the alterations of the structural properties, whereas the higher values (T2b), between 20-60 ms, indicate the water present in the most mobile domains, the withdrawal of which causes weight loss (Proietti et al., 2018).

In the case of MAP, T2a value, which represents more than 80%, is almost constant, with a variation of less than 1 ms, while the T2b shows an irregular profile with a maximum (38.8 ms) corresponding to the minimum (6.8 ms) of T2a at 45 days indicating the transformation of the bound water into free water (figure 2). As already seen for T1, the high humidity values reached in the package seem to alter the normal mobility of water in the starch granules and between them and the outside. The reabsorption of moisture by roasted

chestnuts, because of the high humidity in the packaging, slows down the transpiration process, reduces the weight loss of fruits as shown in Table 2. At the same time, MAP creates an unfavorable environment for microbial growth up to 90 days. Similar results were found by Panagou et al. (2006) who kept chestnuts in various films in a modified atmosphere for 110 days at low temperatures ($<10^{\circ}\text{C}$).

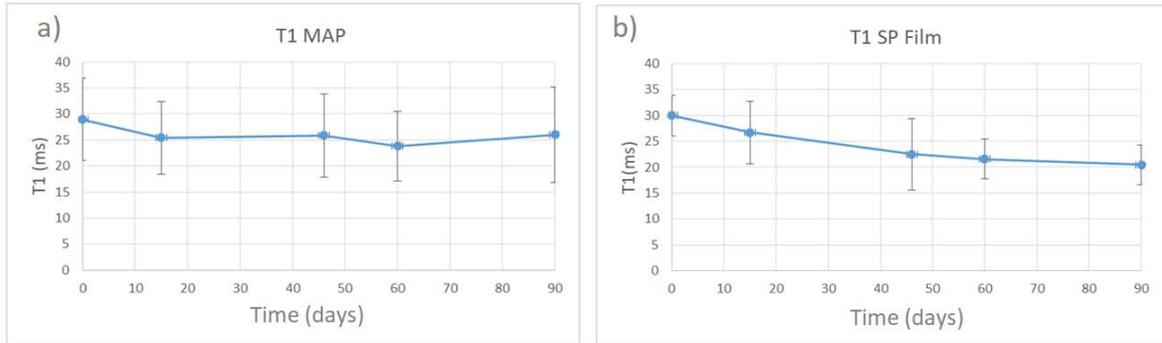


Figure 1. Profiles of longitudinal relaxation time T_1 for roasted chestnuts in a) MAP and b) SP film during storage.

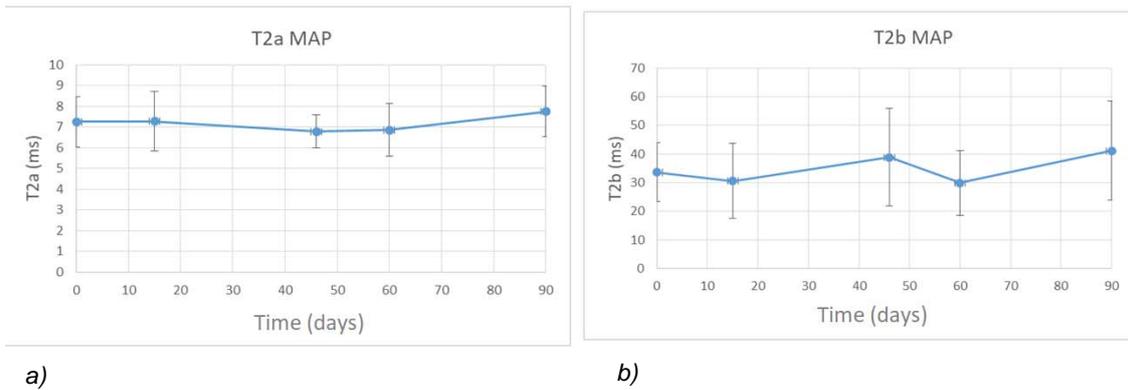


Figure 2. Profiles of the effective transverse relaxation time T_{2a} (a) and T_{2b} (b) for roasted chestnuts in MAP during storage.

Table 2. Moisture (%), water activity (a_w) and weight loss of roasted chestnuts during storage in MAP, SP film.

Storage time (days)	MAP			SP		
	Moisture (%)	water activity (a_w)	weight loss (%)	Moisture (%)	water activity (a_w)	weight loss (%)
0	46.1 ± 0.10^d	0.944 ± 0.005^c	0^a	46.1 ± 0.10^a	0.944 ± 0.005^a	0^a
15	42.0 ± 0.10^b	0.919 ± 0.005^a	0^a	40.0 ± 0.10^a	0.922 ± 0.005^b	1.39^b
30	46.0 ± 0.10^d	0.953 ± 0.005^c	0.005 ± 0^b			
45	43.2 ± 0.10^c	0.919 ± 0.005^a	0.005 ± 0^b			
60	40.1 ± 0.10^a	0.932 ± 0.005^b	0.005 ± 0^b			
90	41.9 ± 0.10^b	0.928 ± 0.005^b	0.080 ± 0^b			

a, b, c, d Different letters indicate statistical difference which was considered significant with $p < 0.05$

On the other hand, in the case of the semipermeable film a simultaneous increase in T_{2b} and a decrease in T_{2a} are observed in all samples. Unlike what is generally indicated with T_1 , roasted chestnuts lose the bound water and the immobilized water while a partial replenishment of liquid takes place outside the starch granules (Figure 3). The overall effect is a 1.4% weight loss (Table 2), after 15 days, due to moisture transmission through the package holes. Chestnuts, on the other hand, being particularly rich in humidity, up to 50% by weight of fresh fruit, naturally tend to dehydrate rather quickly compare to common dried fruit with inevitable loss of firmness (Blaiotta et al., 2014). In this case, high moulds infection was observed for nuts packaged in

SP film after 30 days of storage. Probably the absence of peel has caused a greater perishability of the nuts which reduces the storage time in the ambient air.

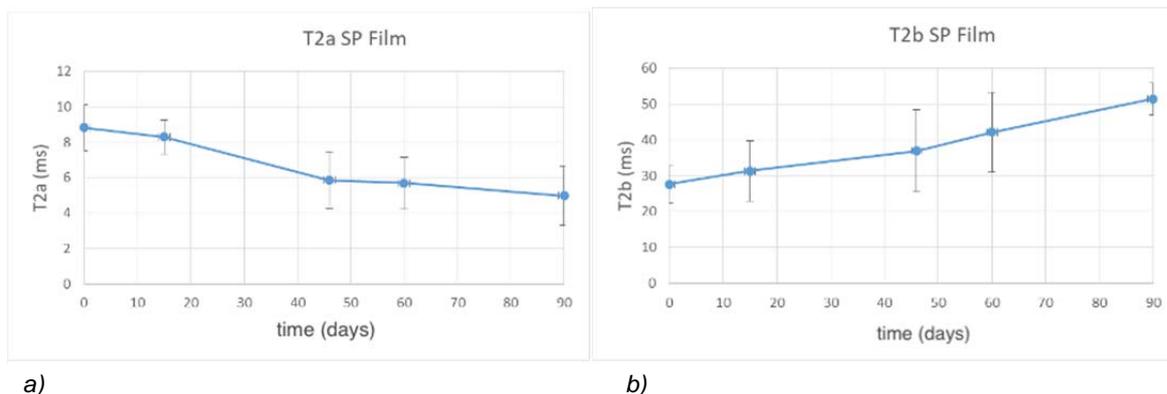


Figure 3. Profiles of the effective transverse relaxation time $T2a$ (a) and $T2b$ (b) for roasted chestnuts in SP film during storage.

The evolution of CO_2 and O_2 concentrations inside the modified atmosphere package is shown in Figure 4a. During storage, a slight and rapid decrease of CO_2 is observed, which after 15 days of storage reached a constant concentration until the end of storage. Likewise, no significant differences are observed in oxygen concentration ($< 0.5\%$ vol) over time.

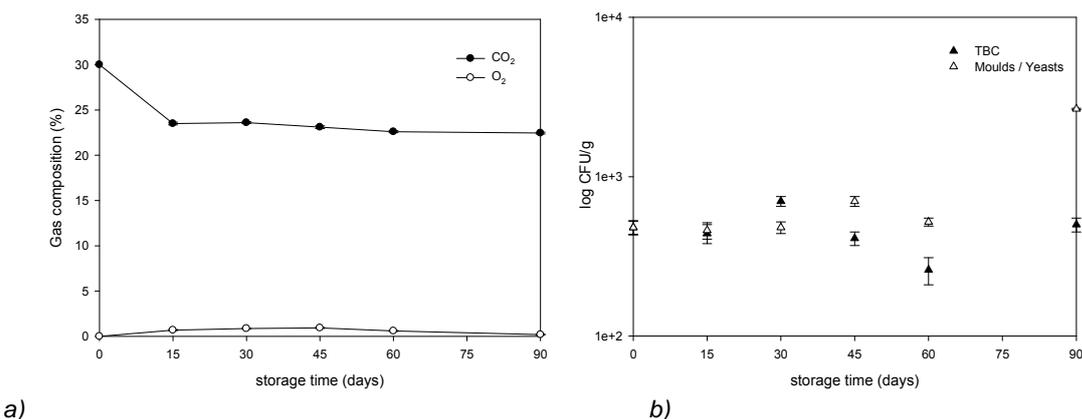


Figure 4. Changes of a) CO_2 and O_2 concentrations and b) total bacteria count and moulds and yeasts in the packages of roasted chestnuts in modified atmosphere (MAP) during the storage.

The microbial results of the total bacterial count and moulds and yeasts in the MAP during storage are shown in Figure 4b. The initial population of TBC and yeasts and moulds is approximately $2 \log CFU/g$ and it is nearly the same order of magnitude during storage. A significant change is observed for yeasts and moulds after 90 days of refrigerated storage, but population increase is considered satisfactory for the safety of ready-to-eat foods, the limit of which should not exceed the maximum of $5 \log CFU/g$ (NSW, 2012). The results are in agreement with those reported by Bhisnbut et al. (2008) who studied MAP and vacuum packaging on shells on and pre-engraved chestnuts in a shorter period of time (30 days): during this period no changes were observed.

4. Conclusions

1H NMR single sided provided information on water compartments and the movement of moisture contained in roasted chestnuts during storage in two different packages: modified atmosphere and semipermeable film. The longitudinal relaxation time $T1$ provides information about the water present in the larger clusters, mainly free water. The $T1$ trend agrees with chestnut weight loss which is lower in MAP than in SP package system. This trend is due to the high humidity values that can be reached in the package due to the poor permeability

of the polymeric films to water vapor. As a result, the reduction of the water content is slowed down and hindered. The MAP package also creates an unfavourable environment for microbial growth allowing a marketability of roasted fruits for up to 90 days, with respect to 30 days for SP film.

Acknowledgments

The project was funded by the Italian Ministry of Agricultural, Food and Forestry Policies.

References

- Adiletta G., Russo P., Proietti N., Capitani D., Mannina L., Crescitelli A., Di Matteo M., 2015, Characterization of pears during drying by conventional technique and portable non invasive NMR, *Chemical Engineering Transactions*, 44, 151-156
- Bhisanbut A, Shin J., Harte J., Fullbright D., Dolan K., Harte B., 2008, The extension of chestnut product quality using modified atmosphere packaging and vacuum skin packaging. IAPRI world conference on packaging
- Blaiotta G., Di Capua M., Romano A., Coppola R., Aponte M., 2014, Optimization of water curing for the preservation of chestnuts (*Castanea sativa* Mill.) and evaluation of microbial dynamics during process *Food Microbiology*, 42, 47-55.
- Capitani D., Mannina L., Proietti, N., ...De Salvador F.R., Delfini M., 2013, Metabolic profiling and outer pericarp water state in zespri, Cl.GI, and hayward kiwifruits. *J. Agric. Food Chem.* 61(8), pp. 1727–1740
- Capitani D., Sobolev A.P., Delfini M., ...Salvador F.R.D., Mannina L., 2014, NMR methodologies in the analysis of blueberries. *Electrophoresis*, 2014, 35(11), pp. 1615–1626
- Capitani D., Sobolev A.P., Di Tullio V., Mannina L., Proietti N., 2017, Portable NMR in food analysis. *Chem. Biol. Technol. Agric.* 4-17
- De Vasconcelos M.C.B.M., Bennett R.N., Rosa E.A.S., Ferreira-Cardoso J.V., 2010, Composition of European chestnut (*Castanea sativa* Mill.) and association with health effects: fresh and processed products. *J. Sci. Food Agric.*, 90, 1578–1589.
- Di Matteo M., Albanese D., Liguori L., 2012, Alternative methods for hazelnuts peeling. *Food Bioprocess Tech*, 5, 1416-1421.
- Jermeni M., Conedera M., Sieber T.N., Sassella A., Schärer H., Jelmini G., Höhn E., 2006, Influence of fruit treatments on perishability during cold storage of sweet chestnuts. *J. Sci. Food Agric*, 86, 877–885.
- Kan L., Li Q., Xie S., Hu J., Wu Y., Ouyang J., 2016, Effect of thermal processing on the physicochemical properties of chestnut starch and textural profile of chestnut kernel. *Carbohydrate Polymers*, 151, 614–623.
- Liu C., Wang S., Chang X., Wang, S., 2015, Structural and functional properties of starches from Chinese chestnuts. *Food Hydrocolloids*, 43, 568–576.
- Moreira R., Chenlo F., Torres M.D., Vázquez G., 2007, Effect of stirring in the osmotic dehydration of chestnut using glycerol solutions. *LWT*, 40, 1507–1514.
- Panagou E.Z., Vekiri S.A., Mallidis C., 2006, The effect of modified atmosphere packaging of chestnuts in suppressing fungal growth and related physicochemical changes during storage in retail packages at 0 and 8°C. *Advances in Horticultural Science*, 20 (1), 82-89.
- Pinter MD, Harter T, McCarthy MJ, Augustine MP., 2014, Towards using NMR to screen for spoiled tomatoes store in 1,000 L aseptically sealed. *MetalLined Totes Sensors*, 14, 4167–76.
- Proietti N., Adiletta G., Russo P., Buonocore R., Mannina L., Crescitelli A., Capitani D., 2018, Evolution of physicochemical properties of pear during drying by conventional techniques, portable-NMR, and modelling, *Journal of Food Engineering* 230, 82-98
- Silva A.P., Santos-Ribeiro R., Borges O., Magalhães B., Silva M.E., Gonçalves B., 2011, Effects of roasting and boiling on the physical and mechanical properties of 11 Portuguese chestnut cultivars (*Castanea sativa* Mill.). *Journal of food*, 9, 214–219
- Stilbs P., 1987. Fourier transform pulsed-gradient spin-echo studies of molecular diffusion. *Progr. Nucl. Magn Reson. Spectr* 19, 1-45.
- Veliyulin E, van der Zwaag C, Burk W, Erikson U., 2005, In vivo determination of fat content in Atlantic salmon (*Salmo salar*) with a mobile NMR spectrometer. *J Sci Food Agric.* 85, 1299–304.
- Xu Z, Morris RH, Bencsik M, Newton MI., 2014, Detection of virgin olive oil adulteration using low field unilateral NMR. *Sensors*, 14, 2028–35.
- Yang B., Jiang G., Gu C., Yang H., Jiang Y., 2010, Structural changes in polysaccharides isolated from chestnut (*Castanea mollissima* Bl.) fruit at different degrees of hardening. *Food Chemistry*, 119, 1211–1215.