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CHARACTERIZATION and COMPARISON of TURKISH TABLE OLIVE VARIETIES with NMR RELAXOMETRY and MAGNETIC RESONANCE IMAGING

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Abstract

Olive is one of the fruits that is mostly consumed in the Mediterranean region. Depending on the variety, oil quality of the olive changes significantly. In this study, Nuclear Magnetic Resonance (NMR), Relaxometry and Magnetic Resonance Imaging (MRI) experiments were used to characterize different Turkish table olive varieties, (*Ayvalik, Mega, Sele, Light*) in terms of tissue structure, fat and water contents. Moisture and fat content were measured using an infrared moisture analyzer and soxhlet extraction, respectively. NMR Relaxometry and MRI experiments were performed with a 0.32 T and 3T system, respectively. Longitudinal relaxation time (T_1) and transverse relaxation time (T_2) signals were acquired. Furthermore, non-negative least square (NNLS) method was implemented to T2 decay curves to investigate water and oil distributions. Turbo Spin Echo sequence with fat and water suppression was used for MRI experiments. Significant correlation existed between the physical measurements and NMR relaxation times.

Key Words: Table Olives, magnetic resonance relaxometry, magnetic resonance imaging

NMR RELAKSOMETRİ ve MANYETİK REZONANS GÖRÜNTÜLEME ile SOFRALIK TÜRK ZEYTİN ÇEŞİTLERİNİN KARAKTERİZASYONU ve KARŞILAŞTIRILMASI

Özet

Zeytin, çoğunlukla Akdeniz bölgesinde tüketilen bir meyvedir. Çeşidine bağlı olarak, kalitesi önemli ölçüde değişir. Bu çalışmada, Nükleer Manyetik Rezonans (NMR), Relaksometre ve Manyetik Rezonans Görüntüleme (MRG) deneyleri, farklı sofralık zeytin tiplerini (Ayvalık, Mega, Sele, Light) yapı, yağ ve su içerikleri açısından karakterize etmek için kullanılmıştır. Su ve yağ içerikleri, sırasıyla, kızılötesi nem analizörü ve soxhlet çıkarma kullanılarak ölçülmüştür. NMR relaksometre ve MR deneyleri sırasıyla 0.32 T ve 3 T sistemi ile yapılmıştır. T1-longitudinal salınım relaksasyon zamanı ve T2- transverse salınım değerleri elde edilmiştir. Ayrıca, T2 relaksasyon eğrisine NNLS yöntemi uygulanarak su ve yağ dağılımlarına dair bilgi elde edilmiştir. Turbo Spin Eko sekansı MRG ile yağ ve su baskılama deneyleri için kullanılmıştır. Fiziksel ölçümler ve NMR T1 ve T2 süreleri arasında ilişkiler bulunmuştur.

Anahtar kelimeler: Sofralık zeytin, manyetik rezonans relaksometre, manyetik rezonans görüntüleme

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INTRODUCTION

Olive has been cultivated in Aegean coast of Turkey for over 8000 years. Around 75-80% of the total olive oil production in Turkey is located in the Aegean region (1). Olive is a rich source of nutrients, bioactives and phenolic compounds as antioxidants, antimicrobials and has protective impact against cardiovascular diseases (1). Olive fruit is obtained from the olive tree (*Olea europaea* L.) which is a native of Mediterranean basin. *Olea europaea* L. tree is a small tree and mainly distributed in coastal regions of eastern Mediterranean Basin, coastal areas of southeastern Europe, Western Asia and Northern Africa. 98% of the world's olive cultivation takes place in Mediterranean region (2). Olive became more and more popular because of its promoting health aspects. Olive oil which is extracted from the olive fruit has also importance due to its known health effects. Olive oil production and consumption increased significantly over the past decades. Thus, characterization of oil contents of olive varieties and quality assessments became more important than ever (3).

The oil and water content of olives contribute to the acceptability and quality parameters of olives in a great extent. Oil content is usually determined by conventional extraction methods. Soxhlet extraction is one of the methods used and new methods are being sought for this purpose (4-6).

Magnetic Resonance Imaging (MRI) is a nondestructive technique which allows to observe internal structures of materials (7-9). This method can be used determine quality parameters of foods (10). MRI was also used to observe the internal structure of olive samples. Nuclear Magnetic Resonance (NMR) is a nondestructive method which is a reliable method to determine moisture and fat content of substances (11-13). In recent studies, NMR technique was started to be preferred over other conventional techniques because of its fast and easy use. NMR relaxometry is based on T_1 and T_2 measurements. T_1 (longitudinal relaxation time) characterizes the increase in the applied RF (Radio Frequency) waves in different planes while T_2 characterizes the decrease of applied RF pulses. Proton pools of samples are also determined by NMR relaxometry (14–16). NMR relaxometry technique takes advantage of different relaxation properties of water and oil. Therefore, determination of moisture and oil contents of food can be achieved by low field NMR (17, 18).

In this study, four table olive types (*Ayvalik, Mega, Light and Sele*) were chosen to determine oil and water contents through the use of NMR Relaxometry and MRI. Different NMR sequences (one pulse, inversion recovery, CPMG) and different MR imaging sequences (Turbo Spin Echo w/wo suppression) were used throughout the study to quantify the oil and water content of samples.

MATERIALS and METHODS

4 different table olive varieties were bought from a local store and they were stored at refrigeration temperature. The table olive varieties *Sele* and *Mega* are Gemlik type olives. Sele is obtained by a salt treatment of Gemlik olive.

Moisture Analysis

Moisture content of the table olives was determined using an infrared moisture analyzer (RADWAG MAC 50, Poland). Table olive samples were cut into pieces after the removal of the stone to dry the sample uniformly before placing in the moisture analyzer. Five measurements were recorded for each type olives.

Free Induction Decay (FID)

In Tiwari and Burk's study, oil contents of mustard, sunflower and soybean were correlated to Free Induction (FID) signal as 0.988, 0.945 and 0.931 respectively (19). This study showed that FID could be used for oil content determination of food materials. In order to utilize this method, moisture content of the olive samples were lowered below 5% via drying. FID was performed by using a 0.32 T (13.52) MHz low resolution system (Spin Track, Russia) to low-moisture olive samples to obtain the signal that was excluded from water. FID parameters were set as 7 ms echo time and 512 scans. Each olive samples were put into a tube separately and then measurements were conducted. For each type of olive, five measurements were taken.

To determine the oil contents of the samples through NMR, a calibration curve was established for the FID. Commercially available olive oil was obtained from the local markets and used in different amounts for different amounts of olive

oil in the tube, the signal intensity obtained from the sample changes. Signal intensity versus the amount of oil (ml) was plotted and calibration curve equation (R^2 =0.99) was found as shown Figure 1.

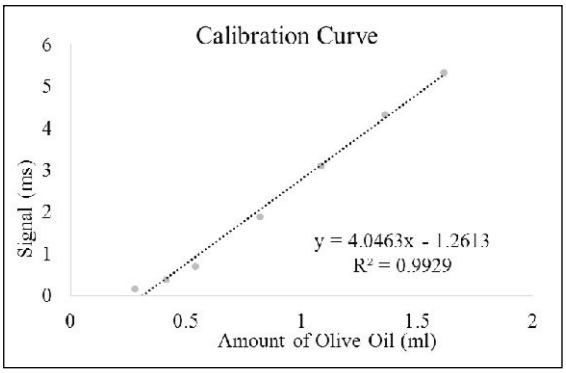

Figure 1: Calibration Curve prepared with Olive Oil in NMR

Oil Content Determination by Soxhlet Method

Soxhlet method was used to determine oil contents of olive types. *n*-Hexane was used as the extracting solvent for 6 hours (20). Moisture free samples were weighed before and after extraction. The differences of weights were attributed to the olive contents of table olive varieties.

T_1 (longitudinal relaxation time) & T_2 (transverse relaxation time) Determination

Each table olive samples with stones were put into tubes and tubes were placed in the 0.32 T system properly. For T_1 measurements, Saturation Recovery sequence was used with delay times changing between 10 us to 4 ms for 16 different times with 32 scans. For T_2 measurements, Carr-Purcell-Meiboom-Gill (CPMG) sequence was used with parameters of 1ms echo time, 400 echoes and 32 scans. 5 replicates were used for the measurements.

NNLS (Non-Negative Least Square) Analysis

Prospa 3.1 (Magritek Inc., Wellington, New Zealand) software was used to perform Non-Negative Least Square Analysis (NNLS). Amplitude and number of peaks of the relaxation spectrum of olives and their relative areas were analyzed in this method. NNLS macro is based on Lawson and Hanson algorithm that depends on regularization function that seeks to find a smooth spectrum of exponentials satisfying the data in a chi-squared sense (7).

Magnetic Resonance Imaging Experiments

For MRI experiments, a 3.0 T system (123.5MHz, SIEMENS, Germany) at Bilkent University National Magnetic Resonance Research Center (Ankara, Turkey) was used. Turbo Spin Echo sequence of 15 ms echo time and 1000 ms repetition time with water and fat suppression options were used for image acquisition. To calculate the proton density (M_0) of the samples, the signal intensity equation given below was used by substituting the T_1 and T_2 times acquired for each sample.

$$SI = M_0\left(1 - 2\exp\left(-\frac{TR - \frac{TE}{2}}{T1}\right) + \exp\left(-\frac{TR}{T1}\right)\right)\exp\left(-\frac{TE}{T2}\right)$$

Equation 1: Signal intensity equation TE: Echo Time, TR: Repetition Time, T1: longitudinal relaxation time, T2: transverse relaxation time

From the above equation proton densities for each table olive type were calculated with help of fat and water suppression images. MR ımages were analyzed using MATLAB (2013b).

Statistical Analysis

In order to correlate water and oil content with NMR results and MR signal intensity, linear regression analysis was conducted for all data. One-way ANOVA was used to detect if there was significant difference between the factors studied.

RESULTS

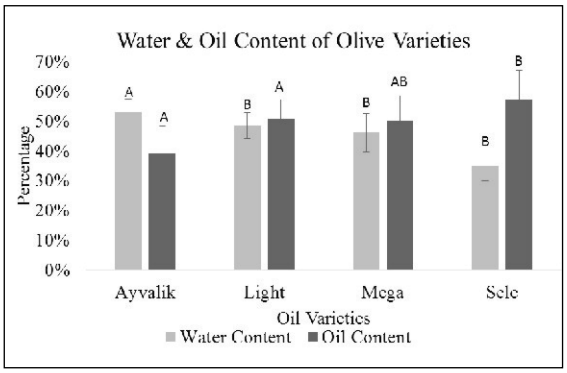

Figure 2: Water and Oil Content for Ayvalik, Light, Mega, Sele Olive Varieties

According to water content analysis in Figure 2, *Ayvalik* had the highest water content of 52%, and *Sele* had the lowest water content of 34%,

among all varieties. However, when compared with oil content in Figure 2, *Sele* had the highest content, 57%, and *Ayvalik* had the lowest oil content, 39%, among all varieties. In terms of water content *Ayvalik* was significantly different from other table olive varieties whereas oil content of Sele was significantly different from others. (P<0.05)

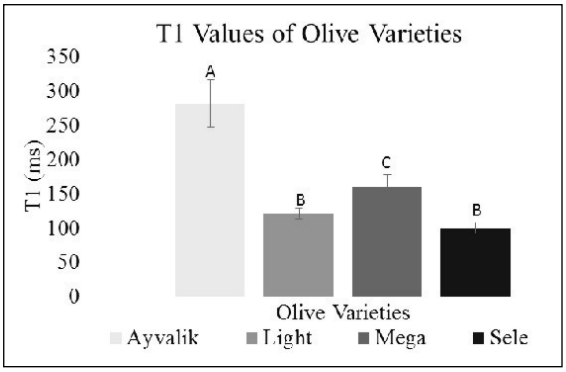

Figure 3: NMR T1 Values for Ayvalik, Light, Mega, Sele Olive Varieties

NMR T1 results in Figure 2 showed that *Ayvalik* had the longest T1 with 281 ms, among all varieties, whereas T1 times of *Light, Mega* and *Sele* were found to be 120, 159 and 100 ms respectively. T1 times of Ayvalik and Mega were significantly different among all varieties. (P<0.05)

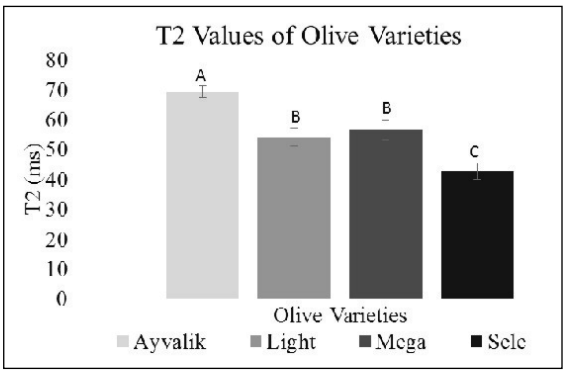

Figure 4: NMR T2 Values for Ayvalik, Light, Mega, Sele Olive Varieties

NMR T2 results in Figure 3 indicated that *Ayvalik* had the longest T2 with value of 69 ms. T2 times of Light, *Mega* and *Sele* were found to be 54 , 56, 42 ms, respectively. Ayvalik and Sele were significantly different while Light and Mega were not found different. (P<0.05)

Using the calibration curve in Figure 4, theoretical oil content of *Sele* was found to be the highest,

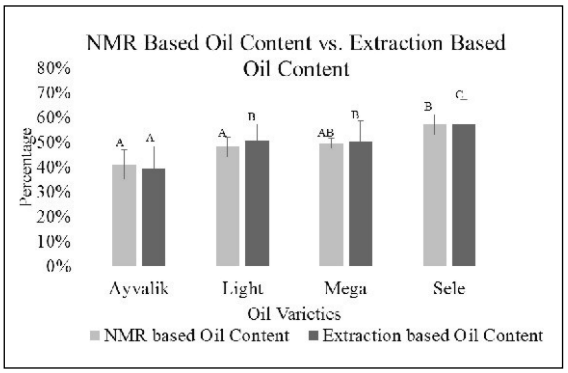

Figure 5: Oil Content prepared with Calibration Curve

with 57%, while *Ayvalik* had 41%, Light had 48% and *Sele* had 57% as seen in Figure 5. In extraction based oil content, *Mega* and *Light* samples were not significantly different from each other but they were significantly different in terms of their oil contents from *Ayvalik* & *Sele* samples (P<0.05). In addition, *Ayvalik* and *Sele* varieties had significantly different oil contents from each other (P<0.05)

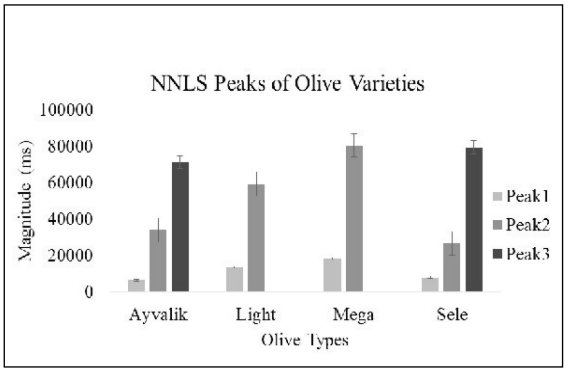

Figure 6: NNLS Peak results for Ayvalik, Light, Mega, Sele Olive varieties

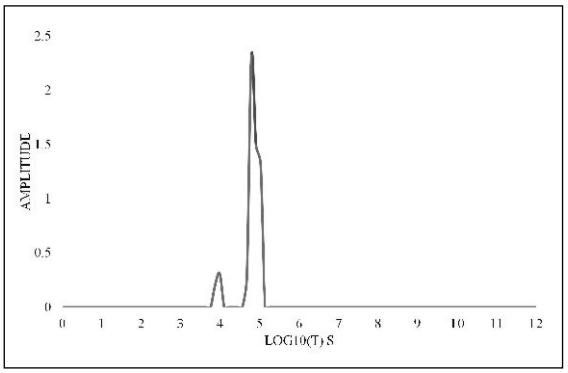

Figure 7: Representative image of NNLS spectrum of olive varieties, Ayvalik

NNLS analysis in Figure 6 showed that all olive types had two peaks but *Ayvalik* and *Sele* had one additional peak. Figure 7 shows a representative NNLS spectrum of *Ayvalik* variety.

T_1 and T_2 correlation with oil content in Table 1 was significant (P<0.05). M_0 found with water suppression equation was also significant (P<0.1). However, signal intensities obtained from MR images were not found to be proper for determination of water content as shown Table 1.

MR fat suppression images in Figure 8 show distribution of water in all varieties.

MR water suppression images in Figure 9 display distribution of oil in all varieties.

DISCUSSION

T_1 Relaxation Times (Oil & Water Content of Table Olive Varieties)

In Figure 3, the highest T_1 value observed in *Ayvalik* that had the highest water content. This justified that T_1 value was mostly related to water

Table 2: Correlation Table of MR results for all varieties with linear regression, R^2 and P value.

	Moisture Correlation	Oil Correlation
T_1	Water Content = 0.336 + 0.000721 x T1 R^2 = 0.576 P=0.241	Oil Content = 0.637 – 0.000877 x T1 R^2 = 0.943 P=0.029
T_2	Water Content = 0.0875 + 0.0066 x T2 R^2 = 0.875 P=0.065	Oil Content = 0.861 – 0.00661 x T2 R^2 = 0.971 P=0.014
Peak1	Water Content = 0.441 + 0.000001 x Peak1 R^2 = 0.008 P=0.912	Oil Content = 0.448 + 0.000004 x Peak1 R^2 = 0.077 P=0.723
Peak2	Water Content = 0.406 + 0.000001 x Peak2 R^2 = 0.099 P=0.685	Oil Content = 0.486 + 0.0000001 x Peak2 R^2 =0.002 P=0.955
Peak1 Area	Water Content = 0.434 + 0.0179 x Peak1 Area R^2 = 0.033 P=0.818	Oil Content = 0.467 + 0.214 x Peak1 Area R^2 = 0.053 P=0.771
Peak2 Area	Water Content = 0.43 + 0.047 x Peak2 Area R^2 = 0.033 P=0.819	Oil Content = 0.455 + 0.068 x Peak2 Area R^2 = 0.077 P=0.723
M_0 (Water Suppression)		Oil Content= 0.53 – 0.121 x $M_{0\ (Water\ Suppression)}$ R^2 = 0.855 P=0.075
M_0 (Oil Suppression)	Water Content = 0.426 + 0.123 x $M_{0\ (Oil\ Suppression)}$ R^2 = 0.442 P=0.33	

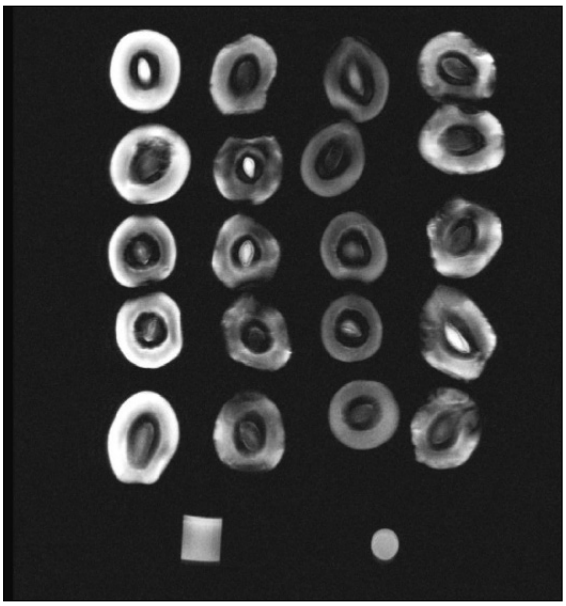

Figure 8: MR fat suppression images for all varieties with 5 replica; *Mega* is first column at the left, *Light* is second column at the left, *Ayvalik* is second column at the right, *Sele* is first column at the right. Above the MR image, there are two reference samples to increase signal to noise ratio.

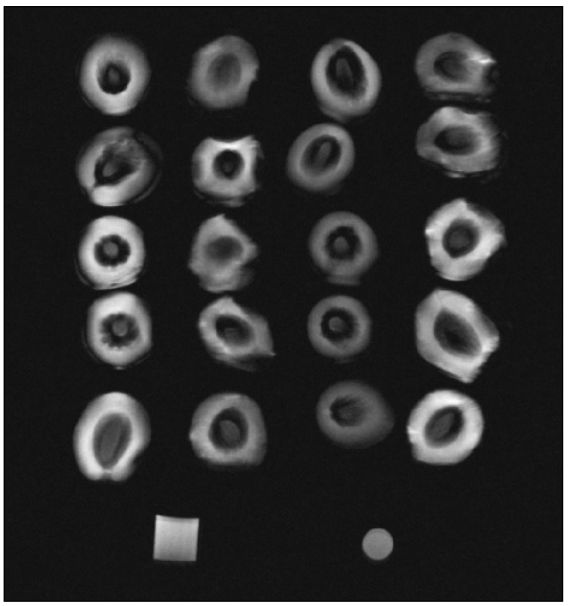

Figure 9: MR water suppression images for all varieties with 5 replica; *Mega* is first column at the left, *Light* is second column at the left, *Ayvalik* is second column at the right, *Sele* is first column at the right. Above the MR image, there are two reference samples to increase signal to noise ratio.

content. The lowest T_1 value was observed for Sele which had the least amount of water. T_1 values were also attributed to oil contents. The highest amount of oil containing table olive type which was *Sele* had the shortest T_1 time. This reverse relation was also applicable for *Ayvalik* since it had little amount of oil it had a long T1 value. Due to high salt concentration of Sele, water molecules bind to salt ions resulting in decrease of T_1 value. Since the relaxation time depends on proton relaxation properties, in the presence of salt ions there is less free water to relax.

T_2 Relaxation Times (Oil & Water Content of Table Olive Varieties)

In Figure 4, it was observed that with increase in oil content the T_2 values decreased. When the oil content in a water-oil system gets higher, T_2 value of the sample decreases since the T_2 value of oil is shorter than the T_2 value of water (21, 22). In this study this hypothesis was justified. T_2 value of *Sele* was lower than the other table olive varieties and it was related to binding of free water to ions. A similar trend was also observed in T_1 values of *Sele* samples but relatively small decrease in T_2 values in Sele compared to T_1 values originated from the high oil contribution to T_2 values. High oil concentration triggers a rapid decrease in T_2 values.

FID Measurements

In Figure 5, which shows the oil contents of table olive varieties based on NMR experiments, it was observed that *Light* & *Mega* samples were not significantly different from each other in terms of oil content. The same relation was observed for *Mega* & *Light* samples in conventional method. In addition, Ayvalik & Sele olive samples were found significantly different from each other in terms of oil contents (P<0.05). The conventional method again gave the same relation for *Ayvalik* & *Sele* which shows that NMR based experiments are reliable and consistent when compared to conventional method in determining oil contents (23).

Figure 6 indicates the T_2 times of different compartments that are present on olive tissue.

NNLS Analysis

NNLS shows the T_2 values of individual proton pools that come from oil and water. The presence of the third peak in *Ayvalik* & *Sele* samples affected the oil vs. peak area correlations in an undesirable manner as shown in Table 1 (24). 3rd peak indicates that there is another proton pool in those olive types which is related with the microstructure of the samples.

CONCLUSION

This study indicated that NMR relaxometry was a good method to determine the oil contents of different table olive samples (25). T_1, T_2 and M_0 values gave satisfactory correlations with oil contents. However, for moisture contents, NMR results were not satisfactory correlations as they were in oil contents (26). It was confirmed with the study that NMR was a sufficient and promising nondestructive method and it could be preferred over conventional oil determination methods in olives.

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