Development and applicability of a tart cherry agarose phantom for computer tomography (CT) imaging

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Abstract

Computer tomography (CT) imaging is an effective method for in vivo characterization of object internal attributes including fresh agro-food product quality. Limitations to move CT technology forward into the development of an inline system include the lack of standardized tools (phantoms) for image quality analysis, cross-sharing and consistent evaluation.

The objective of this study was to develop a set of agarose phantoms suited to study cherry pit and pit fragment detection, using CT imaging. Efficiently sorting out these undesirable features during handling and processing will be extremely beneficial to the tart cherry industry. These phantoms can be used on several CT devices (including ultra-fast CT systems) to quantify CT performance, reproducibility, and applicability. This article describes how the phantoms were created, using agarose, a broadly available and inexpensive material.

Developed phantoms allow studying the measurement of CT image parameters that are relevant to detect fresh cherry pits/pit fragments and helps in the development toward inline CT equipment. Measured phantom CT image parameters include simulated flesh and pit X-ray CT attenuation properties (HU-values), which are statistically similar (P=0.05) to fresh cherries, and inferring of the pit/pit fragment size, using CT images, with a 99 % accuracy rate.

Keywords: Nondestructive, non-invasive, postharvest-quality, sucrose, hydrogel.

1 Introduction

Tart cherry (Prunus cerasus c.v. Montmorency) is one of the most important fruits in Michigan, as well as in the United States (Xing & Guyer 2008a). Tart cherry postharvest internal issues result in a considerable amount of loss and profit decrease for growers and processors, and can impact the desired final product quality and safety. Postharvest internal problems, and major concerns to the industry, include the infestation of plum curculio (Conotrachelus nenuphar) (Leskey & Wright 2007; Xing & Guyer 2008a, 2008b) and remaining pits or pit fragments after cherry mechanical pitting (pit removal) to generate cherry juice concentrate, dried tart cherries, or frozen tart cherries for pastries (Haff, Jackson, & Pearson 2005; Qin & Lu 2005). Efficiently sorting out these undesirable features during handling and processing will be extremely beneficial to the industry. Current sorting technologies can elimi-
nate some of the non-desired fruits, but can also result in dramatic increase in losses, due to a large number of false positives (loss of good/healthy fruits) (Qin & Lu 2005; Xing & Guyer 2008a, 2008b).

In post-harvest, computed tomography (CT) has proven to be an accurate descriptor of internal attributes. Donis-González et al. (2012a; 2012b) found a significant relationship between fresh chestnut’s (Castanea spp.) CT images and their components, including the presence of decayed tissue. Kotwaliwale et al. (2006) distinguished between components of fresh pecans (Carya illinoinensis) (i.e. nutmeat and shell). Barcelon et al. (1999a; 1999b) used CT to study internal quality of peaches (Prunus persica) and mango (Mangifera indica), and Sornsrivichai et al. (2000) determined pineapple fruit (Ananas comosus) translucency and ripeness. CT has never been applied to detect the presence of pits, pit fragments and culculio larvae in tart cherries.

This will enable the optimal design and potential inline application of this imaging technique. The development of test objects (phantoms) goes along with the creation of the CT equipment, CT image quality optimization, and the design of image scanning procedures/protocols. In general, a CT phantom is an artificially and specially designed object that is imaged to analyze, calibrate and tune the performance of CT devices. The main advantage of a phantom is that it provides consistent measurable features in comparison with live subjects (e.g. fresh agricultural commodities). Phantoms used to assess CT imaging equipment respond in a similar manner to how real subjects (e.g. fresh fruits) behave in a specific imaging modality (Aitkenhead, Rowbottom, & Mackay 2013; Birnbaum et al., 2007). In addition, phantoms ought to be shelf-stable, differently to fresh tart cherries, which can only be freshly stored for less than 5-days (Remón et al., 2004). Phantoms need to be suited for use in different CT devices, providing images that are relevant to fresh agriculture internal issues, and subsequently compared between CT systems.

The objective of this study was to develop a set of agarose phantoms suited to study pit and pit fragment detection, using traditional CT imaging. These phantoms can be used on, and across, several CT devices (including ultra-fast CT systems) to quantify CT performance, reproducibility, and applicability. Created phantoms also allow studying the measurement of CT image parameters that are relevant to detect fresh cherry pits and pit fragments (i.e. flesh and pit X-ray CT attenuation properties and digital pit size measurements) and move forward in the development of inline CT equipment. In addition, a brief and preliminary evaluation of the created cherry phantoms against fresh cherry samples, using CT imaging, is also included.

2 Materials and methods

2.1 Cherry sample CT imaging scans

A total of 532 physiologically mature tart cherries (Prunus cerasus c.v. Montmorency, which represent over 95% of tart cherry production in the United States of America) were collected by hand, directly from tart cherry trees in a Michigan orchard in the year 2012 (June). Samples were immediately stored at 4 °C. After 1 d, cherries were attached (toward the same direction – stem perpendicular to the board) to rectangular polyethylene boards (915 mm x 335 mm x 2.8 mm), in 19 rows containing 7 cherries (133 total), using approximately 0.003 kg of transparent silicone per cherry and wrapped using a resinite stretch wrap film, as seen in Fig. 1. CT scans were then conducted on each board.

CT scans were performed using a GE BrightSpeed®¹ RT 16 Elite, multi-detector CT instrument (General Electric Healthcare, Buckinghamshire, UK) as described in Donis-González et al. (2012b). Scanning parameters were optimized using the procedure in Donis-González et al. (2012b). Scanning parameters were optimized using the procedure in Donis-González et al. (2012b). Scanning parameters were optimized using the procedure in Donis-González et al. (2012b).

¹ BrightSpeed® and Volara® are registered Trademarks of General Electric Healthcare.
al. (2012a), 2D CT images were acquired every 1.25 mm (d), at a voltage, current, and image resolution (XY plane) equal to 120keV, 170mA, and 1.369 px/mm respectively.

2.2 Cherry flesh and pit testa HU measurements

HU-values (CT grayscale values) were acquired from fresh cherry flesh and cherry pit testa (cherry seed external hard coat). Two-hundred random measurements, each including the mean HU-value of a 14.5 mm² (27.2 pixels² (px²)) circular region containing healthy fresh cherry flesh at different transverse 2D CT images (slices) and samples were obtained. Similarly, 200 random measurements, each containing the mean HU-value of 1-pixel containing pit testa at different transverse 2D CT images (slices) and samples were acquired. HU-value acquisition is exemplified in Fig. 1. HU-values were acquired and statistically compared following the steps described in Donis-González et al. (2012a).

2.3 Agarose phantom development, CT image acquisition and HU measurements

Gel like phantoms were developed using agarose hydrogel (Sigma-Aldrich, St. Louis, MO, USA). Agarose is a sub-fraction of a mix referred to as agar, a natural polysaccharide de-
rived from red seaweed (Arnott et al., 1974; Aitkenhead et al., 2013). Agarose phantoms of different agarose concentrations and dissimilar sucrose \((C_{12}H_{12}O_{11})\) (J.T. Baker, Phillipsburg, NJ, USA) levels were created, as specified in Table 1. Three phantoms each for the different agarose and sucrose concentration (Table 1) were created in an attempt to closely resemble tart cherry flesh (sections 2.1 and 2.2). Agarose preparation was done following similar steps as to develop electrophoresis gels (Philip, 1983) by following the procedure described in Lynam et al. (2013) using flat bottom cell culture plates (Corning Life Sciences DL, Tewksbury, MA, USA) as molds that resemble tart cherry size. Phantoms that contain sucrose are stored in similar conditions in an equivalent sucrose solution.

**Table 1. Phantom ingredient concentration**

<table>
<thead>
<tr>
<th>Name</th>
<th>Agarose % (w/w)</th>
<th>Sucrose % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P_0.7W</td>
<td>0.7</td>
<td>0</td>
</tr>
<tr>
<td>P_1W</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>P_3W</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>P_5W</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>P_6W</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>P_12W</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>P_5W.132Su</td>
<td>5</td>
<td>13.2</td>
</tr>
<tr>
<td>P_3W.144Su</td>
<td>3</td>
<td>14.4</td>
</tr>
</tbody>
</table>

Resemblance between cherry flesh and each of the phantom’s HU-values (tissue density) was evaluated by acquiring CT images from the phantoms, following the protocol in section 2.1, and then comparing the mean HU-value from 200 measurements, which were acquired following the steps in section 2.2. Statistical significant difference between HU-value measurements from cherry flesh and each phantom was determined as in Donis-González et al. (2012a). The phantom with the agarose/sucrose concentration closest, and not statistically different, to cherry flesh was selected to create the finalized cherry phantom.

### 2.4 Fresh cherry pit extraction, pit fragment preparation, and maximum length pit/fragments Ground-Truth Measurements (GTM)

100 physiologically mature whole fresh tart cherries were obtained and similarly treated as described in section 2.1. From these tart cherries, pits were manually extracted by pressing and then thoroughly cleaned. Pit fragments of different sizes were generated by gently pressing and breaking a total of 80 pits (1 pit at a time) using a machinist bench table vise (manual press) (The Chas, Parker Co., Merien, CT, USA). All of the fragments were then preliminary sized by passing them through a set of four (No. 4: 4.75 mm, No. 5: 4 mm, No. 10: 2 mm, and No. 18: 1 mm) standard testing sieves ASTM E-11 specified (Gilson Company Inc., Worthington, OH, USA). Eight randomly selected whole pits, and 8 randomly selected pit fragments from each sieve were collected to develop the final agarose phantoms (total of 40), as will be described in section 2.5. Maximum length of whole pits and pit fragments used to develop phantoms was discerned using a caliper (Model 500-196-20 6", Mitutoyo Co., Takatsu-ku, Kawasaki, Kanagawa, Japan) generating the Ground-Truth Measurements (GTM) value.

### 2.5 Embedded pit/pit fragments phantom development and pit/pit fragments maximum length Digital Measurements (DM)

To create the finalized set of phantoms with embedded pits and pit fragments, after determining the best agarose/sucrose phantom concentration (section 2.3), pits/pit fragments, prepared and previously selected in section 2.4, were inserted into the approximate centroid of the phantom, using stainless steel precision tweezers, (before phantom solidification (~ 65°
C). In addition, phantoms that did not contain pits/fragments (total of 8) were also produced. Final phantoms are indefinitely stored in a solution, as previously described in section 2.3.

Maximum length Digital Measurements (DM) for each pit and pit fragment seen in the phantom CT images was visually and manually deduced using the Osirix Imaging Software V3.9.2 (64-bit). To control for pit/pit fragment orientation, original 2D trans-axial plane (X-Y directions) CT images were viewed in 5 additional planes, including 90° and 45° from the XY-plane toward the longitudinal axis (Z) – YZ-plane-slice, as well as 90° and 45° toward the horizontal axis (X) – XZ-plane-slice as seen in http://en.wikibooks.org/wiki/Online_OsiriX_Documentation/Multi-planar_reconstruction_(MPR). Example of color and maximum intensity CT images of a set of phantoms reconstructed at 90° from the XY-plane toward the longitudinal axis (Z) – YZ-plane-slice, can be found in Fig. 4. Pit and pit fragment DM was inferred using the Osirix Imaging Software length measurement tool, as seen in http://www.osirix-viewer.com/Manual/. GTM and DM were ultimately compared by using a simple linear regression (Ott and Longnecker, 2001). Calculations were performed using the language and environment for statistical computing software R V2.11.0.

3 Results and discussion

Fig. 2 shows the summary of the statistical analysis comparing the HU-values of fresh tart cherry flesh with different agarose phantoms from dissimilar agarose and sucrose (C12H22O11) concentrations, as enumerated in table 1. In Fig. 2, box-plots followed by the same letter are not significantly different between each other at p = 0.05 (ANOVA) (Tukey multiple comparison of means). Significant changes, spread and high variability of HU-values representing differences in tissue density, based on the phantoms with different agarose and sucrose levels were observed. From Fig. 2, it could be proven that the phantom that accurately reflects fresh cherry flesh has a 5% (w/w) agarose + 13.2% (w/w) sucrose concentration (Table 1). Therefore, this phantom (P_5W.132Su) was selected to accurately resemble fresh tart cherry flesh. All of the other phantoms are significantly different to cherry flesh. If desired, agarose and sucrose concentration can be modified to accurately reflect other tissue densities (HU-values), which can be useful when developing phantoms for other applications and/or commodities.

Figure 2: Box-plots showing the HU-values from fresh cherry flesh and different phantoms (Table 1). A thick black line represents the median of 200 data points per box-plot. Upper and lower quartiles are represented as a box, with the maximum and minimum measurement lines protruding from these.

Fig. 3a includes box-plots of a completely independent data set of flesh and pit testa HU-values, from CT images obtained from fresh tart cherries and finalized agarose phantoms. An example of a YZ-plane 2D CT image slice of a fresh tart cherry and a final agarose phantom, both containing whole pits, can be seen in Fig. 3b and 3c, respectively. An overlying
HU-values-profile containing the HU-values acquired at the grey profile-lines (PLs) are also seen. PLs in Fig. 3 offer a clear presentation of the changes and variation of HU-values, reflected by internal characteristics changes within a fresh cherry and a phantom. PLs are visually similar between the fresh cherry and the phantom. Overall, the highest HU-value (~171) can be observed in regions containing pit testa. Lowest HU-values (~22) can be seen between the pit testa (pit embryo), while the mean HU-value of fresh tart cherry and phantom flesh is approximately 37 HU (± 8.1).

Figure 3: (a) Box-plots showing the HU-values from fresh cherry flesh and finalized phantom (P_5W.132SU in Table 1). A thick black line represents the median of 400 data points per box-plot. Upper and lower quartiles are seen as a box, with the maximum and minimum measurement lines projecting from these. HU-values-profile taken at the grey profile-line (PL), typifying the variation within a (b) fresh cherry and (c) finalized phantoms.

Examples of maximum intensity CT images of a fresh cherry and a set of six finalized agarose phantoms with embedded pits, reconstructed at 90° from the XY-plane toward the longitudinal axis (Z) – YZ-plane-slice are included in Fig. 4. Pixels in the maximum intensity CT images found in Fig. 4 correspond to the maximum intensity value of all pixels at the same planer location (y,z) in the YZ-plane-slices stack (approximately 22 YZ-plane CT slices). Parallel, at the top of every CT image, color images, which correspond to the scanned cherry and the phantoms, can also be observed. Images in Fig. 4 show the similarity between the CT images obtained from phantoms and a fresh tart cherry, as well as how different pit fragment sizes and shapes appear in the CT images.

Results from this study indicate that changes in CT image HU-values could be used as a reference to measure internal characteristics of fresh cherries, cherry phantoms and their pit/pit fragments. More specifically, these cherry phantoms accurately represent fresh tart cherry properties, during CT scanning. Fig. 5 includes the univariable linear regression model, which describes the relationship between the GTM (using calipers) and the DM meas-
urements (using CT images). The correlation coefficient (R = 0.99) indicated a high positive relationship between GTM and DM. P-value, which is highly significant (< 0.01), indicates a significant probability of GTM being explained by DM, and low residual standard error reflects a high precision linear regression model. In other words, measuring pit/pit fragment maximum size using CT images (DM) is highly accurate and comparable with the real pit/pit fragment maximum size measurements (GTM). Therefore, CT images accurately represent the size and shape of whole pits and pit fragments in both fresh tart cherries and the phantoms.

Figure 5: Linear relationship (—) between the maximum pit digital measurements (DM – mm) and Ground-truth measurements (GTM) obtained from developed phantoms with embedded pits. 95% linear regression confidence intervals (CI) are shown as dashed lines (---).

In this study, a set of comprehensive tart cherry phantoms for the purpose of routine performance evaluation, in the field of fresh postharvest cherry quality evaluation, of CT systems were created. These phantoms (standardized tools) can be used to investigate multiple parameters related to the performance of different CT systems (e.g. image quality and pit/pit fragment detection), and aid in the development of an inline CT system, which could be used to sort fresh agricultural fruits, including tart cherries. These phantoms are inexpensive, easy to develop using highly available materials (agarose and sucrose), are shelf stable, and can be adapted to be used on different CT devices (including ultra-fast CT systems) to quantify CT performance, reproducibility, and applicability. The designs of the phantoms facilitate routine, reproducible evaluation of system performance, allowing image-quality and other quantitative measurements (pit/pit fragment size) to be repeated on a regular basis.

4 Conclusions

This study showed the steps to develop a set of comprehensive phantoms that are capable of providing quantitative and accurate measurements of multiple parameters related to the performance evaluation of different CT systems. Parameters include the accurate measurement of pit/pit fragments size (R = 0.99, p-value < 0.01) and the in-depth study of CT HU-value (density) changes and distribution. The design of these phantoms allows these measurements to be repeated on a regular basis, as part of an effort in the development of a fresh fruit inline CT sorting system. The same phantom design methodology can be applied to other similar applications, including the development of phantoms to study internal quality attributes of other fresh agricultural commodities.
5 Acknowledgements

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6 References


