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DIFFERENCES IN NUCLEAR MAGNETIC RESONANCE IMAGES BETWEEN CHILLED AND NON-CHILLED ZUCCHINI SQUASH

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WANG C. Y. and WANG P. C. *Differences in nuclear magnetic resonance images between chilled and non-chilled zucchini squash.* ENVIRONMENTAL AND EXPERIMENTAL BOTANY **32,** 213–219, 1992. Nuclear magnetic resonance (NMR) imaging techniques provide a non-destructive method for detecting changes in the internal structure of zucchini squash (*Cucurbita pepo* L., Ambassador) during exposure to chilling temperatures. Whole, freshly harvested zucchini squash were stored at either 2.5 (chilling) or 12.5°C (non-chilling). A 4.7 Tesla, 33 cm bore size, NMR imaging system was used to generate transverse images of the squash. Chilled squash produced an image with high signal intensity in the epidermal region. The T1 (spin–lattice relaxation time) weighted images, obtained by the inversion recovery technique, showed that cortex tissue of the chilled squash also had higher signal intensity than that of the non-chilled squash, indicating a shorter T1 relaxation time and a greater mobility of water in the chilled tissue. The T2 (spin–spin relaxation time) weighted images, obtained by the spin–echo technique, also showed higher intensity in the chilled squash than in the non-chilled samples, implying a longer T2 relaxation time for the chilled tissue. These results suggest that differences between chilled and non-chilled squash can be discerned non-destructively by the intensity of the NMR images or the T1 and T2 relaxation times.

INTRODUCTION

NUCLEAR magnetic resonance (NMR) imaging has been used to study water distribution and transport in plant tissues.[1,6,12] This technique uses radio-frequency waves to interact with the magnetic moments of hydrogen atoms to produce images. High resolution images of plant tissues have been shown and used to investigate the water content and flow in stems and roots.[2,4,8] NMR images have also been obtained to detect physiological disorders of fruits such as watercore in apples,[13] core breakdown in pears,[11] and bruises in various fruits.[3] This technique is particularly useful in revealing internal structure and detecting incipient deterioration of fruits or other horticultural products without destroying their wholeness. Information obtained from NMR imaging can benefit basic research and practical applications, and more studies should be conducted to explore its possible use as a diagnostic tool for different internal disorders in various commodities.

Many fruits and vegetables of tropical and sub-

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tropical origin are injured when stored at low but non-freezing temperatures.[5] Exposure of these commodities to chilling temperatures causes cellular breakdown and loss of membrane integrity.[7] These alterations can lead to an increase of membrane permeability, electrolyte leakage, and exudation of cellular fluid into the intercellular spaces. If the chilling continues, the injury symptoms further develop into a water-soaking appearance, discoloration and pitting of the surface, dysfunction of the metabolism, and collapse of the tissues. Treatments for alleviating chilling injury need to be applied before the injury becomes irreversible. Therefore, early detection of changes caused by exposure to chilling temperature is important in order to ascertain the necessity and correct timing for these treatments. Early changes induced by chilling injury in tissue include an increase in permeability of cell membrane. Since NMR imaging reflects signal intensity produced by hydrogen density, this study was undertaken to test the hypothesis that NMR imaging can non-destructively detect early changes induced by chilling injury through measuring the increase in water mobility.

MATERIALS AND METHODS

Plant material

Zucchini squash used for this study were freshly harvested from a local farm in Beltsville, MD. Fifty squash were selected for their uniformity of size (18–22 cm in length) and randomly divided into two lots. The first group was placed in a constant cold storage room at 2.5°C and the second group at 12.5°C. Six samples from each treatment were taken for NMR imaging at harvest and 3 days after storage.

Evaluation of chilling injury

The degree of chilling injury was judged mainly by the extent of surface pitting. Three squash from each treatment were transferred from storage chambers (2.5 and 12.5°C) to a 25°C room and allowed to warm up for 5 hr before evaluation. The severity of the injury was rated on a scale of 1–5, with 1 = no abnormality, 2 = trace, 3 = slight, 4 = moderate, and 5 = severe chilling injury.

NMR imaging

A 4.7 Tesla magnetic resonance imaging system (Spectroscopy Imaging System, Co., Fremont, CA) with a 33 cm bore was used. The sample was positioned at the center of a saddle coil which was used as a radio-frequency (RF) transmitter and receiver. A spin–echo (SE) imaging technique was used to obtain high resolution images such as shown in Fig. 1.

An inversion recovery (IR) technique was used to measure the T1 relaxation times. Six IR images were used to compute the T1 relaxation time of the samples. The inversion times (TI) used were 0.01, 0.5, 0.9, 1.4, 2.2 and 3.0 sec. The repetition time (TR) was kept constant at 7 sec. The image intensities from the same location in cortex tissue just beneath the epidermis were fitted into a single exponential curve to calculate the T1 relaxation time. Similarly, the T2 relaxation times were measured using five spin–echo images taken by different echo times (TE): 27, 54, 91, 118, 145, and 172 msec. The repetition time was also set to 7 sec. For the high resolution images in Fig. 1, the field-of-view was 5.5×6 cm. The spatial resolution was 0.1×0.3 mm. The slice thickness was 1.5 mm. Identical pulse sequences and parameters were used to acquire the NMR images of chilled and non-chilled squash.

RESULTS

Chilling injury

After 3 days of exposure to 2.5 or 12.5°C, zucchini squash appeared normal without symptoms of chilling injury (Fig. 2). Surface pitting (an incipient symptom of chilling injury in squash) was not detected until after 6 days of storage at 2.5°C. The development of chilling injury symptoms progressed rapidly in this treatment. Severe pitting and slight decay were detected on the squash after 12 days of exposure to 2.5°C. However, no symptoms of chilling injury appeared on squash stored at 12.5°C throughout the experiment (Fig. 2).

NMR imaging

The image intensity is a function of several NMR properties of the sample such as T1 and T2 relaxation times, hydrogen density, and the mobility of the water molecules. These are phys-

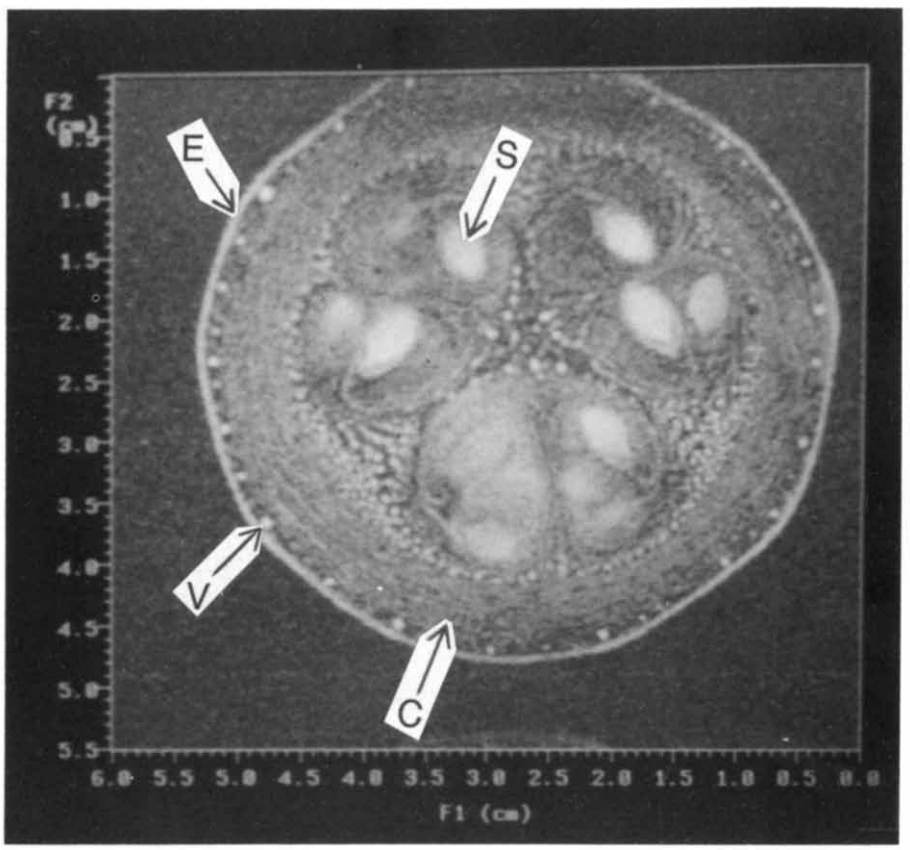

FIG. 1. A high resolution NMR image of zucchini squash. A spin–echo technique was used to obtain this image with TR = 2.4 sec and TE = 23 msec. E, Epidermis; V, vascular tissue; C, cortex; S, seed.

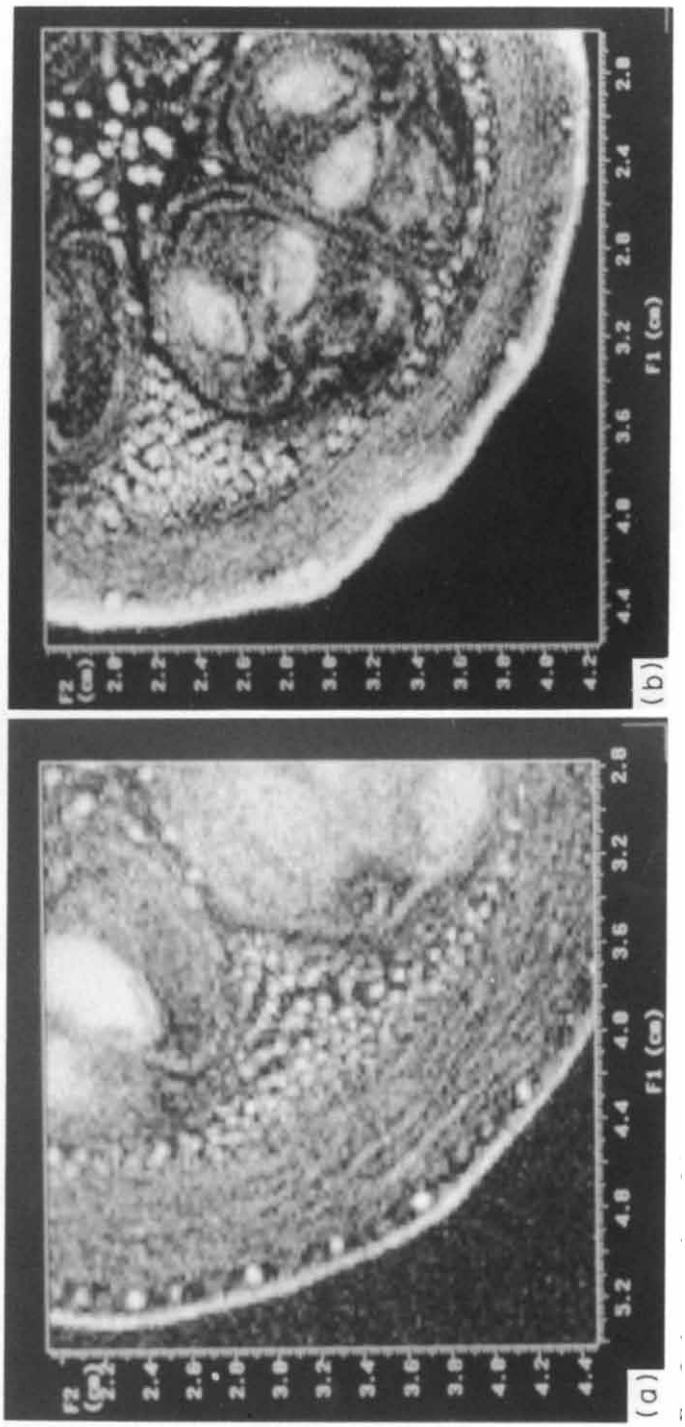

FIG. 3. A comparison of detailed NMR images of two zucchini squash after storage for 3 days at (a) 12.5°C (left) and (b) 2.5°C (right), respectively.

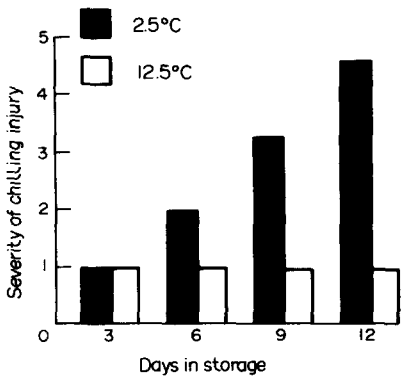

FIG. 2. Severity of chilling injury in zucchini squash stored at 2.5 or 12.5°C. The degree of chilling injury was rated on a scale of 1–5, with 1 = no abnormality, 2 = trace, 3 = slight, 4 = moderate, and 5 = severe injury.

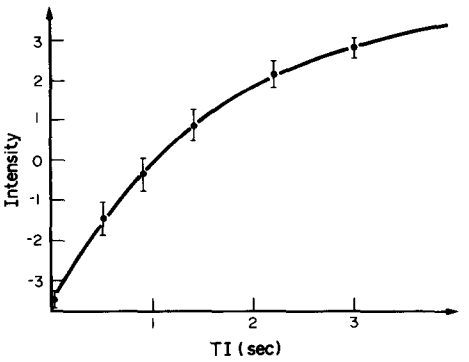

FIG. 4. T1 measurement of zucchini squash cortex tissue. The image intensity is plotted as a function of inversion time (TI). T1 relaxation time is the reciprocal of the exponential constant. Each point is the average of six independent measures. The vertical bars represent the standard errors of the means.

ical parameters of the tissues which reflect the physiological and pathological conditions of the samples. In the healthy non-chilled squash, there was a well-defined high intensity layer in the epidermal region (Fig. 3a). In the chilled squash, however, the high intensity layer was thicker and was not well defined (Fig. 3b). The high intensity area extended into the cortex and vascular bundles.

Measurements of T1 and T2 relaxation times from the cortex area revealed that the chilled squash had a shorter T1 relaxation time and a longer T2 relaxation time than the non-chilled squash (Table 1). In measuring T1 relaxation time, the image intensity was plotted as a function of inversion time (TI) (Fig. 4). Six images were taken at different inversion times. T1 relaxation time is the reciprocal of the exponential constant.

Table 1. *T1 and T2 relaxation times of zucchini squash cortex tissue after* 3 *days of storage at* 12.5°C *(non-chilled) and* 2.5°C *(chilled)**

Treatment	T1	T2
Non-chilled (12.5°C)	1.413 b	0.031 a
Chilled (2.5°C)	0.993 a	0.046 b

* Means separation within column by Duncan's multiple range test. Measurements followed by different letters are significantly different at $P = 0.05$.

A shorter T1 means a greater mobility of water in the tissue and a brighter signal on the image. In measuring T2 relaxation time, the image intensity was plotted as a function of echo time (TE) (Fig. 5). Six images were taken at different echo times. T2 relaxation time is the reciprocal of the exponential decay constant. A longer T2 reflects a higher amount of free water and a stronger image intensity. Therefore, changes in water status in

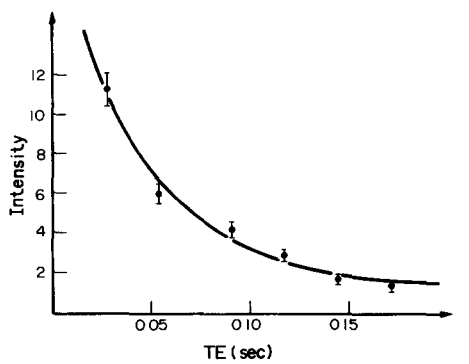

FIG. 5. T2 measurement of zucchini squash cortex tissue. The image intensity is plotted as a function of echo time (TE). T2 relaxation time is the reciprocal of the exponential decay constant. Each point is the average of six independent measures. The vertical bars represent the standard errors of the means.

the tissue as induced by chilling can be detected with T1 and T2 measurements.

DISCUSSION

In this study, chilling injury symptoms were not observed until after 6 days of storage at 2.5°C (Fig. 2). However, a distinct difference was detected between NMR images of chilled and non-chilled squash only after 3 days of storage (Fig. 3a, b). An early detection of chilling injury is of great value both academically and commercially. Low temperature-stressed products are known to recover after short exposure to chilling temperatures. This recovery can occur only when the chilling-induced changes are still at the reversible stage. The conventional way of evaluating chilling injury by visual inspection of the symptoms is usually too late to be of any value for reverting the development of symptoms. Physiological alterations and internal structural changes occur much earlier before the appearance of external symptoms of injury. An early detection of chilling injury before the changes become irreversible is useful to help determine the timing of postharvest treatments, such as intermittent warming, to reduce chilling injury.[10] Our study showed that NMR imaging technique could serve this purpose.

The NMR images of the chilled squash showed higher intensity in the epidermal region than those of the non-chilled squash (Fig. 3A, B). This indicates that the skin tissue and adjacent areas had higher water mobility and diffusion in the chilled squash than in the non-chilled samples. Similar results were obtained from the measurements of T1 and T2 relaxation times in cortex tissue just beneath the epidermis. Chilled squash had a shorter T1 relaxation time and a longer T2 relaxation time than the non-chilled squash indicating that chilling increased water mobility in the tissues. This high water mobility was apparently caused by increased membrane permeability and possibly the breakdown of compartmentation in the chilled squash as a result of chilling injury. Visible symptoms of chilling injury in zucchini squash are water-soaking appearance and surface pitting.[5] The changes that we detected with NMR imaging and T1, T2 relaxation time measurements during the early stages of chilling exposure served as precursory events for these visible symptoms.

The greatest advantage of using NMR imaging is its non-destructiveness. Repeated measurements are possible without destroying the intact plants or organs. It is a powerful research tool to monitor physiological processes over time. It has been demonstrated that the NMR imaging technique is not only useful in investigating water movement in plant tissues and diagnosing internal disorders in fruits as mentioned in the introduction, but also feasible in monitoring the ripening process of fruits.[9] Our study has shown that differences between chilled and non-chilled squash can be discerned with NMR imaging. Further investigation using time-course study is needed to determine how early chilling injury can be detected by this technique, and if NMR images and/or T1 and T2 relaxation times can be used as a non-invasive indicator of the degree of chilling injury.

REFERENCES

1. Bottomley P. A., Rogers H. H. and Foster T. H. (1986) NMR imaging shows water distribution and transport in plant root systems *in situ*. *Proc. natl Acad. Sci., U.S.A.* **83,** 87–89.
2. Brown J. M., Johnson G. A. and Kramer P. J. (1986) *In vivo* magnetic resonance microscopy of changing water content in *Pelargonium hortorum* roots. *Pl. Physiol.* **82,** 1158–1160.
3. Chen P., McCarthy M. J. and Kauten R. (1989) NMR for internal quality evaluation of fruits and vegetables. *Trans. Am. Soc. Agric. Engnrs* **32,** 1747–1753.
4. Connelly A., Lohman J. A. B., Loughman B. C., Quiquampoix H. and Ratcliffe R. G. (1987) High resolution imaging of plant tissues by NMR. *J. exp. Bot.* **38,** 1713–1723.
5. Hardenburg R. E., Watada A. E. and Wang C. Y. (1986) *The commercial storage of fruits, vegetables, and florist and nursery stocks*. U.S. Department of Agriculture, Agriculture Handbook 66.
6. Ishida N., Kobayashi T., Koizumi M. and Kano H. (1989) ^{1}H-NMR imaging of tomato fruits. *Agric. biol. Chem.* **53,** 2363.
7. Lyons J. M. (1973) Chilling injury in plants. *A. Rev. Pl. Physiol.* **24,** 445–466.
8. Omasa K., Onoe M. and Yamada H. (1985) NMR imaging of measuring root system and soil water content. *Envir. Control Biol.* **23,** 99–102.

9. PECH J. C., LATCHE A., ANDRIEU M. H., RAYNAL J. and PRADERE J. (1990) *In vivo* monitoring of fruit ripening by proton NMR imaging. *Abstr. XXIII Int. Hort. Congress* **1,** 635.
10. WANG C. Y. (1990) Alleviation of chilling injury of horticultural crops. Pages 281–302 *in* C. Y. WANG, ed. *Chilling injury of horticultural crops*. CRC Press, Boca Raton, FL.
11. WANG C. Y. and WANG P. C. (1989) Non-destructive detection of core breakdown in 'Bartlett' pears with nuclear magnetic resonance imaging. *HortScience* **24,** 106–109.
12. WANG P. C. and CHANG S. J. (1986) Nuclear magnetic resonance imaging of wood. *Wood Fiber Sci.* **18,** 308–314.
13. WANG S. Y., WANG P. C. and FAUST M. (1988) Non-destructive detection of watercore in apple with nuclear magnetic resonance imaging. *Scientia Hort.* **35,** 227–234.