1 **Computed tomography imaging-based bitter pit evaluation in apples**

2 Si Yongsheng^{1,2†}, Sindhuja Sankaran^{2†*}

3 ¹College of Information Science & Technology, Agriculture University of HeBei, Baoding, HeBei,
4 China.

5 ²Department of Biological Systems Engineering, Washington State University, Pullman, WA.

6 † Equal contributing authors

7 * Corresponding author: Sindhuja Sankaran; Email: sindhuja.sankaran@wsu.edu; Phone: 509-335-
8 8828.

10 **Abstract**

11 Bitter pit is an economically important physiological disorder in apples resulting in serious
12 economic losses. Current visual assessment techniques are not completely useful in evaluating the
13 extent of bitter pit development as they are limited to external symptoms. An X-ray computer
14 tomography (CT) based imaging and associated image processing algorithm was used to assess the
15 number of bitter pits inside the fruit and on the surface of apples. Samples of 40 healthy and 40
16 bitter pit affected 'Honeycrisp' apples were selected from two different field sites, and scanned with
17 CT equipment on 0, 21 and 63 days after harvest. The results showed that the average number of
18 bitter pits increased both on the surface and inside the fruits with increasing storage period. An
19 average of 42-66% pits was present inside the apple fruits in bitter pit-affected apples. In addition,
20 most of the newly developed bitter pits in healthy apples appeared within the fruit. Therefore, the
21 usefulness of X-ray CT imaging as an effective phenotyping tool in identifying internal bitter pit and
22 on the surface in the automated manner was demonstrated in this study.

23 *Keywords:* Phenotyping, Honeycrisp; Image Processing; Apple Disorder

25 Nomenclature

26	a_i	constant used in estimating output of an order statistic filter
27	$CentroidX$	X coordinate of the centre
28	$CentroidY$	Y coordinate of the centre
29	CT	computer tomography
30	DAH	days after harvest
31	$I_{(i,j)}$	greyscale value at the point (i, j) in the image I
32	I_{bw}	binary image
33	I_{min}	minimum greyscale value in the image I
34	$J_{(i,j)}$	greyscale value at the point (i, j) in the contrast enhanced image
35	$(I_{(i,j)} - I_{min})_{max}$	maximum greyscale value in the image $(I_{(i,j)} - I_{min})$
36	P_{num}	total number of the points on the apple boundary
37	QTL	quantitative trait locus
38	WSU	Washington State University
39	y_k	output of an order statistic filter of length N
40	ρ	radius of each boundary from centre of mass of the apple
41	ρ -difference	difference in radius
42	ρ -pulseA	Normalised ρ -difference
43	ρ -pulseB	ρ -pulseA after noise removal

45 1. Introduction

46 Bitter pit is a physiological disorder affecting apple fruit production worldwide (Zupan et al.
47 2013; Buti et al., 2015). It is presumed that the bitter pit starts developing during the pre-harvest
48 period and external symptoms may appear during postharvest storage. The typical symptoms
49 include soft, circular, and discrete pits in the cortical flesh resulting from plasma membrane
50 breakdown with regions surrounding the pits turning brown and desiccated (Faust & Shear, 1968;
51 Fuller, 1979). Several factors are believed to influence the bitter pit development in fruits that

include calcium uptake, crop load, and environmental factors such as location, elevation, and temperature differences (Perring & Jackson, 1975; Van der Boon, 1980; Ferguson & Watkins, 1983, 1992; Perring, 1986; Failla et al., 1990; Witney et al., 1991). Moreover, the susceptibility is also cultivar dependent (Ferguson & Watkins, 1989). The disorder can result in serious economic losses as the affected fruits cannot be sold in the fresh market.

The current bitter pit detection is limited to visual assessment (Pesis et al., 2010; de Freitas et al., 2013). However, this assessment is not completely useful as bitter pit may be present or can develop inside the fruit without any external symptoms during transportation and storage in the packaging facility. Similarly, visual ratings are used to phenotype apples for identifying genes associated with bitter pit susceptibility or tolerance (Voltz et al., 2006; Evans et al., 2011; Kumar et al., 2013; Brazek, 2013; Buti et al., 2015; de Freitas et al., 2015). For example, Buti et al. (2015) scored the bitter pit susceptibility in 364 seedlings (cross between ‘Redchief’ and ‘X6688’ with ‘M.9’ rootstock) on a scale of 0-4, ranging from non-symptomatic to very highly symptomatic for two seasons at full apple maturity. The marker-based restricted multiple quantitative trait locus (QTL) method was utilised with bitter pit incidence association as cofactor to discover minor QTL through linkage. The study found that putative loci influencing the bitter pit symptoms were not stable, which was believed to be an artefact of phenotyping process. The research recommended the development of a more precise phenotyping technique as one of the potential ways that will allow identification of the genes associated with the disorder. Therefore, there is a need for non-destructive sensing of bitter pit in apples for both precise phenotyping and sorting on the commercial packaging lines.

The non-destructive sensing techniques that are being investigated include fluorescence imaging (Lötze et al., 2006), hyperspectral imaging (Nicolai et al., 2006), and computer tomography (CT) imaging (Jarolmasjed et al., 2016). Nonetheless, these techniques are useful for evaluating the presence and absence of bitter pit, rather than a quantitative data representing internal (non-

symptomatic) and external (symptomatic) bitter pits. This data can especially be very useful for genetics and breeding applications. Such information may also be used to decide the effective storage index and duration of harvested fruits in storage by fruit packaging units. With this goal, the specific study objective was to develop a robust image processing algorithm to assess the number of internal and external bitter pits in apples scanned using X-ray CT equipment.

2. Materials and methods

2.1. Fruit sampling and data acquisition

Healthy and bitter pit affected ‘Honeycrisp’ apples (20 each) were selected from two different field sites in Walla Walla County, WA, USA for this study; Burbank (location-1) and Prescott (location-2). The freshly harvested fruits were transferred to Washington State University (WSU), Pullman, WA, cleaned, and sorted in a packaging box. The apples were stored at 5 °C for up to 63 days. X-ray CT imaging was performed at Veterinary Medicine Hospital, WSU using Toshiba Aquilion™ 16 CT tomograph (Tustin, CA, USA). The fruits were scanned multiple times during 63 days of storage. 0, 21, and 63 days after harvest (DAH) data were used for analysis. More details on data collection can be found in Jarolmasjed et al. (2016). CT images were taken from three different directions: longitudinal, transversal and sagittal, with 3 mm thickness between each scan and same number of total scans from each box. Images from longitudinal CT scan were used in this study.

2.2. Image analysis

Pertinent CT images were processed and analysed using MATLAB (2014b) and the Image Processing Toolbox. Images were segmented manually for identifying bitter pits present in individual apple. The total number of pits on the surface (cuticle) and inside the fruit was determined for healthy and bitter pit apples (Fig. 1) based on the method described below.

2.2.1. Bitter pit on the apple surface

The pixel intensities of CT images were normalised using linear transformation such that the intensity ranged from 0 to 1 using following equation:

$$I_{(i,j)} = \frac{I_{(i,j)} - I_{min}}{(I_{(i,j)} - I_{min})_{max}} \quad (i \in (1, m), j \in (1, n)) \quad (1)$$

where, CT image I is $m \times n$ pixel image, $I_{(i,j)}$ is the greyscale value at the point (i, j) in I ; I_{min} is the minimum greyscale value in the image I . $(I_{(i,j)} - I_{min})_{max}$ is the maximum greyscale value in the image $(I_{(i,j)} - I_{min})$. Following this step, contrast enhancement was applied to increase CT image quality.

$$J_{(i,j)} = \begin{cases} 0 & I(i, j) \leq a \\ \frac{I(i,j)-a}{b-a} & I(i, j) \in (a, b) \\ 1 & I(i, j) \geq b \end{cases} \quad (2)$$

where, $J_{(i,j)}$ is the greyscale value at the point (i, j) in the contrast enhanced image. $(0, a)$ and $(b, 1)$ denote the bottom 1% and the top 1% of all pixel values, respectively.

The apple contour image (Fig. 1a) was utilized for bitter pit detection on the surface. To achieve this, Otsu thresholding technique (Otsu, 1975) was applied to create a binary image (Fig. 1b) from the original greyscale image for each apple. Noise from packaging materials (tray and box) was eliminated by removing small objects (<600 pixels) from the binary image. The pit regions in a fruit resulting from fruit core and bitter pits inside the fruit were eliminated by filling in those pixels. Fig. 1c shows the apple image without small objects, fruit core, and pits inside the fruit. The boundary of the fruit was traced from the image (Gonzalez & Woods, 1975). The centre of mass of the apples (i.e. centre of the apple) was determined using the following equations, in which the size of the binary image I_{bw} is $m1 \times n1$:

$$CentroidX = \frac{\sum_{i=1}^{m1} \sum_{j=1}^{n1} i I_{bw}(i,j)}{\sum_{i=1}^{m1} \sum_{j=1}^{n1} I_{bw}(i,j)} \quad (3)$$

$$CentroidY = \frac{\sum_{i=1}^{m1} \sum_{j=1}^{n1} j I_{bw}(i,j)}{\sum_{i=1}^{m1} \sum_{j=1}^{n1} I_{bw}(i,j)} \quad (4)$$

where, *CentroidX* and *CentroidY* are the coordinates of the centre. $I_{bw(i,j)}$ is the pixel value (0 or 1) at the point (i, j) in the binary image. Following this, the radius (ρ , polar distance) of each boundary point from the centre of mass of the apples (origin of distance coordinate) was calculated. During continuous calculation of the polar distances (radii), presence of bitter pit will result in a sudden decrease in the radius. Therefore, to estimate this sudden change in radius, the difference between the radius of first boundary point (i) and every fourth boundary point ($i+3$) was estimated, using the following equation:

$$\rho\text{-difference}(i) = \rho(i+3) - \rho(i) \quad (5)$$

where, $\rho\text{-difference}$ refers to difference in radius, and i can range from 1 to P_{num} . P_{num} is the total number of the points on the apple boundary. For example, the difference between i and $i+3$ boundary points as shown in Fig. 2a, will be $x-y$. The $\rho\text{-difference}$ was normalised as $\rho\text{-pulseA}$. $\rho\text{-pulseA}$ was 1 when $\rho\text{-difference}$ was greater than or equal to 2, else $\rho\text{-difference}$ was 0 (Fig. 2b). In regions of calyx and stem end, a gradual decrease in radius ρ was observed with few noises resulting from anomalies. For example, $\rho\text{-pulseA}$ between -30 and -150 represents stem end region of the apple, while $\rho\text{-pulseA}$ between 70 and 120 represents calyx end, as shown in Fig. 2b. The $\rho\text{-pulseA}$ was converted into $\rho\text{-pulseB}$ peaks by removing some of these noises and finally utilising the data for detecting number of bitter pits on the surface.

2.2.2. Bitter pit inside the apple

Greyscale image was used to determine the total number of pits inside the apples (Fig. 1d). To isolate the background from each apple, grayscale image was multiplied with the contour image. The pixel values outside the apples were converted to zero. To detect the bitter pits, 3-by-3 maximum and 3-by-3 minimum filters were implemented to the multiplied apple greyscale image separately. Both the maximum filter and minimum filter are a form of the order statistic filter. The output of an order statistic filter of length N operating on a sequence (x_i) for N odd is given by

$$y_k = OSF(\{x_i\}_{j=k-M}^{k+M} = \sum_{i=1}^N a_i x_{(i)}^k \quad (6)$$

where $M=(N-1)/2$ and $x_{(i)}^k$ are the order statistics of $x_{k-M} \dots x_k \dots x_{k+m}$. The a_i values are constants that may be chosen for a particular application (Bovik et al., 1983). A sample calculation of maximum and minimum filters is shown below:

$$\begin{bmatrix} 12 & 2 & 5 \\ 5 & 7 & 5 \\ 10 & 2 & 3 \end{bmatrix} = \begin{bmatrix} 12 & 2 & 5 \\ 5 & 12 & 5 \\ 10 & 2 & 3 \end{bmatrix} \quad (7)$$

$$\begin{bmatrix} 12 & 2 & 5 \\ 5 & 7 & 5 \\ 10 & 2 & 3 \end{bmatrix} = \begin{bmatrix} 12 & 2 & 5 \\ 5 & 2 & 5 \\ 10 & 2 & 3 \end{bmatrix} \quad (8)$$

Minimum filter image was subtracted from the maximum filter image to acquire the differential image, which clearly presented the pit areas, boundary, and apple core region with higher pixel values (Fig. 1e). Following this step, all the pixels on the boundary and within the 3-by-3 neighbourhood of the boundary pixels were converted into background pixel values. The resulting image was with pits and apple core only (without boundary, Fig. 1f). No bitter pit was observed in the fruit core. Therefore, the core was eliminated from the central region of the apple. The central region was defined as centre of the individual apple image having a radius one-fifth of the apple diameter (the diameter of a circle with the same area as the apple, Fig. 1g). An area threshold of 5,000 pixels was applied to judge if there is a core inside the apple. Resulting image without the core was converted into binary image using a pixel value threshold of 30 (Fig. 1h). The area of each bitter pit was then estimated. For the pits with area less than 4 pixels, the bitter pit pixels were converted into background. Additionally, length and width ratio of individual bitter pit was calculated with rectangular box fit (Fig. 1i) and pits with ratio greater than 4 were considered for further analysis. It eliminated possibilities of noise near the boundary region and some extent of mechanical bruise injury. Following which, grey difference image (Fig. 1j) was also developed to confirm the bitter pit regions. The average grey pixel value of each bitter pit in the differential image was calculated. The complement of the differential image was used to simplify the image representation.

In addition to core, the stem end and calyx was eliminated from the images (strip removal). The minimum radius in the contour image was utilized to identify the stem and calyx end for determining the strip region. Stem end and calyx end determination was based on the difference between the average radius and the minimum radius. The coordinates of the points on the boundary were converted to polar coordinates. In the range of 0 to 180° (bottom half of apple), the minimum radius caused by calyx was estimated. If the difference between the average and minimum radius was larger than a threshold (> 6 units), the point was marked as calyx end. Similarly, the stem end was detected in the range of 0 to -180°. After both ends (calyx and stem) were detected, a line was drawn through the two ends, and termed as the centre line. Two parallel lines on either sides of the centre line were developed such that the distance between them was 20 pixels. This region between the parallel lines was defined as the strip region (Fig. 1k) and eliminated as background. After removing all the background, the remaining spots were bitter pits inside the apples (Fig. 1l). The total number of pits and pit area (number of pixels showing anomalies) was computed as the output from the image processing algorithm. Using the developed algorithm, The CT Scan images were batch processed and the resulting data was analysed using the SAS® statistical software (ver. 9.2, SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Bitter pit on the apple surface

The minimum, average, and maximum number of bitter pits on the apple surface (20 samples) at different time periods after harvest for two field locations are presented in Fig. 3. At the beginning of the study (DAH=0), the healthy apples did not have any bitter pits on the surface, while the number of bitter pits on the affected apples ranged up to a maximum of 40 and 28 at locations-1 and -2, respectively. The maximum number of bitter pits eventually increased up to 60 in location-2 at 63 DAH. Interestingly, the maximum number of pits on the surface of the fruit

remained almost constant at location-1. However, the average number of bitter pits increased from about 5 to 15 pits at 63 DAH at location-1. In case of location-2, the average number of pits increased from about 7 to 23 during 63 DAH storage. The trend clearly indicated that bitter pit affected apples could further develop bitter pits during storage. In a real-world scenario, if bitter pit affected apples were identified, the produce will be sent to processing immediately before the condition becomes worse.

In bitter pit affected apples, the maximum number of bitter pits decreased between 21 DAH to 63 DAH in few samples. The possible reason for this observation could be because of two neighbouring bitter pits merging into a single pit during disorder progression in storage. This would lead to single count as predicted using the developed algorithm. This decrease in total number of bitter pits from 21 DAH to 63 DAH was only observed in 5 (of 20) apples in location-1 and 4 (of 20) apples in location-2.

In regard to the healthy apples, the average number of bitter pits was less than 1 in both locations. However, in case of location-2, an apple showed 6 bitter pits on 21 DAH, which reduced to 2 pits at 63 DAH. The pits close to each other may have merged. After 63 days of storage, 3 (of 20) and 2 (of 20) healthy apples had developed bitter pits from location-1 and -2, respectively. Such image processing based trends were consistent with ground reference data (visual observation). This indicates the potential of utilising CT imaging based technique for identifying the bitter pits on the surface in an automated manner.

3.2. Bitter pit inside the apple

Table 1 summarises the data on the total number of bitter pits per apple as detected by the image processing algorithm. Average number of pits in bitter pit-affected apples increased from 9 to 19 and 18 to 19 during 63 DAH storage in location-1 and location-2 sampled fruits, respectively. Note that the average number of pits detected increased from 0 to 21 DAH, which again decreased

from 21 to 63 DAH. The decrease in number of bitter pits could be attributed to merging of pits as observed in the bitter pits present in the surface. In addition, the pits present in apple subsurface may have merged into the cuticle surface. The algorithm would detect these newly developed pits as surface pits rather than internal pits. The number of apples with a decrease in the total number of bitter pits inside from 21 DAH to 63 DAH were 13 and 14 of 20 apples in location-1 and -2, respectively. This trend also indicates that the development of bitter pits inside the fruit is at a much faster rate than the surface bitter pits.

In case of healthy apples, the average number of bitter pits was less than 1 at 0 DAH. The average number of bitter pits inside the fruit increased up to 7 at 63 DAH. One of the challenges in the image processing was the complete elimination of regions with internal bruising. The bruising was significant in the fruits from location-1. Fruit bruising could have occurred during harvest and transportation of fruits from field to the laboratory. Usually, the bruising symptoms develops few days after harvest. In regard to the apple production and management practices, bruised apples would result in similar economic losses as that of bitter pit-affected apples, and CT-based image acquisition and processing to identify all internal defects can be beneficial. Ideally, such fruits will be sorted on the packaging line to be sent to processing industry rather than to the fresh market. For healthy samples from location-2, 10 of the 20 apples developed internal bitter pits during 63 days of storage, while only 2 of those apples developed external symptoms. This further signifies the potential of CT-based imaging technique towards identifying internal bitter pit symptoms.

The algorithm also estimated the pit area in each layer of an apple (Fig. 4). For samples from location-1, the total pit area inside the affected apples ranged from 7-2309 pixels at 0 DAH and 20-3287 pixels at 63 DAH. Similarly, for location-2, the corresponding range was from 10-3573 at 0 DAH and 91-3533 at 63 DAH. In regard to the healthy apples in location 2, the total area of newly developed bitter pits ranged from 6 to 203 pixels, indicating that these pits were much fewer and smaller than those in the affected apples. Between 21 DAH and 63 DAH, there was some decrease in

the pit area, which could be associated with shrinkage of pits caused by loss of moisture during storage or merging of internal subsurface pits to the surface as mentioned above.

3.3. Bitter pit distribution within a fruit

Overall, 42 to 66% of the pits in the affected fruit were present inside rather than on the fruit surface (Fig. 5). It indicates that pits seen on the surface of bitter pit affected apples, only represent about 50% of the total bitter pits presents in the apple fruit. In some apples, the number of internal pits could range close to 100% of the all the bitter pits present. In the healthy apples from location 2 at 63 DAH, except 2 samples (having surface symptoms), 100% of newly developed bitter pits were from inside as discussed above. This signifies the importance of CT-based imaging for high-throughput accurate phenotyping to support breeding efforts to develop bitter pit tolerant varieties. In addition, the technique can also be confidently applied for identifying bitter pits inside the fruit during initial stage of development in the processing lines.

4. Conclusions

This work evaluates the feasibility of acquiring qualitative and quantitative data on bitter pit occurrences in apples using X-ray CT-based imaging with a custom-developed image processing algorithm. The CT-based imaging has not been used for acquiring quantitative information during post-harvest produce evaluation. The acquired results indicate the potential of utilizing the non-destructive CT-based imaging technique for thorough evaluation of internal produce quality, which can be very useful during processing as well as in plant breeding studies. The results indicated that most of bitter pit starts developing inside the fruit, while the external symptoms are absent. Identification of such internal symptoms can be greatly beneficial to the processing industry as they can prevent packaging and transportation of defective products. In addition, the technique can also be applied to other specialty crops for internal disorder evaluation.

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Fig. 1- Apple images generated for bitter pit detection on the surface and inside the fruit. (a) Raw segmented image, (b) binary image derived from segmented image, (c) binary image after elimination of small objects (<600 pixels), core, and pits inside the apple (this image was used for estimating the number of bitter pit on the outer surface by calculating the radius from mass centre to the boundary), (d) raw greyscale image used for estimating bitter pits inside the apples, (e) differential image after applying minimum and maximum

353 filter on the greyscale image, (f) image after boundary elimination from the differential
354 image, (g) image after core elimination, (h) binary image acquired from differential image
355 after boundary and core elimination, (i) image determining length-width ratio using
356 rectangular fit, (j) grey difference image, (k) image determining the strip region in the apple
357 image, and (l) the final binary image used for calculating total number of bitter pits and their
358 area inside the fruit.

Fig. 2- (a) Schematics illustrating the ρ -difference estimation from the radius (ρ) between the boundary and the centre of the apple. In an event of bitter pit occurrence on the apple surface, radius y will be less than radius x . ρ -difference will be equal to $y-x$. (b) Pit identification from ρ and ρ -difference values. Radius of each boundary point from the mass centre of the apples, the regions between -100 to -50 and 75 to 125 (θ) represent stem and

365 calyx ends of the apple, respectively. ρ -difference showing possible pit locations, ρ -pulseA
366 after normalisation (0 or 1) of ρ -difference, and ρ -pulseB after noise removal.

Fig. 3- Bitter pit progression on the surface of healthy and bitter pit apples from both locations.

Fig. 4- Total pit area (sum of all pits from 20 samples in each category) inside healthy and bitter pit affected apples from both locations.

Fig. 5- Boxplot showing the number of bitter pits inside the fruit with respect to total number of pits in a given bitter pit-affected apple from both locations. The 'central box' represents the central 50% of the data with lower and upper boundary lines representing the 25% and 75% quantile of the data. A circular dot indicates the median of the data; while the two vertical lines extending from central box indicate the remaining data range. The outliers are marked as '+'.

380 **Table 1- Total number of bitter pits detected by the developed image processing algorithm**
381 **inside the healthy (H) and bitter pit (BP) affected apples from both field locations during 63**
382 **DAH storage period.**

		Number of pits inside the fruit											
Location		1						2					
DAH		0		21		63		0		21		63	
Apple No.	H	BP	H	BP	H	BP	H	BP	H	BP	H	BP	
1	1	3	9	9	6	12	0	23	0	39	2	47	
2	0	0	7	27	12	66	0	94	1	100	2	38	
3	0	6	3	4	6	3	0	30	0	15	0	8	
4	0	1	1	5	4	10	0	40	2	19	5	8	
5	0	2	1	8	6	3	0	28	1	18	1	8	
6	0	0	1	13	1	27	0	34	3	60	0	31	
7	0	0	13	9	22	27	0	6	0	6	3	4	
8	0	4	0	31	0	26	0	2	1	6	1	6	
9	0	0	3	1	5	0	0	18	0	17	0	11	
10	0	52	8	151	6	71	0	15	0	31	0	23	
11	0	54	1	42	2	24	0	1	0	2	0	9	
12	0	0	18	3	9	1	0	15	0	48	0	41	
13	0	0	8	3	10	5	0	2	0	25	1	8	
14	0	6	0	3	0	3	0	7	0	11	0	7	
15	0	4	9	9	16	3	0	2	0	6	0	9	
16	0	1	0	2	1	4	0	8	0	88	1	26	
17	0	5	1	13	0	7	0	7	2	7	3	5	
18	0	17	26	69	13	30	0	11	0	15	0	18	
19	0	13	14	18	5	10	0	24	1	35	2	18	
20	0	18	28	39	11	47	0	1	0	7	0	49	
Minimum	0	0	0	1	0	0	0	1	0	2	0	4	
Average	0	9	7	23	7	19	0	18	1	28	1	19	
Maximum	1	54	28	151	22	71	0	94	3	100	5	49	

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