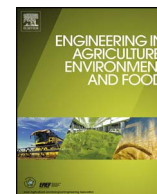




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## Observation and analysis of internal structure of cucumber fruit during storage using X-ray computed tomography

Fumihiko Tanaka<sup>a,\*</sup>, Kohei Nashiro<sup>b</sup>, Wako Obatake<sup>b</sup>, Fumina Tanaka<sup>a</sup>, Toshitaka Uchino<sup>a</sup><sup>a</sup> Laboratory of Postharvest Science, Faculty of Agriculture, Kyushu University, 6-10-1, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan<sup>b</sup> Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 6-10-1, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

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## ABSTRACT

In this study, X-ray computed tomography (CT) was used as a non-destructive technique to characterize and quantify the internal structure of cucumber fruit during storage. The physical properties of cucumber fruit were also measured destructively and related to X-ray absorptivity, and also changes in three-dimensional heterogeneous internal structure were visualized during storage at 15 °C and 25 °C and 90% RH for 7 days. As a result, the average gray scale (GS) value calculated from X-ray CT scanned tissue images indicated good correlations with the density, porosity, and elastic modulus of cucumber fruit. The peak height of the GS value related to the density and porosity. Standard deviation of the GS value was also related to the moisture content of the fruit. These results indicated that X-ray CT can be used to estimate physical properties related fruit quality. It was also revealed that the radiodensity of cucumber fruit changed in the mesocarp tissue but not change in the placenta tissue. GS level in the mesocarp tissue changed from white to dark from the fruit pedicel towards the apex at 25 °C. This result is useful to understand the expansion of low density part in fruit during storage.

## 1. Introduction

The evaluation of internal quality of fresh produce is extremely important for the agricultural industry and consumers, and numerous non-destructive imaging methods have been developed in recent years. X-ray computed tomography (CT) is a useful technique for non-destructive mapping of radiodensity distribution, and it provides high-quality images of the internal structure of agricultural produce because the differences in X-ray attenuation of different materials creates contrast to differentiate low- and high-density materials (Karathanasis and Hajek, 1996). X-ray imaging techniques have been widely used in the agricultural and food industry for foreign material detection and quality control. Morita et al. (2003a, 2003b) proposed a foreign material detection method for food by spectral analysis of reflected soft X-rays, and Arendse et al. (2016, in press) measured the geometrical characters (volume and size) of pomegranate fruit. In addition, Kelkar et al. (2015) proposed a method to determine the density of foods using X-ray linear attenuation. Moreover, Jha et al. (2010) reported the potential of X-ray CT to characterize the internal and external properties of mango. Donis-González et al. (2014a) investigated the internal decay of chestnuts, internal defects in pickling cucumbers, translucency disorder in pineapple, pit presence in tart cherries and plum curculio infestation of tart cherries. They suggested that there is a potential for non-destructive

inline sorting of the internal quality of several agricultural products. Donis-González et al. (2014b) developed the classification method based on CT images for fresh chestnuts. Donis-González et al. (2015) also investigated the presence of undesirable fibrous tissue in carrots through CT images. Using high resolution CT, the pore space within apple (Mendoza et al., 2007), core breakdown in pears (Lammertyn et al., 2003a, 2003b), microstructure in kiwifruit (Cantre et al., 2014) and bread (Demirkesen et al., 2014) were investigated. Verboven et al. (2008) visualized three-dimensional gas exchange pathway in pome fruit using synchrotron radiation X-ray tomography. Kuroki et al. (2004) investigated that gas-filled intercellular spaces in cucumber fruit were developed after harvest; however, there is no investigation of internal structure change in whole fruit during storage. And previous studies have so far not focused on using X-ray imaging to directly determine the physical properties of fruit during storage.

The objectives of this study were to relate the physical properties of cucumber fruit to X-ray CT attenuation characteristics and to visualize three-dimensional heterogeneous structural changes in the fruit during storage.

\* Corresponding author.

E-mail address: [fumit@bpes.kyushu-u.ac.jp](mailto:fumit@bpes.kyushu-u.ac.jp) (F. Tanaka).<https://doi.org/10.1016/j.eaef.2017.12.004>

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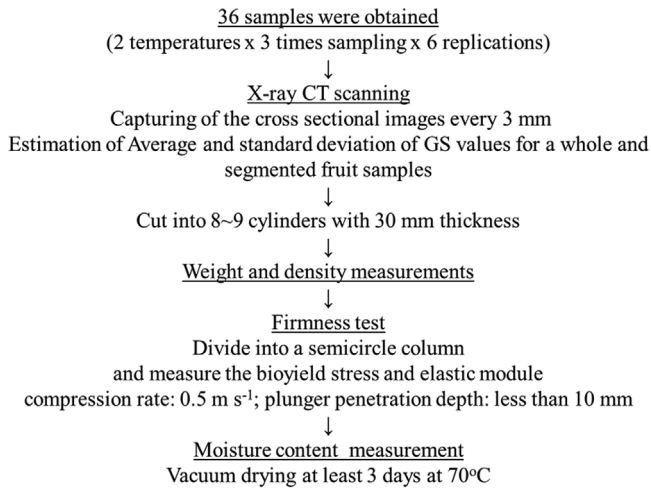


Fig. 1. Flowchart of experiments.

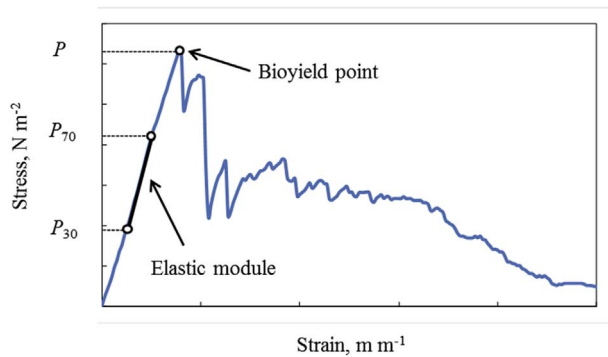


Fig. 2. Definition of firmness of cucumber fruit.

## 2. Materials and methods

### 2.1. Material and storage conditions

Fresh cucumber fruit (*Cucumis sativus* L.) picked at the proper stage of maturity were obtained from a local market and stored in a low-temperature and humidity chamber (TPAV-120-20, ISUZU, Niigata, Japan) at 15 °C or 25 °C with 90% relative humidity (RH) for 7 days. Storage experiment at 25 °C was employed as an accelerated aging test. All samples were assessed for density, porosity, moisture content, flesh firmness, and X-ray attenuation at day 1 (initial day), day 4, and day 7 (some data were also obtained at day 3 and day 5). The samples for measuring physical properties were cut into 30 mm lengths in order to describe the differences along the fruit from pedicel to apex and were divided into 8–9 segments. Fig. 1 shows a flowchart of the X-ray CT scanning and physical properties experiments. Totally, 36 cucumbers were used to relate the X-ray CT and physical properties. The procedures and methods were detailed in the following sections.

### 2.2. Measurement of physical properties

#### 2.2.1. Density and porosity

Density was measured by the water replacement method and a pycnometer method, respectively. To estimate the density of segmented cucumber, the sample volume  $V_s$  (m<sup>3</sup>) was measured by the water replacement method at 25 °C. Density  $\rho_s$  (kg m<sup>-3</sup>) was calculated using the following equation (1):

$$\rho_s = m_s/V_s \quad (1)$$

where  $m_s$  is the sample mass (kg).

True density  $\rho_{tr}$  (kg m<sup>-3</sup>) was measured using a pycnometer method with toluene as the displacement medium at 25 °C. Then, porosity  $\varepsilon$  was calculated using the following equation (2):

$$\varepsilon = 1 - \rho_s/\rho_{tr} \quad (2)$$

#### 2.2.2. Flesh firmness

Bioyield stress and elastic modulus of samples were measured using a creep meter (RE-3305, YAMADEN, Tokyo, Japan) equipped with a cylindrical plunger (diameter: 3 mm) was used for the puncture test. The samples were cut into 30 mm thickness along its length and the shape of a semicircle column was obtained by cutting a column in half in the direction of height to measure flesh firmness. The sample was placed on the stage of the creep meter with the cut surface on the base plate and the plunger penetrated up to 10 mm into the sample at a speed of 0.5 mm s<sup>-1</sup>. In accordance with the texture analysis method proposed by Mallikarjunan (2011), the bioyield stress  $P_b$  (N m<sup>-2</sup>) and elastic modulus  $E$  (N m<sup>-2</sup>) of sample were estimated from the stress-strain curve as described in Fig. 2.  $E$  was calculated from the slope between the points given the stress  $P_{70}$  and  $P_{30}$  in the stress-strain curve (see Fig. 2).

#### 2.2.3. Moisture content

In order to measure the moisture content (wet basis, w.b.) of cucumber, each cut samples with 30 mm thickness was placed in an aluminum cup and dried for at least 3 days at 70 °C in a vacuum oven (ADP 300, Yamato Scientific Co., Tokyo, Japan) with a vacuum pump (Minivac PD-138, Yamato Scientific Co., Tokyo, Japan: Attainable vacuum 0.062 Pa, effective exhaust speed 162 L min<sup>-1</sup>).

### 2.3. X-ray computed tomography measurement

X-ray CT images were generated using an X-ray CT scanner (Latheta LCT-100, Hitachi Aloka Medical, Tokyo, Japan) with X-ray source operated at 50 kV and 1 mA. Entire cucumber was placed in a holder with a 120 mm inner diameter and scanned using a slice thickness of 3 mm at the scanning and computation time of 18 s/slice. A series of sliced images was captured from the fruit apex towards the pedicel. About 70 images were obtained from each sample and the pixel resolution was 250  $\mu$ m (480  $\times$  480 pixels/image). The Hounsfield Unit (HU) scale was employed to express X-ray attenuation through the sample. In the HU scale, the radiodensity of distilled water at standard pressure and temperature is defined as zero HU, while the radiodensity of air at standard pressure and temperature is defined as -1000 HU. The corresponding HU value can be defined as:

$$HU = 1000 \times (\mu - \mu_{water})/(\mu_{water} - \mu_{air}) \cong 1000 \times (\mu - \mu_{water})/\mu_{water} \quad (3)$$

where  $\mu$  (cm<sup>-1</sup>),  $\mu_{water}$  (cm<sup>-1</sup>) and  $\mu_{air}$  (cm<sup>-1</sup>) are the linear attenuation coefficients of sample, air and water, respectively (Huda, 2010). After adjusting the HU scales range, each slice image was stored as 8-bit bitmap (480  $\times$  480 pixels). The scanning was replicated three times for each condition.

### 2.4. Image processing

Image J software (NIH) was used for digital image processing. HU can be simply converted to 8-bit digital data; however, it is necessary to identify an adequate range of HU values in order to visualize the slice image without noise. To obtain a clear X-ray CT image, an adequate HU range was identified. When the binary image was obtained without limiting the HU range, noise could not be removed. When a lower limit was set at -850 HU, a void area appeared inside the cucumber fruit on day 1. As for a higher HU limitation, 300 HU was employed because there was no noise data surrounding the fruit over than this threshold value. Therefore, a range from -900 to 300 HU was employed to

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