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Determining tomato fruit maturity with nondestructive in vivo nuclear magnetic resonance imaging

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ABSTRACT

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Nuclear magnetic resonance (NMR) images were taken of freshly harvested tomato fruit (*Lycopersicon esculentum* cv. Castlemart). Measurements were also made of the stage of ripeness, rate of carbon dioxide and ethylene production, lycopene and chlorophyll content, density of the pericarp wall, and consistency of the locular tissue. An NMR image at 256×256 pixel per image and 16 levels of intensity per pixel clearly showed many structural details of the fruit. Increased intensity was associated with liquefaction of the placental tissue during ripening, while graininess of the pericarp wall was associated with a decrease in wall density. In vivo NMR imaging of mature-green fruit produced images in which differences in maturity could be seen. The long time required to produce an image and the cost of operation currently precludes the use of NMR imaging to sort mature-green tomato fruit.

INTRODUCTION

Tomato fruit undergo an orderly series of physiological and morphological changes as they progress in development from mature-green (MG) to red-ripe fruit. These changes include the development of red color (i.e., lycopene synthesis), loss of chlorophyll, softening, increased respiration and increased production of the plant hormone ethylene. Undetected by nondestructive physiological and visual measurements are changes in the firmness and consistency of the locular tissue. This tissue contains the developing seeds and the associated placental tissue. Locular tissue rapidly changes during the early stages of ripening from a very firm tissue, similar in consistency to the pericarp wall, to an amorphous gel. This liquefaction of the placental tissue is one of the first changes that heralds the onset of ripening and its measurement could be an effective means of differentiating among the maturities of fruit harvested at the various MG stages of development

(Kader and Morris, 1976). Currently, the time from harvest of MG fruit to the initiation of ripening, i.e., the attainment of the breaker stage with the development of external red coloration, is impossible to predict accurately unless each fruit was tagged at anthesis. Once ripening has been initiated and the breaker stage attained, ripening progresses in a predictable fashion controlled almost exclusively by the ambient temperature since internal levels of endogenously produced ethylene are near saturation levels.

The ability to determine rapidly and nondestructively the developmental stage of freshly harvested MG fruit would reduce the variability in ripening experiments with whole fruit and could be used commercially to produce more uniform packs. Most fresh market tomatoes are harvested in California at the MG stage of development which encompasses fruit of varying degrees of maturity. Harvested MG fruit are sorted on the basis of shape and size, and packed. Boxes of MG tomato fruit are usually gassed with ethylene to accelerate ripening and to make ripening more uniform. However, since MG fruit can not now be sorted on the basis of the number of days they will take to initiate ripening (i.e., reach the breaker stage), even boxes of gassed fruit can contain a mixture of maturities from green to red-ripe fruit when they arrive at the wholesale market. An additional handling step is required to sort these tomatoes into the uniform ripeness classes required for retail sale. This added step increases both the cost of the fruit and is a source of mechanical damage that reduces quality. A rapid, nondestructive measure of maturity would allow segregation and packing of uniform MG tomato fruit. These fruit could be gassed and sent directly to retail markets without the need for repacking.

A number of nondestructive techniques have been investigated to determine the maturity of harvested tomato fruit. They have included delayed light emission (Chuma et al., 1982; Abbott et al., 1986) transmission of visible light (Worthington et al., 1973) and sound (Abbott et al., 1968; Saltveit et al., 1985), and changes in the vibrational mode of the fruit (Stephenson et al., 1973). Nuclear magnetic resonance (NMR) imaging has been used to study in vivo changes in water content of pear fruit during core breakdown (Wang and Wang, 1989), of roots in various media (Bottomley et al., 1986) or in synthetic foam medium (Brown et al., 1986), and in wood (Wang and Chang, 1986). High resolution NMR permits close examination of plant tissue (Eccles and Callaghan, 1986; Connelly et al., 1987). Experiments using NMR imaging to detect ripening associated changes in the locular tissue of freshly harvested MG tomato fruit are reported in this paper.

MATERIALS AND METHODS

Tomato fruit (*Lycopersicon esculentum* Mill., cv. Castlemart) were grown under standard cultural practices at the University of California, Davis, Vegetable Research Farm. Fruit at various stages of maturity were hand harvested, and only fruit free of external defect and of uniform size and shape were selected for experimentation. The rate of carbon dioxide and ethylene production were meas-

ured after holding the fruit overnight at 20C in an ethylene-free, humidified atmosphere (Saltveit, 1982; Saltveit and Yang, 1987).

An NMR instrument uses a strong static magnetic field and weak radio frequency radiation to detect protons, chiefly those associated with water (Brown et al., 1986). Transverse images were taken of the fruit with a General Electric CSI-2, 85 MHz, Spectrometer with a 310 mm bore horizontal 2.0 T magnet. A 10-cm diameter solenoidal coil, oriented perpendicular to the static magnetic field, was used as the receiver. The timing for the acquisition of the spin-lattice relaxation time (T1) and the spin-spin relaxation time (T2) were adjusted to produce an image whose intensity was proportional to the water content of the tissue. A 3-mm diameter Tygon tube filled with water was attached to the equator of the fruit with tape to help orientate the fruit in the NMR cavity so that the image plane was known for subsequent cutting and photographing of the fruit. Computations to produce the image were done with a dedicated 32-bit Motorola 68000 CPU within the CSI-2 running proprietary software. Initial imaging at 128×128 pixels per image did not provide sufficient resolution so subsequent imaging was done at 256×256 pixels per image with 16 levels of intensity per pixel.

Ripeness of the whole fruit was scored on external visual appearance with 1 = mature-green, 2 = breaker, 3 = turning, 4 = pink, 5 = light-red, and 6 = red-ripe (USDA, 1975). After imaging, the fruit were cut in half through the equator and photographs were taken. Four additional stages of development have been identified for MG fruit on the basis of internal color and tissue consistency (Kader and Morris, 1976). These stages are MG1, characterized by firm green locular tissue and seeds that are cut when the fruit is sliced with a sharp knife; MG2, soft green locular tissue with seeds that are pushed out of the way when the fruit is cut with a sharp knife; MG3 some gel in the locule, but no red color visible inside the cut fruit; and MG4, locular tissue predominantly gel with some red visible in the columella and locular tissue. In some experiments 1-cm diameter pericarp discs were excised with a cork borer and their density determined by dividing their weight by their volume. The volume was determined by immersing the discs into a tared beaker of water and measuring the buoyant force that was equivalent to the volume, i.e., the weight of water displaced by the disc.

RESULTS AND DISCUSSION

An NMR image of a MG tomato fruit revealed many structural details (Fig. 1). The pericarp and locular walls were easily seen in all the images. Vascular tissue in the pericarp wall appeared as bright spots. Seeds and associated placental tissue in the locule were visible in MG1 fruit but became less distinct as ripening progressed. Air cavities within the fruit appeared as dark areas because they were devoid of water.

As fruit progressed in ripening from MG to breaker to turning to red-ripe, a number of physical and physiological changes occur (Table 1). Subjective ratings of external color advance from 1 to 6 as chlorophyll was degraded and lycopene

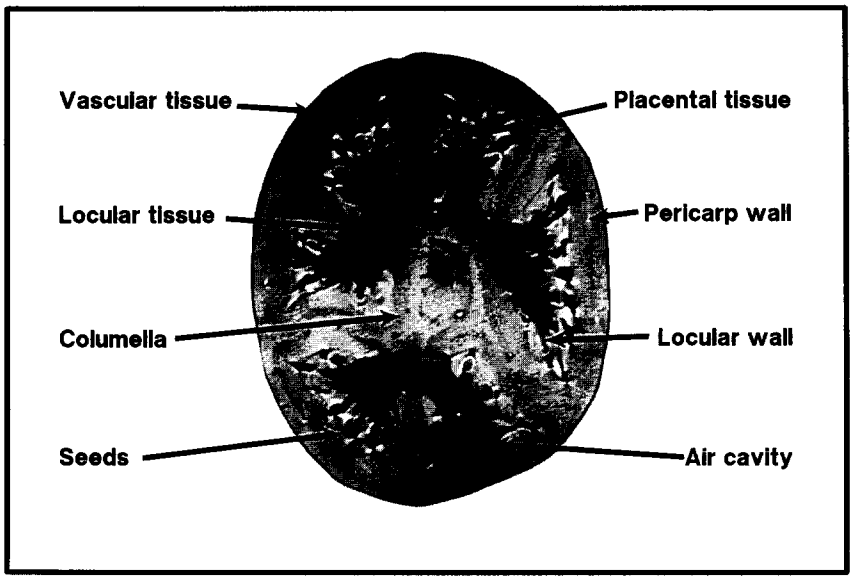

Fig. 1. An NMR image through the equator of a mature-green tomato fruit.

TABLE 1

Comparison among subjective external and internal visual ripeness scores, ethylene and carbon dioxide production, and firmness of freshly harvested tomato fruit

Maturity	External color	Internal maturity	nl C_2H_4/(g h)	μl CO_2/(g h)	Firmness
Red-ripe	6 [X]	– [Y]	9.8 a [W]	24.5 b	1.8 [Z] a
Turning	3	–	5.7 b	23.9 b	1.6 ab
Breaker	2	–	2.6 c	28.8 a	1.3 b
Mature-green	1	3	0.4 d	17.9 c	0.8 c
Mature-green	1	1	0.03 e	11.7 d	0.7 c

[W] Means within each column followed by the same letter are not significantly different by Duncan's multiple range test, $P = 0.05$.

[X] External color subjectively rated as mature-green equals 1, breaker equals 2, turning equals 3, pink equals 4, red-ripe equals 6.

[Y] Internal maturity subjectively rated on firmness of locular tissue and color of seeds with 1 being green, firm tissue and seeds cut with sharp knife, and 3 being green, reddish tissue with seeds not cut by knife.

[Z] Firmness was measured as the displacement in mm of a 500 g weight resting on the fruit for 10 s.

synthesized. Firmness did not significantly change as internal maturity progressed from 1 to 3 with the softening and changes in the locular tissue. Evolution of both carbon dioxide and ethylene from the fruit significantly changed during ripening and those changes can be used to differentiate among the stages of ripening. In fact, those changes were used in this study to select MG fruit at various stages of development for imaging.

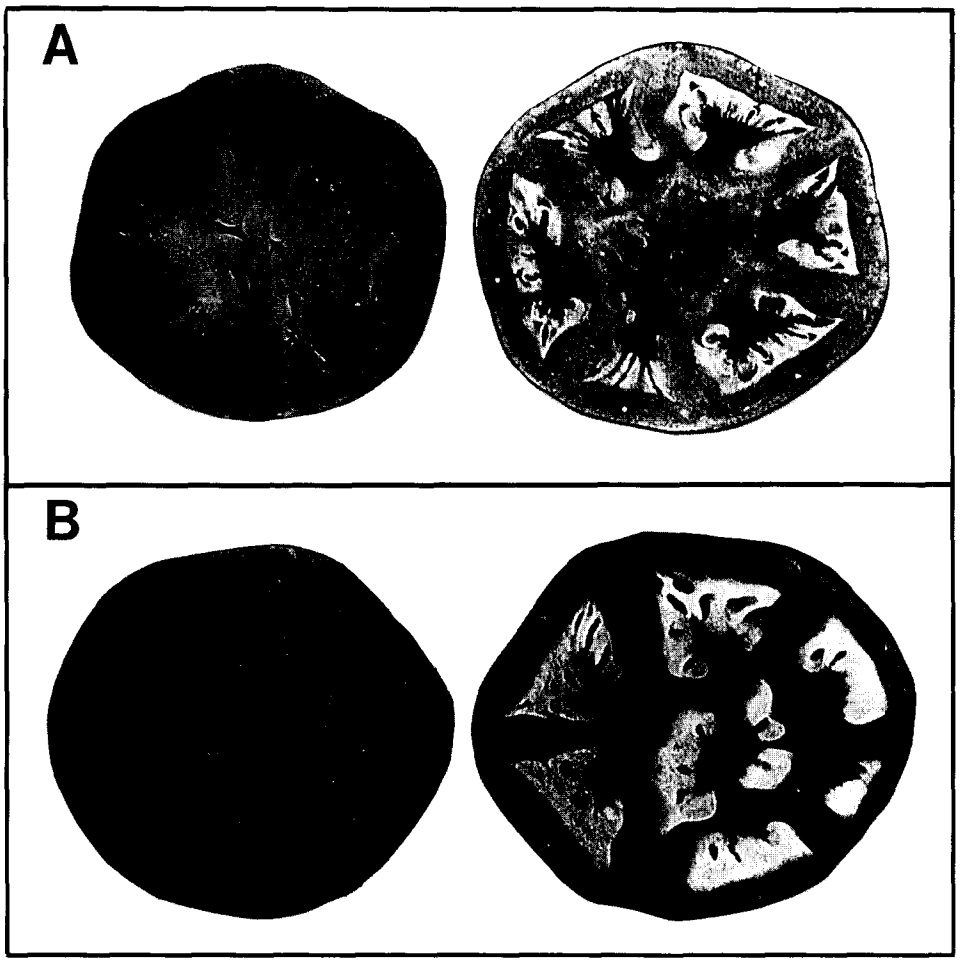

Fig. 2. Comparison between photographs (left side) and NMR images (right side) of transverse sections through the equator of mature-green (MG) tomato fruit at two stages of development: (A) MG1 stage. (B) MG3 stage of development.

As fruit ripened from MG1 to MG3, the image of the locular tissue intensified and became less like that of the pericarp wall. Increased intensity of the image from the locular region of the fruit indicates an increase in water content which coincided with liquefaction of the locular tissue (Fig. 2). During this change there were no changes in the external appearance or color of the fruit. The appearance of red color at the MG4 stage in the columella and locule has been measured by light transmittance and used to separate MG4 fruit from less mature fruit (Worthington et al., 1973).

The image of the pericarp wall became 'grainy' as the fruit continued development. This development of 'graininess' was associated with a decrease in the

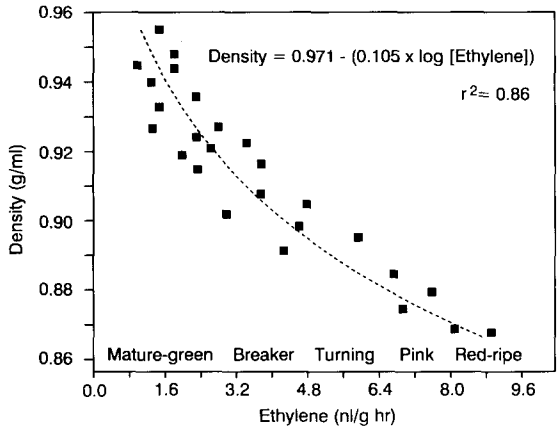

Fig. 3. Relationship between ethylene production by whole field-grown tomato fruit (cv. Castlemart) harvested at various stages of ripening from mature-green to fully red ripe and density. The density measurements were made on excised pericarp discs removed from the same fruit.

density of the pericarp tissue during the latter stages of ripeness (Fig. 3). The equation:

$$\text{Density} = 0.971 - (0.105 \times \log [\text{Ethylene}])$$

defines the relationship, with an r^2 of 0.86, between density in g/ml and ethylene production in nl/(g h). The pericarp tissue became spongy as small air pockets developed in the tissue during ripening. This was evident both as 'graininess' in the NMR images and as decreased density of excised pericarp discs. It should be noted, however, that the decrease in density with fruit ripening was only observed in fruit grown in the field during the summer. The same cultivar grown under cooler conditions, i.e. in the greenhouse and in the field during the spring, showed an increase in density with ripening. Greenhouse grown whole fruit increased in density from 0.957 to 0.981 g/ml and pericarp discs excised from the same fruit increased from 0.987 to 0.995 g/ml during development from mature-green to ripe fruit, respectively. Care must therefore be exercised in correlating changes in the in vivo NMR image with changes taking place during ripening.

NMR images of MG fruit could be used to differentiate among MG ripeness classes. The greatest advantage to using NMR imaging is that it is nondestructive. Repeated images can be made during development and ripening of the same fruit. The spectrometer used in this study produced a useful image in 15–20 min per fruit: 6–11 min to position the fruit and to adjust the magnetic fields (i.e., shim the fields) within the bore of the magnet for each new fruit or for repositioning the same fruit, 6 min to acquire the NMR signals, and 3 min to compute and display the image. The magnetic fields were shimmed to reduce field inhomogenity caused by differences in the size, shape and position of the fruit. More advanced NMR models can produce useful images in under a minute, but the time necessary to hand position each fruit and manually shim the field extends the time required for imaging to over 5 min per fruit. At present, the time required to produce a useful

image and the cost of operating the instrument precludes using NMR imaging to sort MG fruit for general physiological experiments, much less for commercial application. Continued advancement of NMR technology coupled with robotic positioning of the fruit and computer expedited shimming could reduce the time required for imaging and make the technique economical for specialized markets. Presently, the main application of in vivo NMR imaging is for the intensive study of time-dependent changes in difficult to obtain, or produce fruit.

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