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Abstract

Pulsed field gradient spin echo (PFGSE) methods have been investigated as potential candidates for off-line and on-line determination of fruit quality. A single shot pulse sequence which uses diffusive attenuation to suppress the water signal in avocado is shown to give a good correlation with oil content. Following from this result, another pulse sequence was used to measure Brix in intact cellular tissue of apple and strawberry. Given the signal to noise ratio, the correlation for avocado and apple was established without repeated acquisition, so this protocol should also be useful for rapid, on-line measurements at low spectrometer frequencies. Water suppression by the T_1 -null method fails with cellular tissue because of the considerable variation in the longitudinal relaxation times of vacuolar and cytoplasmic water.

Results

The T_2 -D correlation spectrum [1] of ripe avocado tissue (figure 1) shows that the effective water diffusion coefficient is about 30 times greater than that of the oil [2], suggesting that a fast, single shot sequence could provide useful correlations with oil content. The schematic diagram of such a pulse sequence is shown in figure 2. The first echo (S_1) acquired with a short echo time (2 ms) is proportional to the total mobile proton signal, water and oil. The second echo (S') acquired with a longer echo time (40 ms) arises only from oil because of the diffusive attenuation of the water signal. This is achieved by applying strong suppression gradients, namely 0.4 T/m. A weak constant background gradient ($8.86 \cdot 10^{-3}$ T/m) was imposed to help sharpen the echo and improve signal to noise. The linear correlation between the ratio of the second and first echo with oil content is shown in figure 3. The oil content in avocado tissue is measured by the standard dry weight method and converted to oil content [3].

Following these encouraging results, the diffusivity of the water and sugars in the different cell compartments apple tissue was also investigated with T_2 -D spectroscopy. Such spectrum of fresh apple tissue has three peaks as shown in figure 4. Peak 1 is mainly associated with the water in the vacuole as it has the longest relaxation time [4]. It seems to have two overlapping components with slightly different relaxation times; which may be a consequence of a cell size distribution. Peak 2, with a short T_2 is likely to be associated with water in the cytoplasm. Peak 3, with a slower effective diffusion coefficient is associated with the non-exchanging protons in sugar [5]. The observation that the sugar diffusivity, even intact tissue, is significantly less than that of the vacuolar or cytoplasmic water strongly suggests that selective water suppression in cellular tissue can be achieved with pulsed gradient methods. Accordingly, the pulsed gradient area in a simple PGSE Hahn echo pulse sequence was first increased sufficiently to completely suppress the signal from a sample of pure water. This corresponded to a pulse area and diffusion time, $q^2\Delta$ of $1.016 \times 10^6 \text{ m}^{-2} \text{ s}$. These same gradient parameters were first used with sucrose solutions and the spin-echo intensity recorded as a function of concentration resulting in satisfactory linear correlations [5]. The spin-echo intensity against Brix value as measured by hand held refractometer for fresh apple tissue is shown in figure 5. In this experiment a small constant background gradient was applied to sharpen the spin echo and thereby increase the signal/noise ratio. The signal to noise ratio was reasonably good so the data was acquired with only a single scan and no phase cycling and no signal accumulation, which suggests that the method can also be used as a single-shot protocol for on-line Brix measurement.

The robustness of the method was further tested by repeating the correlation with strawberries. The parenchyma of strawberry is much softer than that of apple and cutting pieces of the same dimensions is not possible, therefore the echo intensity was normalised against weight. Nevertheless a satisfactory linear correlation is still obtained (figure 6) though it was necessary to increase the number of scans to 4 to obtain sufficient signal/noise with the smaller sample size.

Conclusions

Our results have established that it is possible to use water suppression by diffusive attenuation to measure indices of fruit quality, namely the oil content in avocado and Brix in intact cellular tissue of apple and strawberry. Subject to sufficient signal/noise, the correlations were established without the benefits of phase cycling or repeated acquisition, so this protocol should also be useful for rapid, on-line measurements at low spectrometer frequencies. Water suppression by the T_1 -Null method fails with cellular tissue because of the considerable variation in the T_1 's of vacuolar and cytoplasmic water.

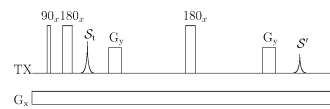


Figure 2: Water-suppression pulse sequence for oil content determination.

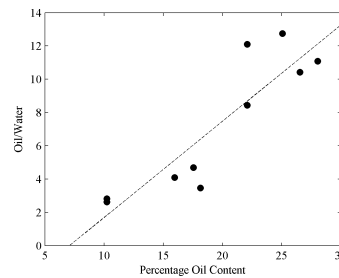


Figure 3: Linear Correlation between the second and first echo amplitudes with the percentage of oil content determined by the dry weight method for ripe avocado tissue ($y = 0.58x - 4.1$).

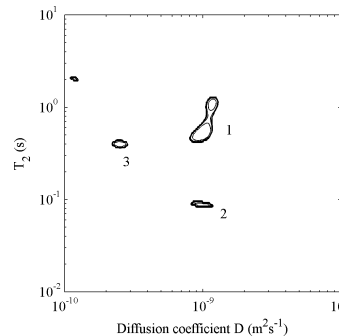


Figure 4: T_2 -D spectrum of fresh Red Delicious parenchyma acquired at spectrometry frequency of 23.4 MHz with a CPMG 90-180° pulse spacing of 4 ms.

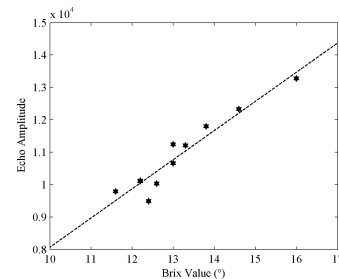


Figure 5: Spin echo amplitude of apple parenchyma tissue against Brix values as measured by hand held refractometer ($y = 901x - 934$). The experimental conditions were those of water suppression for sucrose.

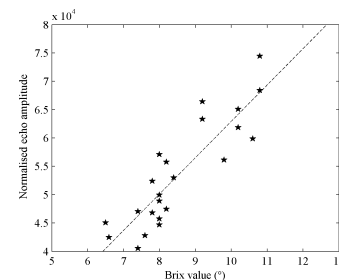


Figure 6: Normalised spin echo amplitude of strawberry parenchyma tissue against Brix values as measured by hand held refractometer ($y = 6410x - 1122$).

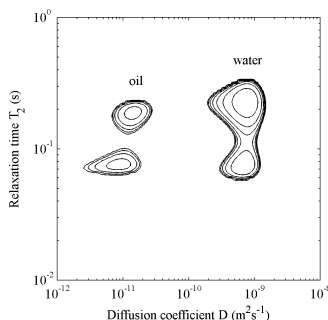


Figure 1: T_2 -D correlation spectrum of ripe avocado tissue acquired at 23.4 MHz with a 90-180° pulse spacing of 200 μs . After removing the skin, the sample was bored towards the stone and represents an average of outer, middle and inner layer.

References

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Acknowledgments

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