

# Evaluation of Low Field (5.40-MHz) Proton Magnetic Resonance Measurements of $D_w$ and $T_2$ as Methods of Nondestructive Quality Evaluation of Apples

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**ADDITIONAL INDEX WORDS.** *Malus × domestica*, nuclear magnetic resonance, self-diffusion, spin-spin relaxation, watercore, internal browning, bruising, soluble solids, titratable acids

**ABSTRACT.** A 5.40-MHz NMR system was used for measuring the self-diffusion coefficient of water ( $D_w$ ) and the spin-spin relaxation constant ( $T_2$ ) in apple (*Malus × domestica* Borkh.) tissue. The pulsed field gradient spin echo (PFGSE) technique was used to measure  $D_w$ , and the Carr-Purcell-Meiboom-Gill (CPMG) technique was used to measure  $T_2$ .  $T_2$ ,  $T_2$  and  $D_w$  values were compared for apples with differing amounts of soluble solids concentration (SSC) and with and without internal defects, such as bruising, watercore, and internal browning. ‘Granny Smith’, ‘Golden Delicious’, and ‘Delicious’ apples were tested. In ‘Golden Delicious’,  $D_w$  highly correlated with apple tissue SSC ( $P < 0.002$ ,  $r^2 = 0.68$ ). This indicates that  $D_w$  could potentially be used for sorting ‘Golden Delicious’ apples based on SSC, but the coefficient of determination needs to be improved before it would be commercially viable. There were no measurable differences in  $D_w$  among healthy apple tissue and tissue affected by either watercore or internal browning.  $T_2$  values showed no relationship between healthy apple tissue and bruised tissue in ‘Golden Delicious’ and ‘Granny Smith’. However, in ‘Delicious’ tissue,  $T_2$  values were statistically different between healthy and bruised tissue ( $P < 0.02$ ). Further comparisons in ‘Delicious’ between watercore and healthy apple tissue showed no differences. But, there were statistical differences found between  $T_2$  in healthy apple tissue and tissue with internal browning ( $P < 0.01$ ). These results indicate that  $T_2$  could potentially be used for separating ‘Delicious’ apples with internal browning or with bruising from healthy apples. Titratable acids and pH were correlated for ‘Golden Delicious’ ( $P < 0.08$ ). This correlation is significant because one may be able to noninvasively measure pH in ‘Golden Delicious’ apples using NMR, which could then be correlated to titratable acids.

Apple production in the United States in 1995 was 10 million metric tons with a value of 1.1 billion dollars (U.S. Dept. of Commerce, 1995). Currently, external apple quality is determined using visual inspection or optical equipment. Random samples are used to evaluate firmness and to detect internal defects such as watercore and internal browning. Apple lots with high percentages of defects are rejected. This means the good apples within rejected lots are discarded or sold for less than their intrinsic market value. Apples sold for processing are worth \$40/t (U.S. Dept. of Commerce, 1994) whereas apples sold on the fresh market may be worth \$200/t (U.S. Dept. of Commerce, 1995). Therefore, even if a rejected lot of apples could be sold for processing, the loss of income to the seller would be substantial. Nondestructive detection of apples within rejected lots could be used to sort good apples from defective apples, which could be sold at the appropriate market prices.

To date, nondestructive testing of the soluble solids concentration (SSC) of apples has not been possible. Advantages of sorting apples by soluble solids level have yet to be determined, because the nondestructive testing techniques needed for this type of research have not been available. However, it seems reasonable

that separation of apples into two or three SSC categories (e.g., high, medium, and low) would offer advantages for marketing, processing, or subsequent management of apples in storage.

Watercore is a quality disorder associated with particular apple cultivars. Before harvest, while the fruit is still on the tree, intercellular air spaces in flesh adjacent to the vascular bundles around the core become saturated with a sorbitol solution (Meheriuk et al., 1994). As the fruit matures, the saturated region expands into the cortex and outer cortex (Clark et al., 1997, 1998; Marlow and Loescher, 1984). Watercore tissue lacks the ability to convert sorbitol to fructose and is characterized by a glass-like appearance. Watercore has been detected by magnetic resonance imaging using proton density distribution (Wang et al., 1988). High signal intensity was found in watercore regions. Wang et al. (1988) were able to characterize the distribution and severity of watercore at individual locations within the sample.

Some apple cultivars with watercore tend to develop flesh browning during storage (B. Upchurch, personal communication). With this defect the interior of the apple becomes brown and mushy. This can then develop into senescent breakdown where the fruit becomes dry, soft, and mealy (Meheriuk et al., 1994). Deterioration starts at the core and spreads outward. Except in the most severe cases, this condition has no effect on an apple’s external appearance. However, flesh browning or senescent breakdown renders the fruit unmarketable. ‘Fuji’ apples with watercore do not develop flesh browning or senescent breakdown (Clark et al., 1998). After 3 or more months in storage ≈30% of ‘Delicious’ apples with watercore may develop flesh browning and/or senescent breakdown. In the remaining 70% of

Received for publication 27 July 1998. Accepted for publication 4 Jan. 1999. Purdue Agricultural Experiment Station journal paper 15413. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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the fruit, the condition ameliorates itself. Because of the difficulty in distinguishing severe flesh browning and senescent breakdown, the word internal browning (IB) has been used as a collective term to describe apples that have either flesh browning or senescent breakdown.

The spin-spin relaxation constant ( $T_2$ ), measured by magnetic resonance sensors, is a function of chemical composition, molecular mobility, temperature, diffusion rate, species concentration, and structure (Schrader et al., 1992). Three main causes of  $T_2$  relaxation in fruit have been suggested. Using aqueous solutions of varying pH, Meiboom (1961) determined that  $T_2$  depends on the amount of  $^{17}\text{O}$  present at a given pH. More recently Hills and Duce (1990) and Duce et al. (1992) have determined that fast proton exchange between water and solutes and diffusion through internally generated field gradients affect  $T_2$ . The fast proton exchange mechanism is dependent upon the stage of the ripening process. As starches are broken down into sugars, more solute hydroxyl groups are available to exchange with the water protons. This increases exchange, causing  $T_2$  to decrease with ripening. The third factor, which affects  $T_2$  and is discussed by Duce et al. (1992), is the presence of internally generated field gradients. These gradients develop as a result of magnetic susceptibility variations at air-liquid interfaces.

Although some investigators have proposed that the  $T_2$  relaxation curve for apple tissue is comprised of three exponential decay terms (Snarr and Van As, 1992), such multiexponential relaxation curves should be interpreted with caution because several different mechanisms can be involved (Belton and Ratcliffe, 1985; Brownstein and Tarr, 1977; Hills et al., 1989; Snaar and Van As, 1992). Analyses of Carr-Purcell-Meiboom-Gill (CPMG) data for apple samples from this system showed a single exponential behavior ( $r^2 > 0.98$ ). Multiexponential fitting of the data produced no additional information. The CPMG data were sampled for 2 s with a time delay between consecutive  $180^\circ$  pulses of 2 ms (1000 data points).

Wai et al. (1995) used a static gradient in a proton magnetic resonance ( $^1\text{H}$ -MR) sensor to measure signal parameters affected by self-diffusion of water. They developed a modified Hahn Echo pulse sequence, which gave an Echo Ratio (ER) that was affected by the self-diffusion coefficient of water ( $D_w$ ). They

reported that  $D_w$  decreased with an increase in percent sucrose in aqueous sucrose solutions and that ER was correlated to the weight percent sucrose in these solutions. Tests on core samples from fruit showed a correlation between ER and refractometer measurements of the SSC of juice squeezed from the fruit samples.

Previous researchers have reported relationships between these proton magnetic resonance parameters and SSC. Callaghan et al. (1994) found that magnetic resonance images of kiwifruit immediately after harvest and after 1 month storage showed significant decreases in  $D_w$  occurred and they attributed these to the conversion of starch to soluble sugars, which increased viscosity. They also observed from images that CPMG- $T_2$  values in the flesh and the locules were smaller in the ripened fruit. They suspect that this is the result of increased rotational correlation times due to more frequent interactions with the sugar molecules. Birch and Karim (1992) also saw a decrease in CPMG- $T_2$  with increasing glucose concentration and an increase in CPMG- $T_2$  with dextrose equivalent. This work also supports the conclusion that increased correlation times due to increased sugar content and degree of polymerization (100/DE) reduced CPMG- $T_2$  values. Work by Cho et al. (1993) showed that, based only on chemical exchange,  $T_2$  should decrease linearly with increasing SSC. Their results suggest that a more detailed analysis is needed to adequately describe the relationship between  $T_2$  and SSC.

The objective of this research was to evaluate the acceptability/accuracy of using proton magnetic resonance for the nondestructive measurement of apple soluble solids concentrations and the detection of internal defects in apples using measurements of the self-diffusion coefficient of water ( $D_w$ ) and the spin-spin relaxation constant ( $T_2$ ) of apples. The Pulsed Field Gradient Spin Echo pulse sequence (PFGSE) was used to measure  $D_w$  and the CPMG pulse sequence was used to measure  $T_2$ .

## Materials and Methods

**MATERIALS.** Three apple cultivars were used in these tests: 'Golden Delicious', 'Delicious', and 'Granny Smith'. Collectively, they give a set of apple tissue samples with a wide range of SSC and titratable acidity (TA). 'Granny Smith' apples have higher acid and less sugar than 'Golden Delicious' and 'Deli-

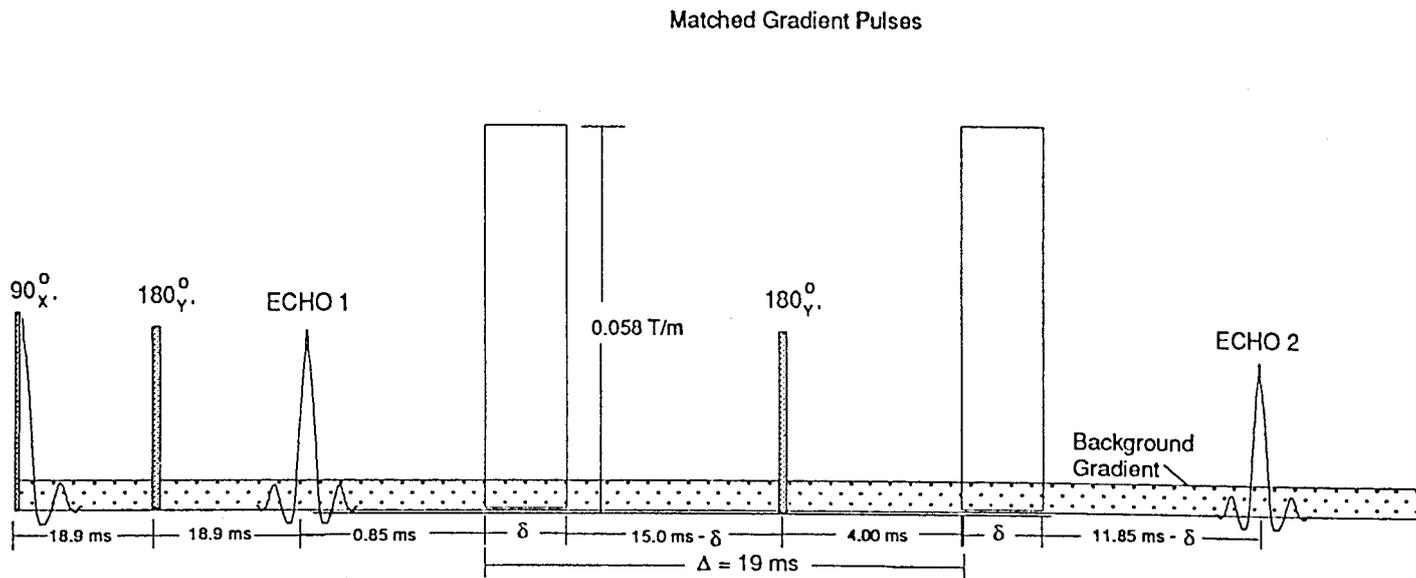


Fig. 1. PFGSE sequence used for measuring  $D_w$  of apple tissue. The background gradient ( $\approx 0.0013 \text{ T}\cdot\text{m}^{-1}$ ) was caused by magnet inhomogeneity and sample heterogeneity. Gradient pulses were applied to quadrupole coils located on the perimeter of the solenoid coil, which produced  $90^\circ$  and  $180^\circ$  pulses (Keener, 1996).

cious'. 'Golden Delicious' have the highest sugar content of the three cultivars and acid levels comparable to those of 'Granny Smith'.

Apples were peeled and sliced longitudinally into quarters with a knife. The core was removed and the quarters were cut parallel to the stem-calyx axis into  $\approx 3$  mm thick slices. Sixty milliliter cylindrical sample bottles 40 mm in diameter were filled with fruit segments from 'Golden Delicious', 'Delicious', and 'Granny Smith' apples.

**MAGNETIC RESONANCE TESTS.** The samples were evaluated using the LF-1A Magnetic Resonance Sensor (Stroshine et al., 1994), and a specially built magnet, the MAR-7 (Keener et al., 1996). As described by Keener et al. (1996), the system was adapted for  $D_w$  measurements using the Pulsed Field Gradient Spin Echo (PFGSE) technique. The CPMG pulse sequence,  $90^\circ\text{-}\tau\text{-}[180^\circ\text{-echo-}\tau]_n$ , was used to measure  $T_2$ . These were tested in the 44 mm diameter probe with a  $0.058 \text{ T}\cdot\text{m}^{-1}$  gradient.  $D_w$  was measured using the PFGSE test (Fig. 1).  $T_2$  was measured using the CPMG test with a tau time of 1.0 ms.

In the PFGSE tests, the gradient duration varied from 0.5 to 6.0 ms, and an echo ratio was measured for each. Equations 1 and 2 are obtained from a mathematical analysis of the PFGSE sequence (Stejskal and Tanner, 1965).

$$\frac{A(2\tau)}{A_0(2\tau)} = \exp[-kD_w] \quad [1]$$

$$k = [(\gamma G \delta)^2 (\Delta - \frac{\delta}{3})] \quad [2]$$

where  $A(2\tau)$  = echo amplitude at time  $t$ ,  $A_0(2\tau)$  = echo amplitude at time  $t$  with no pulsed gradient,  $D_w$  = self-diffusion coefficient of water ( $\text{m}^2\cdot\text{s}^{-1}$ ),  $\gamma$  = gyromagnetic ratio,  $\gamma_H = 2.6752 \times 10^7 \text{ T}^{-1}\cdot\text{s}^{-1}$ ,  $G$  = applied gradient,  $0.058 \text{ T}\cdot\text{m}^{-1}$ ,  $\delta$  = length of gradient pulse,  $s$ ,  $\Delta$  = time between matched gradient pulses,  $s$ .

On a plot of the natural logarithm of echo ratio versus the applied gradient ( $k$ ) the slope is equal to  $D_w$  (Stilbs, 1987). Sample data are shown in Fig. 2.

**SOLUBLE SOLIDS CONCENTRATION AND TITRATABLE ACIDS.** After completion of MR tests, juice was squeezed from each sample and a hand-held, temperature corrected refractometer (0 to 32 °Brix range) was used to measure SSC. This was taken to be mass percent soluble solids in the juice. The pH of 5 mL of the juice was measured using an electrode pH meter. Titratable acid level was determined by neutralizing (7.0 pH) the 5 mL of juice with a dilute sodium hydroxide solution ( $\approx 0.02 \text{ mol}\cdot\text{L}^{-1}$ ). This process was monitored with the pH meter. The amount of acid neutralized was converted to a weight percent assuming all of the acid was malic acid. Linear regression analyses were used to determine whether  $D_w$  and  $T_2$  were related to SSC.

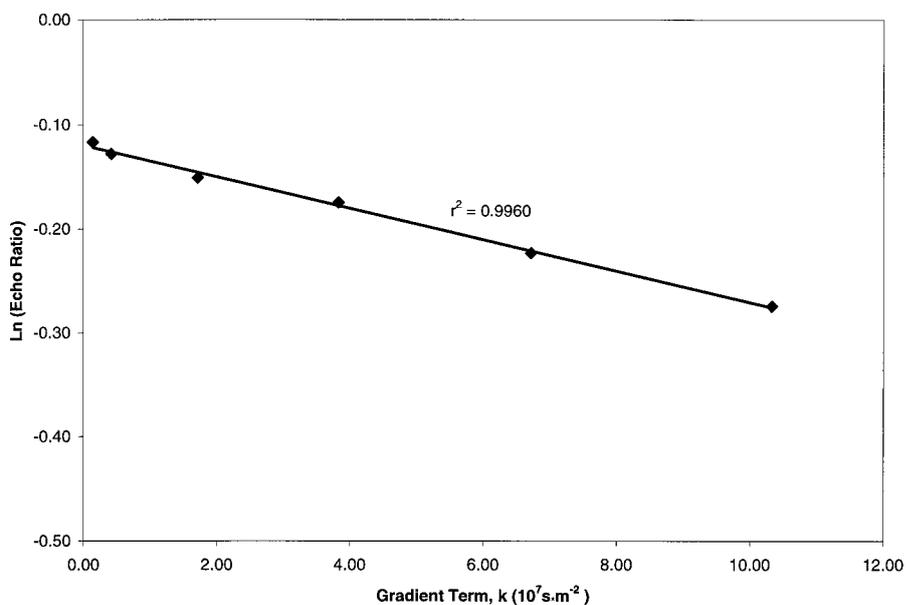
The influence of insoluble solids on  $D_w$  was evaluated in a second set of apple samples prepared in the same manner as the previous samples. Percent soluble solids was determined on a small portion ( $\approx 15$  g) of the apple by refractometer measurement of expressed juice. Additionally, a piece from each apple was weighed (20 to 25 g) and then dried at  $70^\circ\text{C}$  in a convection oven for 72 h. The oven drying gave the total solids and moisture content. Using the total weight of water in the sample and the weight percentage of soluble solids the insoluble solids in each sample were calculated.

**BRUISED AND DAMAGED TISSUE.** Tests were also conducted to investigate whether the  $D_w$  and  $T_2$  measurements could be used to differentiate between bruised and nonbruised apple tissue. 'Golden Delicious', 'Delicious', and 'Granny Smith' apples were dropped 0.76 m (30 inches) onto a concrete floor. By dropping each apple four times, bruises were created in four separate locations  $90^\circ$  apart around the equator of the apple. The apples were kept at room temperature for 3 h after bruising so that characteristic changes could occur. During this time, the bruised areas turned dark brown and browning was visible beneath the skin. Samples of nonbruised, healthy (H) tissue and bruised (B) tissue were removed under normal room conditions ( $20^\circ\text{C}$  and 60% relative humidity) and placed in separate 60-mL glass bottles for testing. NMR testing was done immediately to limit tissue exposure to air. Total testing time including sample preparation was five minutes per sample. All samples in this study were prepared and tested in this same manner.

Comparisons of H tissue with tissue affected by watercore or internal browning were made on 'Delicious' apples. Six different 'Delicious' apples were identified as having watercore. They were taken from apples provided by B. Upchurch, U.S. Dept. of Agriculture Research Laboratory (Kearneysville, W.Va.). He detected watercore by passing light from a high intensity fiber optics system through the stem-calyx axis of the apples. Apples with watercore transmitted more light. The watercore apples were stored temporarily and then shipped to Purdue University. Samples were prepared from six of these apples by cutting and then dicing H tissue, which was near the outside of the apple away from watercore area, healthy tissue near the watercore (NW), and watercore tissue (WC). The diced tissue was placed in 60-mL sample bottles and tested.

Apples with internal browning were also provided by B. Upchurch. He identified apples as having watercore, stored them for  $\approx 5$  months at  $1^\circ\text{C}$  in air, and then removed them from storage and shipped them overnight to Purdue University. Apples were cut open and examined for the presence of internal browning. Samples of discolored tissue taken from apples with internal browning were labeled as IB and samples taken from apples with

Fig. 2. Sample data for an apple sample at  $22.5^\circ\text{C}$  from the PFGSE tests. The gradient term ( $k$ , Eq. 2) was increased by increasing  $\delta$ , the length of the gradient pulse;  $\delta$  was increased from 0.5 to 6 ms.



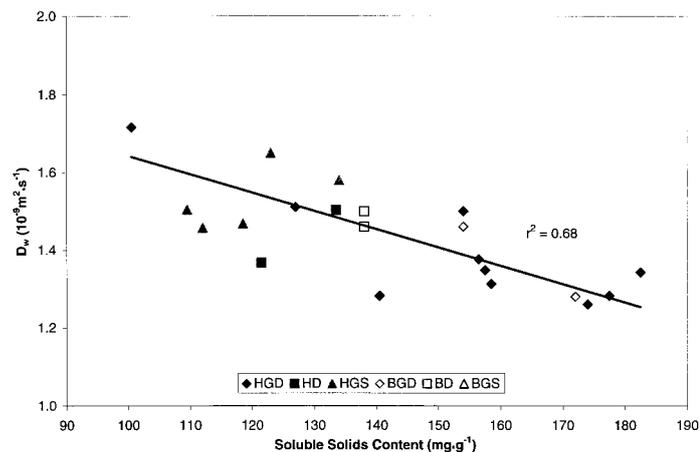


Fig. 3.  $D_w$  versus percent SSC for samples of apple tissue at 22.5 °C. The linear regression of  $D_w$  versus SSC gives a  $r^2$  of 0.68 for 'Golden Delicious'. HGD = healthy 'Golden Delicious', HD = healthy 'Delicious', HGS = healthy 'Granny Smith', BGD = bruised 'Golden Delicious', BD = bruised 'Delicious', and BGS = Bruised 'Granny Smith'.

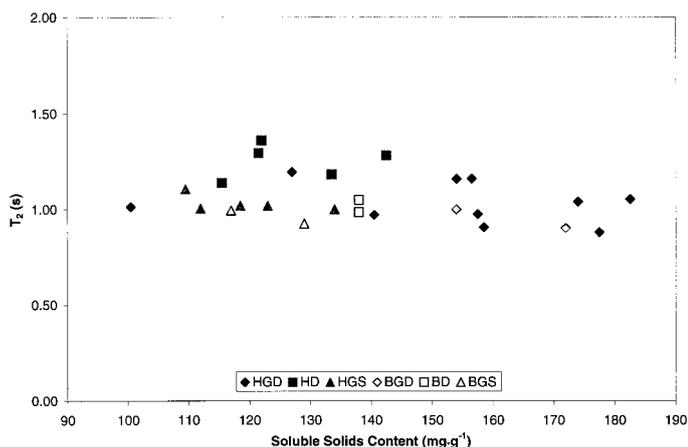


Fig. 4.  $T_2$  versus SSC for samples of apple tissue at 22.5 °C. HGD = healthy 'Golden Delicious', HD = healthy 'Delicious', HGS = healthy 'Granny Smith', BGD = bruised 'Golden Delicious', BD = bruised 'Delicious', and BGS = bruised 'Granny Smith'.

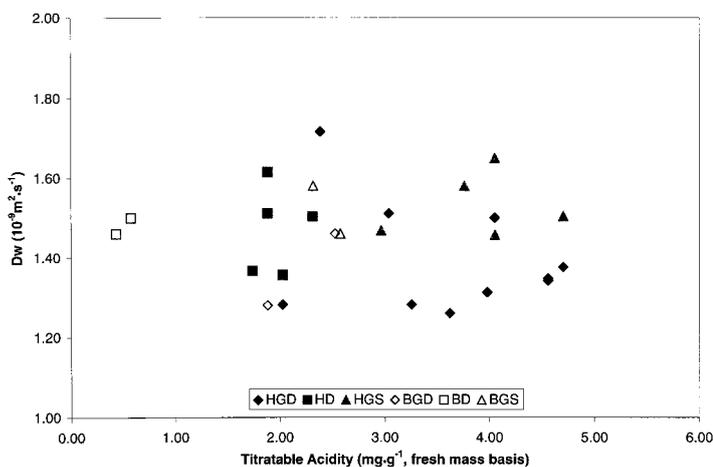


Fig. 5.  $D_w$  versus titratable acidity for samples of apple tissue. HGD = healthy 'Golden Delicious', HD = healthy 'Delicious', HGS = healthy 'Granny Smith', BGD = bruised 'Golden Delicious', BD = bruised 'Delicious', and BGS = bruised 'Granny Smith'.

only healthy tissue were labeled H. Of the 20 apples tested, 6 had internal browning.

Analyses of variance were performed on these data using SAS (Cary, N.C.). Statistical significance was based on a  $P < 0.05$ . Only results with a significance at  $P < 0.05$  are discussed, unless noted otherwise.

## Results and Discussion

**SOLUBLE SOLIDS CONCENTRATION.**  $D_w$  decreased with increasing SSC (Fig. 3). The line shown in Fig. 3 is the linear regression model for  $D_w$  versus SSC for 'Golden Delicious' ( $P < 0.02$ ,  $r^2 = 0.68$ ). There was no statistical significance between  $T_2$  and SSC in any of the three apple cultivars (Fig. 4).  $D_w$  and  $T_2$  showed no statistical significance with TA (Fig 5 and Fig 6) or pH (Fig. 7 and Fig. 8). It was interesting that in the 'Golden Delicious', which had a low pH and high SSC, TA and pH had a statistically significant relationship ( $P < 0.09$ ) (Fig. 9). However, 'Delicious' apples and 'Granny Smith' apples showed no dependence between pH and TA. This indicates that 'Golden Delicious' apples may contain less buffering whereas 'Delicious' apples and 'Granny Smith' apples may contain more. This correlation is significant because one may be able to noninvasively measure pH using NMR, which could then be correlated to TA in 'Golden Delicious' apples.

**BRUISING.**  $D_w$  was appreciably different between B and H apple tissue in only one apple ('Granny Smith') at 120  $\text{mg}\cdot\text{g}^{-1}$  SSC (Table 1). There was no statistically significant change in SSC before or after bruising. However, in 'Granny Smith' when B and H samples were taken from the same apple the B samples were slightly lower in SSC than the nonbruised.  $T_2$  decreased in all the B tissue compared with H tissue and the  $T_2$  values for B apples ranged between 0.12 and 0.135 s lower than  $T_2$  values for H fruit. This effect was only statistically significant in 'Delicious' ( $P < 0.01$ ) and are shown separately in Table 2. This same effect was observed by McCarthy et al. (1995) in  $T_2$  data from B and H apples. However, they saw a decrease in sample heterogeneity as measured by the Hahn Spin Echo Method (Hahn, 1950). It is likely that, upon cell rupture, either the mixing of intracellular with extracellular components or the activity of enzymes released from cells in the bruising process creates or increases relaxation (chemical exchange) even though sample heterogeneity is reduced. This work also supports this because both TA and pH were lower for bruised tissue compared with H tissue (Table 1). These decreases were significant in the 'Golden Delicious' ( $P < 0.02$ ) and 'Granny Smith' ( $P < 0.01$ ) cultivars. Measurement of titratable acids in the samples revealed that, on the average, the percent acid in bruised apple tissue was 43% lower than the percent acid in H tissue from the same apple (Table 1). Previous work with solutions containing 0 to 93  $\text{mg}\cdot\text{g}^{-1}$  organic acid by fresh mass (FM) has shown that  $D_w$  is correlated to organic acid level (Keener, 1996). However, in the samples tested in this study, the TA did not appear to affect  $D_w$  possibly because the levels of organic acid in apple fruit is relatively low ( $< 10 \text{ mg}\cdot\text{g}^{-1}$  fresh weight).

**WATERCORE.** The SSC of the tissue taken near the area where watercore developed (NW) was  $\approx 30$  to 40  $\text{mg}\cdot\text{g}^{-1}$  (fresh weight) higher than the SSC of the healthy tissue (H) near the outside periphery of the apple, and the watercore tissue (WC) was slightly higher in SSC than the H tissue (Table 2) ( $P < 0.04$ ). Tests on healthy apples showed SSC variations from 10 to 20  $\text{mg}\cdot\text{g}^{-1}$  (fresh weight) around the periphery of the cross section. In these tests, the variations in SSC were greater around the periphery of the cross section, 20 to 40  $\text{mg}\cdot\text{g}^{-1}$  (fresh weight). Note that the NW

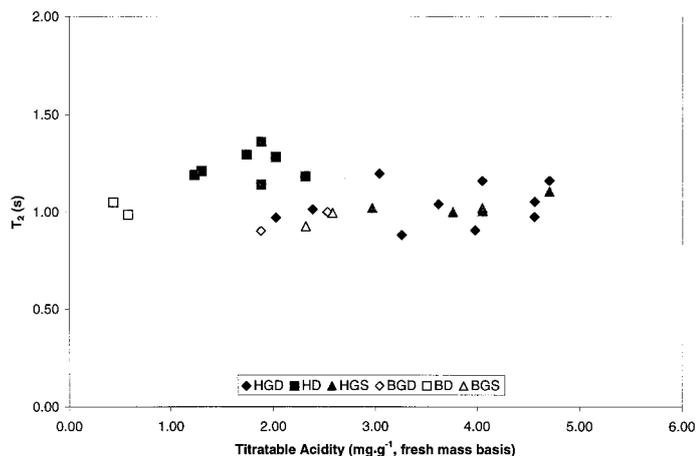


Fig. 6.  $T_2$  versus titratable acidity for samples of apple tissue. HGD = healthy 'Golden Delicious', HD = healthy 'Delicious', HGS = healthy 'Granny Smith', BGD = bruised 'Golden Delicious', BD = bruised 'Delicious', and BGS = bruised 'Granny Smith'.

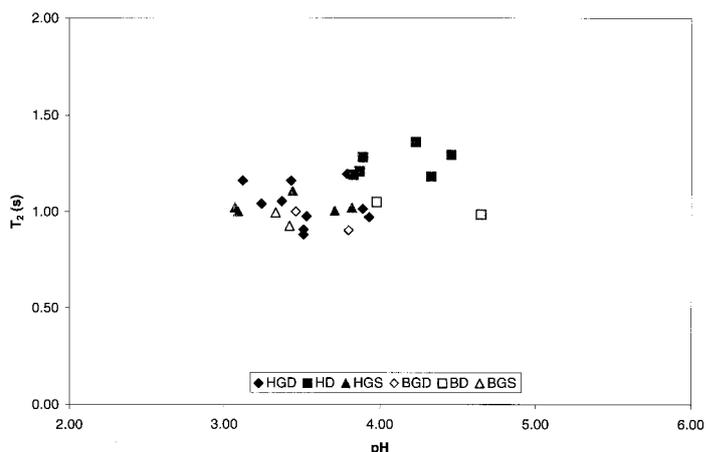


Fig. 8.  $T_2$  versus pH for samples of apple tissue. HGD = healthy 'Golden Delicious', HD = healthy 'Delicious', HGS = healthy 'Granny Smith', BGD = bruised 'Golden Delicious', BD = bruised 'Delicious', and BGS = bruised 'Granny Smith'.

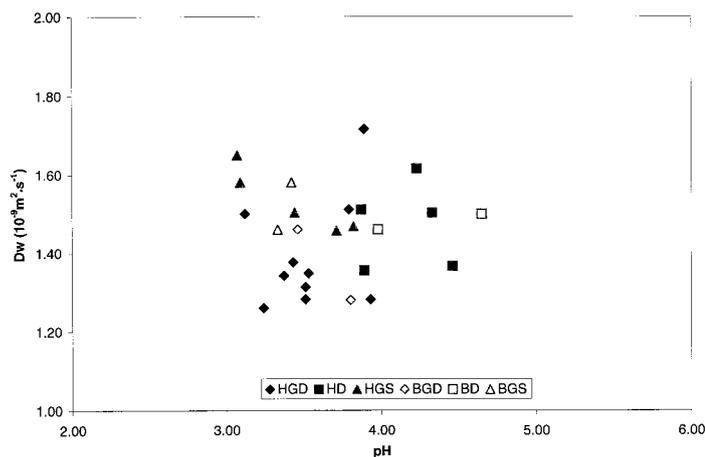


Fig. 7.  $D_w$  versus pH for samples of apple tissue. HGD = healthy 'Golden Delicious', HD = healthy 'Delicious', HGS = healthy 'Granny Smith', BGD = bruised 'Golden Delicious', BD = bruised 'Delicious', and BGS = bruised 'Granny Smith'.

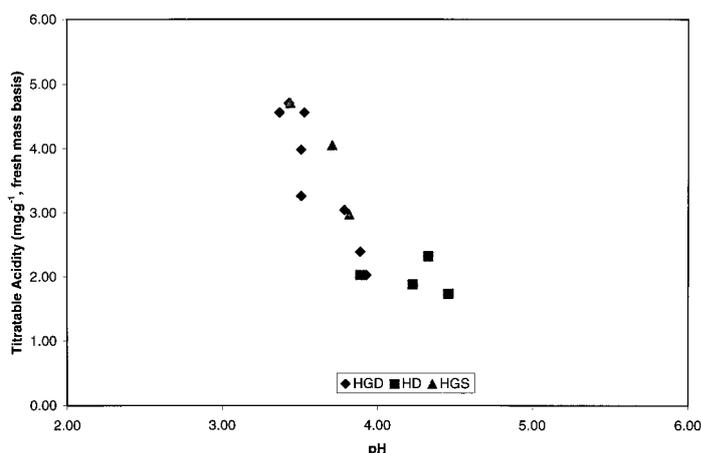


Fig. 9. Dependence of titratable acidity on pH for samples of apple tissue. HD = healthy 'Delicious', HGS = healthy 'Granny Smith' (---), and HGD = healthy 'Golden Delicious' (—).

tissue had a higher SSC than the WC tissue. This suggests that some of the water that fills the air spaces around the cortex may have come from the neighboring tissue.  $D_w$  does not show any clear trends with increasing SSC for these samples (Table 2). However, the  $T_2$  values for watercore tissue were 0.08 to 0.0935 s lower than  $T_2$  values for healthy tissue (Table 2) although this difference was not statistically significant. The mechanisms that control watercore are unknown and are currently being studied (Clark et al., 1998; Marlow and Loescher, 1984).

**INTERNAL BROWNING.** The SSC range for these internal browning (IB) samples is very narrow because they were all 'Delicious' apples and had about the same maturity (Table 2).  $D_w$  varied greatly and showed no statistical significance with SSC measurement (Table 2). While  $T_2$  decreased with increasing SSC for the H tissue, no correlation between  $T_2$  and SSC was observed in the apples with IB (Table 2). The average  $T_2$  for tissue samples with internal browning was 0.25 s lower than the  $T_2$  of H ( $P < 0.01$ ) tissue samples. There was no statistical difference in  $D_w$  between tissue with IB and H. Since there is a loss of structure associated with IB, the decrease in  $T_2$  is consistent with changes observed in bruised tissue. The effect should be similar to bruising with

mixing of extracellular and intracellular fluids in air spaces within the sample.

## Conclusions

The effects of levels of SSC and TA on  $D_w$  and  $T_2$  were investigated using samples consisting of many small slices of apple tissue. Changes in TA were significantly correlated with pH in 'Golden Delicious' ( $P < 0.09$ ), indicating that there may be minimal buffering in these apple tissue. This may be important because pH can be measured noninvasively using NMR, which could then be correlated to TA. The  $D_w$  and  $T_2$  of apple tissue decreased with increasing refractometer SSC. However, the only statistically significant relationship was between  $D_w$  and SSC in 'Golden Delicious' apples. In 'Golden Delicious' apples,  $D_w$  was shown to be dependent on SSC ( $P < 0.05$ ,  $r^2 = 0.68$ ). This indicates that in 'Golden Delicious' apples the measurements of  $D_w$  could be used for noninvasive detection of SSC. However, this correlation between  $D_w$  and SSC was much lower than correlations for sucrose solutions and fruit juices (Keener, 1996) where coefficients of determination ( $r^2$ ) ranged from 0.89 to 0.95 ( $P < 0.05$ ). Nondestructive sorting by

Table 1.  $D_w$  and  $T_2$  versus titratable acidity (TA) and soluble solids content (SSC) for nonbruised, healthy (H) and bruised (B) apple tissue taken from 'Golden Delicious' (GD), 'Delicious' (D), and 'Granny Smith' (GS) apples.

| Cultivar <sup>z</sup> | Sample | Tissue condition | $D_w$ ( $m^2 \cdot s^{-1}$ ) | $T_2$ (s) | pH   | TA ( $mg \cdot g^{-1}$ ) | SSC ( $mg \cdot g^{-1}$ ) |
|-----------------------|--------|------------------|------------------------------|-----------|------|--------------------------|---------------------------|
| GD                    | 1      | H                | $1.50 \times 10^{-9}$        | 1.16      | 3.12 | 4.05                     | 154                       |
| GD                    | 1      | B                | $1.46 \times 10^{-9}$        | 1.00      | 3.46 | 2.53                     | 154                       |
| GD                    | 2      | H                | $1.26 \times 10^{-9}$        | 1.04      | 3.24 | 3.62                     | 174                       |
| GD                    | 2      | B                | $1.28 \times 10^{-9}$        | 0.902     | 3.80 | 1.88                     | 172                       |
| D                     | 3      | H                | $1.41 \times 10^{-9}$        | 1.21      | 3.87 | 1.30                     | 137                       |
| D                     | 3      | B                | $1.50 \times 10^{-9}$        | 0.985     | 4.65 | 0.578                    | 138                       |
| D                     | 4      | H                | $1.40 \times 10^{-9}$        | 1.19      | 3.83 | 1.23                     | 138                       |
| D                     | 4      | B                | $1.46 \times 10^{-9}$        | 1.05      | 3.98 | 0.434                    | 138                       |
| GS                    | 5      | H                | $1.58 \times 10^{-9}$        | 1.00      | 3.09 | 3.76                     | 134                       |
| GS                    | 5      | B                | $1.58 \times 10^{-9}$        | 0.926     | 3.42 | 2.32                     | 129                       |
| GS                    | 6      | H                | $1.65 \times 10^{-9}$        | 1.02      | 3.07 | 4.05                     | 123                       |
| GS                    | 6      | B                | $1.46 \times 10^{-9}$        | 0.994     | 3.33 | 2.58                     | 117                       |

<sup>z</sup>From each apple (samples 1–6) one nonbruised healthy and bruised sample was taken.

Table 2. A comparison of healthy (H) tissue and unhealthy tissue taken from 'Delicious' apples in three studies. The unhealthy tissue was bruised (B), near watercore (NW) or watercore (WC), or had internal browning (IB) in studies 1, 2, and 3, respectively.

|                              | Study                 |                       |                       |                       |                       |                       |                       |
|------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|                              | B <sup>x</sup>        |                       | WC <sup>y</sup>       |                       |                       | IB <sup>z</sup>       |                       |
|                              | B                     | H                     | NW                    | WC                    | H                     | IB                    | H                     |
| $D_w$ ( $m^2 \cdot s^{-1}$ ) | $1.48 \times 10^{-9}$ | $1.41 \times 10^{-9}$ | $1.36 \times 10^{-9}$ | $1.34 \times 10^{-9}$ | $1.44 \times 10^{-9}$ | $1.30 \times 10^{-9}$ | $1.28 \times 10^{-9}$ |
| SD                           | $3 \times 10^{-11}$   | $1 \times 10^{-11}$   | $7 \times 10^{-11}$   | $6 \times 10^{-11}$   | $4 \times 10^{-11}$   | $14 \times 10^{-11}$  | $12 \times 10^{-11}$  |
| $T_2$ (s)                    | 1.02                  | 1.20                  | 1.10                  | 1.01                  | 1.09                  | 0.68                  | 0.94                  |
| SD                           | 0.05                  | 0.01                  | 0.02                  | 0.09                  | 0.02                  | 0.06                  | 0.06                  |
| SSC ( $mg \cdot g^{-1}$ )    | 138                   | 138                   | 148                   | 131                   | 114                   | 145                   | 150                   |
| SD                           | 0.0                   | 1.0                   | 10.5                  | 1.0                   | 7.0                   | 5.0                   | 7.0                   |

<sup>z</sup>Tissue from different apples was compared. The apples were picked at the same time and stored under the same conditions in the same box.

<sup>y</sup>Samples of NW, WC, and H tissue were taken from the same apple.

<sup>x</sup>Samples of H tissue were taken from the same apple as the B tissue.

MR measurements would only be feasible if  $D_w$  versus SSC correlations for apples could be increased to levels similar to those for juices.

B and H tissue was evaluated in all three cultivars and no statistical difference was measured between  $D_w$  or  $T_2$  in B or H tissue except  $T_2$  in 'Delicious' apples (Table 2). 'Delicious' tissue had smaller  $T_2$ s for bruised vs H apple tissue ( $P < 0.02$ ). This indicates that depending upon the level of bruising  $T_2$  may be able to distinguish B and H tissue.

WC tissue samples had, on the average, a lower  $T_2$  (1.01 vs. 1.09 s) than H tissue. However, the difference was not as great as seen in B compared with H tissue, and this difference was not statistically significant. The SSC of the WC tissue [ $\approx 130 mg \cdot g^{-1}$  (fresh weight)] was greater than the H tissue [110 to 120  $mg \cdot g^{-1}$  (fresh weight)] and less than the NW tissue [140 to 160  $mg \cdot g^{-1}$  (fresh weight)]. These differences were statistically significant ( $P < 0.04$ ) and may indicate that the WC tissue is extracting some water from neighboring tissue.

Apple tissue with IB had a  $T_2$ , which was on average 0.25 s lower than the  $T_2$  of H tissue ( $P < 0.01$ ). There appeared to be no difference in  $D_w$  between apple tissue with IB and H tissue. These results suggest that it may be possible to detect IB in 'Delicious' apples by use of  $T_2$  measurements. Detection of watercore may also be possible, but it would be more difficult because watercore affects  $T_2$  much less than does IB.

It is also interesting to note that in Table 2 the  $T_2$  of H tissue varied in each study. Although time from harvest was not recorded, it was known that the B study had the freshest apples

and the IB study had the oldest apples. Even the H tissue in the IB study had a  $T_2$  about the same as the B tissue in the B study. These results suggests that it may be possible to use  $T_2$  measurements to separate 'Delicious' apples that are fresh, B, or have IB.

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