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Proton spin–spin relaxation time of peel and flesh of navel orange varieties exposed to freezing temperature

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Abstract: Proton spin–spin relaxation times (T_2) of peel (albedo and flavedo) and juice sacs (flesh segments) of navel oranges were measured at 10 MHz using a Bruker Minispec PC 110 NMR spectrometer. The oranges were subjected to chilling (5 °C) and freezing (−7 °C) temperatures for 20 h and warmed to room temperature before peeling for T_2 measurements. The exposure to chilling or freezing temperature did not affect the T_2 values of peel, but freezing caused an appreciable decrease (∼15%) in the T_2 values of flesh segments of the varieties of navel oranges studied. When the peel was exposed to −20 °C, the T_2 showed a drastic reduction suggesting that the peel did not freeze at −7 °C. The possible cause of reduction in the T_2 values when exposed to freezing temperature may be damage to the juice sac membrane and leakage of juice out of the sac. The difference in the T_2 values between juice sacs of freeze-affected and normal oranges can potentially be used for detection of freeze-damaged fruits.

Keywords: NMR relaxation time; navel oranges; freeze damage; internal quality

INTRODUCTION

The research and development of quality evaluation measurements of agricultural products and related techniques are becoming increasingly important. Non-destructive detection of external quality factors is more advanced in application than detection of internal factors. Post-harvest handling and processing of citrus fruits are economically important steps in transferring fruit from the orchard to the consumer. Citrus fruits are sorted to remove fruit that is damaged or of poor quality. The detection of internal defects such as freeze damage is problematic. Freeze damage in oranges does not occur every year but it is a common event. It is usually not visible on the surface of the fruit. Over time the freeze-damaged interior portions of the fruits become dry, causing the fruit to become unmarketable. A rapid, non-destructive method of detecting freeze damage would allow the industry to prevent poor-quality fruit from reaching the consumer.

Current sorting systems for quality evaluation of citrus fruit provide significant information on surface defects but are of little use for detection of internal defects such as freeze damage. NMR techniques have been used for examining agricultural products from biological and physiological points of view since the 1960s and engineering-oriented applications of NMR to agricultural products began in the late 1980s. The application of NMR has been reported for evaluation of internal quality of fruits and vegetables.[1,2] In recent years, NMR techniques have also been used to monitor changes in foods at harvest, during post-harvest storage, and during processing.[3] Chen *et al*[4] studied maturity evaluation of avocados by NMR methods, while other researchers have used NMR techniques for studying bruising, browning and loss of water core in apples.[5–8] However, the application of NMR related to changes following freezing of fruit has been rare. Duce *et al*[9] found qualitative changes in NMR images of courgette before and after freeze–thawing. Gamble[10] studied changes in the exchange properties of aqueous protons at discrete locations within the fruit, after freezing and thawing fresh blueberries. Freezing effects in kiwifruit have been studied by NMR imaging.[11] All of these previous studies have reported changes in the proton spin–spin relaxation rates of fruit after freezing and thawing. The purpose of this study was to determine if low-field NMR could

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be used to detect freeze damage in oranges through relaxation time measurements.

MATERIAL AND METHODS

Three varieties (Washington, Summer Gold and Lane Late) of freshly harvested navel oranges were supplied by UC Lindcove Research & Extension Center, Exeter, CA, USA, and nearly 160 oranges were examined for the present study. The oranges were stored in a cold room at 5 °C. For the freeze damage study, all the varieties of oranges were kept for 20 h in a freezer chest maintained at −7 °C. In order to evaluate change in relaxation times for complete frozen state, the Summer Gold oranges were exposed to −20 °C for 20 h in a cold room. The samples were thawed/warmed to room temperature (21 °C) over 24 h under normal laboratory conditions before NMR measurements.

A Bruker Minispec PC 110 NMR spectrometer (Bruker Optics, Minispec Division, Woodlands, TX, USA) operating at 10 MHz and equipped with a 40 mm diameter sample tube was used for all samples. The oranges were carefully peeled to avoid any damage to the segments. The peel (albedo and flavedo) was cut into small pieces to fill the homogeneous region of the radiofrequency coil. About 12 g of fresh peel pieces were taken and gently pressed in the tube to achieve a uniform filling. Two orange segments were gently pushed in the NMR tube. Proton spin–lattice relaxation times (T_1) of representative samples of peel and flesh segments were measured using an inversion recovery pulse sequence. Proton spin–spin relaxation times (T_2) were measured at room temperature employing Carr–Purcell–Meiboom–Gill (CPMG)[12,13] pulse sequence, and 150 even echoes were sampled using Experimental Supervisor software (Bruker Optics). The repetition delay was 4 s, 9 scans were performed, and a pulse spacing of 3 or 5 ms was used for peel and flesh samples, respectively. Figure 1 shows sample data sets from non-frozen and frozen/thawed orange sections. The T_1 and T_2 values were calculated by fitting the decay curves using Experimental Supervisor software. The data were analyzed by statistical analysis of t-test and coefficient of variation. The paired t-test at significance level of 0.05 was performed to assess the effects of different temperature treatments.

RESULTS AND DISCUSSION

The spin–spin and spin–lattice relaxation times are expected to vary from one fruit to the next. This variation is a consequence of slightly varying composition (moisture or sugar content) as well as other variations such as structure of the fruit. The actual quality determination of a fruit will depend upon having a measurement that can adequately differentiate defects from good fruit. Defects in the fruit such as bruising and freeze damage will influence relaxation time constants. In order to differentiate defective fruit from normal fruit using relaxation time measurements, the normal expected variation for these time constants for good quality fruit needs to be known as well as the variation and amount of the change in the time constant resulting from a specific defect.

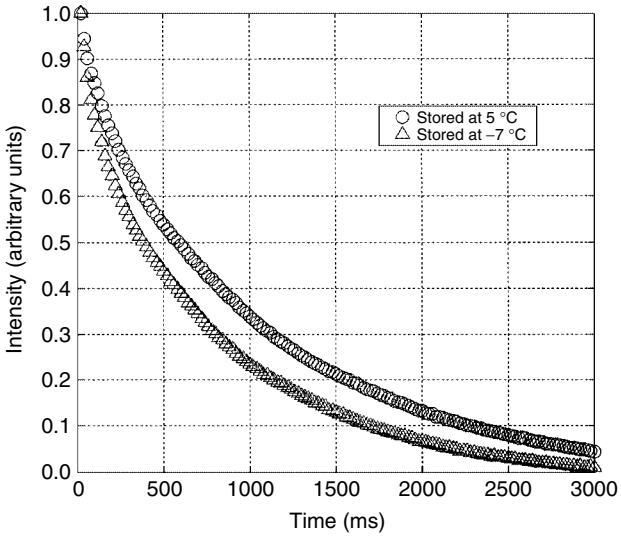

Figure 1. CPMG decay curve from flesh segments of navel oranges (var. Washington) stored for 20 h at 5 °C and −7 °C and thawed.

Natural variation in NMR relaxation times (T_1 and T_2)

The mean T_2 values were ∼340 ms for peel and ∼1010 ms for flesh segments (pulp), whereas T_1 values were 580 and 1620 ms, respectively, for peel and flesh segments (Table 1). The coefficient of variation of these measurements ranged from 4% to 9%. These values were used to set optimum acquisition parameters for rapid and reliable T_2 measurements of the subsequent large number of samples. The moisture content of Washington navel oranges ranged from 740 g kg^{-1} w.b. (peel) to 857 g kg^{-1} w.b. (flesh segments).[14] This significantly lower moisture content for peel compared with flesh segments may be one of

Table 1. NMR relaxation times T_1 and T_2 of non-frozen navel oranges (var. Washington)

	Peel		Flesh segments	
Orange	T_2 (ms)	T_1 (ms)	T_2 (ms)	T_1 (ms)
1	310	570	980	1680
2	337	610	1020	1710
3	367	570	1070	1660
4	314	500	990	1540
5	348	620	1040	1630
6	377	650	1040	1730
7	368	620	1050	1630
8	344	600	960	1630
9	327	550	990	1540
10	320	500	960	1490
Mean	341	579	1010	1624
Standard deviation	24	51	39	78

the major factors responsible for the relatively lower values of the observed peel relaxation times.

T_2 of partially frozen and unfrozen navel orange parts (peel and flesh segments)

Exposure of navel oranges to −7 °C for 24 h caused partial freezing. Tan[15] studied freeze temperature profiles of navel oranges (var. Washington) and observed that freezing started after 3–4 h of exposure at −7 °C and 18 h exposure resulted in relative percentage ice mass formation ranging from 60 to 200 mL L^{-1} of the fruit flesh as determined by a calorimetric method. This observed variability may be due to variation in the constituents and structural components of the oranges used for the study. The partial freezing-induced changes in orange peel and flesh segments were measured through relaxation time T_2 in the present study. It is quite evident from Fig 1 that the CPMG signal of flesh segments from the oranges exposed to −7 °C decayed faster compared with that from the oranges exposed to 5 °C. However, a reliable differentiation cannot be made between freeze damage and non-freeze-damaged fruit unless the variability is included. The T_2 values for peel and flesh parts of a large number of Washington navel oranges exposed for 20 h to chilling (5 °C) or freezing (−7 °C) temperatures and thawed/warmed to room temperature are given in Table 2. The data in this table show that exposure to chilling or freezing temperatures did not affect peel T_2 values, but after freezing/thawing an appreciable decrease in T_2 values of flesh was observed. The values of T_2 for peel and flesh samples of two other varieties, namely Summer Gold and Lane Late, exposed to chilling or freezing temperatures are given in Tables 3 and 4. The data confirmed that mean T_2 values of the peel did not change, whereas the values of the exposed flesh decreased significantly (>10%). The paired *t*-test values of data in Tables 2, 3 and 4 at a significance level of 0.05 also clearly show that the exposure to two different temperatures significantly affected the T_2 values of the flesh only. Comparison of the data in Tables 2, 3 and 4 further shows that the T_2

Table 2. NMR relaxation time T_2 (ms) data of navel oranges (var. Washington)

	Chilled (5 °C)			Frozen (−7 °C) and thawed	
Orange	Peel	Flesh segments	Orange	Peel	Flesh segments
1A	360	1070	1B	379	920
2A	396	1000	2B	401	910
3A	340	1080	3B	379	900
4A	350	1070	4B	357	860
5A	402	1120	5B	366	890
6A	331	1010	6B	367	790
7A	343	990	7B	381	986
8A	371	1000	8B	396	940
9A	364	1010	9B	365	880
Mean	362	1039		377	897
Standard deviation	24	47		15	54

Paired *t*-test at significance level of 0.05:

	t-value	*P*-value
Peel	1.86	0.10
Flesh	5.45	0.00

Table 3. NMR relaxation time T_2 (ms) data of freeze-thawed navel oranges (var. Summer Gold)

	Chilled (5 °C)			Frozen (−7 °C) and Thawed	
Orange	Peel	Flesh segments	Orange	Peel	Flesh segments
1A	429	1030	1B	447	910
2A	446	1020	2B	448	960
3A	418	1060	3B	398	910
4A	434	1070	4B	472	910
5A	445	1110	5B	381	870
6A	421	1080	6B	385	960
Mean	432	1062		422	920
Standard deviation	12	33		39	35

Paired *t*-test at significance level of 0.05:

	t-value	*P*-value
Peel	0.68	0.53
Flesh	5.84	0.00

Table 4. NMR relaxation time T_2 (ms) data of freeze-thawed navel oranges (var. Lane Late)

	Chilled (5 °C)			Frozen (−7 °C) and thawed	
Orange	Peel	Flesh segments	Orange	Peel	Flesh segments
1A	428	1140	1B	338	860
2A	447	1080	2B	359	980
3A	400	1010	3B	443	1020
4A	422	1060	4B	393	920
5A	378	1060	5B	356	930
Mean	415	1070		378	942
Standard deviation	27	47		42	61

Paired *t*-test at significance level of 0.05:

	t-value	*P*-value
Peel	1.51	0.20
Flesh	2.76	0.05

of both peel and flesh for the Summer Gold and Lane Late varieties were slightly higher compared with that for the Washington variety. The probable reason for such a trend could be a difference in maturity dates. Summer Gold and Lane Late mature late in the harvest season, whereas Washington is a mid harvest season maturity variety. It is expected that the late navel orange varieties tend to have lower sugar content than early and mid maturity varieties (Arpaia Mary Lu, personal communication). It is also known that NMR relaxation time is inversely related with sugar content in leaves of cereal crops.[16] Therefore, slightly higher values of T_2 are expected for the late maturing navel orange varieties.

T_2 of frozen and unfrozen navel oranges parts (peel and flesh)

Freezing Summer Gold variety navel oranges for 20 h in a cold room maintained at −20 °C and thawing to room temperature drastically reduced (75%) the T_2 values for the peel, indicating that significant freezing had taken place (Table 5), and the T_2 of the flesh also reduced by 30%. The large difference in T_2 values for these well-frozen peel samples suggests that the peel was not frozen at −7 °C exposures.

T_2 of different sections of juice sac of navel oranges

A Bruker Minispec NMR sample tube can accommodate only two sections of flesh segments in the homogeneous radiofrequency coil region. To study the variation between different flesh segments from the same orange, T_2 values of peel (sample taken randomly from cut pieces pooled) and various sections were measured. The data (Table 6) showed that the T_2 of the different sections varied insignificantly (∼5%). The T_2 values of different segments of oranges exposed to −7 °C and thawed also did not vary greatly but were reduced significantly compared with the unexposed values.

In summary for the three navel orange varieties studied, on exposure to −7 °C, the T_2 of peel remained nearly the same whereas the T_2 of flesh was reduced significantly (Table 7). That the T_2 values of peel were not affected on exposure to −7 °C suggests that this temperature is not low enough to freeze the peel, although it does partially freeze the flesh segments. This inference is supported by the results obtained on the freezing temperatures of peel and flesh parts of navel oranges that peel freezes at lower temperature than flesh.[15] The freezing most likely ruptures the flesh membranes and juice leaks out, lowering the moisture content of the flesh and resulting in reduced T_2 values. The consistent trend in the navel varieties studied, that the T_2 values of flesh exposed to freezing temperature were significantly reduced, clearly suggests that the T_2 of flesh can be utilized for identifying freeze-damaged oranges. This method may be further extended to whole oranges by using a wide-bore low-field NMR,

Table 5. NMR relaxation time T_2 (ms) data of freeze-damaged navel oranges (var. Summer Gold) exposed to −20 °C

	Chilled (5 °C)			Frozen (−20 °C) and thawed	
Orange	Peel	Flesh segments	Orange	Peel	Flesh segments
1A	405	1080	1B	100	687
2A	396	1020	2B	84	642
3A	375	1040	3B	98	740
4A	423	1040	4B	94	800
5A	408	990	5B	98	694
Mean	401	1034		95	713
Standard deviation	18	33		6	60

Table 6. NMR relaxation time T_2 (ms) data of different flesh segments of frozen-thawed or chilled navel oranges (var. Washington)

	Frozen (−7 °C) and thawed							
		Flesh segments						
Orange[a]	Peel	A	B	C	D	E	Mean	CV (%)
1A	370	830	920	840	900	860	870	4.5
2A	330	920	910	890	920	920	912	1.4
3A	420	930	920	880	950	940	924	2.9
4A	480	960	960	910	910	910	930	2.9
5A	424	780*	920	840	910	880	888	4.1
Mean	405	910	926	872	918	902	905	
Standard deviation	57	56	19	31	19	32	25	
	Chilled (5 °C)							
1B	370	1030	1040	1040	1020	990	1024	2.1
2B	391	910	920	880	970	970	930	4.2
3B	405	970	1050	980	1000	950	990	3.8
4B	450	1000	920	900	1020	990	966	5.5
5B	412	1050	980	1020	970	970	998	3.6
Mean	406	992	982	964	996	974	982	
Standard deviation	30	55	63	71	25	17	36	

[a] Slices injured while peeling, not included in mean.

Table 7. Mean NMR relaxation time T_2 (ms) data of frozen-thawed or chilled navel oranges

	Chilled (5 °C)		Frozen (−7 °C) and thawed	
Variety	Peel	Flesh segments	Peel	Flesh segments
Washington	360	1040	377	897
Summer Gold	432	1062	422	920
Lane Late	415	1070	378	942
Mean	402	1057	392	920
Standard deviation	38	16	26	23

where this method could be used for rapid detection and separation of damaged oranges.

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REFERENCES

1 Chen P, McCarthy MJ and Kauten R, NMR for internal quality evaluation of fruits and vegetables. *Trans ASAE* **32**:1747–1753 (1989).
2 Clark CJ, Hockings PD, Joyce DC and Mazucco RA, Application of magnetic resonance imaging to pre- and post-harvest studies of fruits and vegetables, *Postharvest Biol Technol* **11**:1–21 (1997).
3 McCarthy MJ, *Magnetic Resonance Imaging in Foods*. Chapman & Hall, New York, p. 110 (1994).
4 Chen P, McCarthy MJ, Kauten R, Sarig Y and Han S, Maturity evaluation of avocados by NMR methods. *J Agric Eng Res* **55**:177–187 (1993).
5 McCarthy MJ, Zion B, Chen P, Ablett S, Darke AH and Lillford PJ, Diamagnetic susceptibility changes in apple tissue after bruising. *J Sci Food Agric* **67**:13–20 (1995).
6 Clark CJ and Burmeister DM, Magnetic resonance imaging of browning development in 'Braeburn' apple during controlled-atmosphere storage under high CO_2. *Hort Science* **34**:915–919 (1999).
7 Gonzalez JJ, Valle RC, Bobroff S, Biasi WV, Mitcham EJ and McCarthy MJ, Detection and monitoring of internal browning development in 'Fuji' apples using MRI. *Postharvest Biol Technol* **22**:179–188 (2001).
8 Clark CJ, MacFall JS and Bieleski RL, Loss of watercore from 'Fuji' apple observed by magnetic resonance imaging. *Scientia Hort* **73**:213–227 (1998).
9 Duce SL, Carpenter TA and Hall LD, Nuclear magnetic resonance imaging of fresh and frozen courgettes. *J Food Eng* **16**:165–172 (1992).
10 Gamble GR, Non-invasive determination of freezing effects in blueberry fruit tissue by magnetic resonance imaging, *J Food Sci* **59**:571–573 (1994).
11 Kerr WL, Clark CJ, McCarthy MJ and de Ropp JS, Freezing effects in fruit tissue of kiwifruit observed by magnetic resonance imaging. *Scientia Hort* **69**:160–179 (1997).
12 Carr HY, Effects of diffusion on free precession in nuclear magnetic resonance experiments. *Physical Review* **94**:630–638 (1954).
13 Meiboom S and Gill D, Modified spin–echo method for measuring nuclear relaxation times. *Rev Sci Instrum* **29**:688–691 (1958).
14 Sinclair WB, *The Biochemistry and Physiology of the Lemon and Other Citrus Fruits*. University of California, Division of Agriculture and Natural Resources, Oakland, p. 946 (1984).
15 Tan E, *Early Detection of Freeze Damage in Oranges Using Gas Sensors*. MS Thesis, Biological and Agricultural Engineering, University of California, Davis (2003).
16 Nagarajan S, Gambhir PN, Chahal SS and Tiwari PN, Proton spin–lattice relaxation time in relation to water content, soluble protein and soluble sugar in leaves of cereal crops, *J Nuclear Agric Biol* **18**:253–255 (1989).