

NONDESTRUCTIVE EVALUATION OF FRESH CHESTNUT INTERNAL
QUALITY USING X-RAY COMPUTED TOMOGRAPHY (CT)

By

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ABSTRACT

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Internal decay is an important quality attribute in chestnuts (*Castanea* spp.). Worldwide, internal decay is mainly caused by microorganism attack and physiological cell breakdown. It is problematic for the industry, and impacts consumer satisfaction, shelf life, and proper storage. Currently, destructive techniques can be employed to evaluate fresh chestnut internal quality. However, clearly not all produce can be evaluated. In commercial situations, decayed chestnuts are eliminated by their proclivity to float in water. Nonetheless, performance significantly varies between species and throughput, making this floating method unreliable for sorting purposes. Thus, the overall objective of the study is to develop the methods to nondestructively visualize and automatically classify fresh chestnuts, based on their internal quality, using X-ray CT imaging.

In this study, medical grade computed tomography (CT) was used to obtain transversal two-dimensional (2D) images from fresh chestnuts (cv. 'Colossal' and Chinese seedlings). If the information obtained by the CT scanning of fresh chestnuts is to be used in an industrial setting for in-line sorting, automated interpretation of CT images is essential. For this purpose: (1) Chestnut CT image quality was optimized by studying the combined effect of

image acquisition parameters (voltage – 120 kV, current – 170 mA and slice thickness – 2.5 mm) using response surface methodology, (2) effective image visualization techniques to infer fresh chestnut internal quality attributes were established, and (3) an image analysis algorithm for the automatic classification of CT images obtained from 2848 fresh chestnuts, during the harvesting years from 2009 to 2012, was developed and tested.

The CT imaging system provided high-resolution and high-contrast images of the internal structure and components of fresh chestnuts. Approximately 50 original CT image slices (stack) were obtained per chestnut, from three different planes (angular orientations) across the longitudinal (Z) (XY-plane-slice), horizontal (YZ-plane-slice) and vertical (XZ-plane-slice) axes. From this image stack, 6 secondary CT images per chestnut sample, including mean and maximum intensity value images for each of the planes were extracted. Thereafter, a total of 1194 grayscale intensity, and textural features were extracted from the 6 secondary CT images per sample. Ultimately, 86, 155 and 126 features were found to be effective in designing a quadratic discriminant analysis classifier with an overall performance accuracy of 85.9 %, 91.2 % and 96.1 % for 5, 3 and 2 classes, respectively.

This study provides a powerful tool to accurately visualize and sort chestnuts based on their internal quality, leading to the improved marketability of attractive, safe, high quality chestnuts. Results show that this method is accurate, reliable, and objective and it is applicable to an automatic noninvasive in-line CT sorting system.

*To those close and far away that I try to tell, every time I can, that I love. You might never know how much your love, encouragement, moral support and sacrifice has meant to me through the development of this dissertation. It has not been easy, but this is an accomplishment from all of us.
With lots of love this is for you...*

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TABLE OF CONTENTS

LIST OF TABLES	xi
LIST OF FIGURES	xii
KEY TO ABBREVIATIONS	xviii
CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW	1
1.1 Introduction	1
1.2 Chestnut fruit	6
1.3 World chestnut industry	9
1.4 Electronic sorting technologies	13
1.5 Basic principles of X-ray computed tomography (CT)	15
1.6 Application of CT in non-medical industries	26
1.7 Objectives and Hypothesis	27
CHAPTER 2. APPLICATION OF RESPONSE SURFACE METHODOLOGY (RSM) TO SYSTEMATICALLY OPTIMIZE FRESH CHESTNUT COMPUTED TOMOGRAPHY (CT) IMAGE QUALITY	29
2.1 Abstract	29
2.2 Introduction	30
2.3 Materials and methods	31
2.3.1 Chestnut sample collection, reference cylinders, and Quality Assurance (QA) phantom CT imaging scans	31
2.3.2 RSM Box-Behnken design for CT image quality optimization	34
2.3.3 Visual based fresh chestnut quality evaluation and SNR (Y1)	39
2.3.4 Volume accuracy (Y2) using Teflon® reference cylinders	42
2.3.5 HCSR (Y3) and LCD (Y4) calculation using a standard QA Phantom	42
2.3.6 Optimized 2D CT image quality validation from an independent data set ..	43
2.4 Results	46
2.4.1 RSM Box-Behnken design for CT image quality optimization	46
2.4.2 SNR (Y1) optimization	52
2.4.3 Volume accuracy (Y2) optimization	56
2.4.4 HCSR (Y3) and LCD (Y4) optimization	60
2.4.5 Optimized CT image quality attributes and validation	67
2.5 Discussion	73
2.6 Conclusions	75
CHAPTER 3. RELATION OF COMPUTED TOMOGRAPHY (CT) HOUNSFIELD UNIT MEASUREMENTS AND INTERNAL COMPONENTS OF FRESH CHESTNUTS	77

3.1 Abstract	77
3.2 Introduction	77
3.3 Materials and methods	78
3.3.1 Sample collection and preparation	78
3.3.2 <i>In vivo</i> CT imaging scans.....	80
3.3.3 Visual based fresh chestnut quality and internal component assessment.	80
3.3.4 HU-value inference using training data set.....	83
3.3.5 Chestnut categories prediction using an independent testing data set.....	87
3.4 Results.....	88
3.4.1 HU-value and category threshold inference using training data set.....	88
3.4.2 Chestnut categories prediction using an independent testing data set.....	92
3.5 Discussion	97
3.6 Conclusion.....	98
CHAPTER 4. POSTHARVEST NONINVASIVE ASSESSMENT OF FRESH CHESTNUT INTERNAL DECAY USING COMPUTED TOMOGRAPHY (CT) IMAGES	99
4.1 Abstract	99
4.2 Introduction.....	100
4.3 Materials and methods	104
4.3.1 Sample collection and preparation	104
4.3.2 CT image preprocessing:.....	105
4.3.3 CT image re-slicing.....	105
4.3.4 Individual chestnut CT image cropping.....	106
4.3.5 Contrast enhancement	111
4.3.6 CT image segmentation (Binary mask):	112
4.3.7 Visual based fresh chestnut quality and internal component assessment:	113
4.3.8 Feature extraction:.....	113
□ Basic intensity features:.....	114
□ Haralick textural (Tx) features:.....	115
□ Intensity local binary pattern (LBP) textural features:	118
□ Intensity Gabor textural features:.....	120
□ Contrast features:	122
4.3.9 Feature selection:	124
4.3.10 Classification (training and validation):	128
4.4 Results.....	138
4.5 Discussion	142
4.6 Conclusions.....	147
CHAPTER 5. DISCUSSION AND FINAL REMARKS.....	149
APPENDICES	160
APPENDIX A. Estimation of SNR, Volume accuracy, High contrast spatial resolution (HCSR), Low Contrast Detectability (LCD), and digital quality assessment (DQA)	161
APPENDIX B. CT imaging in other fresh agricultural commodities	168

REFERENCES	176
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LIST OF TABLES

Table 1. Scanning parameters for the CT – General Electric, BrightSpeed™ RT 16 Elite (GE Healthcare, Buckinghamshire, England, Great Britain)	34
Table 2. Box-Behnken experimental design variables	36
Table 3. Design matrix (un-coded factors) in respect to each response variables	38
Table 4. ANOVA table for Signal-to-noise Ratio (SNR), Volume Accuracy (VA), High Contrast Spatial Resolution (HCSR), and Low Contrasts Detectability (LCD) non-linear response models	48
Table 5. Stationary maximized points in original units for each non-linear response variable model	49
Table 6. Regression coefficients and P values ^a for each non-linear response variable model	51
Table 7. Acquired HU value samples from different chestnut components and air, based on regions of interest (ROIs).....	84
Table 8. Haralick textural (<i>T_x</i>) features (Haralick, 1979)	117
Table 9. Main selected features (seventy five) for the Quadratic discriminant analysis (QDA) classifier using sequential forward selection (SFS) in combination with the Fisher discriminant objective function (<i>J(W)</i>) for the 5-, 3- and 2-class classifiers.....	139
Table 10. Classifier performance using selected features (<i>m</i>) with a 4-folds validation	141

LIST OF FIGURES

Figure 1. World chestnut (<i>Castanea</i> spp.) production in 2011	2
Figure 2. Chestnut fruit morphology. A. Chestnut fruit longitudinal cut, B. Spiny burr attached to chestnut tree with chestnuts. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.....	6
Figure 3. X-ray computed tomography (CT) A. Traditional CT working principal, B. Measuring arrangement of the GE (GE Healthcare, Buckinghamshire, England, Great Britain) BrightSpeed™ RT 16 Elite CT used in these studies, C. Schematic representation of a CT system scanning (5 chestnuts per row) containing several 2D XY-plane CT images (slices).....	18
Figure 4. CT imaging and data acquisition A. Transmission measurements through the objects at numerous angles (black arrows) around the object, B. Back-projected (reverse) transmission measurement onto 2D XY-plane CT image matrix, C. Back-projection reconstruction from projection values (P), for a simple image of four voxels.	19
Figure 5. (a) 3D virtual cross-sections representation of a chestnut at three planes (different angular orientations) along the horizontal (X), vertical (Y), and longitudinal (Z) axes (Figure not to scale). (b) Original series of acquired CT XY-plane-slices. (c) Reconditioned (re-sliced) CT YZ-plane-slices. (d) Re-sliced CT XZ-plane-slices.....	24
Figure 6. (a) Fresh chestnuts samples, and Teflon® reference cylinders, (b) Testing phantom, (c) 16-bit CT images of fresh chestnut samples for Signal-to-noise Ratio (SNR) calculation, and Teflon® reference cylinders for volume accuracy (mm^{-3}) estimate. (d) 8-bit CT image of QA phantom used to infer High Contrast Spatial Resolution (HCSR). (e) 8-bit CT image of QA phantom used to infer Low Contrast Detectability (LCD).	32
Figure 7. (a) Example of chestnut 16-bit CT image slice used for Signal-to-noise Ratio (SNR) calculation, from a region containing fresh uniform chestnut tissue, using reference color image. (b) Example of Teflon® reference cylinder 8-bit CT image used to estimate volume accuracy (mm^{-3}) with binary image after segmentation (simple global threshold of 135). (c) Example of cropped 8-bit CT image used to calculate High Contrast Spatial Resolution (HCSR) with binary image after segmentation (simple global threshold of 134). Each pattern consists of five bars and spaces called line pairs (lp). The sizes of the patterns are	

equivalent to 1.6 mm, 1.3 mm, 1.0 mm, 0.6 mm, and 0.5 mm, respectively. (d) Example of cropped 8-bit CT image used to determine Low Contrast Detectability (LCD) with binary image after segmentation (simple global threshold of 94). Image displays various sized Holes (H). The diameter of each H equal 10.0 mm, 7.5 mm, 5.0 mm, 3.0 mm, and 1.0 mm. 41

Figure 8. Example of images used for Digital Quality Assessment (*DQA*). (a) Reference color image. (b) 16-bit optimized CT gray scale image. (c) Binary image of whole chestnut after segmentation, applying a simple global threshold of 400 HU to Fig. 8(b). (d) Binary image of transition points and pellicle after employing a Sobel filtering method to detect edges in Fig. 8(b). (e) Binary image of healthy tissue after segmentation, using a simple global threshold of 1050 HU to Fig. 8(b). Figure is partially presented in color. 45

Figure 9. Surface plots of *SNR* versus (a) slice thickness (ST - mm) for current (C - mA), (b) slice thickness (ST - mm) for voltage (V - kV), and (c) current (C - mA) for voltage (V - kV). 54

Figure 10. (a) Surface plot of volume accuracy (VA - mm⁻³) versus slice thickness (ST - mm) for current (C - mA). (b) Interaction plot for volume accuracy (VA - mm⁻³) versus slice thickness (ST - mm) for current (C - mA). Data points followed by the same lower case letter within the same slice thickness are not significantly different at P = 0.05 (ANOVA with post-hoc Tukey multiple comparison of means). Surface plots of volume accuracy (VA - mm⁻³) versus (c) slice thickness (ST - mm) for voltage (V - kV), and (d) current (C - mA) for voltage (V - kV). 58

Figure 11. Surface plots of *HCSR* versus (a) slice thickness (ST - mm) for current (C - mA), (b) slice thickness (ST - mm) for voltage (V - kV), and (c) current (C - mA) for voltage (V - kV). 62

Figure 12. Surface plots of *LCD* versus (a) slice thickness (ST - mm) for current (C - mA), (b) slice thickness (ST - mm) for voltage (V - kV), and (c) current (C - mA) for voltage (V - kV). 64

Figure 13. Example of Gray-scale CT image quality for low (-1), medium (0), and high (+1) factor combinations. Note that low (-1) and high (+1) level combinations are not part of the Box-Behnken design, but CT images were acquired for visualization and validation purposes. 66

Figure 14. Box-plots showing experimental results for CT image quality attributes (*SNR*, VA (mm⁻³), *HCSR*, *LCD*) from a completely independent validation data set, using optimized scanning parameters, as seen in Table 5. The median of each experimental quality attribute is represented as a thick horizontal black line, upper and lower quartiles as a box with the maximum and minimum

measurements as lines protruding from these. The mean experimental response for each quality attributes is symbolized as a black solid dot (.). Predicted optimized response using non-linear response surface polynomial models (Table 2) is symbolized as a hollow circle ($^{\circ}$). 69

Figure 15. Relationship between Subjective Quality Rating (*SQR*) values obtained from a 5-experts panel and the Digital Quality Assessment (*DQA*) from their corresponding CT image validation set, obtained through optimized scanning parameters. Black solid line represents the least-square linear regression line ($n = 110$). The two dashed lines indicate 95 % CI of the linear regression. The two dotted lines indicate 95 % prediction intervals of the linear regression..... 72

Figure 16. Cross-sectional XY-plane 2D CT images and color raw image slices (RGB) of chestnuts. (A) shows a healthy chestnut (SL1). (B) shows a partially decayed chestnut (SL2). (C) shows a completely decayed chestnut (SL3). (D) shows a partially decayed chestnut (SL2), illustrating the HU-values-profile taken at the grey line (PL), typifying the HU variation within components in the same chestnut. 82

Figure 17. Box-plots showing the HU values from ROIs obtained using a training data set. The median is represented as a thick horizontal black line, upper and lower quartiles as a box with the maximum and minimum measurements as lines protruding from these. Box-plots followed by the same letter and enclosed by the same rectangle are not significantly different between each other at $P = 0.05$ (ANOVA) (Tukey multiple comparison of means). Solid circles joined by a dashed black line (---) show the relationship between the mean of the HU values for each ROIs and subsequently each category. 89

Figure 18. (a) Category prediction using estimated thresholds (horizontal black lines) with an independent testing data set. HU threshold values are indicated above each threshold line. The selected HU value threshold range for air, void spaces, decay tissue, pellicle tissue, and healthy tissue are -1024 to -975, -974 to -500, -400 to -210, -209 to 24, and 25 to 300; respectively. (b) Confusion matrix corresponding to the category prediction accuracy (overall accuracy rate = 90.6%). (c) Representative images, containing automatically generated color labeled images from their corresponding CT images, based on HU-value threshold intervals. RGB raw image slices are also included as a quality reference. 95

Figure 19. Procedure used to generate the pattern classification algorithm to categorize chestnut quality using CT images. 103

Figure 20. Mean intensity values of pixels in all of the: (a) YZ-plane-slice of the whole board containing chestnuts, (b) YZ-plane-slices for the first chestnut row, (c) XY-plane-slices for the first chestnut row, (d) XZ-plane-slices for the first

chestnut row. Mean intensity values of pixels in final secondary CT cropped chestnut for the (e) YZ-, (f) XY- and (g) XZ-plane-slices. Maximum intensity values of pixels in final secondary CT cropped chestnut for the (h) YZ-, (i) XY- and (j) XZ-plane-slices. From (a) - (d) the beginning of the first chestnut row (Z1-), the end of the row (Z2-), the left side (X1-), the right side (X2-), the bottom (Y1-) and the top (Y2-) of the first chestnut -spatial-location-values are shown in red.

..... 108

Figure 21. (a) Partially decayed fresh raw chestnut slices. (b) Original secondary mean CT image (YZ-plane-slice), (c) adjusted secondary mean CT image (YZ-plane-slice), (d) final contrast enhanced secondary mean CT image (YZ-plane-slice). (e) Segmented CT image from final contrast enhanced secondary mean CT image (YZ-plane-slice) (binary mask). (f) Original secondary maximum CT image (YZ-plane-slice), (g) adjusted maximum secondary maximum CT image (YZ-plane-slice), and (h) final contrast enhanced secondary maximum CT image (YZ-plane-slice). 110

Figure 22. (a) Healthy and (b) partially decayed chestnuts with their corresponding secondary maximum CT image (XY-plane-slice) (55 pixels x 65 pixels). (1) Local Binary Patter (LBP) transformations using different pixel comparison (d,h) was applied to the secondary maximum CT image. (2) Secondary maximum CT image Gabor filtered transformed images ($I_{rs}(i,j)$) at different scales (r) and orientation (s) for an $\theta = 45^\circ$ are included. (3) Example of Haralick textural (T_x) features, contrast and intensity features obtained from included secondary maximum CT image. For visual reference, three-color fresh raw image slices of the evaluated chestnut are included..... 126

Figure 23. (a) Sample distribution used to train and validate the 5-class classifier. Figure also contains an example for each of the 5 categorical classes, representing the quality index levels. (b) Quadratic discriminant analysis (QDA) classifier performance using validation set with 4-folds, in relation to the number of selected features (m). Black line represents the classification mean performance, dotted black line (---) represent 95 % confidence intervals for the validation pool. (c) Validation confusion matrix corresponding to the chestnut quality class prediction using 25 % of samples with 86-m (overall accuracy rate equal to 85.9 %). 130

Figure 24. (a) Sample distribution used to train and validate the 3-class classifier. Figure also contains an example for each of the 3 categorical classes, representing the quality index levels. (b) Quadratic discriminant analysis (QDA) classifier performance using validation set with 4-folds, in relation to the number of m . Black line represents the classification mean performance, dotted black line (---) represent 95 % confidence intervals for the validation pool. (c) Validation confusion matrix corresponding to the chestnut quality class prediction using 25 % of samples with 155-m (overall accuracy rate equal to 91.2 %). 132

Figure 25. (a) Sample distribution used to train and validate the 2-class classifier. Figure also contains an example for each of the 2 categorical classes, representing the quality index levels. (b) Quadratic discriminant analysis (QDA) classifier performance using validation set with 4-folds, in relation to the number of m . Black line represents the classification mean performance, dotted black line (---) represent 95 % confidence intervals for the validation pool. (c) Validation confusion matrix corresponding to the chestnut quality class prediction using 25 % of samples with 126- m (overall accuracy rate equal to 96.1 %). Figure is partially presented in color..... 134

Figure 26. (a) Ultrafast Rossendorf electron beam X-ray tomograph (ROFEX) scanner working principle. (b) Experimental setup of the ROFEX-scanner. 152

Figure 27. Visual preliminary results of CT images (lower row) obtained using the ROFEX-scanner with its corresponding color raw image slices (upper row). (a) Healthy, (b) partially decayed (rotten) and (c) completely decayed chestnuts. (d) 3D reconstruction of two chestnuts, showing a rotten section in one of the chestnuts (white arrow). 153

Figure 28. (a) Commercially available in-line traditional X-ray sorter, and (b) its corresponding 2D X-ray images. 157

Figure 29. Typical unit operations in mechanized packinghouse (Thompson et al., 2002). Vision of future integration of in-line Computed Tomography (CT) for sorting fresh agricultural products*. *: Flowchart in red are the proposed sections that will be potentially improved..... 159

Figure 30. Color raw images of (a) chestnuts, (b) pineapples, (c) tart cherries and (d) pickling cucumbers showing the regions of interest (ROIs) for each fresh commodity. 169

Figure 31. (a) Color raw image slices, cross-sectional 2D CT images acquired using the GE BrightSpeed™ RT 16 Elite CT scanner, and 3D reconstruction of pineapples (b) Black dots showing the HU-values for the mean of 100 data points per each pineapple ROI ($n = 100$). Values followed by the same letter are not significantly different between each other at $P = 0.05$ (ANOVA) (Tukey multiple comparison of means). Vertical bars represent the standard deviation (SD) of each ROI. 170

Figure 32. (a) Color raw image slices, cross-sectional 2D CT images acquired using the GE BrightSpeed™ RT 16 Elite CT scanner, and 3D reconstruction of tart cherries (b) Black dots showing the HU-values for the mean of 100 data points per each tart cherry ROI ($n = 100$). Values followed by the same letter are not significantly different between each other at $P = 0.05$ (ANOVA) (Tukey multiple comparison of means). Vertical bars represent the SD of each ROI... 172

Figure 33. (a) Color raw image slices, cross-sectional 2D CT images acquired using the GE BrightSpeed™ RT 16 Elite CT scanner, and 3D reconstruction of pickling cucumbers (b) Black dots showing the HU-values for the mean of 100 data points per each pickling cucumber ROI (n = 100). Values followed by the same letter are not significantly different between each other at P = 0.05 (ANOVA) (Tukey multiple comparison of means). Vertical bars represent the SD of each ROI. 174

KEY TO ABBREVIATIONS

2D – Two-dimensional.

3D – Three-dimensional.

ANOVA – Analysis of variance.

CGI – Michigan Chestnut Grower Inc.

CI – Confidence intervals.

CS – Chestnut species. Co - ‘Colossal’ (*Castanea sativa* x *C. crenata*) and Ch - Chinese seedlings (*C. mollissima*).

CT – Computed Tomography.

dH₂O – Distilled water.

DQA – Digital Quality Assurance.

df – Degrees of freedom.

FAO – United Nations Food and Agriculture Organization.

Gy - Grays.

HCSR – High Contrast Spatial Resolution.

HU – Hounsfield Units.

IAEA – International Atomic Energy Agency.

kV – Kilovolt.

L and U – Lower and Upper category thresholds.

LCD – Low Contrast Detectability.

mA – Milliamperage.

MRI – Magnetic Resonance Imaging.

MS – Mean square.

NIR – Near infrared.

PB – Peg-board containing chestnut samples attached to it. Boards are used to keep samples in place while scanning. PB1 contained Chinese seedlings and PB2 ‘Colossal’ chestnuts.

PDA – Potato-dextrose-agar.

PL – Profile-line (HU values).

Px – Pixel.

QA – Quality Assurance.

RGB – Sub-index representing images that are originally printed in color. RGB is a universally accepted system for color representation in television, video sets, and CRT displays; using red, green and blue primaries. It is not a perceptually uniform color space (i.e. differences between colors in the 3D RGB space do not correspond to those perceived by humans).

ROI, ROIs – Region of interest, Regions of interest.

SCF – Scientific Committee on Food of the European Commission.

SD – Standard deviation.

SL – Internal disorder severity levels. Where, SL1 contains chestnuts with no internal disorders – Healthy, SL2 chestnuts which are partially disordered and SL3 represents the group of chestnuts, that are completely disordered.

SS – Sum of squares.

SU – Sampling unit. Where, SU1 = 1-pixel per repetition, and SU2 = Mean HU value from a 4 mm^2 (6.6 pixel^2) square region per repetition.

RSM – Response Surface Methodology.

SD – Standard Deviation.

SNR – Signal-to-noise Ratio.

SQR – Subjective Quality Rating.

VA – Volume Accuracy, expressed in mm^{-3} .

WHO – World Health Organization.

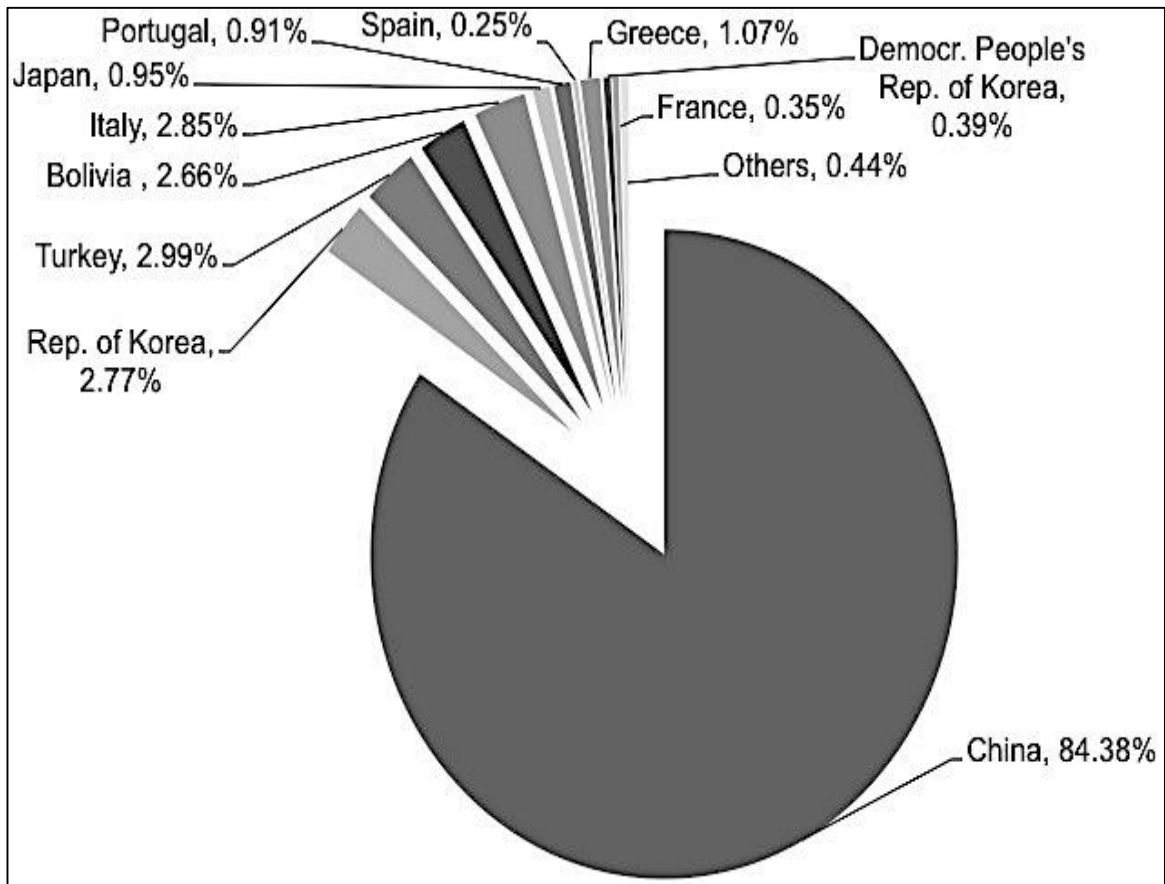
CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Chestnut (*Castanea* spp.) is one of the most popular nut-bearing trees in the countries of Asia, including China, Republic of Korea, and Japan; as well as the Mediterranean region (Ridley, 1999). Worldwide, from 1998 to 2011, chestnut production increased by approximately 92,000 kg per year (Food and Agriculture Organization Statistics Division (FAOSTAT), 2013). As observed in Fig. 1, in 2011, chestnuts grown in Asia and Europe accounted for more than 95% of the worldwide production, with chestnut production increasing in Australia, New Zealand, Chile and Bolivia among others (Grau and France, 1999; Klinac et al., 1999; Ridley, 1999; Fulbright and Mandujano, 2000; Mencarelli, 2001).

In the United States, chestnuts are uncommon; people are more likely to be familiar with the unrelated and poisonous horse chestnut (*Aesculus* spp.) (Fulbright and Mandujano, 2000), than with any of the edible, sweet chestnut species, including *C. dentata*, *C. mollissima*, *C. crenata*, *C. sativa*, *C. seguinii*, *C. pumila*, and *C. henryi* (Anagnostakis et al., 1998; Fulbright and Mandujano, 2000; Miller, 2003). This is partially due to chestnut blight (*Cryphonectria parasitica*, Murril and Barr), a fungus disease that virtually eliminated the once-widespread American chestnut (*C. dentata*) during the first half of the twentieth century (Merkel, 1905; Metcalf and Collins, 1909; Gravatt and Marshall, 1926). Over the past twelve years, with the availability of blight resistant hybrids, as well as tolerant European and Chinese cultivars, commercial orchards, and wild forest

tree populations have significantly increased in the United States, including Michigan (Fulbright and Mandujano, 2000; Jacobs, 2007).



Source: Food and Agriculture Organization Statistics Division (FAOSTAT), 2013
Figure 1. World chestnut (*Castanea* spp.) production in 2011

Before the early 19th-century, especially in the European Mediterranean region and in Asia, chestnut consumption was predominant in rural areas, and was considered to be a food for the poor. Currently, chestnuts are mainly consumed by roasting the fresh product (Harte et al., 2003), but also are used as a cooking ingredient in diverse culinary applications, including pastries, and specialty dishes (Kelley and Behe, 2002; De la Montaña Miguelez et al., 2004; Blackwell, 2006; Borges et al., 2008). Additional to the fresh product, small

portions of chestnuts are consumed peeled. Mechanically peeled and subsequently frozen chestnuts are usually sold as a value-added product to extend the chestnut market beyond seasonal sales, providing the opportunity to expand markets and their utilization (Guyer et al., 2003; Gao et al., 2008). Positively, for the United States, Vossen (2000) claimed that the chestnut industry has a great potential, and a small rise in domestic consumption could generate a net revenue of up to 800 million US\$ annually. Studies performed in Wisconsin and California, between the years 2000 and 2004, evaluating consumer preference for chestnuts, reflected that the majority of the interviewed had never tasted a chestnut, but were interested in exploring them as a new food. Overall quality and nutritional value were listed as the most important attributes influencing purchase and consumption decisions (Gold et al., 2004).

Chestnut quality is measured not only by external factors such as color, shape, size, surface blemish, and surface mold growth, but also by internal disorders and freshness, which are very important for consumer acceptance. It is important to note that the external appearance usually is not altered, at least initially by internal disorders, making disordered chestnuts difficult to detect without destructive evaluation. Internal disorders usually are the result of anatomical and physiological changes within the tissue, such as moisture loss, chemical conversion, discoloration, senescence, microorganism attack, cell breakdown (physiological decay), and insect injury (Upchurch et al., 1993).

In fresh chestnuts, microorganism attack is the most important internal disorders determining internal (kernel) quality. *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp., *Phomopsis castanea*, *Acrospeira mirabilis*, and *Sclerotinia*

pseudotuberosa (syn. *Ciboria batschiana*, *S. batschiana*; anamorphic from *Rhacodiella castanea*, syn. *Myrioconium castanea*) have been persistently identified worldwide, including in the United States, as microorganisms that play an important role as casual agents in postharvest microbiological decay (Ridé and Gudin, 1960; Paglietta and Bonous, 1979; Ellis and Ellis, 1985; Montealegre and Gonzalez, 1986; Vettraino et al., 2005; Jerimini et al., 2006; Sieber et al., 2007; Donis-González, 2008; Donis-Gonzalez et al., 2010). Other issues, like chestnut internal kernel breakdown (IKB) also threatens the industry, especially in Michigan. IKB is defined as physiological kernel decay, near the end of the harvesting season (September), where no fungal pathogens are linked with the condition. Symptomatic IKB chestnuts develop properly and no apparent issue can be observed on the chestnut shell. It has been hypothesized that IKB might be related to pollination incompatibility (Long, 2012). In some cases, these problems can lead to a completely unmarketable product. A certain amount of affected product may be sold in less demanding markets, nonetheless this practice results in important economic and potential market losses due to consumer rejection.

In addition to quality concerns and product appearance, product safety is also a significant issue. Some fungi present in chestnuts are capable of secreting substances that are potent, acute toxins, or carcinogens to both animals and humans. These toxic agents are called mycotoxins, and their impact on domestic animals in terms of decreased growth rate, abnormal reproduction, and early death has long been recognized. The most important mycotoxins are aflatoxins, deoxynivalenol (DON), zearalenone, fumonisin B1, T-2 toxin, and ochratoxin A

(Adams and Moss, 2000; Bullerman and Bianchini, 2007). These substances have been predominately isolated from grains such as wheat and corn, but can also been found in chestnuts (Overy et al., 2003; Donis-González et al., 2010a) as well as in other products including rice (Tanaka et al., 2007), grapes (Varga et al., 2007), beer, wine (Mateo et al., 2007), vegetable oil (Schollenberger et al., 2008), peanuts, pecans (Lillard et al., 1970), and processed foods (Adams and Moss, 2000; Bullerman and Bianchini, 2007).

Currently, random and destructive sampling techniques can be employed to evaluate internal chestnut quality. However, in commercial situations, physiologically disordered, microbiologically decayed, and empty or damaged chestnuts, are eliminated by their proclivity to float in tap water, as healthy chestnuts tend to sink. Nevertheless, performance varies significantly between cultivars and throughput, proving that this floating method is unreliable for sorting purposes. Errors associated with the method might be caused by the large density variations among individual chestnuts and the presence of a perfectly healthy chestnut containing void spaces, thick shell, or pellicle embedded in the healthy kernel. Additionally, the technique is slow, cumbersome, and a significant amount of healthy product is also being discarded. (Donis-González, 2008).

All these issues regarding postharvest mold, decay, safety concerns, and inability to sort chestnuts appropriately, reflect the need to develop a reliable technique for chestnut internal disorder detection, which is fast, practical and appropriate to apply for in-line sorting systems.

1.2 Chestnut fruit

Many nuts, as well as chestnuts, are wrapped in a papery or spiny husk called the involucre, more commonly known as a bur (Fig. 2). This structure is usually mistaken as the fruit, but it is actually a whorl of modified leaves around the flower or flowers. Inside the involucre, depending on the species or cultivar, one to three chestnuts can be found. In chestnuts, the ovule

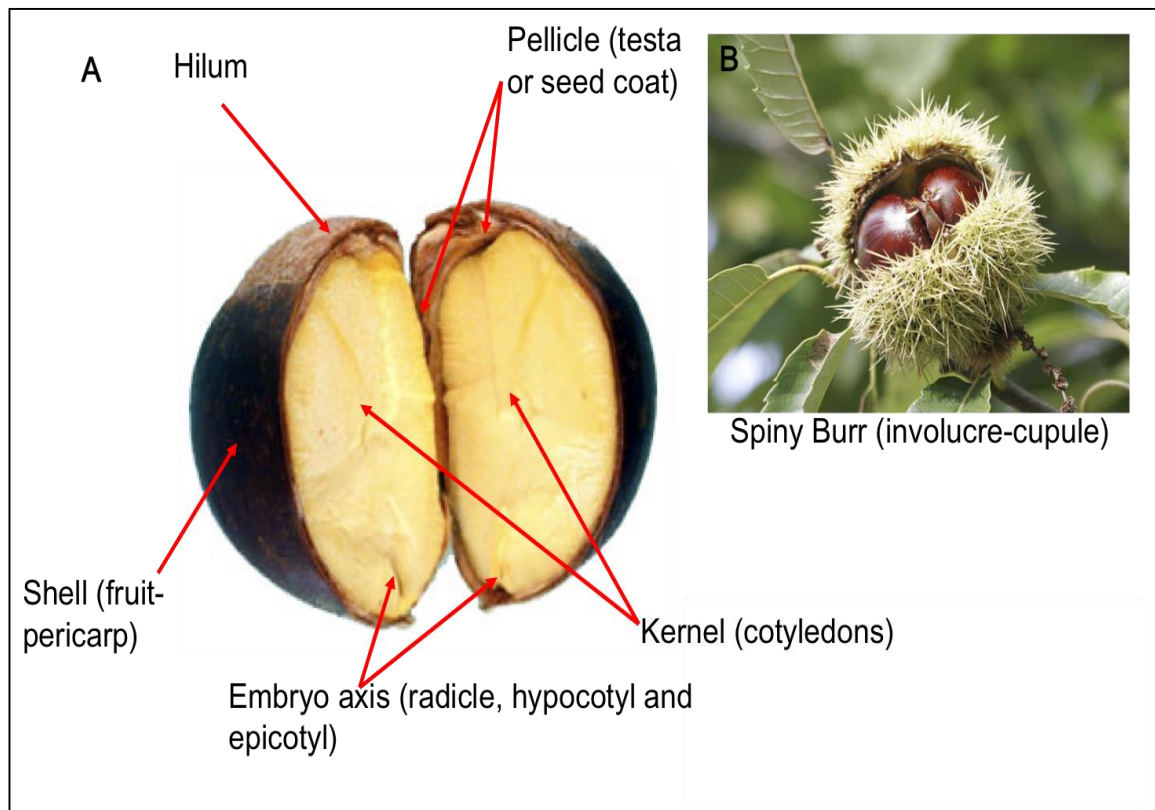


Figure 2. Chestnut fruit morphology. A. Chestnut fruit longitudinal cut, B. Spiny burr attached to chestnut tree with chestnuts. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation

or ovules (inside ovary) that develop into a seed or several seeds are called kernels, structures, which will swell and give rise to what is known as the shell (fruit called an achene or pericarp). The shell has an external brown wall with a

woody and shiny appearance. Two large cotyledons, forming the kernel, surround the embryo, which contains the radicle, hypocotyls and epicotyls. Between the shell and the kernel, is a thin brown papery-like structure, commonly called a pellicle, which is botanically known as a seed coat, testa or episperm (Mencarelli, 2001; Miller, 2003). Usually there is only one kernel per shell, but in certain cases there may be two or more, commonly referred to as a multiple embryo chestnut. In different regions of Europe, including Italy and Spain, the term “marron”, or “marrone” denotes a single kernel nut, while the term “chatâigne”, “castaño” or “castaña” denotes double or even more kernels per shell. Therefore, from a botanical point of view, a chestnut is an achene type fruit, containing one or more seeds, known as a kernel with creamy, yellow-colored edible cotyledons, covered by a membrane called pellicle or episperm (Mencarelli, 2001; Miller, 2003).

When chestnuts begin to ripen in late summer or autumn, the bur changes color from light green to yellow-brown and releases the chestnuts. Sometimes the bur opens on the tree, releasing the chestnuts, but the bur can also drop and open on the ground (Anagnostakis et al., 1998; Mandujano et al., 1998; Miller, 2003; Willis et al., 2007).

Unlike edible nuts such as almonds, hazelnuts, and walnuts, the chestnut kernel is slightly hard, rich in carbohydrates, mainly starch, but also sucrose, glucose and fructose (40 – 90 %), containing high fiber (14 - 19 %), protein (6 - 10 %) and low lipids (0.4 - 10 %), which are 90 % unsaturated fatty acids (nutrients are expressed as the percentage of dry weight). After harvest, chestnuts water content is relatively high (> 50 %) (Anagnostakis and Devin, 1999). Furthermore, chestnuts are a source of vitamin A, calcium, iron, fiber with

and antioxidants (Biomhoff et al., 2006; Gao et al., 2008). Biomhoff et al. (2006) as well as Anagnostakis and Devin (1999) reported that all these characteristics are highly beneficial for human health and are needed for proper nutrition and protection of animal cells, ranking chestnuts as a healthy food.

After harvest, respiration of most seeds is usually characterized by a low and constant rate without any peak, reflecting a non-climacteric respiration pattern. The same relative pattern can be found in chestnuts, but when compared to other seeds and to most other nuts, chestnuts have a higher respiration rate. Subsequently, water loss and starch conversion to sugars is high (Kader, 2002; Willis et al., 2007). Based on several respiration rate studies (Harte et al., 2003), chestnuts were comparable to blueberries (Perkins-Veasie, 2004) and iceberg lettuce (Smyth and Cameron, 1998; Kader, 2002), exhibiting a lower respiration rate than cut broccoli (Talasila et al., 1994), peas, asparagus, sweet corn, and mushrooms (Kader, 2002).

In addition to physiological, and microbiological decay problems arriving from the field, the most common changes that occur in chestnuts after harvest are: moisture loss, starch conversion to sugar, fungal decay, cell breakdown, and insect damage (Wells, 1980). Only starch conversion to sugar, commonly known as "curing", positively affects chestnut quality, enhancing the flavor, sweetness, and acceptability of the product (Harte et al., 2003). The other three major factors have a negative affect on quality and storage potential of the final product (Paglietta and Bonous, 1979; Wells, 1980; Montealegre and Gonzalez, 1986; Harte et al., 2003; Jerimini et al., 2006). Because quality can only be maintained, sometimes monitored or detected, and not improved after harvest (Kader, 2002;

Willis et al., 2007; Kincaid et al., 2008), efforts have focused on increasing the storability of chestnuts by reducing microbes, insects, and maintaining the quality at harvest. Vossen (2000), reported that during three months storage, temperatures between -2 °C to 0.5 °C were recommended, playing an important role in inhibiting fungal decay, increasing storage, and reducing moisture loss. In Michigan, in addition to the recommended storage temperature (Vossen, 2000), a postharvest treatment has recently been incorporated and has proven to maintain chestnut quality during storage. Treatment consists in dipping fresh chestnuts for 5 min in water containing 2700 ppm hydrogen dioxide + 200 ppm peracetic acid (Storox™, BioSafe Systems, Glastonbury, CT, USA) (Donis-González, 2008; Donis-González et al., 2010b). Nonetheless, as mentioned before, because there is no reliable commercially available sorting method, chestnuts that are already decayed at time of harvest, are still being stored among healthy product. The storing of decayed chestnuts inevitably increases costs for the chestnut industry. Furthermore, decayed, bad quality commodity additionally contaminates healthy chestnuts, causing them to decay during storage. Therefore chestnut sorting might be considered pre-storage as well as post-storage, before the product is sent to the client.

1.3 World chestnut industry

According to the Food and Agriculture Organization Statistics Division (FAOSTAT), in 2013 at least 25 countries produced chestnuts. Reports from 1998 to 2011, showed that Chestnut production and harvested area have experienced a continuous average growth. Chestnut production worldwide in

2011 was estimated to be over 2,015,000 metric tons (2.01×10^9 kg) distributed as following: China, ~ 84 %; Republic of Korea, ~ 3 %; Italy, Turkey, and Bolivia, ~ 2-3 % each; France, Portugal, Spain, and Greece, ~ 0.2-1 % each; and the United States, Australia, New Zealand, Chile, Peru, Brazil, Albania, among others, less than 0.1 % each (Fig. 1).

China is the largest low-cost producer and exporter of chestnuts with an estimated production of 1,700,000 metric tons (1.7×10^9 kg). Approximately one third of their chestnut production is exported to Japan. Locally, most chestnuts are consumed fresh or roasted with an unspecified amount used in Chinese cuisine to develop a broad variety of dishes (Vossen, 2000).

Republic of Korea, the second largest producer, yields significantly less chestnuts compared to China per year (55,780 metric tons = 55,780,000 kg), approximately half of which are exported to Japan and another countries like the United States (~ 1 to 3 %) (Vossen, 2000).

Japan is the largest chestnut importer and is among the largest consumer group, even though it is not the biggest producer. In the Republic of Korea and Japan, local or imported chestnuts are primarily stored under refrigerated conditions of 4 – 7 °C, and consumed fresh, boiled, or as an ingredient in diverse dishes. Some are also stored dry or peeled for further use.

Within Europe, Italy is the largest chestnut producer with approximately 57,493 metric tons (57,493,000 kg) and leads the world in production of delicacy-processed chestnut-based products such as *marrone glacé* (preserved chestnuts in sugared liquor). In Europe, the use of dry chestnuts and chestnut flour in

cooking has recently declined, but the popularity of these products is increasing elsewhere, especially in the United States. These value-added products, such as processed, dried, peeled, and frozen chestnut have reached more than 8.00 US\$ per pound, prompting moves to expand the chestnut industry worldwide (Vossen, 2000). Europe's second largest producer is Portugal, with up to 21,990 metric tons (21,990,000 kg) per year. France is one of the biggest importers of chestnuts in Europe, mostly from Italy, Spain, and Portugal.

Recently, the United States, Australia, New Zealand, Chile, and other countries in the Southern Hemisphere have begun to produce chestnuts and have established economically sustainable industries, mainly for export (Mandujano et al., 1998; Fulbright and Mandujano, 2000; Vossen, 2000). The United States has at least 5,000 acres of chestnut tree plantations. Of these, approximately 1,500 acres are less than 10-year-olds. Chestnut trees are mainly in California, Michigan, Oregon, Washington, Iowa, Idaho, Nebraska and Ohio (Vossen, 2000). These commercial plantations are primarily from the cultivar 'Colossal' (European-Japanese hybrid = *C. sativa* x *C. crenata*). This cultivar has been broadly used because it produces large nuts (up to 30 g), high yields (> 55 kg per tree), and is commercially sold at nurseries (Fulbright and Mandujano, 2000; Miller, 2003). Other cultivars such as 'Dunstan Hybrid' (a patented seedling of a third generation cross between American and Chinese chestnut hybrids = *C. dentata* x *C. mollissima*), 'Skookum', 'Layeroka', 'Myoka', 'Skioka', and 'Eaton', which have apparent Chinese characteristics, have also been planted (Miller, 2003).

In recent years chestnut average production has been increasing (Vossen, 2000). As an example, in 2003 approximately 1,500 kg of chestnuts were harvested by the Chestnuts Grower Incorporation (CGI) in Michigan, while in 2007, a total of 22,000 kg were harvested (Blackwell, 2006). An important chestnut state is California, where the oldest commercial plantations are found. Vossen (2000) indicated that most of the early chestnut orchards in California were established by immigrants during the Gold Rush and are mostly seeds from European chestnuts.

Michigan's chestnut harvest starts in mid-September and proceeds through the first week of November, but may start and subsequently end slightly later, in northern or colder locations. Product is currently primarily sold through CGI, a producer owned and controlled marketing cooperative. Most chestnuts are sold from Thanksgiving through Christmas, via sales to specialty ethnic markets, retail stores, food processors, restaurants, holiday festivals, farmers' markets and individual consumers. Fresh and frozen peeled chestnuts make up 60 % and 30 %, of outlet sales respectively. The remaining product (10 %) is sold as flour, breading, and new dehydrated products such as chips and slices, and puree (Blackwell, 2006).

Since freshness and microbial quality are sales factors in chestnut, domestic production has a definite advantage over imported chestnuts. But to maintain this advantage, local quality monitoring, sorting, storage conditions, and postharvest management strategies are all key strategies in preventing the chestnuts from molding and decaying. Regardless of poor quality and lack of freshness, mainly due to long-term storage and transport of imported chestnuts,

the United States annually imports between 5 and 15 million kg of fresh European and Chinese chestnuts, at a retail price of approximately 40 million US\$ (4-8 US\$ per pound). This indicates that the United States chestnut consumption is high and economically significant. Nonetheless, in order for the United States to expand its markets, replace imported chestnuts, and fulfill all its local needs today, more than 20,000,000 m² of production area would be required. Furthermore, if chestnuts are marketed efficiently and domestic consumption increases by only 0.22 kg per capita, the United States would require over 202,000,000 m² of mature production area to meet the demand. Following this growth, the industry would be worth more than 300 million US\$ per year, but may be worth more than 800 million US\$ annually, if the increase in consumption is even higher (Vossen, 2000).

1.4 Electronic sorting technologies

Presently, noninvasive techniques mainly using color computer vision systems are employed to determine external quality attributes (peel color, external defects and shape) in fresh vegetables, nuts and fruits (Brosnan and Sun, 2004; Mery and Pedreschi, 2005; Blasco et al., 2007; Gomes and Leta, 2012; Moreda et al., 2012). In addition, techniques based on optical, magnetic resonance imaging (MRI), near-infrared (NIR), vibration, sonic and ultrasonic, have also been explored for non-destructive determination of internal quality attributes of a variety of agricultural and food products (Milczarek et al., 2009; Cubero et al., 2010; Lorente et al., 2011). Internal quality attributes which have

been explored include apple firmness and soluble solids content (Peng and Lu, 2008), peach firmness (Lu and Peng, 2006), tart cherries pit presence (Qin and Lu, 2005), tomato mechanical damage (Milczarek et al., 2009) and pickle internal defects (Ariana and Lu, 2010). However, because of the morphological characteristics of some commodities, like chestnuts, which contain an external, relatively thick (1.0-2.0 mm), bright and shiny shell (> 70 gloss units), it is not possible to use traditional color or even NIR sorting methods available in the industry to evaluate internal quality traits. Currently, only destructive techniques can be reliably employed to evaluate internal components of a variety of fresh agricultural commodities, including chestnuts (Donis-González et al., 2013). Clearly invasive techniques can't be applied to all produce and, thus there is a need to develop an *in vivo* in-line nondestructive tool capable of better assessing fresh agricultural commodity internal attributes. This will enable the fresh commodity industry to offer better quality products, therefore increasing customer satisfaction, increase market share profit growth and have less customer complaints (Garvin, 1984). One commodity which could extremely benefit from such technologies is the chestnut, due to its propensity toward internal decay, as described in Donis-González (2010).

Though not commercially available to the agricultural field, computed tomography (CT) is an important noninvasive diagnostic tool broadly used in the medical field and material sciences (Bushberg et al., 2002). Despite extensive research efforts and off-line application studies, a real-time in-line CT inspection system for internal quality attributes of fresh produce is not commercially available. However, because of X-ray capabilities of visualizing internal properties

of objects through thick matter, as well as recent advances in high-performance computing systems, new detector technologies including modern graphical processing unit (GPU) computing capabilities (Pratx and Xing, 2011) and high-performance X-ray tubes, non-medical applications are gaining attraction. New detector technologies offer real-time imaging, equipment cost decreases, extended or continuous operation, and significant reduction in image reconstruction time (Butz et al., 2005; Hanke et al., 2008).

1.5 Basic principles of X-ray computed tomography (CT)

X-rays are short wave radiations (10^{-10} - 0.01 nm) with energy between 1.92×10^{-17} and 1.92×10^{-14} J, which can penetrate matter. The wavelengths are shorter than those of visible light rays (390 - 700 nm) and ultraviolet rays (10 - 400 nm), and longer than gamma rays (< 0.01 nm). X-rays are generated by bombarding electrons on a metallic anode (X-ray tube) (Bushberg et al., 2002). CT is an imaging modality where an X-ray tube is rotated around an object(s) and the attenuation is recorded on a detector (Fig. 3). Other equipment may contain a rotating stage in front of a fixed (non-moving) X-ray tube and detector (Bushberg et al., 2002).

Data acquisition in a CT involves making X-ray transmission measurement through the object(s) at various angles around the object(s), as seen in Fig. 4a. Each X-ray that is acquired in CT is a transmission measurement through the object(s) along a line, where the detector measures X-ray intensity (I_t). The un-

attenuated X-ray beam intensity (I_t) is also measured during the scan by a reference detector. The relationship between I_t and I_o is given by:

$$I_t = I_o e^{-\mu t} \quad (1-1)$$

where t is the object(s) thickness and μ is the average linear attenuation coefficient along the X-ray path. I_t and I_o are equipment dependent values, but the product μt (parameter of interest), is related to the internal structure of the object(s) along a given X-ray. When Eq. 1-1 is rearranged, the measured values I_t and I_o can be used to calculate μt , as seen in Eq. 1-2.

$$\mu t = \ln \left(\frac{I_o}{I_t} \right) \quad (1-2)$$

where $\ln$ is the natural logarithm (constant approximately equal to 2.718281828), t is canceled out, and the attenuation coefficient (μ) for each X-ray is used in the CT reconstruction algorithm. This computation, which is a preprocessing step performed before two-dimensional (2D) CT image reconstruction, reduces the dependency of the CT image of the equipment-dependent parameters, resulting in a 2D CT image that depends primarily on the object(s) density (Bushberg et al., 2002). After preprocessing of the raw data, a CT reconstruction algorithm is used to produce the 2D CT images. There are multiple reconstruction strategies, however, filtered back-projection reconstruction is the most widely used. The back-projection method uses planar projection data sets (preprocessed

sinogram) to build up the 2D CT image in the computer by reversing the acquisition steps, as seen in Fig. 4b. During acquisition, a detector integrates μ along a known path of each X-ray beam. During back-projection reconstruction, μ for each X-ray is smeared along the X-ray path in the image of the object(s). In addition to μ for each X-ray path, the reconstruction algorithm also records the acquisition angle and position in the detector array. The back-projection algorithm begins with an empty image matrix (512 x 512 pixels), and as the data from a large number of X-ray paths (180 up to 1000) are back-projected onto the 2D CT image matrix, a 2D CT image slice is generated (XY-plane-slice). In other words, μ is added to each pixel in a line through the image corresponding to the X-ray paths, as exemplified in Fig. 4c (van-Daatselaar et al., 2004; Goldman, 2008).

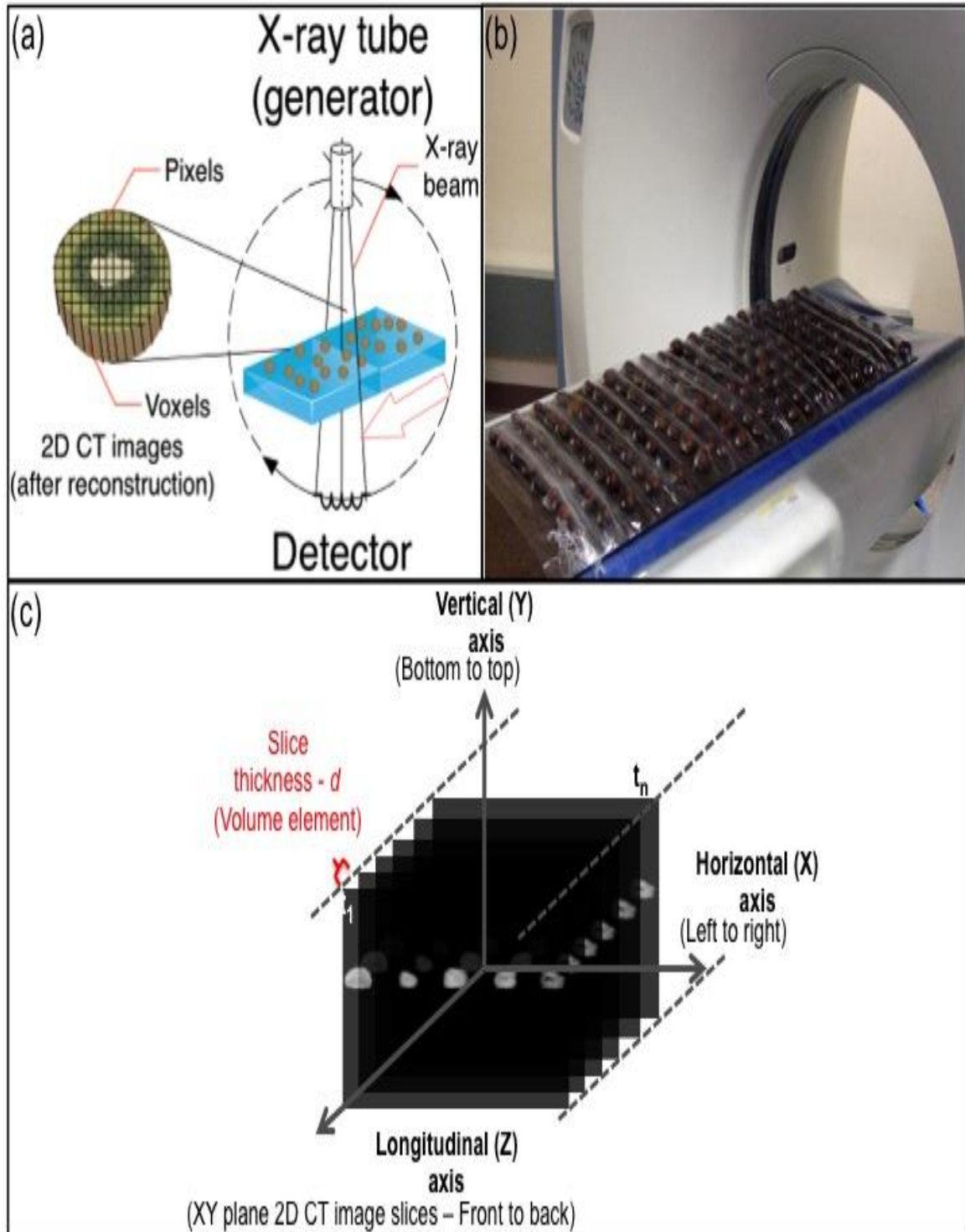


Figure 3. X-ray computed tomography (CT) A. Traditional CT working principal of conveying objects through system, B. Measuring arrangement of the GE (GE Healthcare, Buckinghamshire, England, Great Britain) BrightSpeed™ RT 16 Elite CT used in these studies, C. Schematic representation of a CT system scanning (5 chestnuts per row) containing several 2D XY-plane CT images (slices).

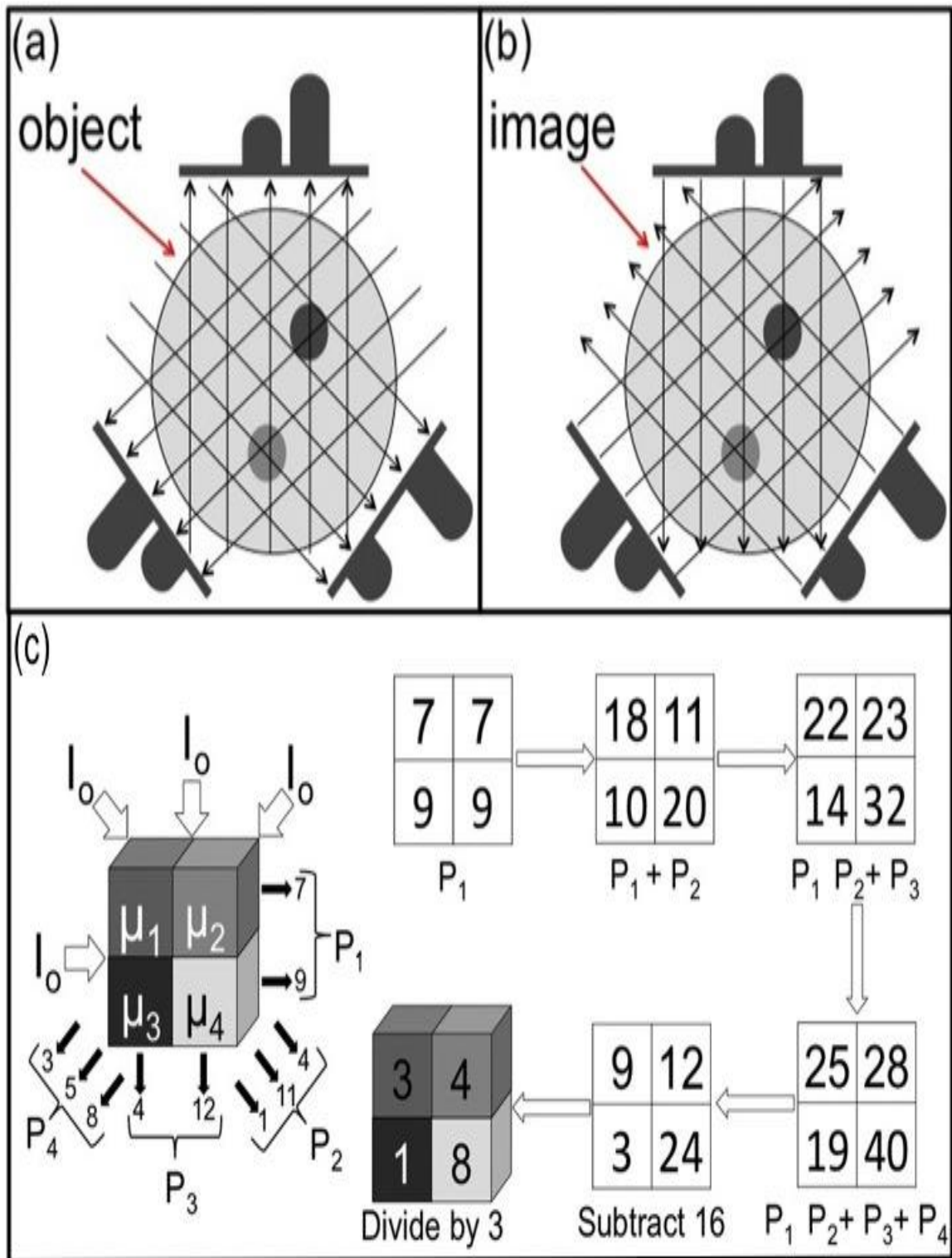


Figure 4. CT imaging and data acquisition A. Transmission measurements through the objects at numerous angles (black arrows) around the object, B. Back-projected (reverse) transmission measurement onto 2D XY-plane CT image matrix, C. Back-projection reconstruction from projection values (P), for a simple image of four voxels.

In CT the difference in physical density of materials is visualized by changes in image intensity and it is expressed in 'Hounsfield-Units' (HU) (or 'CT-number'). Hounsfield-Units represent the X-ray attenuation capabilities of a specific material. In a 2D CT X-ray image, the $HU_{(x,y)}$ in each pixel (x,y) , of the image is generated through a combination of X-ray projection images and by using Eq. 1-3,

$$HU_{(x,y)} = 1,000 \frac{\mu_{(x,y)} - \mu_{water}}{\mu_{water}} \quad (1-3)$$

where $\mu_{(x,y)}$, is the floating point number of the (x,y) -pixel before the XY-plane-slice reconstruction, μ_{water} is the attenuation coefficient of water (approximately 0.195), and the $HU_{(x,y)}$ is the Hounsfield Unit observed in the final 2D CT image. Therefore, objects with a low-density like air, at standard temperature and pressure, have a low-HU (-1000 HU), and high-density materials like bone will have a high-HU (up to 3000 HU). In general, a HU-value equal to 0 stands for the density of distilled water (1.0 g cm^{-3}); values in the positive range represent materials with a mass density above 1.0 g cm^{-3} ; and values in the negative range stand for those below 1.0 g cm^{-3} (Bushberg et al., 2002).

In the case of several objects conveyed through a CT system, arranged in rows, as seen with chestnuts in Fig. 3b, a single scanning of the CT system consists of a block of 3D data stored as voxels. Voxels (volume elements), have

the same in-plane dimensions as pixels (2D image elements), but also include the slice thickness (d) dimension (Bushberg et al., 2002) as observed in Fig. 3c. However, not the entire block of data is acquired at once. Instead, each XY-plane-slice is processed as the objects (chestnuts), previously arranged in rows and attached to the boards, are passing through the CT scanner. A XY-plane-slice is analogous to a virtual cross-section of the chestnuts imaged through the CT scanner. Therefore, the imaging procedure is done one XY-plane-slice (cross-section) at a time, starting with t_1 - and ending with t_n -XY-plane-slice, as outlined in Fig. 3c. In addition, newer helical technology and multi-slice detectors are available to allow multiple image slices to be obtained simultaneously while constantly moving the samples through the imaging aperture (Bushberg et al., 2002).

The originally acquired CT XY-plane-slices; containing images from several chestnuts per row, moving through the Z-axis (longitudinal direction) are stored in memory using a digital imaging and communications in medicine (DICOM) standard format. DICOM images are composed exclusively of shades of gray (16 bits), varying from black (low-density like air) at the weakest intensity to white at the strongest (high-density materials like bone). When visualized in traditional DICOM visualization software's, like the Osirix Imaging Software V3.6.1 (<http://www.osirix-viewer.com/>) image pixels are expressed in HU. On the other hand, when imported into other software's like MATLAB (2012a, The MathWorks, Natick, MA, USA) (<http://www.mathworks.com>), DICOM images contain 65536 shades of gray (intensity values) per pixel, ranging from 0 to

65535, which are mapped to the original HU-values. In addition, DICOM files contain metadata that can easily be accessed, providing detailed information about the image, such as the size, dimensions, modality used to create the image, and equipment settings used to capture the image. For comprehensive information about the standard the reader can refer to the official DICOM web site (<http://medical.nema.org/>).

There are multiple advantages of CT compared to traditional 2D projection X-ray imaging. First, CT completely eliminates the superimposition of images of structures within the samples and outside the region of interest. This is because in 2D X-ray imaging, only one projection image (X-ray transmission through sample) is acquired per object of interest. In contrast, depending on the CT scanner type, a CT image is acquired at different angles by reconstructing information obtained from 180 up to 1000 X-ray 2D transverse projection images (van-Daatselaar et al., 2004; Goldman, 2008). Second, because of the intrinsic contrast and high resolution of CT, physical density differences between materials as low as 0.5 % can be easily differentiated (Bushberg et al., 2002). Thirdly, the data from one CT imaging procedure can be reconditioned to be observed in various planes (different angular orientations) as seen in Fig. 5, or even observed volumetrically by creating a 3D image and merging the information from several 2D slices. Using a chestnut as a model in Fig. 5, each chestnut contains between 8 to 17 XY-plane-slices representing virtual cross-sections of a chestnut along the longitudinal (Z) axis. This number of slices is

dependent on chestnut physical size and d , which in this case $d = 2.56$ mm. CT image slices can be viewed in different planes, such as 90° from the XY-plane toward the longitudinal axis (Z) – YZ-plane-slice, and 90° toward the horizontal axis (X) – XZ-plane-slice. This process of reconditioning CT images so that they can be observed in various planes (different angular orientations) is commonly known as re-slicing (Bushberg et al., 2002). As before, depending on chestnut size and d , each chestnut contains 8 to 17 YZ-plane-slices and XZ-plane-slices.

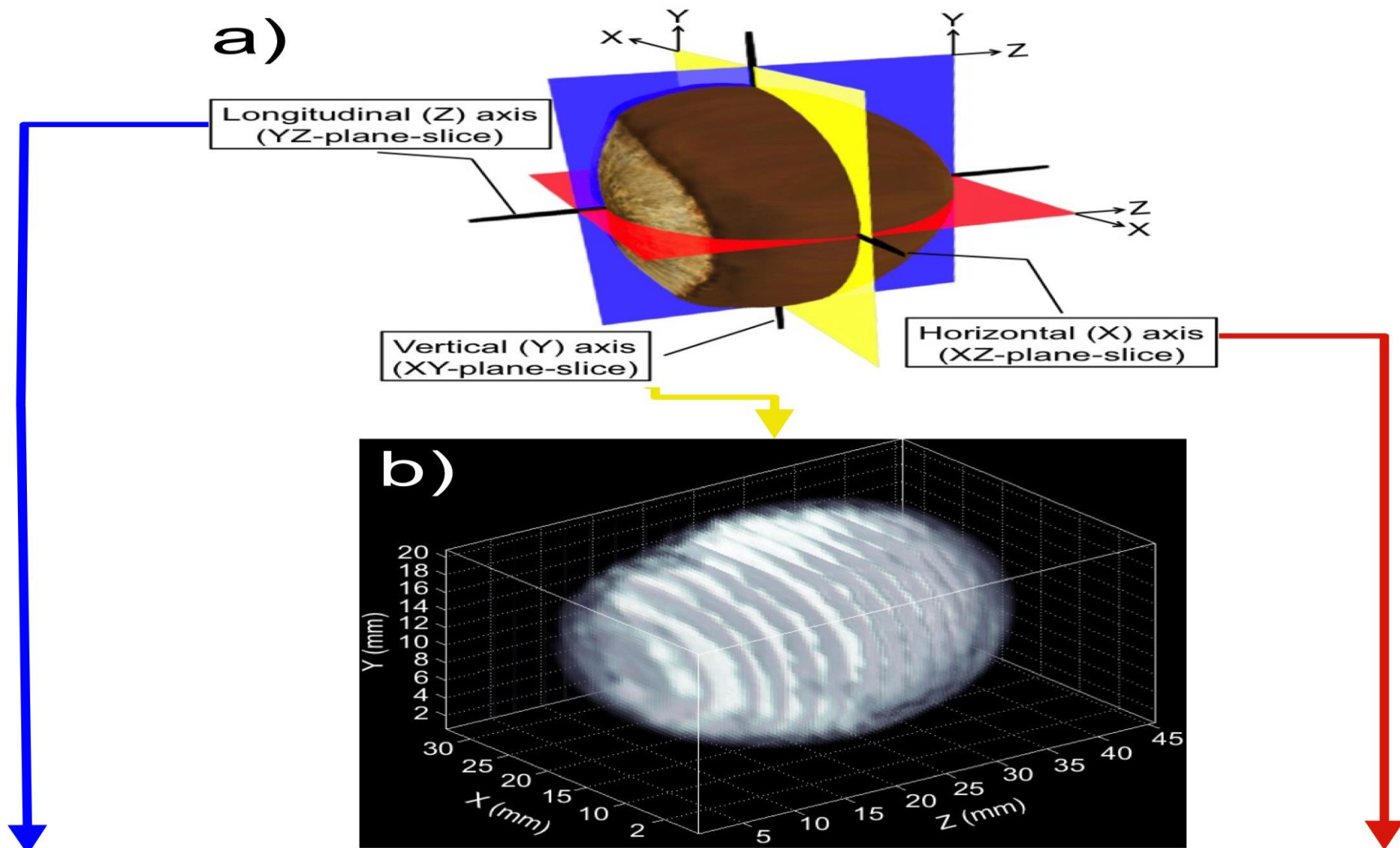
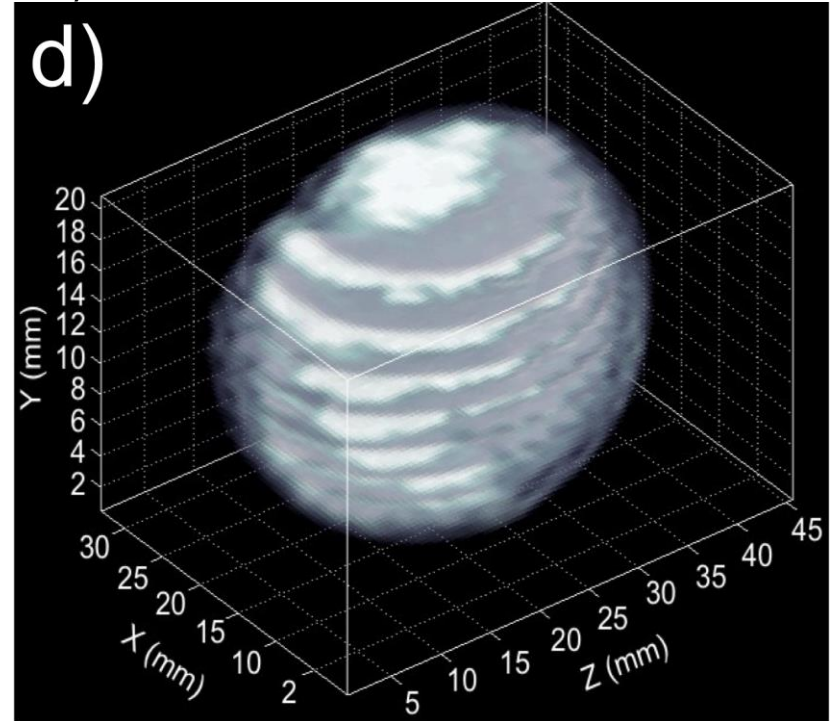
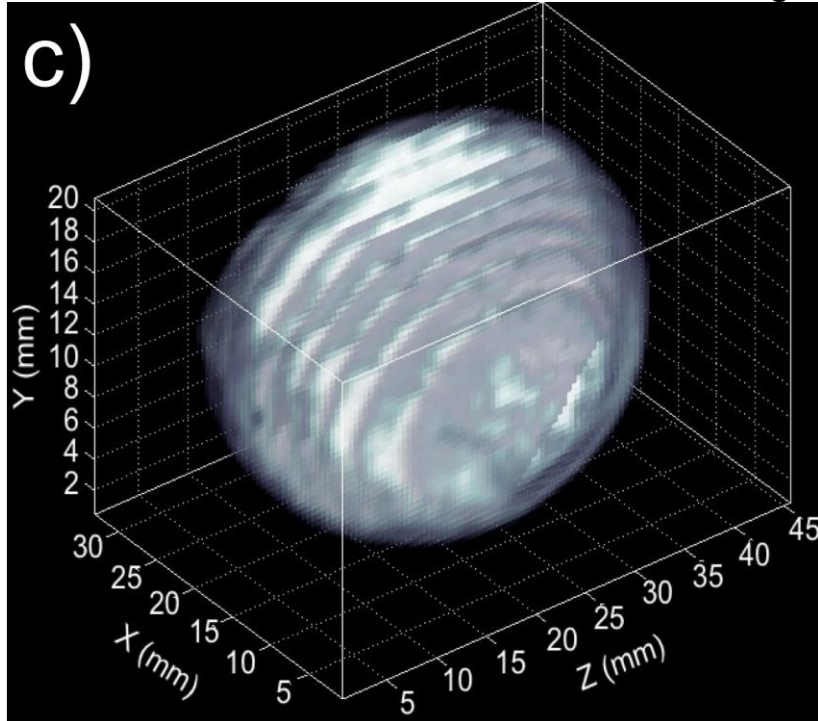


Figure 5. (a) 3D virtual cross-sections representation of a chestnut at three planes (different angular orientations) along the horizontal (X), vertical (Y), and longitudinal (Z) axes (Figure not to scale). (b) Original series of acquired CT XY-plane-slices.

Figure 5 (cont'd).



(c) Reconditioned (re-sliced) CT YZ-plane-slices. (d) Re-sliced CT XZ-plane-slices.

1.6 Application of CT in non-medical industries

CT has proven to be an invaluable diagnostic tool for numerous applications in the medical field and material sciences (Bushberg et al., 2002), ranging from tumor diagnosis and segmentation (Stadler et al., 2004; Deglint et al., 2007) to osteoporosis screening (Bushberg et al., 2002) to differentiation of encountered foreign bodies in corpses (Bolliger et al., 2009).

In agriculture and animal science fields, off-line adaptation of CT technology has proven to be an accurate nondestructive descriptor of inner properties. For example, water content of oak (*Quercus robur*) and spruce (*Picea abies*) wood was accurately determined by Fromm et al. (2001) and an estimation of lean meat content and quality was made in pigs (*Sus spp.*) by Furnols et al. (2009). In agriculture, has been shown to be an accurate descriptor of internal characteristics.

Postharvest internal evaluation of several nuts, fresh vegetables, and fruits has been recently shown to be achievable as well. Jha et al. (2010) described the potential of nondestructive techniques, including CT, to measure mango (*Mangifera indica*) internal quality (i.e. size, shape, pulp and moisture), and Kotwaliwale et al. (2006) differentiated nutmeat from shell components in fresh pecans (*Carya illinoensis*). Barcelon et al. (1999b; 1999a) used CT to study internal quality of peaches (*Prunus persica*) and mango, including fruit density, moisture content, soluble solids, and acidity. In addition, studies done by Sornsrivichai et al. (2000) determined pineapple fruit (*Ananas comosus*)

translucency and ripeness. Moreover, using high resolution CT, which is a sampling tool that combines less than 1 to 2 mm thick CT image slices with a high spatial frequency reconstruction algorithm to generate CT images that show small details (Kelly et al., 2003), Mendoza et al. (2007) measured pore space in apples (*Malus domestica*), Lammertyn et al. (2003) obtained three dimensional (3D) spatial distribution of 'Conference' pear (*Pyrus* sp.) core breakdown, and Verboven et al. (2008) studied 3D gas exchange pathways in pears and apple

1.7 Objectives and Hypothesis

The comprehensive objective of the study is to develop the methods to nondestructively visualize and classify fresh chestnuts, based on their internal quality, using X-ray CT imaging. This study will provide a powerful tool to sort chestnuts based on their kernel quality, leading to the improved marketing of attractive, safe, high quality chestnuts.

The specific objectives of the research are:

1. To determine the combined effect of image acquisition parameters, which include voltage (kV), current (mA), and slice thickness (mm) on optimizing CT image quality (signal to noise ratio, volume accuracy, high contrast spatial resolution and low contrast detectability), using response surface methodology (CHAPTER 2).

2. To establish effective image visualization techniques that will infer internal quality attributes of fresh chestnuts (healthy and decayed tissue, pellicle, void spaces and air), using X-ray CT images (CHAPTER 3).

3. To apply image pre-processing techniques (cropping and image enhancement), CT image segmentation, image feature extraction, feature selection, multivariate discrimination algorithms and artificial neural network (ANN) classifiers, which will automatically categorize fresh chestnuts based on their internal quality, using X-ray CT images (CHAPTER 4).

The hypothesis of this research is that by measuring multiple X-ray CT image features, using different image analysis techniques, in combination with multivariate statistical discrimination algorithms and ANNs classifiers, it will be possible to categorize chestnuts based on their internal quality. The long-term goal is to develop a system that automatically classifies chestnuts by their internal quality, after harvest. This nondestructive classification method will eventually replace the questionable water floating technique, currently used worldwide, to separate decayed from healthy chestnuts.

CHAPTER 2. APPLICATION OF RESPONSE SURFACE METHODOLOGY (RSM) TO SYSTEMATICALLY OPTIMIZE FRESH CHESTNUT COMPUTED TOMOGRAPHY (CT) IMAGE QUALITY

2.1 Abstract

The objective of this chapter is to describe a method that was developed to systematically and efficiently obtain a model of CT scanning factor levels, which returns optimized high quality chestnut (*Castanea* spp.) CT images. Chestnut two-dimensional CT images were used to describe this optimization procedure, considered to be a critical step in the development of a fast, nondestructive technique, capable of assessing fresh internal quality attributes and components of chestnuts, and other agricultural commodities. Response Surface Methodology (RSM), using a three-factor, three-level Box-Behnken statistical design, was used to optimize the factors affecting image quality, which include X-ray voltage, current, and slice thickness. Response variables representing image quality were digitally and automatically inferred from fresh chestnut image signal-to-noise ratio, Teflon® cylinder reference volume accuracy, a Quality Assurance (QA) high contrast spatial resolution phantom, and a QA low contrast detectability phantom. Second-order RSM prediction models for each response variable reflected a combined maximized CT image quality at a voltage, current, and slice thickness equal to 120 kilovolts, 170 milliamps, and 2.5 millimeters respectively. The experiment yielded optimal chestnut CT images that can accurately reflect internal decay of fresh chestnuts with an overall accuracy rate equal to 96 %,

taking as reference data the subjective quality rating of five trained chestnut experts.

2.2 Introduction

Even though image quality is an important factor that has been addressed in the medical and veterinary fields (Mayo et al., 1994; Ford et al., 2003; Du et al., 2007) little is known about how to statistically, routinely, systematically, and reliably optimize CT parameters.

Response Surface Methodology (RSM) is a compilation of mathematical and statistical methods suitable to optimize, develop and improve processes (Myers et al., 2008), which can be helpful to quantitatively and routinely adjust parameters that influence CT image quality. RSM has never been applied to optimize CT image quality, but other engineering fields heavily rely in this technique to optimize processes, including the optimization of protease production (Braga et al., 2010), monitoring of ball bearings (Patil et al., 2010), and ultrasonic-stimulated solvent extraction (Wang et al., 2011). The RSM makes usage of factorial designs when a few significant parameters or factors are involved in optimization (Myers et al., 2008), including three-level complete factorial design, central composite design, equiradial designs, and Box-Behnken design among others (Myers et al., 2008). The Box-Behnken design is a balanced incomplete three-level block design, useful to fit second-order response surfaces, and in certain cases, it is as powerful as a three-level complete factorial design, only that it requires 15 runs instead of 27 without repetitions (Myers et al., 2008).

Therefore, the aim of this study is to describe RSM using a Box-Behnken design as a reliable method to accurately and systematically optimize CT scanning parameters of utmost importance, which include voltage, current, and slice thickness, essential to obtain good quality chestnut CT images as described in theory by Renaudin et al. (1993), Mayo et al. (1994), Sharma et al. (2006), Goldman (2007), Sellakumar et al. (2007) and Arnold et al. (2010). This research offers a tool that will methodically yield useful, and high quality CT images in any field related with CT. Optimized chestnut CT images are obtained without the biased aid of human intervention as traditionally done in practice (General Electric Company, 2007), and without using unnecessary scanning runs, which are time consuming and computationally inefficient. More specifically, this method reliably yielded maximized quality CT images, which accurately reflect inner components of chestnuts.

2.3 Materials and methods

2.3.1 Chestnut sample collection, reference cylinders, and Quality Assurance (QA) phantom CT imaging scans

A total of 50 physiologically mature and apparently healthy Chinese chestnuts (*C. mollissima*) were collected by hand, directly from chestnuts trees in a Michigan orchard. Following common postharvest procedures, samples were immediately submerged for 300 s in 5 L of room temperature distilled water containing 2700 $\mu\text{L L}^{-1}$ hydrogen dioxide plus 200 $\mu\text{L L}^{-1}$ peracetic acid

(Storox®¹, BioSafe Systems, Glastonbury, CT, USA) with the objective of reducing mold contamination, and stored at 4 °C. During this process, the majority of decayed, empty or damaged chestnuts were eliminated by their proclivity to float, as healthy chestnuts tend to sink (Donis-González, 2008; Donis-González, 2008; Donis-Gonzalez et al., 2010). As it was important to obtain uniform healthy tissue matter for the signal-to-noise estimation, after 20 d, 9 of the apparently healthiest chestnuts were attached to a rectangular acrylic transparent board (2.75 mm x 300 mm x 24 mm), using approximately 50 g of transparent silicone per chestnut, as seen in Fig. 6a.

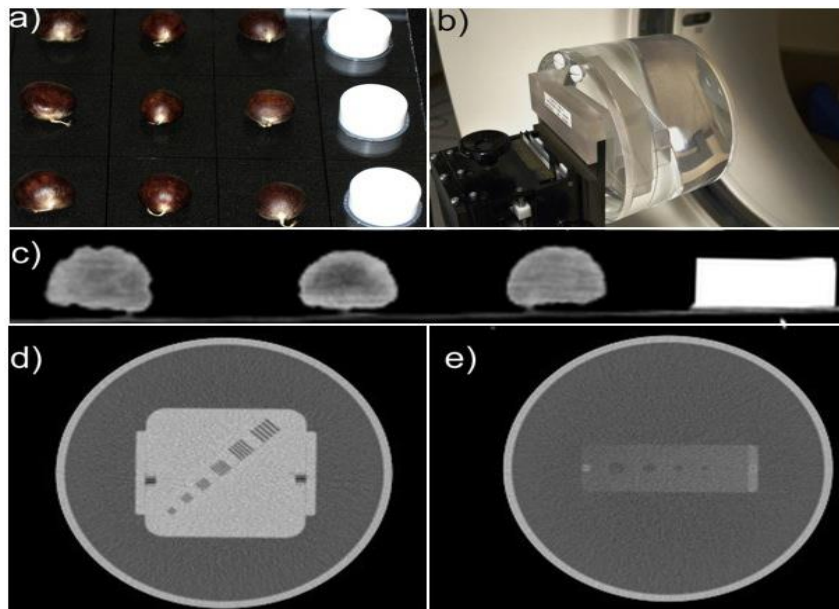


Figure 6. (a) Fresh chestnuts samples, and Teflon® reference cylinders, (b) Testing phantom, (c) 16-bit CT images of fresh chestnut samples for Signal-to-noise Ratio (SNR) calculation, and Teflon® reference cylinders for volume accuracy (mm^{-3}) estimate. (d) 8-bit CT image of QA phantom used to infer High Contrast Spatial Resolution (HCSR). (e) 8-bit CT image of QA phantom used to infer Low Contrast Detectability (LCD).

¹ Storox® is a registered Trademark BioSafe Systems.

To avoid sample confusion individual chestnuts were numbered. Parallel to the chestnuts, one row containing three Teflon®² (Polytetrafluoroethylen) reference cylinders (38 mm diameter x 13 mm height) (Applied Plastics Technology Inc., Bristol, RI) were also attached to the board, using approximately 85 g of transparent silicone per Teflon® cylinder (Fig. 6a).

CT scans were performed in a GE BrightSpeed®³ RT 16 Elite, multi-detector CT instrument (General Electric Healthcare, Buckinghamshire, United Kingdom) to the board containing chestnuts and Teflon® reference cylinders, resulting in a set of 2D CT images as seen in Fig. 6c, as well as to a standard QA phantom (Series QA-Phantom, GE BrightSpeed®, General Electric Healthcare, Buckinghamshire, United Kingdom) (Fig. 6b), resulting in a set of 2D CT images (e.g. Fig. 6d and Fig. 6e), as specified by General Electric Company (2007). Even though, not used to its fullest capabilities, because of fast CT scanning protocols, the CT instrument contains a Volara®³ digital data acquisition system (24-bit), which has the capability of acquiring images with a 0.65 mm isotropic image resolution (smallest detected structure), meaning that the spatial resolution in the horizontal-vertical plane (XY) and that in the longitudinal planes (XZ and YZ) can be the same (Tsukagoshi et al., 2007). Scanning parameters are in Table 1.

² Teflon® is a registered Trademark of E.I. du Pont de Nemours and Company.

³ BrightSpeed® and Volara® are registered Trademarks of General Electric Healthcare.

Table 1. Scanning parameters for the CT – General Electric, BrightSpeed™ RT 16 Elite (GE Healthcare, Buckinghamshire, England, Great Britain)

Units	Parameter
Voltage (kV)	80, 100, 120
Current (mA)	10, 90, 170
Slice thickness (pixel size in the Z-axis) (mm) – d	0.625, 2.5, 5
Pixel size (mm) in the X- and Y-axes	0.73
Resolution in the XY-plane (pixels mm ⁻¹)	1.37
Pixel area in the XY-plane (mm ²)	0.53
Resolution in the XZ- and YZ-planes (pixels mm ⁻¹)	0.625 ^a , 2.5 ^b , 5.0 ^c
Pixel area in the XZ- and YZ-planes (mm ²)	1.82
Voxel volume (mm ³)	0.46 ^a , 1.82 ^b , 3.65 ^c
Pitch (table movement – mm : rotation)	17.5:1
Time per rotation (s)	1.2 ^{b,c} , 1.7 ^a
Scan time (s) per 1000 mm of board-length	48 ^{b,c} , 96 ^a
Reconstruction matrix	512 x 512
Field of view (FOV) (mm)	500
Original image intensity resolution	16-bit
CT images per 1000 mm of board-length (t_n)	1600 ^a , 400 ^b , 200 ^c

^aSlice thickness (d) = 0.625 mm, ^b d = 2.5 and ^c d = 5.0 mm.

2.3.2 RSM Box-Behnken design for CT image quality optimization

Box-Behnken three-factor, three-level statistical design was used to optimize and evaluate main effects, interaction effects, and quadratic effects of the CT scanning parameters on image quality. In this study, image quality is characterized by four of the most important CT image quality measurements, including fresh chestnut image Signal-to-noise Ratio (SNR), reference cylinders Volume Accuracy (VA), High Contrast Spatial Resolution (HCSR), and Low

Contrast object Detectability (LCD) using a standard QA phantom (Goldman, 2007). Definition and description of how each of the image quality attributes was calculated is included in sections 2.3.3 through 2.3.5.

The Box-Behnken design is specified by a set of points at the midpoint of each edge of a multidimensional cube and a center point triplicate, being appropriate to construct a second order polynomial model of the combined effect of scanning parameters (factors) to each response variable (Myers et al., 2008). The computer-generated models for every response variable representing image quality were developed using the language and environment for statistical computing software R (V2.10.0, R Development Core Team, Vienna, Austria). Models with coded-factors are specified as Eq. 2-1.

$$Y_n = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (2-1)$$

where Y_n is each measured response or dependent variable representing image quality analyzed separately, associated with each factor level combination; b_0 is the regression intercept; b_1 through b_{33} are the regression coefficients; and X_1 , X_2 , and X_3 are the independent variables or factors. Levels for each independent scanning variable, which represent values for each factor were coded as low (-1), medium (0), and high (+1) (Myers et al., 2008) (Table 2 and 3). Levels were selected based on equipment capabilities, prior preliminary experimentation, chestnut morphology, and size (Miller, 2003). The selected dependent, coded, and un-coded independent variables are shown in Table 3, showing how each

independent variable changes from one run to another. Un-coded voltage (X_1), current (X_2), and slice thickness (X_3) parameter combinations used to perform CT scanning for each of the 15 runs are specified in Table 3. One run is considered as the procedure where all the CT images in total are acquired from the board containing fresh chestnuts, Teflon® reference cylinders, and the standard QA phantom using the combination of specified parameters. The QA phantom images were acquired separately, from the board.

Table 2. Box-Behnken experimental design variables

Variables (units)	Notation	Levels (coded) / un-coded		
		Low (-1)	Medium (0)	High (+1)
<i>Indep. variables (Factors – X_f)</i>				
X_1 - Voltage (kV)	V	80	100	120
X_2 - Current (mA)	C	10	90	170
X_3 - Slice thickness (mm)	ST	0.625	2.5	5.0
<i>Dependent variables (Response - Y_n)</i>				
Y_1 - Signal-to-noise Ratio	SNR			
Y_2 - Volume Accuracy (mm ⁻³)	VA			
Y_3 - High Contrast Spatial Resolution	HCSR			
Y_4 - Low Contrast Detectability	LCD			

After generating the RSM models presented in Table 2, the process was individually optimized for each response Y_n , obtaining an optimized model per

response variable. Optimization was performed to find the levels of voltage (X_1), current (X_2), and slice thickness (X_3) maximizing each Y_n .

Table 3. Design matrix (un-coded factors) in respect to each response variables

Run	Independent variables (Factors)			Dependent variables (Response) ^a			
	Voltage (kV)	Current (mA)	Slice thickness (mm)	SNR $\pm$ SD	Volume accuracy $\pm$ SD (mm ⁻³)	HCSR $\pm$ SD	LCD $\pm$ SD
1	100	10	0.625	0.14 $\pm$ 0.19b	1.56 $\pm$ 0.35bc	0.08 $\pm$ 0.01a	0.16 $\pm$ 0.01a
2	80	90	0.625	0.27 $\pm$ 0.54ab	2.31 $\pm$ 0.47c	0.19 $\pm$ 0.02b	0.21 $\pm$ 0.03b
3	120	90	0.625	0.52 $\pm$ 0.41ab	2.39 $\pm$ 0.53c	0.30 $\pm$ 0.04c	0.28 $\pm$ 0.08b
4	100	170	0.625	0.49 $\pm$ 0.38ab	2.58 $\pm$ 0.36c	0.39 $\pm$ 0.06c	0.24 $\pm$ 0.06b
5	80	10	2.5	0.17 $\pm$ 0.32ab	0.67 $\pm$ 0.30ab	0.10 $\pm$ 0.02a	0.20 $\pm$ 0.06ab
6	120	10	2.5	0.38 $\pm$ 1.14ab	0.68 $\pm$ 0.26ab	0.21 $\pm$ 0.03c	0.30 $\pm$ 0.02b
7	100	90	2.5	0.59 $\pm$ 0.56ab	0.61 $\pm$ 0.25ab	0.40 $\pm$ 0.08c	0.52 $\pm$ 0.04c
8	100	90	2.5	0.62 $\pm$ 0.57a	0.62 $\pm$ 0.54ab	0.43 $\pm$ 0.09c	0.46 $\pm$ 0.09c
9	100	90	2.5	0.61 $\pm$ 0.52a	0.65 $\pm$ 0.22ab	0.44 $\pm$ 0.07c	0.46 $\pm$ 0.08c
10	80	170	2.5	0.51 $\pm$ 0.33ab	0.63 $\pm$ 0.52ab	0.44 $\pm$ 0.13c	0.42 $\pm$ 0.08c
11	120	170	2.5	0.66 $\pm$ 0.45a	0.60 $\pm$ 0.14ab	0.52 $\pm$ 0.03d	0.67 $\pm$ 0.15cd
12	100	10	5.0	0.39 $\pm$ 0.43ab	0.61 $\pm$ 0.02b	0.27 $\pm$ 0.08bc	0.32 $\pm$ 0.03b
13	80	90	5.0	0.59 $\pm$ 0.52ab	0.43 $\pm$ 0.24ab	0.39 $\pm$ 0.13c	0.47 $\pm$ 0.08c
14	120	90	5.0	0.69 $\pm$ 0.62ab	0.45 $\pm$ 0.04a	0.50 $\pm$ 0.13cd	0.80 $\pm$ 0.16d
15	100	170	5.0	0.70 $\pm$ 0.59ab	0.42 $\pm$ 0.01a	0.53 $\pm$ 0.15cd	0.72 $\pm$ 0.17d

^aANOVA for each run at P = 0.05 and Tukey multiple comparison of means post-hoc test. Values in each run followed by the same letter within dependent variable are not significantly different.

2.3.3 Visual based fresh chestnut quality evaluation and SNR (Y1)

Immediately after scanning, each fresh chestnut was transversely sliced in 4-sections using a sharp hand knife. Slice sizes varied depending on chestnut size (5 to 7.5 mm thickness). All internal kernel faces between each slice (total of 6) were then qualitatively assessed for disorders. Slice internal faces, were scanned using a 48-bit color, 9600 x 4800 dots-per-inch (DPI) charge-coupled-device (CCD) scanner (Scan Maker S400, Microtek International Inc., China), using the ScanWizard 5 (Microtek International Inc., China) standard image acquisition software, yielding a tagged image file format (tiff) color image, with a resolution of 816 x 1123 pixels. Color scanning was performed for record keeping and to use as references, to accurately determine uniform healthy fresh tissue for SNR calculation from the 2D chestnut CT images. Before every scan, the scanner was thoroughly cleaned, using compressed air in combination with wiping the scanning glass with delicate task wipes, which had been previously soaked in mild non-streak glass cleaner. To avoid variability between images, and to stabilize the intensity of the scanner lamp, the scanner was on for at least 15 min before scanning. It is important to mention that the scanner, which was used in this study, is internally calibrated every time it is tuned on, so no calibration and/or calibration targets are required (http://support.microtek.com/product_dtl_2.phtml?prod_id=38).

The SNR is defined as the measurement that compares the level of signal in the fresh chestnut CT image to the level of background noise (graininess). It is calculated as the ratio of the mean (μ) Hounsfield Unit (HU) value of a 9 pixels²

square region of interest (ROI) (5.42 mm^2), as seen in Fig. 7a containing fresh, healthy, uniform chestnut tissue to the standard deviation (SD) of the ROI, using Eq. (A. 1) in the appendix section A. Higher values imply higher signal in comparison to noise therefore yielding better quality images with less graininess (Goldman, 2007).

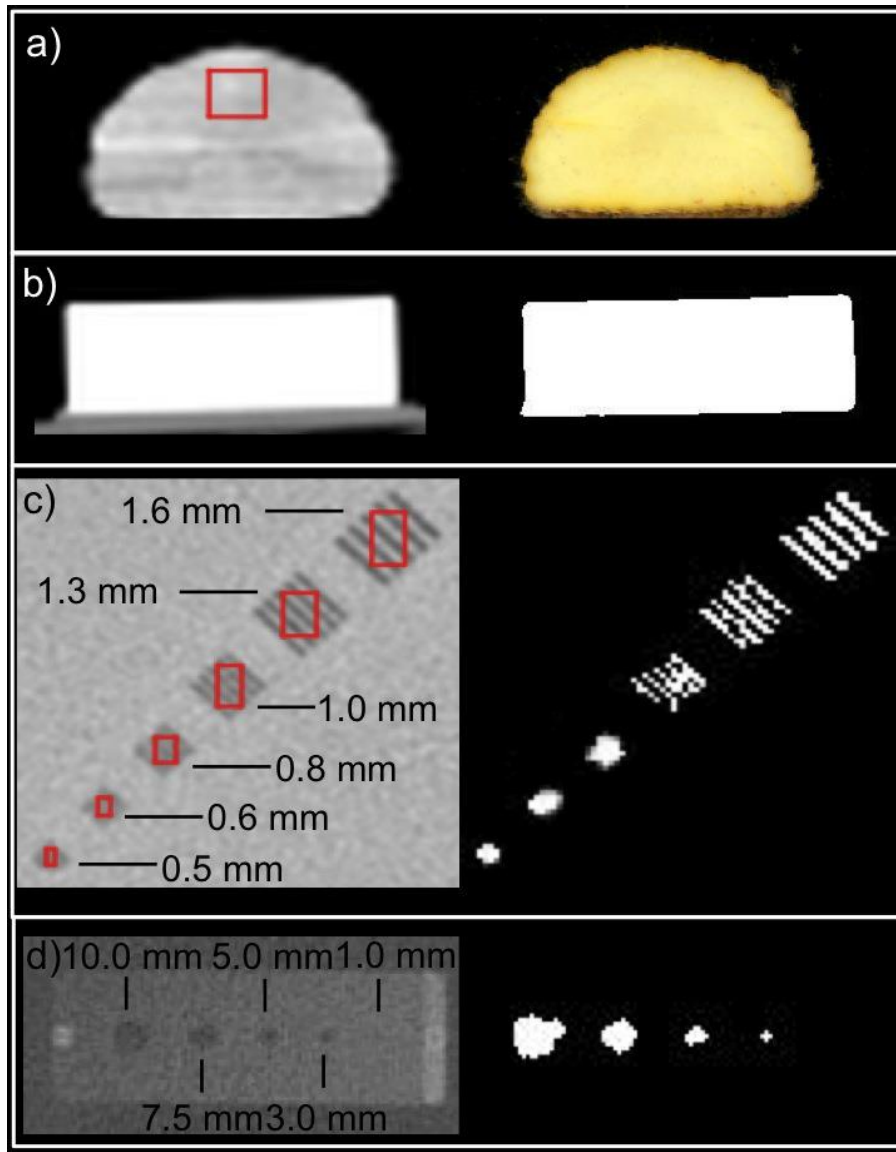


Figure 7. (a) Example of chestnut 16-bit CT image slice used for Signal-to-noise Ratio (SNR) calculation, from a region containing fresh uniform chestnut tissue, using reference color image. (b) Example of Teflon® reference cylinder 8-bit CT image used to estimate volume accuracy (mm^3) with binary image after segmentation (simple global threshold of 135). (c) Example of cropped 8-bit CT image used to calculate High Contrast Spatial Resolution (HCSR) with binary image after segmentation (simple global threshold of 134). Each pattern consists of five bars and spaces called line pairs (lp). The sizes of the patterns are equivalent to 1.6 mm, 1.3 mm, 1.0 mm, 0.6 mm, and 0.5 mm, respectively. (d) Example of cropped 8-bit CT image used to determine Low Contrast Detectability (LCD) with binary image after segmentation (simple global threshold of 94). Image displays various sized Holes (H). The diameter of each H equal 10.0 mm, 7.5 mm, 5.0 mm, 3.0 mm, and 1.0 mm.

2.3.4 Volume accuracy (Y2) using Teflon® reference cylinders

Volume accuracy estimation was calculated from Teflon® reference cylinders CT images, following Eq. (A. 2) found in the appendix section. Volume accuracy (mm^{-3}), is defined as the extent to which the estimated volume of each of the Teflon® reference cylinders, using 8-bit CT images agrees with the mean standard true volume ($\sim 14,743 \text{ mm}^3$) of three reference cylinders (Fig. 7b). Higher volume accuracy values represent better overall 3D information from a stack of 2D CT images.

Volume accuracy estimate is assumed to be accurate, because the reference cylinders were precisely molded using compression technology (Applied Plastics Technology Inc., Bristol, RI) with pure, high HU (900), high quality Teflon®. In addition, after cylinders were precisely and carefully cut, dimensions of each cylinder were confirmed using a digital caliper (ABSOLUTE Digimatic Caliper Series 500, Mitutoyo, Singapore) with a resolution equal to 0.01 mm.

2.3.5 HCSR (Y3) and LCD (Y4) calculation using a standard QA Phantom

The HCSR and LCD are computed using a QA phantom, which is optimized to be used in the CT imager used in the study (General Electric Company, 2007); and applicable to this study, because its size and densities resemble those of the commodity of interest (i.e. fresh chestnuts) (Miller, 2003; Du et al., 2007).

In practice HCSR is estimated visually, with the aid of some quantitative measurements of resolution (General Electric Company, 2007). In this study, HCSR was measured using image analysis techniques (e.g. Fig. 7c) applying the derived Eq. (A. 3) in the appendix section. The HCSR of a CT image is a measurement that defines how well two high contrast objects placed close together are distinguished, and how small can these objects be visualized (Du et al., 2007; General Electric Company, 2007; Sande et al., 2010). In this study, higher HCSR values represent images that can better distinguish between two high contrast objects placed close together.

The LCD represents the ability of a CT image to discriminate objects that vary slightly from their background (low contrast), defined by the smallest visible object (Du et al., 2007; General Electric Company, 2007). Conventionally LCR is determined visually as specified by General Electric Company (2007). In this study, LCD was calculated without the help of a human observer using derived Eq. (A. 4), as found in the appendix section, and using images as the one exemplified in Fig. 7d (General Electric Company, 2007; Sande et al., 2010). High LCD values represent images that can better discriminate small objects (sensitivity) (Du et al., 2007; Goldman, 2007).

2.3.6 Optimized 2D CT image quality validation from an independent data set

A completely different set of 2D CT images were acquired with optimized scanning parameters as summarized in Table 5, from 266 'Colossal' (*C. sativa* x *C. crenata*), 266 Chinese independent chestnut samples, three Teflon® reference cylinders, and the QA phantom. CT image acquisition and processing

was the same as described in sections 2.3.1 and 2.3.2, except that in the case of the chestnuts, scanning boards were larger (915 mm x 335 mm), and naturally decayed chestnuts were not eliminated. After scanning, all chestnuts were sliced and color scanned for record keeping, and to use as samples for CT image quality validation, as described in section 2.3.3.

The purpose was to first determine experimental CT image quality attributes (SNR, VA, HCSR, and LCD) from this independent set of 2D CT images, using ten repeated measurements ($n = 10$: 5-‘Colossal’ and 5-Chinese) for SNR, and three repeated measurements ($n = 3$) for reference cylinder VA, HCSR, and LCD. These experimental values of response were then compared with predicted values, using RSM models (Table 6) and optimized scanning parameters (Table 5).

Second, CT images were used to study their effectiveness in reflecting fresh chestnut internal decay levels, in comparison with color image slices (optimized CT image quality validation). Fresh chestnut quality was elucidated, using the Subjective Quality Rating (SQR) of a 5-expert panel of individuals working closely with chestnut production and research. Panelists were experienced in detecting, identifying, and quantifying quality attributes (decay) in fresh chestnuts. Each expert was presented with 11 randomized chestnut color image slices per cultivar (e.g. Fig. 7a and Fig. 8a), for subjective quality rating (total of 110 - n). The SQR was expressed as the apparent percentage of decay tissue in relation to the total area of each color image. A value equal to 100 represents a chestnut slice that is completely decayed, while 0 indicates that the

chestnut does not contain decay. The SQR values obtained from the panel of experts were then linearly modeled in relationship to the Digital Quality Assessment (DQA) from their corresponding 2D CT images (Fig. 8b to Fig. 8e). The DQA was calculated using image analysis techniques as explained the appendix section, by using Eq. (A.1). DQA was expressed as the percentage of decay tissue in relation to the total area of each CT image. A value equal to 100 represents a chestnut slice that is completely decayed, while 0 describes a chestnut that does not contain decay.

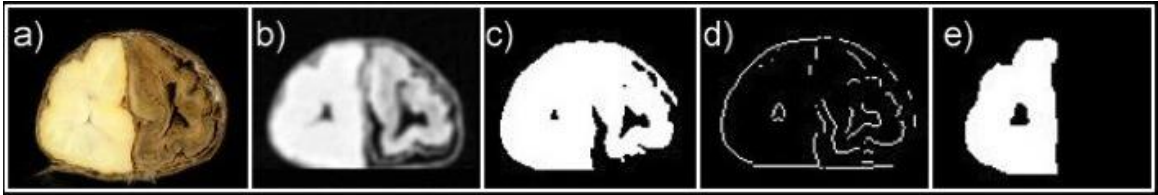


Figure 8. Example of images used for Digital Quality Assessment (DQA). (a) Reference color image. (b) 16-bit optimized CT gray scale image. (c) Binary image of whole chestnut after segmentation, applying a simple global threshold of 400 HU to Fig. 8(b). (d) Binary image of transition points and pellicle after employing a Sobel filtering method to detect edges in Fig. 8(b). (e) Binary image of healthy tissue after segmentation, using a simple global threshold of 1050 HU to Fig. 8(b). Figure is partially presented in color.

Calculations, image processing and analysis, from sections 2.3.1 through 2.3.6 were done in MATLAB (2009a, The MathWorks, Natick, MA). Data analysis for section 2.3.6 was performed using R (V2.10.0, R Development Core Team, Vienna, Austria).

2.4 Results

2.4.1 RSM Box-Behnken design for CT image quality optimization

The RSM using Box-Behnken design proved to be a reliable method that offered a clear perception in how the diverse factor level combinations of X-ray tube voltage, current, and slice thickness resulted in different responses for each of the CT image quality measurements (SNR, VA, HCSR, and LCD), as seen in Table 3. The Box-Behnken design presented sufficient information to test the lack-of-fit for each of the non-linear response models, as presented in the Analysis of Variance Analysis (ANOVA) (Table 4). This affirms that this design is as robust in terms of prediction performance, in comparison with a complete factorial design or others (Myers et al., 2008).

Table 4 shows that all P values for the lack-of-fit criteria are non-significant (p > 0.05), suggesting that the second order non-linear models can accurately predict the effect of the independent variables (factors) for each of the response variable (dependent) independently. Furthermore, the approach indicates that the non-linear models are optimally designed to provide an ideal insight on the behavior of a second-order response surface, including the inference of maximized stationary points (optimized conditions) as presented in Table 5 (Myers et al., 2008). The relationship between the dependent and independent variables was completely interpreted, displayed, and studied in depth, using a set of response surface and interaction plots (Fig. 9-12), in combination with the regression coefficients for each response model, as summarized in Table 6. The values in Table 6 corresponding to the coefficients for each coded factor (voltage

(X_1), current (X_2), and slice thickness (X_3)) are related to the effect of each of the factors on the change to each response variable (Y_n). In other words, coefficients are used to study the mathematical relationship in the form of a polynomial equation for each of the measured response (Y_n), as seen in Eq. 2-1 (Myers et al., 2008).

Table 4. ANOVA table for Signal-to-noise Ratio (SNR), Volume Accuracy (VA), High Contrast Spatial Resolution (HCSR), and Low Contrasts Detectability (LCD) non-linear response models

Term	df	Sum of squares (SS)				Mean square (MS)				F-value			
		SNR	VA	HCSR	LCD	SNR	VA	HCSR	LCD	SNR	VA	HCSR	LCD
Linear	3	0.01	7.2	0.40	4717.0	0.00	2.39	0.14	1572.4	210.4	143.7	182.38	236.8
Quadr	3	0.00	0.2	0.01	327.3	0.00	0.06	0.00	109.1	6.4	3.3	2.79	16.4
Polyn.	3	0.00	0.4	0.02	536.8	0.00	0.12	0.01	178.9	24.0	7.1	11.63	26.0
Res. Err.	5	0.00	0.1	0.01	46.5	0.00	0.02	0.00	6.64				
Lack-of-fit	3	0.00	0.1	0.00	41.5	0.00	0.02	0.00	8.30	4.4	8.2	1.59	3.32
Pure error	2	0.00	0.0	0.00	5.0	0.00	0.00	0.00	2.50				

Table 4. Cont'd

Term	df	P-value ^a			
		SNR	VA	HCSR	LCD
Linear	3	0.0a	0.0a	0.0a	0.0a
Quadr	3	0.0a	0.1	0.1	0.0a
Polyn.	3	0.0a	0.0a	0.0a	0.0a
Res. Err.	5				
Lack- of-fit	3	0.2	0.1	0.4	0.3
Pure error	2				

^aANOVA for each dependent variable at P = 0.05

Table 5. Stationary maximized points in original units for each non-linear response variable model

Response	Factors			Eigenvalues		
variable	Voltage	Current	Slice	Voltage	Current	Slice
SNR	113	140	5.0	-0.003	-0.005	-0.012
VA (mm ⁻³)	> 120 ^a	> 170 ^a	< 0.625 ^a	0.757	0.085	-0.041
HCSR	113	170	5.0	-0.034	-0.038	-0.066
LCD	120	117	4.4	-9.502	-7.497	-1.250
Max	> 120^a	> 170^a	5.0			
Min	113	140	< 0.625^a			
Optimized	120	170	2.5			

^aMaximum stationary point was not discerned.

Table 6. Regression coefficients and P values^a for each non-linear response variable model

Term (code)	SNR		Volume accuracy		HCSR		LCD	
	Coeff. ^b	p-value	Coeff.	p-value	Coeff.	p-value	Coeff.	p-value
Intercept (b_0)	0.5970	0.000a	0.5985	0.000a	0.4238	0.000a	0.4893	0.000a
Voltage (X_1)	0.1100	0.000a	0.0000	0.767	0.0458	0.001a	0.1695	0.000a
Current (X_2)	0.1533	0.000a	0.0814	0.085	0.1465	0.000a	0.1243	0.000a
Slice thickness (X_3)	0.0113	0.000a	-0.8775	0.000a	0.0868	0.000a	0.0837	0.000a
Voltage x Current (X_1X_2)	-0.0003	0.811	-0.0316	0.962	-0.0030	0.813	0.0584	0.125
Voltage x Slice thickness	-0.0019	0.202	-0.0279	0.387	0.0035	0.784	0.0531	0.123
Current x Slice thickness	0.0002	0.896	-0.2595	0.009a	-0.0074	0.567	0.0243	0.058
Voltage ² (X_1^2)	-0.0040	0.033a	0.0830	0.834	-0.0367	0.032a	-0.0446	0.114
Current ² (X_2^2)	-0.0120	0.000a	-0.0170	0.219	-0.0652	0.002a	-0.0883	0.009a
Slice thickness ² (X_3^2)	-0.0036	0.004a	0.7553	0.000a	-0.0366	0.033a	-0.0103	0.688

^aANOVA for each term at P = 0.05, giving a statistical significance summary of the main dependent variable effects their interactions. ^bNote that Non-linear coefficients are scaled to the coded factors, as specified in Eq. (2-1).

Coefficients with higher values represent a higher probability for statistical interaction effects and quadratic relationship. A positive value signifies an effect that favors the response, while a negative value indicates an antagonistic effect (Myers et al., 2008).

2.4.2 SNR (Y_1) optimization

Regression coefficients for the SNR (Table 6) indicate that the individual effect of voltage, current, and slice thickness are statistically significant, since the corresponding P values are ≤ 0.05 . No interaction effects between any of the factors appear to be statistically significant. Table 4 shows the result of ANOVA for the CT image SNR, in addition it was determined that the R^2 and R^2 (adjusted for df) are 99.04 % and 97.81 %, respectively. This indicates that the factors remarkably explain the amount of variation in the observed values of SNR. Additionally, it is clear that the first, second, and polynomial order terms contribute significantly to the model (P value ≤ 0.05), so the canonical analysis or the relationships between factors in the data set is relevant (Myers et al., 2008). The stationary point is near the experimental region, and the three eigenvalues are negative, indicating that the stationary point is a maximum SNR (Table 5); clear evidence a set of optimal scanning conditions was inferred. Optimized response for SNR was established to be at a voltage, current, and slice thickness equal to 113 kilovolts (kV), 140 milliamperage (mA), and 5.0 mm respectively. The surface response plots shown in Fig. 9 (a-c) indicate that SNR increases with an increase in all factors (voltage, current, and slice thickness), consequently

increasing image quality. This can be visually confirmed in Fig. 13, where the combination of low voltage, current, and slice thickness yield CT images with low SNR, hence CT images have poor quality (i.e. high graininess) (Bushberg et al., 2002; Du et al., 2007; Goldman, 2007). This graininess phenomenon is consistent with the theoretical fact that specifies that by increasing voltage, current, and slice thickness, a higher number of X-rays will be detected by the CT equipment, yielding better quality images with a higher SNR, and therefore less graininess (Bushberg et al., 2002; Du et al., 2007; Goldman, 2007).

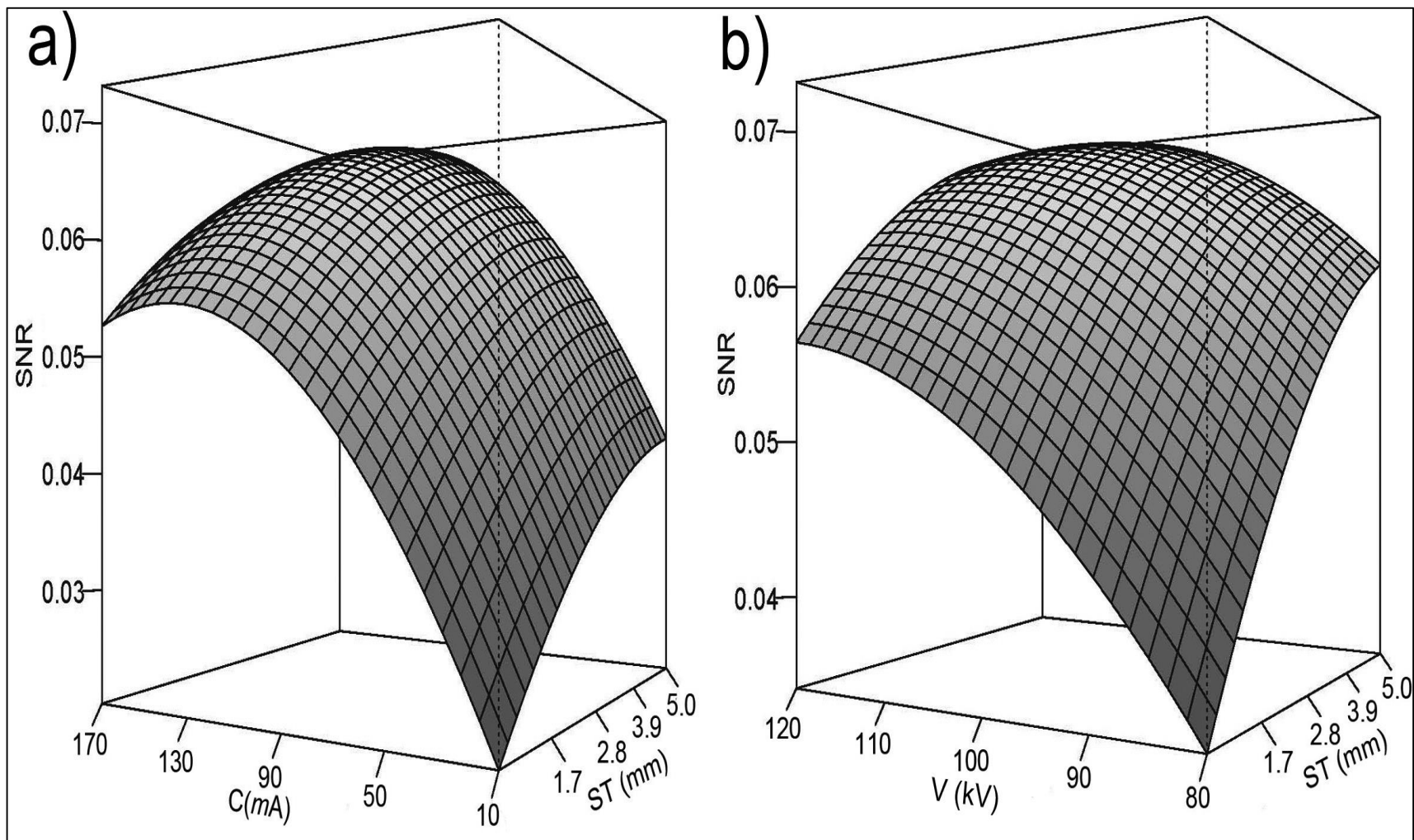
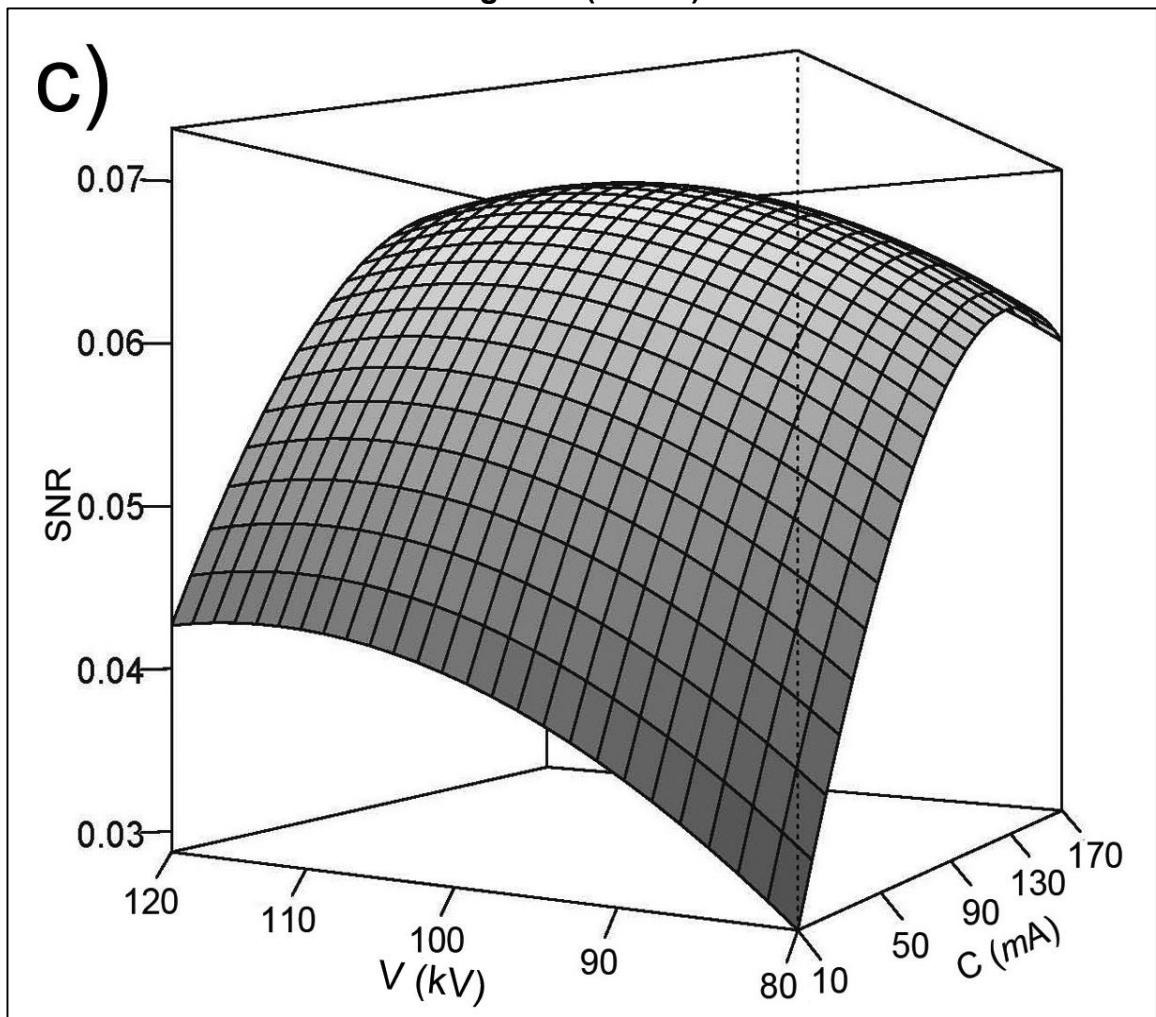


Figure 9. Surface plots of SNR versus (a) slice thickness (ST - mm) for current (C - mA), (b) slice thickness (ST - mm) for voltage (V - kV).

Figure 9 (cont'd).



(c) current (C - mA) for voltage (V - kV).

2.4.3 Volume accuracy (Y_2) optimization

Regression coefficients for VA (Table 6) indicate that the individual effect of slice thickness is the only statistically significant factor affecting volume accuracy, since the corresponding P value is ≤ 0.05 . It is also observed that the interaction effect between slice thickness and current also has a significant contribution (P value ≤ 0.05). Table 4 shows the result of ANOVA for the CT image volume accuracy model that yielded an R^2 and R^2 (adjusted for df) of 98.51 % and 96.59 %, respectively. This indicates that the factors highly explain the amount of variation in the observed values of volume accuracy. Additionally, it is clear that the first, and polynomial order terms contribute significantly to the model (P value ≤ 0.05), so the canonical analysis or the relationships between factors in the data set is relevant (Myers et al., 2008). The stationary point is not near the experimental region, and only one out of the three eigenvalues are negative, indicating that the stationary point could not be determined (Table 5). Therefore, clear evidence of a set of optimal scanning conditions could not be inferred (Myers et al., 2008).

Even though optimized conditions could not be determined, the surface response plots shown in Fig. 10 (a, c, and d) indicate that volume accuracy is directly proportional to the voltage and the current, while volume accuracy is inversely proportional to slice thickness. Consequently, the combination of high slice thickness, low current, and voltage yield CT images with low volume accuracy, hence CT images under these conditions offer low 3D information.

Additionally, by statistically slicing, therefore evaluating the slice thickness and current interaction effect (Fig. 10-b), it can be seen that only low (0.625 mm) and high (5 mm) slice thickness causes a significant change in volume accuracy dependent of current. Medium (2.5 mm) slice thickness does not seem to significantly change volume accuracy with the variation of current. These results are consistent with other studies in the medical (Bushberg et al., 2002; Goldman, 2007) and agricultural (Mendoza et al., 2007) fields, where it has been found that by reducing slice thickness, better overall 3D information or volume accuracy will be digitally perceived from CT images.

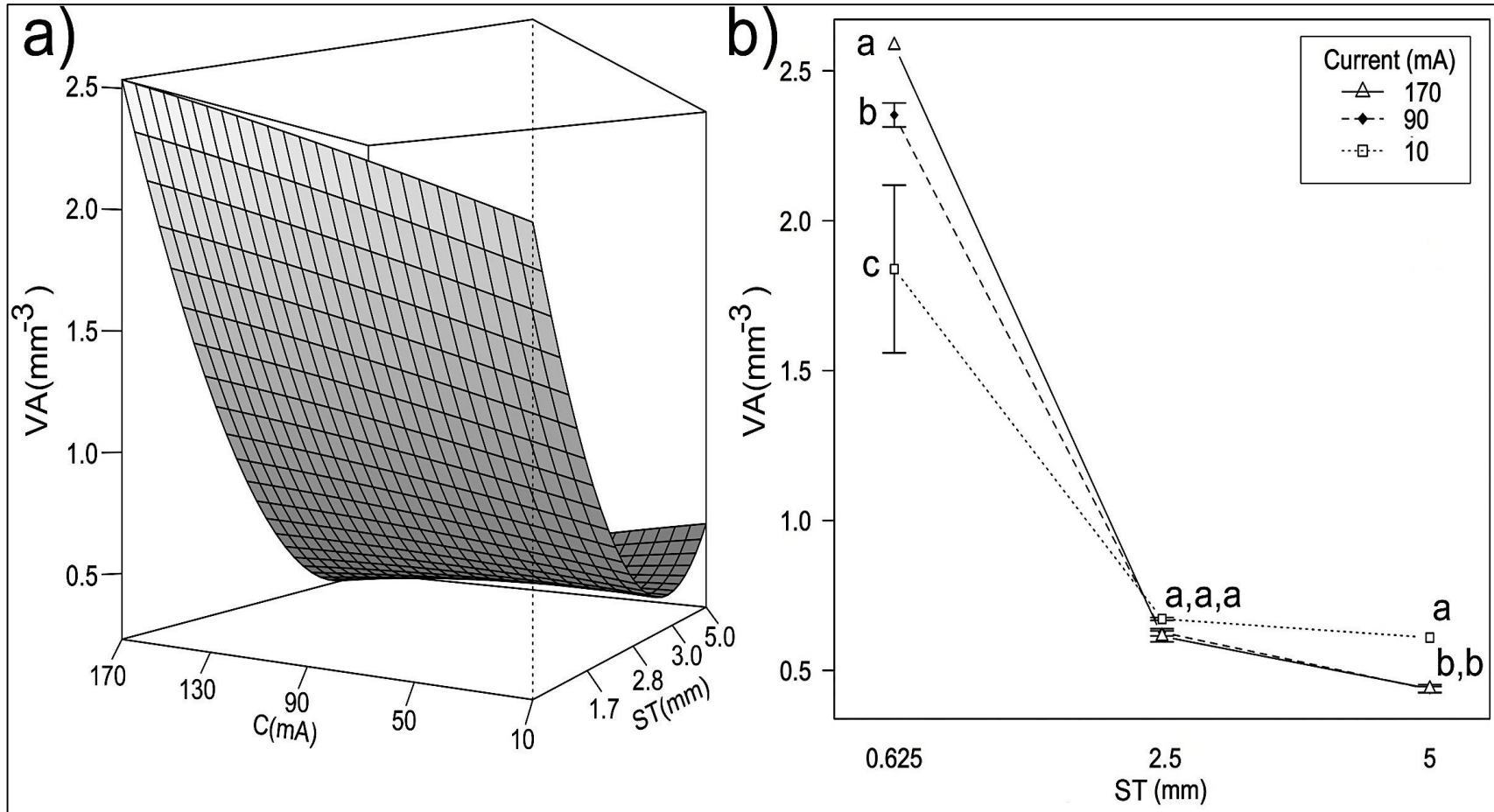
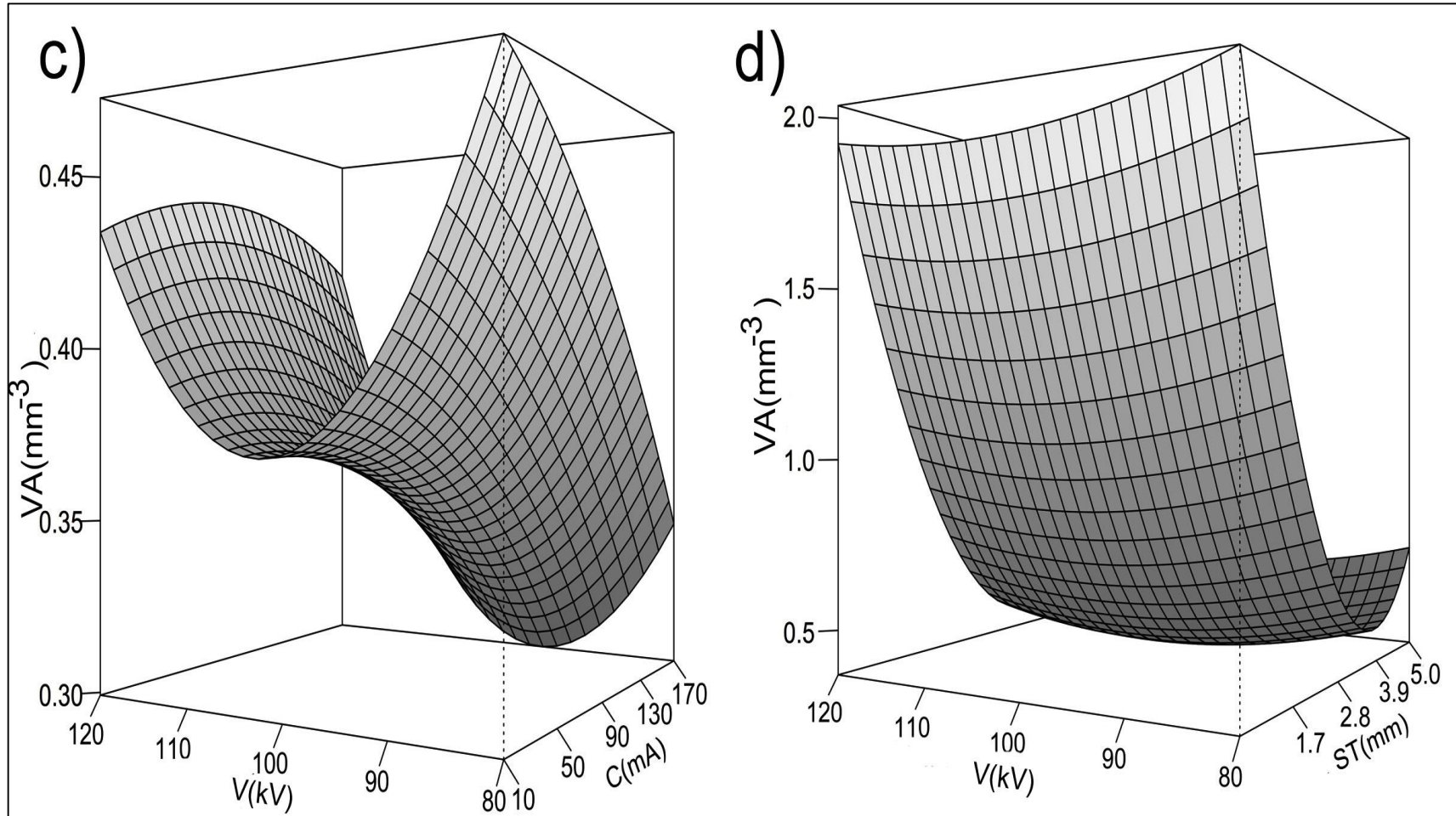


Figure 10. (a) Surface plot of volume accuracy (VA - mm^{-3}) versus slice thickness (ST - mm) for current (C - mA). (b) Interaction plot for volume accuracy (VA - mm^{-3}) versus slice thickness (ST - mm) for current (C - mA). Data points followed by the same lower case letter within the same slice thickness are not significantly different at $P = 0.05$ (ANOVA with post-hoc Tukey multiple comparison of means).

Figure 10 (cont'd).



Surface plots of volume accuracy (VA - mm^{-3}) versus (c) slice thickness (ST - mm) for voltage (V - kV), and (d) current (C - mA) for voltage (V - kV).

2.4.4 HCSR (Y3) and LCD (Y4) optimization

Regression coefficients for both HCSR and LCD (Table 6) indicate that the individual effects of voltage, current, and slice thickness are statistically significant (P values ≤ 0.05). No interaction effects between any of the factors appear to be statistically significant.

Table 4 shows the result of ANOVA for HCSR, in addition it was determined that the R^2 and R^2 (adjusted for df) are 98.83 % and 97.32 %, respectively. This indicates that the factors highly explain the amount of variation in the observed values of HCSR. Additionally, it is clear that the first, and polynomial order terms contribute significantly to the model (P value ≤ 0.05), so the canonical analysis (relationships between factors) in the data set is relevant (Myers et al., 2008).

Table 4 displays the result of ANOVA for LCD, where the R^2 and R^2 (adjusted for df) equaled 99.17 % and 98.11 %, respectively. This indicates that the factors remarkably explain the amount of variation in the observed values of LCD. Additionally, it is clear that the first, second, and polynomial order terms contribute significantly to the model (P value ≤ 0.05), so relationships between factors is relevant (Myers et al., 2008).

The stationary points for both HCSR and LCD are within the experimental region, and the three eigenvalues are negative, indicating that the stationary point is a maximum (Table 5); therefore clear evidence of a set of optimal scanning conditions was inferred. Optimized response for HCSR was established to be at a

voltage, current, and slice thickness equal to 113 kV, 170 mA, and 5.0 mm respectively. Optimized response for LCD was established to be at a voltage, current, and slice thickness equal to 120 kV, 117 mA, and 4.4 mm respectively. The surface response plots shown in Fig. 11-12 (a-c) indicate that HCSR and LCD increase with an increase in all factors (voltage, current, and slice thickness). Analysis shows that increasing voltage, current, and slice thickness will result in an increase of HCSR and LCD. This can be visually confirmed in Fig. 13, where the combination of low voltage, current, and slice thickness yield CT images with low HCSR and LCD, hence CT images have difficulty distinguishing between two high contrast objects placed close together, and the ability of discriminating objects that vary slightly from their background is limited (Goldman, 2007). It could be confirmed that both HCSR and LCD follow a similar response trend as the SNR, where objects placed close together and small low-contrast objects are obscured by high noise or graininess (Fig. 13) (Goldman, 2007).

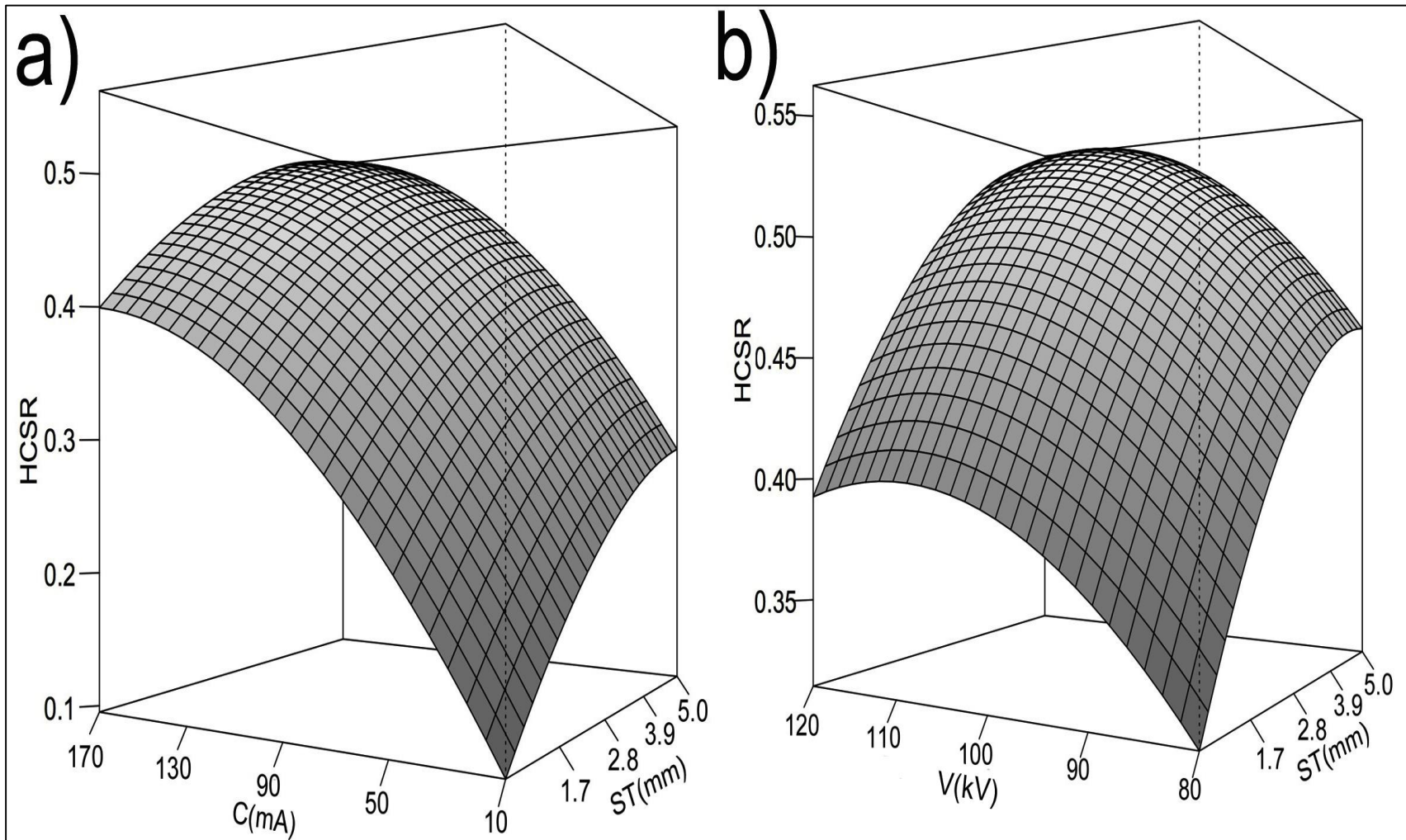
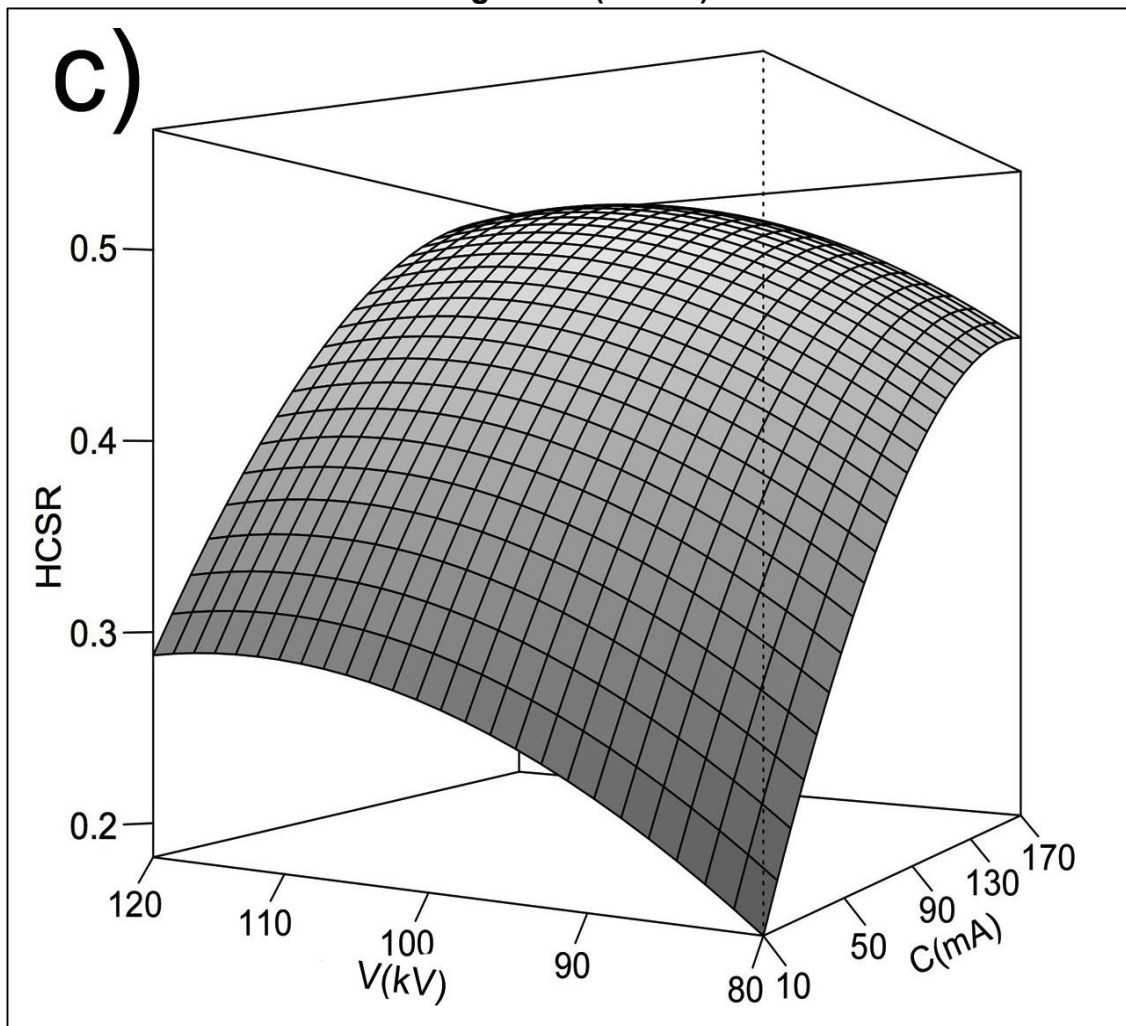


Figure 11. Surface plots of $HCSR$ versus (a) slice thickness (ST - mm) for current (C - mA), (b) slice thickness (ST - mm) for voltage (V - kV).

Figure 11 (cont'd).



(c) current (C - mA) for voltage (V - kV).

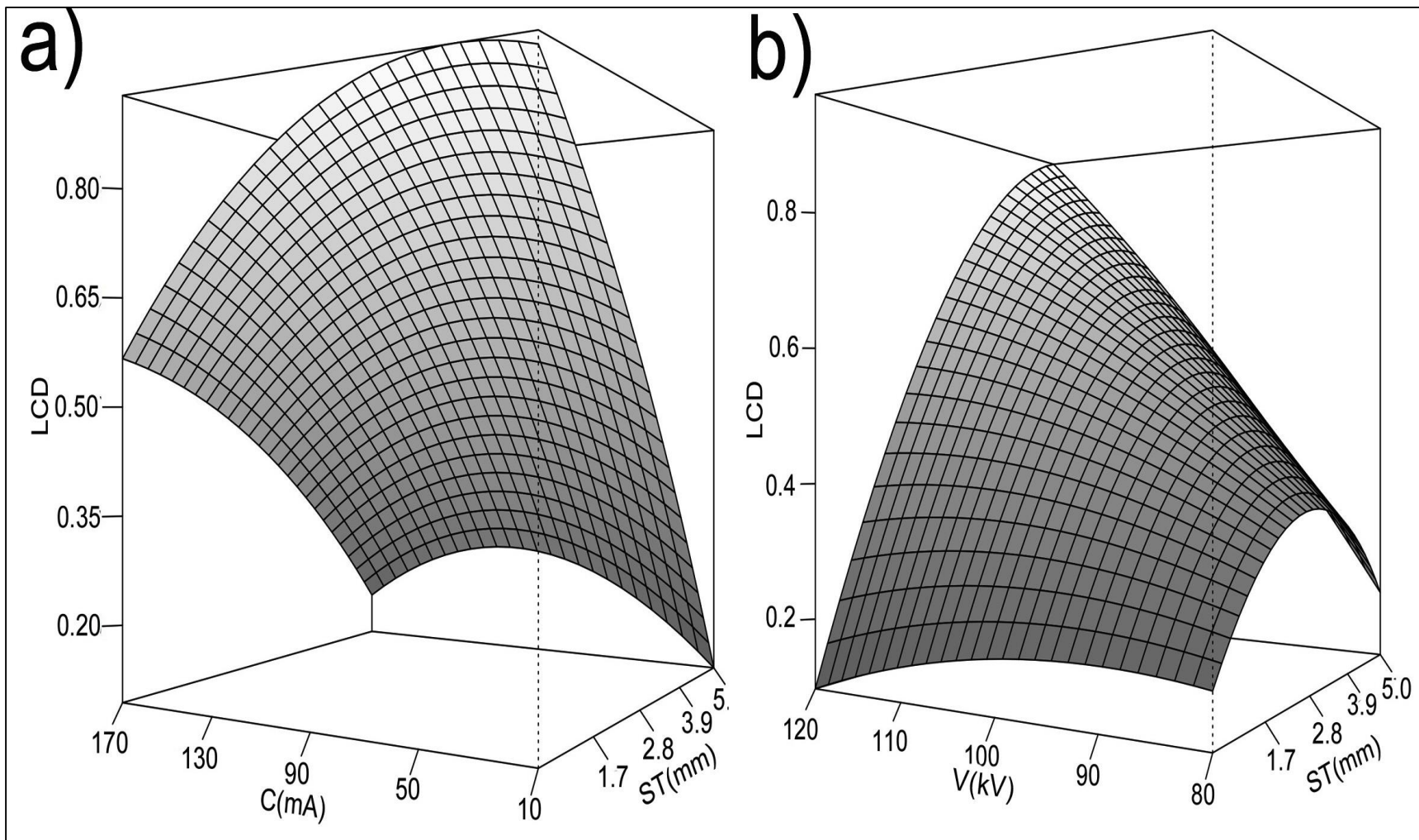
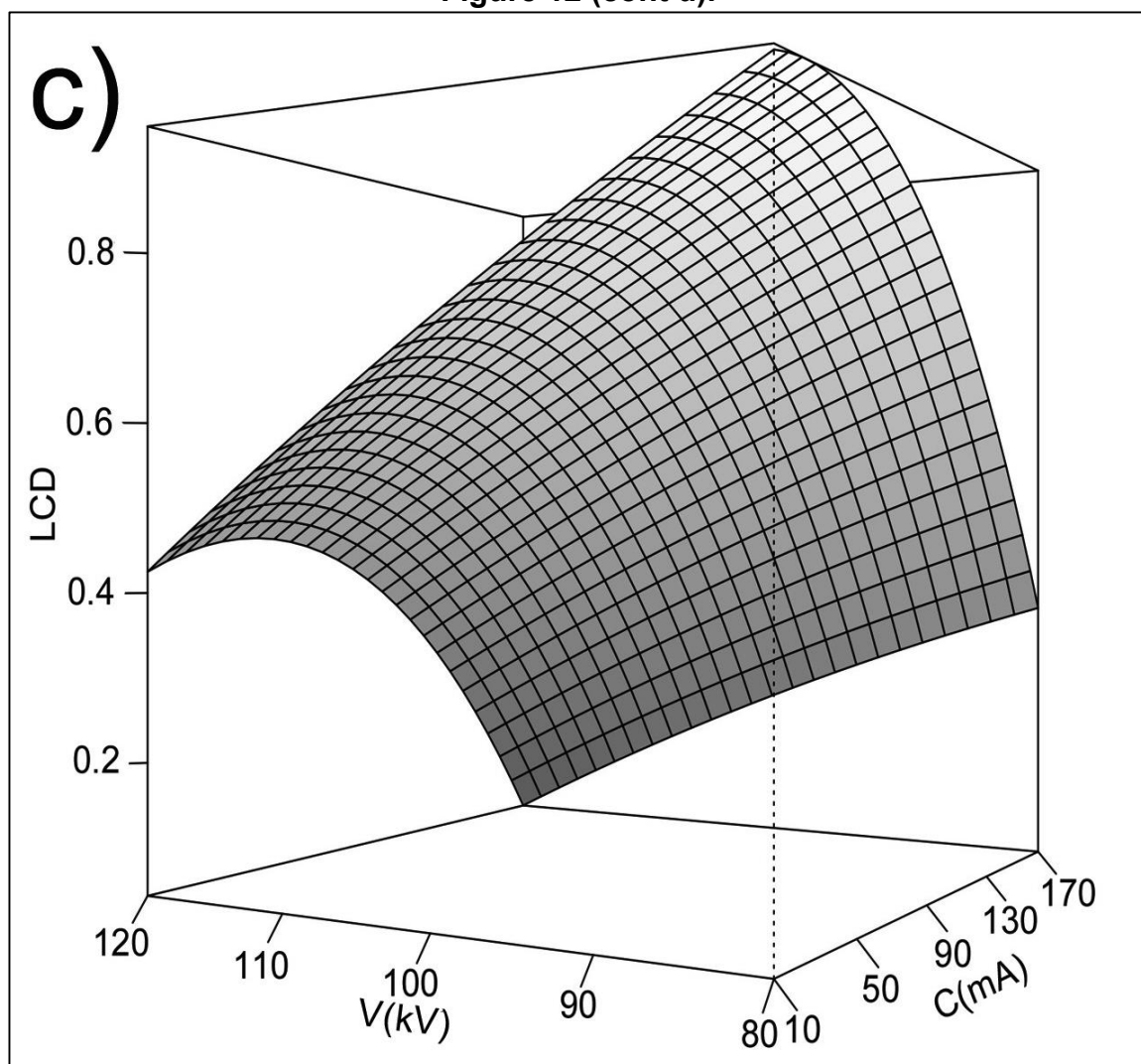


Figure 12. Surface plots of LCD versus (a) slice thickness (ST - mm) for current (C - mA), (b) slice thickness (ST - mm) for voltage (V - kV).

Figure 12 (cont'd).



(c) current (C - mA) for voltage (V - kV).

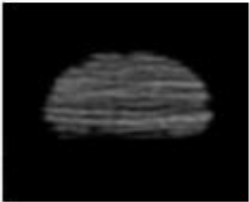
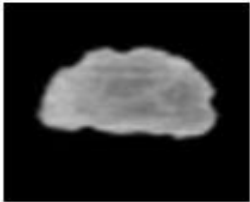
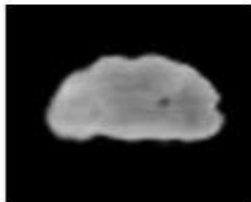
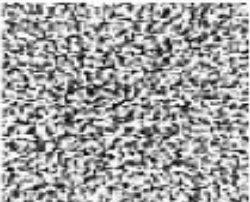
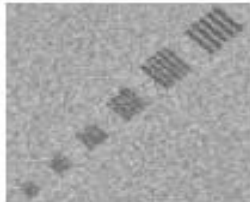
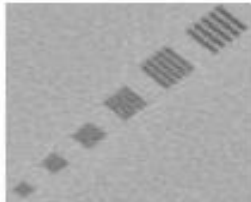
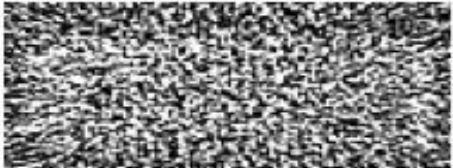
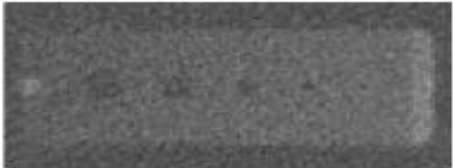
		Independent variables (Coded Levels)		
		V(-1), C(-1), ST(-1)	V(0), C(0), ST(0)	V(+1), C(+1), ST(+1)
Dependent variables - Response	SNR			
	VA (mm ³)			
	HCSR			
	LCD			

Figure 13. Example of Gray-scale CT image quality for low (-1), medium (0), and high (+1) factor combinations. Note that low (-1) and high (+1) level combinations are not part of the Box-Behnken design, but CT images were acquired for visualization and validation purposes.

2.4.5 Optimized CT image quality attributes and validation

The study of the effect of independent variables on each of the response variables individually facilitated the determination of optimized conditions to acquire maximized quality images. Final optimized parameters for voltage, current, and slice thickness were set at 120 kV, 170 mA, and 2.5 mm (Table 5). These values were chosen, because analysis showed that increasing current and voltage result in a rise of all response variables, including SNR, VA, HCSR, and LCD. Even though it might seem tempting to exponentially increase voltage, and current even higher (above experiment upper boundary), it would not be recommended, due to the fact that it can be predicted that the gain in image quality will be minimum in comparison with a significant increase in X-ray dose, and decrease the usable life of the X-ray source, as specified by studies in medical field (Boone et al., 2003; Woodford et al., 2007), and CT hardware performance studies (Prokop, 2003; McCollough et al., 2006; Goldman, 2007). Based on the previously discussed RSM models, it is not recommended to decrease voltage, and current below 113 kV, and 140 mA respectively, because a significant loss in image quality, due to a significant increase in CT image noise would be observed.

In contrast, it could be seen that if slice thickness increases, volume accuracy will decrease while SNR, HCSR, and LCD increase. Therefore a slice thickness optimized value of 2.5 mm was chosen, with the objective of finding an intermediate value, between maximized volume accuracy (slice thickness ≤ 0.625 mm), while keeping high SNR, HCSR, and LCD (slice thickness ≥ 4.4 mm). In

addition, setting scanning slice thickness at 0.625 mm or lower, will not only lower 2D CT image quality significantly, by reducing SNR, HCSR, and LCD; but will increase scanning time (Table 1), a critical factor in the development of fast CT scanning systems (Hampel et al., 2005; Du et al., 2007; Sellakumar et al., 2007; Bierberle et al., 2009).

Final experimental optimized response for each image quality attribute; from the independent CT image validation set, is summarized in Fig. 14. These experimental values for optimized CT images indicated that the mean SNR, VA, HCSR, and LCD equaled 0.69 ± 0.25 (SD), $0.43 \pm 0.13 \text{ mm}^{-3}$, 0.52 ± 0.03 , and 0.74 ± 0.25 respectively. In comparison, the predicted response, using RSM models (Table 6) and optimized scanning parameters (Table 5) equaled 0.74, 0.32, 0.54, and 0.75 for SNR, VA, HCSR, and LCD respectively (Fig. 14). Experimental values are in close agreement to predicted values; hence, the RSM prediction models are accurate.

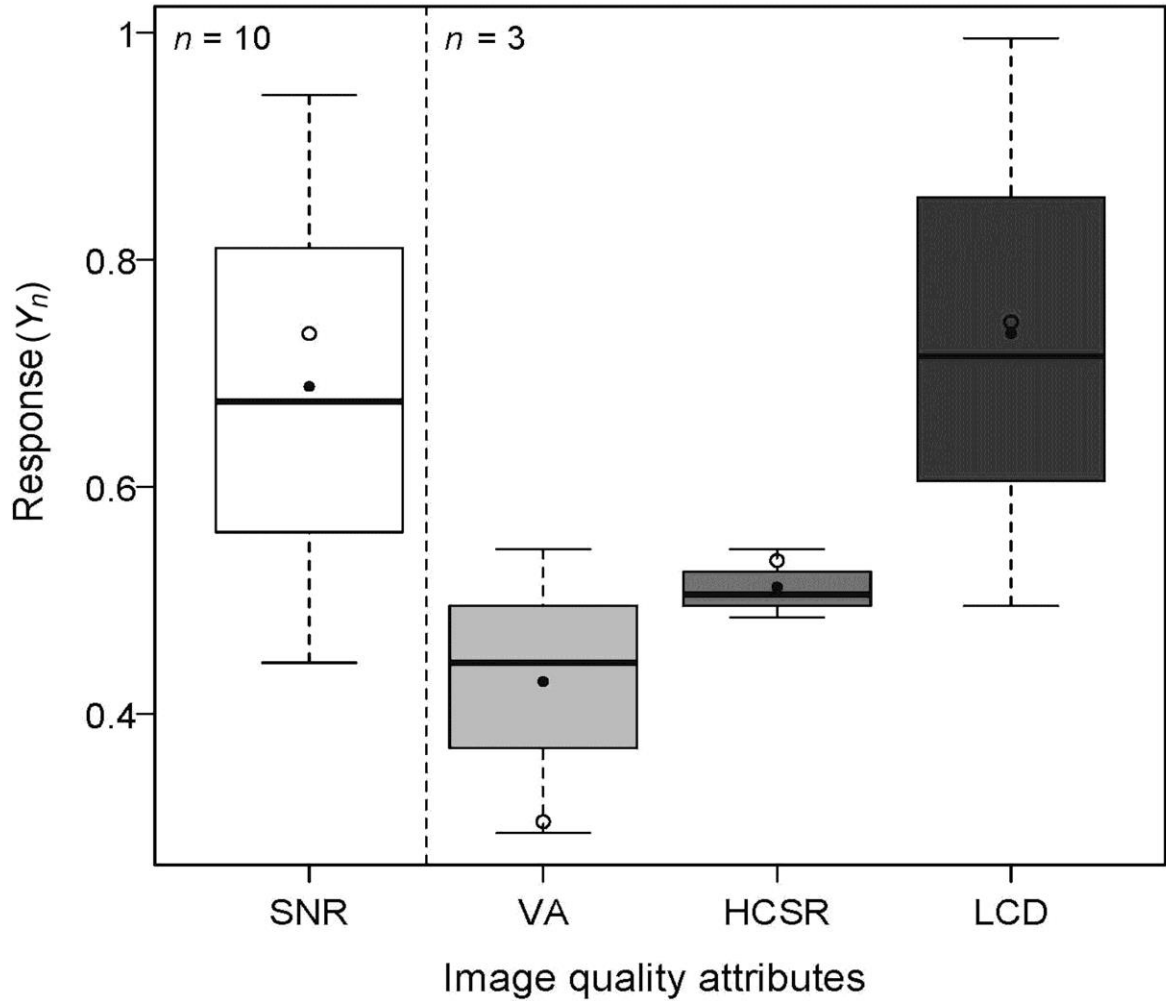


Figure 14. Box-plots showing experimental results for CT image quality attributes (SNR, VA (mm^{-3}), HCSR, LCD) from a completely independent validation data set, using optimized scanning parameters, as seen in Table 5. The median of each experimental quality attribute is represented as a thick horizontal black line, upper and lower quartiles as a box with the maximum and minimum measurements as lines protruding from these. The mean experimental response for each quality attributes is symbolized as a black solid dot (•). Predicted optimized response using non-linear response surface polynomial models (Table 2) is symbolized as a hollow circle (°).

It could be visually confirmed in Fig. 13, that SNR is one of the most important and determinant response variables that will enhance the detection of ROIs in fresh chestnuts (e.g. Slight decayed tissue vs. healthy tissue in chestnuts) and is highly related to the HCSR and LCR (Du et al., 2007; Goldman,

2007). SNR does not seem to have a relationship to the 3D overall volumetric information, mainly because the scanned Teflon® reference cylinders (900 HU) have a high contrast in relation to their background (Air = -1000 HU). Volume accuracy might play a higher role if the difference in contrast between the Teflon® reference cylinders and the background wouldn't have been as high (i.e. Fresh chestnut tissue). Nonetheless, it was observed that the smaller the slice thickness the higher the observed volume accuracy.

The HCSR represents the detection of edges of structures and small foreign objects when a significant difference in contrast exists (Du et al., 2007; Goldman, 2007), for example to determine the presence of foreign objects in the chestnuts or void spaces. Resulting optimized 2D CT images showed that the smallest discernable line pair (lp) is the 1.0 mm bar pattern, which equals a spatial resolution of $1.5 lp\ mm^{-1}$ (General Electric Company, 2007).

LCD results indicated that optimized CT images provide the ability to detect structures as small as 3 mm diameter (General Electric Company, 2007). More specifically for chestnut sorting purposes, this means that the smallest discernable section of tissue with a slight contrast difference (e.g. decay versus healthy), will be equal to 3 mm diameter in all directions, due to the intrinsic isotropic image resolution of the scanner (Tsukagoshi et al., 2007). Therefore, a section of decay, pellicle, void space, or other region of interest smaller than 3 mm is practically undetectable using optimized CT images.

Relationship between SQR values obtained from a 5-expert panel and the DQA from their corresponding 2D CT image validation set, obtained through

optimized scanning parameters (Fig. 15) demonstrated a significant positive correlation ($R = 0.98$, $P < 0.05$), with a coefficient of determination that accounts for 96.6 % of total variability explained by linear regression. This indicated a high match between the digital estimation of decay (DQA) and the human visual perception of decay (SQR), ultimately reflecting good scanning parameter optimization/selection.

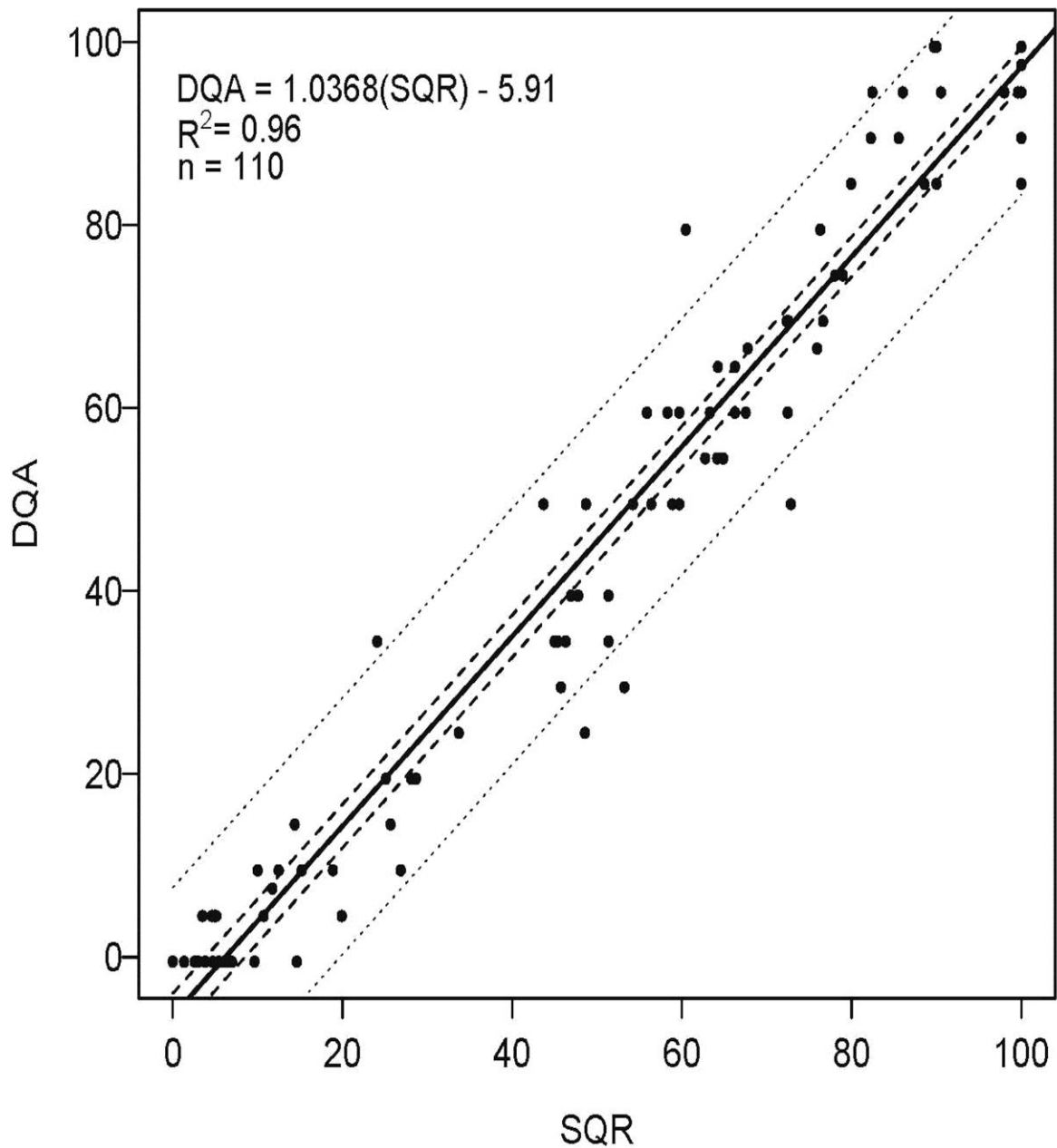


Figure 15. Relationship between Subjective Quality Rating (SQR) values obtained from a 5-experts panel and the Digital Quality Assessment (DQA) from their corresponding CT image validation set, obtained through optimized scanning parameters. Black solid line represents the least-square linear regression line ($n = 110$). The two dashed lines indicate 95 % CI of the linear regression. The two dotted lines indicate 95 % prediction intervals of the linear regression.

2.5 Discussion

The study applied RSM using Box-Behnken design as a systematic tool to optimize CT scanning settings (voltage, current, and slice thickness), using CT images of fresh chestnuts, Teflon® reference cylinders, and a specific QA phantom, as imaging models for CT image quality (SNR, VA, HCSR, and LCD). Currently, CT image quality assurance for different application is done by visually assessing image appearance, in combination with statistical parameters, and human aided quantitative measurements to validate visual tests, as described by several scientists including Goldman (2007), Ledenius et al. (2009), Prokop (2003), Arnold et al. (2010), and companies like General Electric Company (2007). The issue is that image quality assurance mainly relies in repetitive subjective measurements of quality, which is time consuming, prone to high variability, and bias dependent on the human observer capabilities and expertise. Contrarily, in this study, custom formulas were derived in an attempt to provide a new automatic and objective way to measure CT image quality by mimicking the procedure that is usually done by human raters.

If necessary, the same tool can be applied and slightly modified to evaluate the effect of other determinant scanning factors critical for image quality, additional scanning response attributes, and further applications that will significantly advance the field of CT and especially fast CT scanning. Determinant factors for image quality that were not evaluated in this research, because of their minor effect in image quality in comparison to the studied factors can also be studied, like the effect of X-ray detector spacing, image

reconstruction algorithms, detector failure, X-ray tube focal spot, and number of projection images (Bushberg et al., 2002; Goldman, 2007). Other applications of the proposed tool include the systematic optimization of parameters for micro-CT system (Badr et al., 1997; Du et al., 2007), clinical applications (Badr et al., 1997; Huda et al., 2000; Woodford et al., 2007; Wang et al., 2011), X-ray CT dose reduction (Mayo et al., 1994; Boone et al., 2003; McCollough et al., 2006; Goldman, 2007; Ledenius et al., 2009), ultra-fast CT parameter optimization (Hampel et al., 2005; Bierberle et al., 2009), and potential image optimization for in-line postharvest sorting of agricultural commodities (Barcelon et al., 1999b; Barcelon et al., 1999a; Sornsrivichai et al., 2000; Butz et al., 2005).

Optimized high-resolution 2D CT images, as obtained from this study, can accurately reflect subjective internal characteristics of fresh chestnuts with a high accuracy rate (96.6 %), with high contrast between internal chestnut structures. This information is useful to study chestnut optimum storage conditions, quality standards (Mencarelli, 2001), the effect of mechanical harvesting, pre-harvest treatments (Mandujano et al., 1998; Monarca et al., 2005; Sieber et al., 2007; Donis-González, 2008; Donis-Gonzalez et al., 2010), fresh chestnut *in vivo* fruit morphology for cultivar characterization (Ertan, 2007), and chestnut peelability (Guyer et al., 2005). In addition, fast image processing, pattern recognition (Wulfshohn et al., 1993; Kavdir and Guyer, 2007) and feature extraction (Kim and Schatzki, 2000) applied to more than one 2D CT image per sample, will be a requirement to develop in-line quality sorting algorithms and systems. Based on the size of possible discerned structures using optimized CT images, which is equal to objects larger than 3 mm (founded on LCD results) separated by at least

1 mm (established *HCSR* results) between them, it can be inferred that the minimum number of acquired images per sample (i.e. ~ 8-17 CT images per chestnut) are sufficient for the development of future quality-sorting algorithms, critical to offer premium quality fresh chestnuts.

2.6 Conclusions

The *RSM* using Box–Behnken design has proved to be a successful technique to assess the significance of three of the most important CT equipment scanning parameters (voltage, current, and slice thickness), in combination with a digital and automatic procedure of measuring CT image quality (SNR, VA, *HCSR*, and LCD). It is a reliable method or tool that will yield useful, and high quality CT images in any field related with CT, without the biased aid of human sight. Second-order (polynomial) *RSM* prediction models for each response variable reflected a combined maximized CT image quality at voltage, current, and slice thickness equal to 120 kV, 170 mA, and 2.5 mm respectively, for this specific postharvest application. More specifically, optimized scanning parameters provided fresh chestnut CT images with high-resolution and high-contrast with a high accuracy rate (96.6 %) between the digital estimation of decay (DQA), and human visual perception of decay (SQR). In addition, experimental response values from optimized CT images are in close agreement to optimized predicted response values using non-linear response models; hence, the models are accurate.

Results obtained in this experiment not only offer an advanced technique that can statistically, routinely, systematically, and reliably optimize CT

parameters, but also progress in the study of fresh chestnut quality, as well as the development of an in-line CT sorter for chestnuts and other agricultural commodities.

CHAPTER 3. RELATION OF COMPUTED TOMOGRAPHY (CT) HOUNSFIELD UNIT MEASUREMENTS AND INTERNAL COMPONENTS OF FRESH CHESTNUTS

3.1 Abstract

In this study, a medical grade computed tomography (CT) was used to obtain XY-plane 2D CT images from decayed and healthy fresh chestnuts, from the hybrid cultivar 'Colossal' and Chinese seedlings. Attenuation coefficients, referred to as Hounsfield-units (*HU*) or CT numbers, were acquired from different 2D CT image regions including air, and several chestnut components containing decayed tissue, healthy tissue, various imperfections such as pellicle invagination into healthy kernel, and void spaces. Results offer an *in vivo* accurate insight of fresh intact chestnuts, and suggest that CT technology is appropriate for in-line sorting. HU measurements can be used as a nondestructive predictor of fresh chestnut internal components with a 90.6% overall accuracy rate.

3.2 Introduction

CT methods for accurate visualization, segmentation and inner component identification of fresh chestnuts, which include the presence of decayed tissue, pellicle, void spaces and healthy tissue, are not available. It is hypothesize, that CT technology can be used as a tool to study fresh *in vivo* chestnut components. Additionally, with appropriate equipment and classification algorithms, the information gathered in this study will indicate if the technique is practical and suitable to apply for in-line sorting systems to accurately determine fresh chestnut

quality. For that reason, the objective of this study was to evaluate the HU values (Section 1.5) from different components of fresh chestnuts and their scanning environment (air) from images of the hybrid cultivar 'Colossal' and from Chinese chestnut seedling.

3.3 Materials and methods

3.3.1 Sample collection and preparation

A total of 100 kg of physiologically mature chestnuts (*C. sativa* x *C. crenata*) cv. 'Colossal' and Chinese chestnut seedlings (*C. mollissima*), were obtained from Chestnut Growers Inc. (CGI; Grand Haven, Michigan, USA). These chestnuts were previously collected from seven commercial farms in Michigan. Postharvest treatment (dip) was done as described in section 2.3.1. In addition, physiological internal disorders were stimulated in 25% of the apparently healthy chestnuts (sinkers) by submerging them for 300 s in 80 °C dH₂O. This procedure induces chestnut kernel damage by heat-shock, causing it to subsequently degrade during storage. Internal microbial decay was artificially induced in another 25% group of sinkers. This was done by manually injecting 100 µL of *Penicillium expansum* spore suspension, containing 3.8×10^6 spores L⁻¹, through the shell and into the chestnut kernel, using a 1 mL sterile medical grade tuberculin syringe with a 0.21 mm x 15.9 mm needle (BD™, NJ, USA). *P. expansum*, used for the experiment, was previously isolated from infected chestnuts and cultured onto a semi-selective medium containing potato-dextrose-

agar (PDA) (BD™), 20 $\mu\text{L L}^{-1}$ streptomycin (Sigma-Aldrich, Mo, USA) and 50 $\mu\text{L L}^{-1}$ ampicillin (Sigma-Aldrich) in a Petri dish. Spores were collected from a two week old fungal culture, growing at 25 °C. To prepare the inoculum, 15 mL of sterile dH₂O was added to the culture and the spores were gently removed from the surface with a sterile bacteriological loop. Spore were enumerated with a hemocytometer and then adjusted to the desired concentration, by dilution with sterile dH₂O. Previous steps provided four groups of chestnuts per species, 1) Apparently healthy chestnuts (sinkers), 2) Apparently naturally damaged chestnuts (floaters), 3) Heat-induced-damaged chestnuts, and 4) *Penicillium*-inoculated chestnuts. The purpose of dividing the ‘Colossal’ and ‘Chinese seedling’ into four groups was to guarantee a uniform distribution of a diverse range of internal characteristics needed for the experiment.

All chestnuts from each group were stored in mesh bags at 4 °C. After 90 d, 40 chestnuts from each group were randomly picked and attached to two rectangular wood pegboards (PB1 = 930 mm x 380 mm and PB2 = 1060 mm x 380 mm), in 20 rows containing 8 chestnuts (160 total), using approximately 0.005 kg of 100% transparent silicone per chestnut (General Electric, Waterford, NY, USA). Each pegboard (PB1 and PB2) contained a unique chestnut species (CS); PB1 contained Chinese seedlings and PB2 ‘Colossal’ chestnuts. Additionally, to avoid sample displacement, individual chestnuts were numbered and whole PBs containing chestnuts were wrapped using a 0.0033 mm stretch (shrink) polyethylene wrap (Just Packing Supplies Inc., New York, NY, USA) (Fig. 3b). Immediately after, CT scans were conducted.

3.3.2 *In vivo CT imaging scans*

CT scanning was performed on whole PBs, placed on the GE BrightSpeed™ RT 16 Elite, multi-detector CT instrument (General Electric Healthcare, Buckinghamshire, United Kingdom), as seen in Fig. 3b. The experimental conditions (scanning parameters) for the CT observations are shown in Table 1, using the parameters corresponding to a slice thickness (d) of 0.625 mm. This d was used to have a higher resolution in the Z-axis. A total of 1505 and 1729 XY-plane 2D CT images (slices) from PB1 and PB2 were obtained, respectively. Fewer images were acquired from PB1, because it was shorter in length due to the fact that Chinese seedlings are smaller in comparison to 'Colossal' chestnuts. Total scanning time for PB1 equaled 89 s, and PB2 102 s, which corresponds to a scanning time of approximately 0.6 s per chestnut.

3.3.3 *Visual based fresh chestnut quality and internal component assessment*

Raw color sliced internal faces were color scanned, as described in section 2.3.3, for record keeping and to use as references to accurately determine HU values from the XY-plane CT images as described in section 3.3.4. All chestnuts were then categorized based on their internal disorder severity level (SL), irrespective of their group; where SL1 contained all chestnuts that are healthy, SL2 chestnuts which are partially disordered, and SL3 represented the group of chestnuts which are completely disordered. A representative example of cross-sectional (XY-plane) CT 2D images in the fresh state, which reflects the distribution of healthy and disordered tissue among chestnut samples, is shown in Fig. 16. In these images, healthy - SL1 (e.g. ACT), partially affected (decayed) -

SL2 (e.g. BCT and DCT), and completely affected by internal disorder - SL3 (e.g. CCT) chestnuts can be viewed. Parallel, at the right side of every CT image, freshly sliced raw color images (RGB), which correspond to approximately the same CT scanned slices, can also be observed (e.g. ARGB, BRGB, CRGB and DRGB). These images proved useful when judging internal quality, and to accurately resolve the HU values and ROIs on the CT images. Figure 16-DCT also exemplifies an image of a partially decayed fresh chestnut, affected by *P. expansum*, with an overlying HU-values-profile containing the HU values acquired at the grey profile-line (PL). As visual reference a HU-standard-bar can also be noticed.

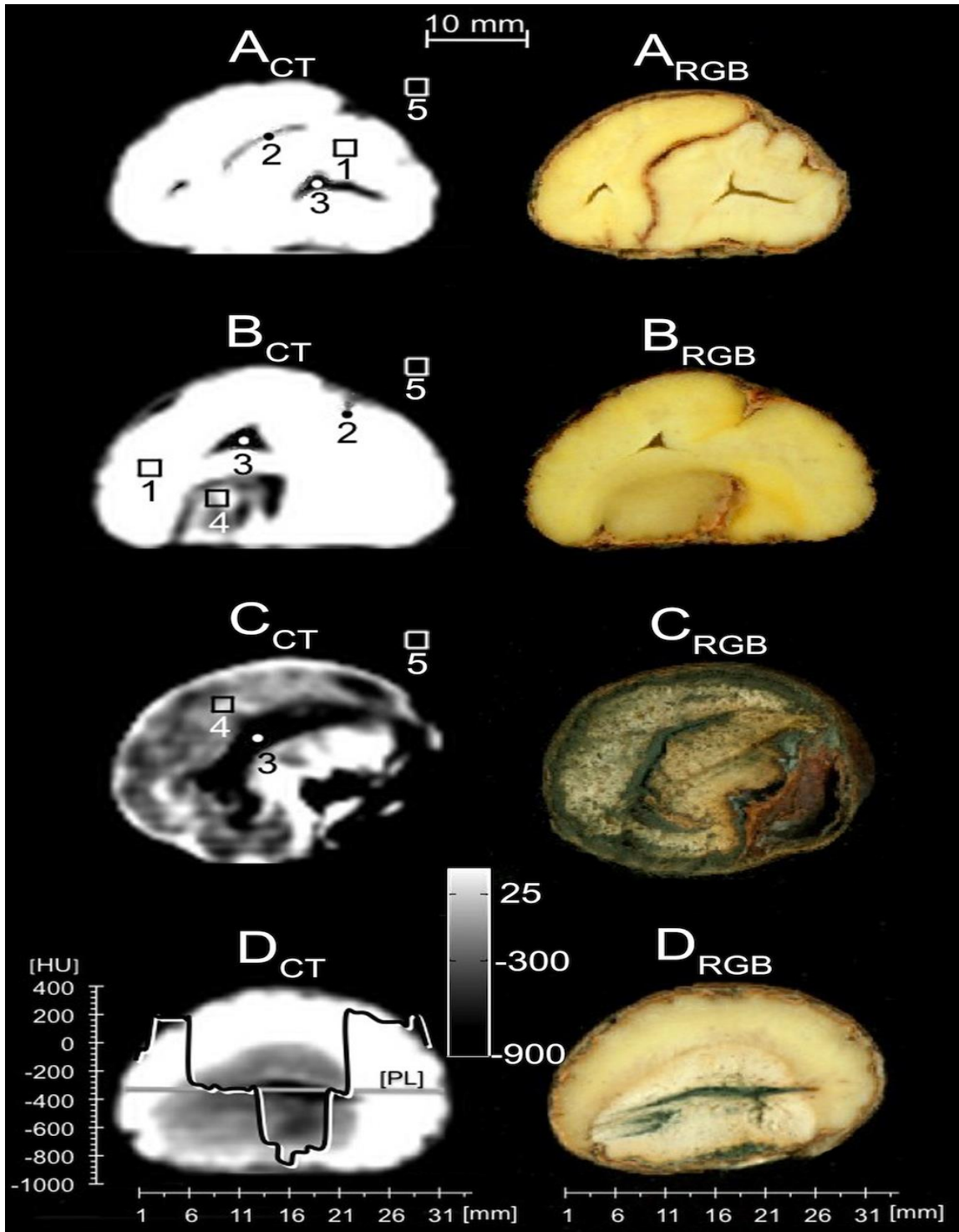


Figure 16. Cross-sectional XY-plane 2D CT images and color raw image slices (RGB) of chestnuts. (A) shows a healthy chestnut (SL1). (B) shows a partially decayed chestnut (SL2). (C) shows a completely decayed chestnut (SL3). (D) shows a partially decayed chestnut (SL2), illustrating the HU-values-profile taken at the grey line (PL), typifying the HU variation within components in the same chestnut.

3.3.4 HU-value inference using training data set

HU-values from eighteen regions of interest (ROIs) were acquired from different internal tissues (components) of fresh chestnuts and their scanning environment (air), including decayed tissue, healthy tissue, pellicle, and void spaces from XY-plane 2D CT images of the chestnut sample. ROIs were designed to include all possible combinations where different chestnut components and air are present, as seen in Table 7.

Table 7. Acquired HU value samples from different chestnut components and air, based on regions of interest (ROIs)

ID	SU ^a	Examples	ROIs			Mean HU value ^d	Threshold ^f	
		In Fig. 16	CS ^b	SL ^c	Region (Categ. ^e)		L	U
A	SU1	A,B,C CT 5	Ch	-	Air	-1000.94	-1024	-975
B	SU1	A,B,C CT 5	Co	-	Air	-1001.15		
C	SU2	ACT 3	Ch	SL1	Void space	-830.21	-974	-500
D	SU2	ACT 3	Co	SL1	Void space	-826.64		
E	SU2	BCT 3	Ch	SL2	Void space	-836.98		
F	SU2	BCT 3	Co	SL2	Void space	-837.58		
G	SU2	CCT 3	Ch	SL3	Void space	-825.48		
H	SU2	CCT 3	Co	SL3	Void space	-847.88		
I	SU1	BCT 4	Ch	SL2	Decayed	-273.35	-499	-210
J	SU1	BCT 4	Co	SL2	Decayed	-293.67		
K	SU1	CCT 4	Ch	SL3	Decayed	-299.40		
L	SU1	CCT 4	Co	SL3	Decayed	-313.73		
M	SU2	ACT 2	Ch	SL1	Pellicle	-118.68	-209	24

Table 7. (cont'd)

ID	SU ^a	Examples	ROIs			Mean HU value ^d	Threshold ^f	
		In Fig. 16	CS ^b	SL ^c	Region (Categ. ^e)		L	U
N	SU2	ACT 2	Co	SL1	Pellicle	-137.73		
O	SU1	ACT 1	Ch	SL1	Healthy	138.99	25	300
P	SU1	ACT 1	Co	SL1	Healthy	115.82		
Q	SU1	BCT 1	Ch	SL2	Healthy	125.56		
R	SU1	BCT 1	Co	SL2	Healthy	112.84		

^aSampled Unit (SU) to infer mean HU value^d. SU1 = 4 mm² (6.6 pixels²) square region/repetition (300), SU2 = 1-pixel/repetition (300). ^bChestnut species (CS) = cv. 'Colossal' – Co, Chinese – Ch. ^cSeverity level (SL) = SL1, SL2 and SL3 represents healthy, partially decayed and completely decayed chestnuts, respectively. ^eCategories were inferred after performing ANOVA at P = 0.05 and Tukey multiple comparison of means test (Fig. 17). ^fLower (L) and upper (U) thresholds for each category estimated from maximum and minimum HU.

The first two ROIs included the HU-values of air, defined as the scanning environment, from different scans. Both ROIs, were acquired to evaluate the repeatability between CT scans in the 2D CT reconstructed slices and to determine the intrinsic difference between HU values acquired in a similar medium. To confirm this, 300 repeated measurements, each including the mean HU value of a 4 mm^2 (6.6 pixel^2) square region containing only air, from PB1 (Table 7, ID-A) and PB2 (Table 7, ID-B) at different transverse XY-plane 2D CT images (slices) were obtained, as exemplified in Fig. 16 -ACT 5, -BCT 5 and -CCT 5. This first step, after acquiring the images was to determine if significant variability between XY-plane 2D CT images (slices) occurred. This is important to corroborate because it will indicate if observed differences between HU values obtained from different ROIs are due to internal changes in physical and/or chemical characteristics of chestnut samples within evaluated ROIs or if changes are due to unknown problems in the equipment or image acquisition procedure.

Thereafter, HU values of another sixteen different ROIs from chestnuts samples were acquired, based on CS, and disorder SL (Table 7). In total, three hundred random repetitions per measurement from dissimilar XY-plane 2D CT images were conducted for every ROI type. Depending in the ROIs size, each measurement either included the mean HU value of a 4 mm^2 square region, or the HU value acquired from an individual pixel. The sampled region for each ROI can be found in Table 7 under the sampled unit (SU) column. All HU values were acquired using the Osirix Imaging Software V3.6.1, developed by Dr. Antoine Russet's Software Team (<http://www.osirix-viewer.com/>). Due to artifacts that

occur near sample transitions (beam hardening effects), care was taken to avoid sampling near changeover regions, between the different media types.

HU values comparing the ROIs were analyzed using one-factor analysis of variance (ANOVA). Significance difference between ROI means was determined using the Tukey post-hoc multiple comparisons of means test at the 95% family-wise confidence level ($P = 0.05$) (Ott and Longnecker, 2001). Calculations were performed using the language and environment for statistical computing software R V2.10.0 (<http://cran.r-project.org/>).

3.3.5 Chestnut categories prediction using an independent testing data set

XY-plane 2D CT images were acquired from a completely different and independent set of 266 ‘Colossal’ and 266 Chinese chestnuts, with the objective of testing the accuracy of how well the five categories can be forecasted (blindly classified), using only HU value measurements. From these CT images, 50 independent HU value random measurements per each of the 5 categories and per CS were acquired, yielding a total of 500 independent testing data points. Accuracy refers to the percentage of correct category predictions inferred by acquired HU value, when compared with the true category label in the testing data set (Shapiro and Stockman, 2001). For example, a misclassification would occur if the sampled data point from pellicle tissue (e.g. Fig. 16-ACT 2) (true label) would yield a HU value equal to -300, automatically and mistakenly assigning this data point to the decay category, based on HU values thresholds summarized in Table 7, and further explained in sections 3.4.1 and 3.4.2. Chestnut sample

handling, CT scanning imaging parameters and procedure, HU value tissue sampling, and data handling followed the procedure described in section 3.3.4.

3.4 Results

3.4.1 HU-value and category threshold inference using training data set

CT image acquisition reliability and repeatability was confirmed by comparing HU values acquired from air, considered as a homogeneous standard medium. As expected and observed in Fig. 17, HU mean values of air including ROIs identified as A (Chinese data set) and B ('Colossal' data set) are not significantly different ($P < 0.05$) and fall under the same category (air). Analysis supported that under the specified experimental conditions (Table 1), the CT used in the study, has a HU value SD for a homogeneous standard medium equal to ± 1.80 HU. In other words, the equipment will be able to accurately discern between tissues only when the HU value difference is higher than 1.8 HU.

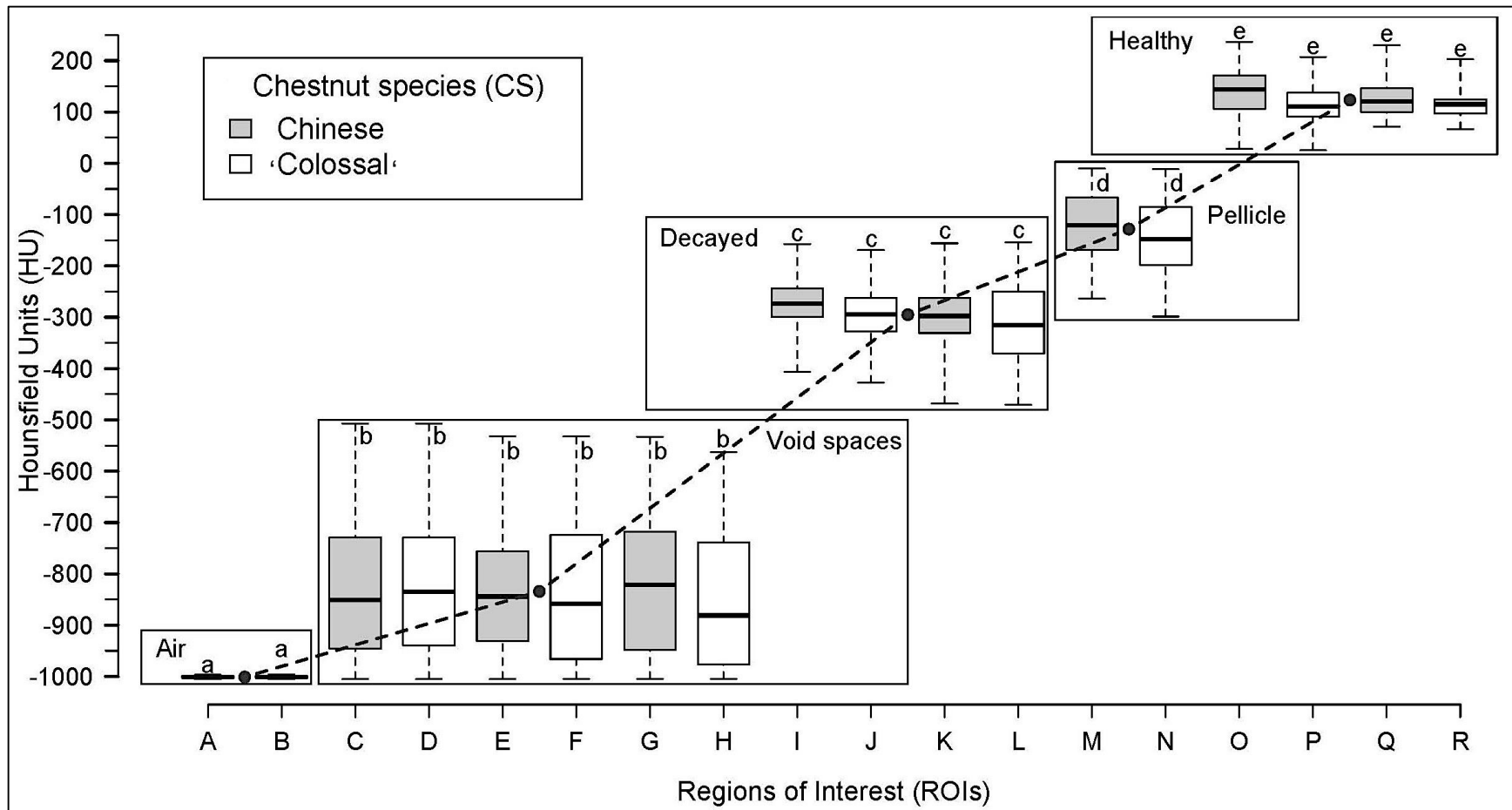


Figure 17. Box-plots showing the HU values from ROIs obtained using a training data set. The median is represented as a thick horizontal black line, upper and lower quartiles as a box with the maximum and minimum measurements as lines protruding from these. Box-plots followed by the same letter and enclosed by the same rectangle are not significantly different between each other at $P = 0.05$ (ANOVA) (Tukey multiple comparison of means). Solid circles joined by a dashed black line (---) show the relationship between the mean of the HU values for each ROIs and subsequently each category.

Fig. 17 offers a clear outline of the changes and variation of HU values associated with changes in internal characteristics within samples and between different SL. Supporting these observations, data showed that positive values, higher than 24.89 HU (density > 100.0 kg m⁻³), could be observed in areas containing healthy tissue. Negative HU-values, between -2.52 and -1001.08 HU (density < 100.0 kg m⁻³), correspond to the existence of pellicle, decayed and void spaces. Similar results were described in other studies, where moisture content and density changes in fruits (Barcelon et al., 1999b; Barcelon et al., 1999a) and wood (Fromm et al., 2001), were reflected in significant HU value differences.

Regions of interest that are not statistically different can be grouped in the same categorical variables as seen in the boxes around the ROIs in Fig. 17 and summarized in Table 7. Information from Fig. 17 was used to generate preliminary thresholds separating each neighboring category. These thresholds were developed by taking in account the minimum and maximum HU values for each category. ROIs followed by the same letter and enclosed by the same rectangle in Fig. 17 are encompassed in the same category since they are not significantly different between each other at P = 0.05. Fig. 17 also includes solid black dots located in the mean HU value per category, joined by a dashed line (--) to observe the change in HU values in between categories.

Analyzing the statistical difference among ROIs in Fig. 17 revealed five categories, which separately categorize sampled ROIs containing air, void spaces, decayed tissue, embedded pellicle and healthy tissue as summarized in

Table 7. Significant changes, spread and high variability of HU values, based on the different ROIs can also be observed in Fig. 17, which indicated an increase in HU value across the categories. From Fig. 17, it is shown that the HU values obtained from the ROIs are independent of CS and SL, due to the fact that only the sampled region, which reflects changes on internal characteristics or chestnut internal components, determines the changes in categories. Seeing that it has been repetitively demonstrated that HU values are highly correlated to matter bulk density, as reported by several scientists, including Fromm et al. (2001) and Bushberg et al. (2002), it can then be confirmed that the significant change in HU value means between the categories, is due to the changes in tissue density within the chestnut. Even though this result seems obvious, because of the principals of CT X-ray technology, it is important to mention that it indicates that the HU values can be used as an index to segregate and group chestnuts, not only based on their internal characteristics or components, but also because the technology is sensitive enough to accurately quantify chestnut kernel density changes. Density is considered an important quality parameter for chestnut as well as for other commodities, as previously expressed by Barcelon et al. (1999a). By rapidly and accurately measuring commodity density, some other problems might also be predicted such as insect damage, mechanical injuries, and firmness.

By observing Fig. 17, it can be concluded that physical and/or chemical changes in the chestnut kernel, some caused by internal disorders and others by the presence of void spaces and pellicle embedded in the healthy kernel tissue, result in the HU values declining in relationship with degraded tissue health. It

can also be seen that by comparing between categories and evaluating the mean HU value in combination with the total HU value range for each of the categories, a threshold can be set to accurately separate healthy tissue from pellicle as well as healthy tissue from decayed or disordered tissue. Pellicle can somewhat be separated from decayed tissue but can accurately be segregated from void-spaces and air. Decayed tissue can additionally be separated from both void-spaces and air. Finally, no well-defined threshold can be specified between air and void-spaces.

3.4.2 Chestnut categories prediction using an independent testing data set

Threshold value testing, summarized in Fig. 18 was useful to determine how well the predicted HU values can forecast chestnut components. Fig. 18a shows the prediction of the categories from an independent testing HU values data set, using the estimated thresholds. The selected HU value thresholds, which include lower (L) and upper (U) HU values for all the categories, can also be found in Table 7. Figure 18b includes the confusion matrix corresponding to this prediction procedure. Each column of the matrix represents the occasions in a predicted category, while each row represents the instances in an actual category. This matrix is beneficial, because it visibly documents the misclassified categories (Shapiro and Stockman, 2001). Additionally, Fig. 18c shows representative images, containing a labeled image generated from its corresponding CT image, based on HU value thresholds. In Fig. 18c, color images are also included as a quality reference. Labeled images were automatically generated using the region-growing tool in Osirix Imaging Software

V3.6.1 (<http://www.osirix-viewer.com/>). This tool is used in the medical field to isolate or label structures in a CT image automatically after the user has indicated lower and upper HU value bounds that will be labeled, as specified by Wang and Smedby (2010). As an example, the healthy tissue is yellow labeled (white in gray scale image), after setting the lower bound at 25 HU and the higher bound at 300 HU. All other categories are comparably labeled, with respective threshold intervals and varying colors.

Results from Figs. 18a and 18b, show that the overall accuracy rate for all the categories prediction, using independent testing data is equal to 90.6 %. The highest accuracy rates were obtained from the prediction of the air category, and the healthy category, where 100.0 % of the samples can be separated from all of the other categories. Void spaces can be separated from the other categories with a 96.0 % accuracy rate, because 4.0 % of the data points were recognized as air. The lowest accuracy rate is present when trying to differentiate between decayed tissue and pellicle, with an average accuracy value equal to 78.6 %. Here, 20.0 % of the decayed tissue was mistakenly predicted as pellicle, and 23.0 % of the pellicle was wrongly categorized as decayed tissue.

Fig. 18c, indicated that regardless of the SL, XY-plane 2D CT images could be used to accurately categorize chestnut components and air, based on selected thresholds. Positively, decayed tissue is always isolated from healthy tissue and both categories are never misclassified. On the other hand, if closely observed, it can be seen that slight misclassification can be observed between decayed tissue and pellicle, as well as void spaces and air, especially if categories are mixed within each other, as seen in Fig. 18c(SL2) and 18c(SL3).

In addition, Fig. 18c(SL1) clearly states that certain tissues could be categorized incorrectly (healthy tissue as pellicle), due to artifacts and noise that occur near category transitions; broadly known as beam hardening effects (Bushberg et al., 2002).

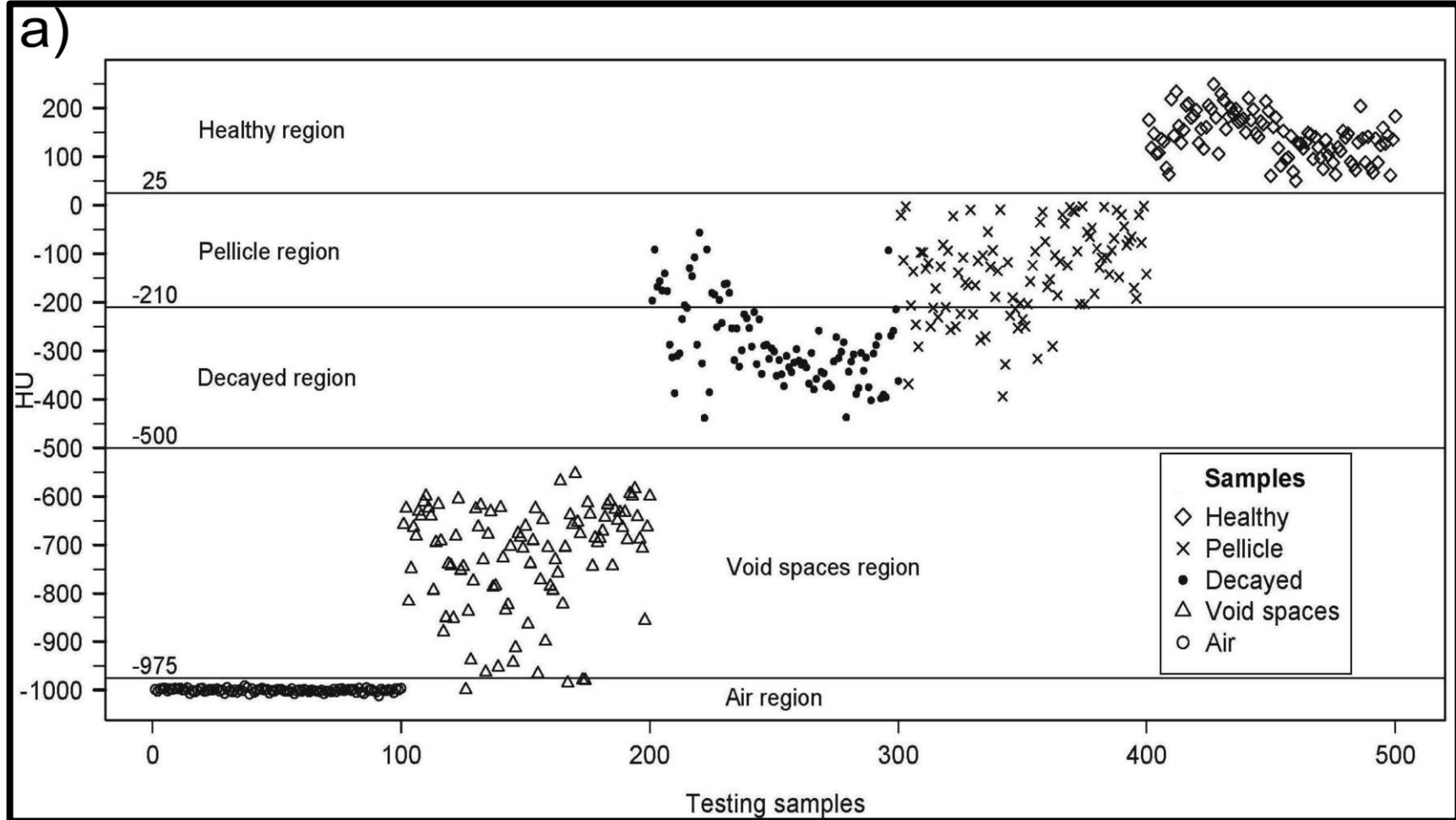
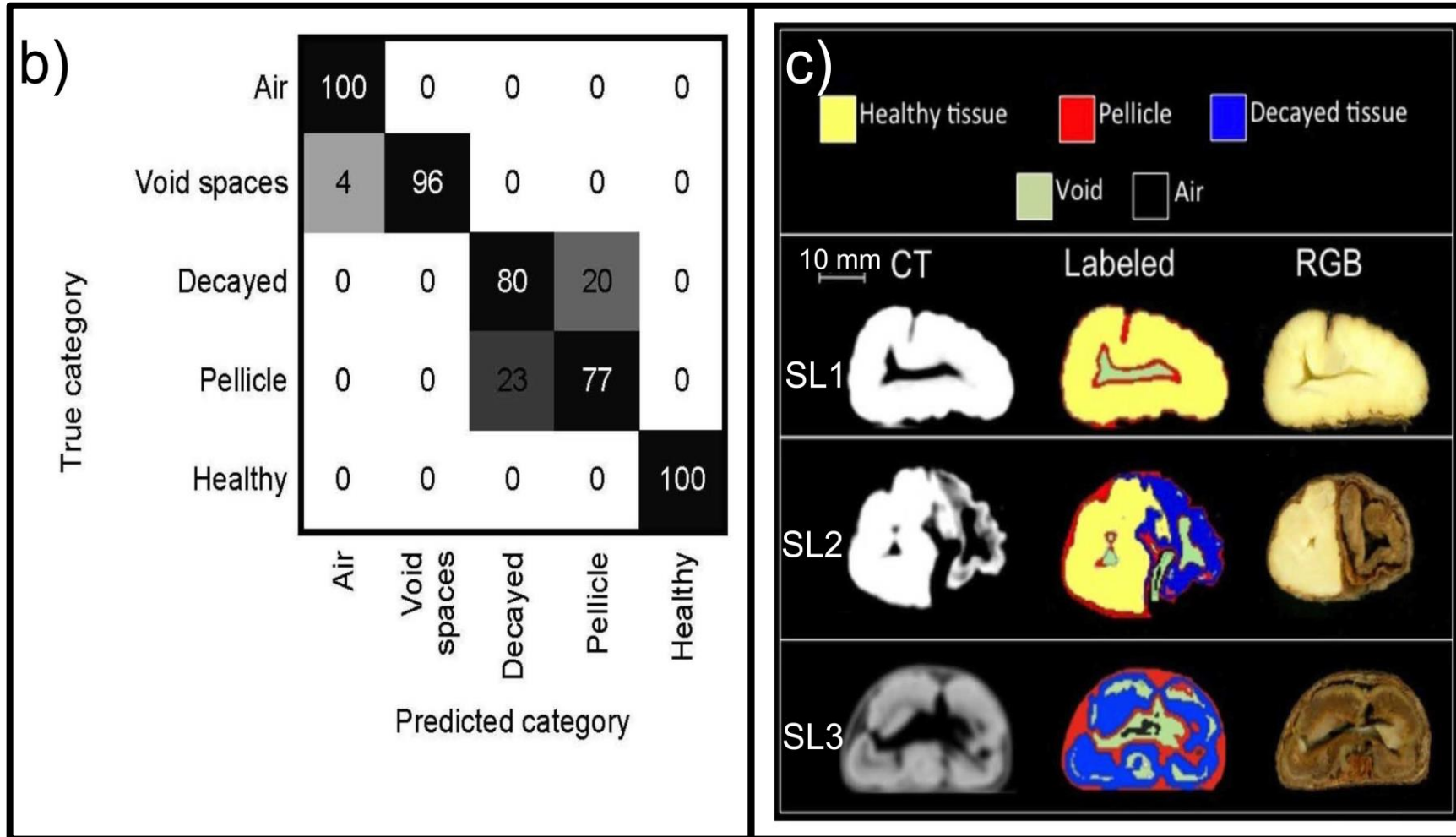


Figure 18. (a) Category prediction using estimated thresholds (horizontal black lines) with an independent testing data set. HU threshold values are indicated above each threshold line. The selected HU value threshold range for air, void spaces, decay tissue, pellicle tissue, and healthy tissue are -1024 to -975, -974 to -500, -400 to -210, -209 to 24, and 25 to 300; respectively.

Figure 18 (cont'd)



(b) Confusion matrix corresponding to the category prediction accuracy (overall accuracy rate = 90.6%). (c) Representative images, containing automatically generated color labeled images from their corresponding CT images, based on HU-value threshold intervals. RGB raw image slices are also included as a quality reference.

3.5 Discussion

Results show that CT technology can be used as a technique that will be able to visualize and measure macroscopic changes in chestnuts components. By doing this, the technique might be able to detect possible negative effects of mechanical harvesting and pre-harvest treatments, cultivar characterization and morphology (chestnut peelability), physiological changes during storage, and early pathogen development. In addition, the data presented in this study is essential for developing classification algorithms to sort chestnuts based on their internal characteristics. HU values obtained from XY-plane 2D CT images are used as a reference to determine the presence of internal components of chestnuts, which will be useful for developing future prediction models of chestnut internal quality. Nonetheless, results clearly indicated, that in addition to raw HU values, other methods related to image processing, pattern recognition (Wulfshohn et al., 1993; Kavdir and Guyer, 2007), and feature extraction (Kim and Schatzki, 2000), will be a requirement to aid the development of future sorting algorithms, and will be necessary to accurately separate void-spaces from air as well as pellicle from decayed tissue. Transferring this tool to different commodity industries will enable them to control and promote the quality of their products. In addition, for the chestnut and other fruits, vegetables, and nuts, this application will provide the opportunity to select products according to their final use.

Further research is needed, to develop sorting algorithms (see Chapter 4) and to investigate optimal equipment settings for real-time sorting potential, as

well as determine hardware capabilities, sorting speed, and system cost considerations (see Chapter 5).

3.6 Conclusion

CT imaging provides high-resolution and high-contrast images of the internal structure and components of fresh chestnuts. Internal component prediction accuracy equal to 90.6% was achieved by using HU values as predictors. In future studies, sorting algorithm speed, equipment cost and characteristics, as well as other methods related to image processing, feature extraction and pattern recognition will be useful for the development of reliable sorting algorithms and CT sorting systems. More studies must be pursued to evaluate the accuracy of sorting algorithms and in-line classification (Chapter 4).

The main advantage of using HU values with the aid of other algorithms will be that CT is a fast, non-invasive procedure that has the potential to be adapted for quality evaluation and in-line sorting. In general, this study indicated that CT has a high potential for nondestructively evaluating internal components of fresh chestnuts, which are attributes associated with chestnut kernel quality.

CHAPTER 4. POSTHARVEST NONINVASIVE ASSESSMENT OF FRESH CHESTNUT INTERNAL DECAY USING COMPUTED TOMOGRAPHY (CT) IMAGES

4.1 Abstract

X-ray CT is an effective noninvasive tool to visualize fresh agricultural commodities' internal components and quality attributes, including those of chestnuts. There is no reported procedure to automatically, effectively and efficiently classify fresh commodities from a continuous in-line flow through a CT system. If the information obtained by CT scanning of fresh agricultural commodities is to be used in an industrial application (e.g. in-line sorting), automated interpretation of CT images is essential. For this purpose, an image analysis method (algorithm) for the automatic classification of CT images obtained from 2848 fresh chestnuts (cv. 'Colossal' and Chinese seedlings), during the harvesting years from 2009 to 2012, was developed and tested. Classification accuracy was evaluated by comparing the classes obtained from six secondary CT images per chestnut, derived from raw CT images, to their internal quality assessment. Internal quality assessment was done by an experienced human rater by visually and invasively rating fresh chestnut internal decay severity (quality) into 5-, 3- and 2- classes.

After CT image preprocessing, cropping and segmentation, 1194 grayscale intensity and textural features were extracted from the six resultant CT images per sample. Relevant features were selected using a sequential forward selection algorithm with the Fisher discriminant objective function. 86, 155 and 126

features were effective in designing a quadratic discriminant classifier with a 4-fold training for the 3 different class scenarios. Performance accuracy of 85.9 %, 91.2 % and 96.1 % for 5, 3 and 2 classes was found, respectively. Results show that this method is accurate, reliable, and objective in determining fresh chestnut internal quality, and it is applicable to an automatic noninvasive in-line CT sorting system.

4.2 Introduction

Researchers agree that the absence of sorting commodities in industry using in-line CT systems is primarily due to their limitations in gathering useful information, and optimum data handling, when operating at high-speed (Butz et al., 2005). More specifically, if CT in-line systems were to be developed, little is currently known about how to efficiently handle the high amount of acquired data, while continuously scanning. In addition, there is no available procedure reported to automatically, effectively and efficiently classify fresh agricultural commodities from a continuous in-line flow of entities going through a CT scanning system.

Pattern recognition algorithms, which are an intrinsic and an important part of computer vision systems, offer a mechanism of classifying commodities based on their quality attributes, and can be applied to CT systems. In general, systems are trained from patterns of interest extracted from a set of images, representing different quality categories. A pattern or feature is represented by a group of textural and image intensity features, which are able to define all of the quality categories. The system then assigns a new image series to a specific quality category or class (Duda et al., 2000). The first step consists of extracting a high

number of features (patterns) from the different categories of known images. Features then must be selected by their capacity of correctly separating the images into different categories, therefore training the system, and allowing it to automatically classify new images. Classification is done using statistical and clustering algorithms by assigning images per sample, to its corresponding class (Duda et al., 2000; Mery and Soto, 2008). The reader may refer to complete information regarding statistical pattern recognition methods, which have been extensively described in several publications, including Jain et al. (2000), Duda et al. (2000), Bishop (2007), and Holmström and Koistinen (2010). Steps used to generate the pattern classification algorithm to categorize chestnut quality using CT images are illustrated in Fig. 19, and in depth explained in the materials and methods section.

Therefore, the aim of this study is to describe a reliable method that will systematically, rapidly, and efficiently classify a fresh agricultural commodity (i.e. chestnuts) using CT images, obtained from a commercially available medical grade CT system. This classification method is unique because it automatically uses a set of CT images to determine the overall quality of fresh chestnuts, without the aid of a human sorter. This research offers a novel tool, which can methodically classify chestnuts from high-throughput 2D CT images. In addition, the developed algorithm can be used to study the potential development of an in-line CT system, and can be accessed by scientists to automatically and efficiently classify CT acquired images, in other domains. This automatic classification algorithm is a critical step in the development of a fast, in-line, nondestructive CT system, capable of assessing fresh internal quality attributes

and components of individual chestnuts, other agricultural commodities, in addition to other objects of interest (e.g. airplane parts).

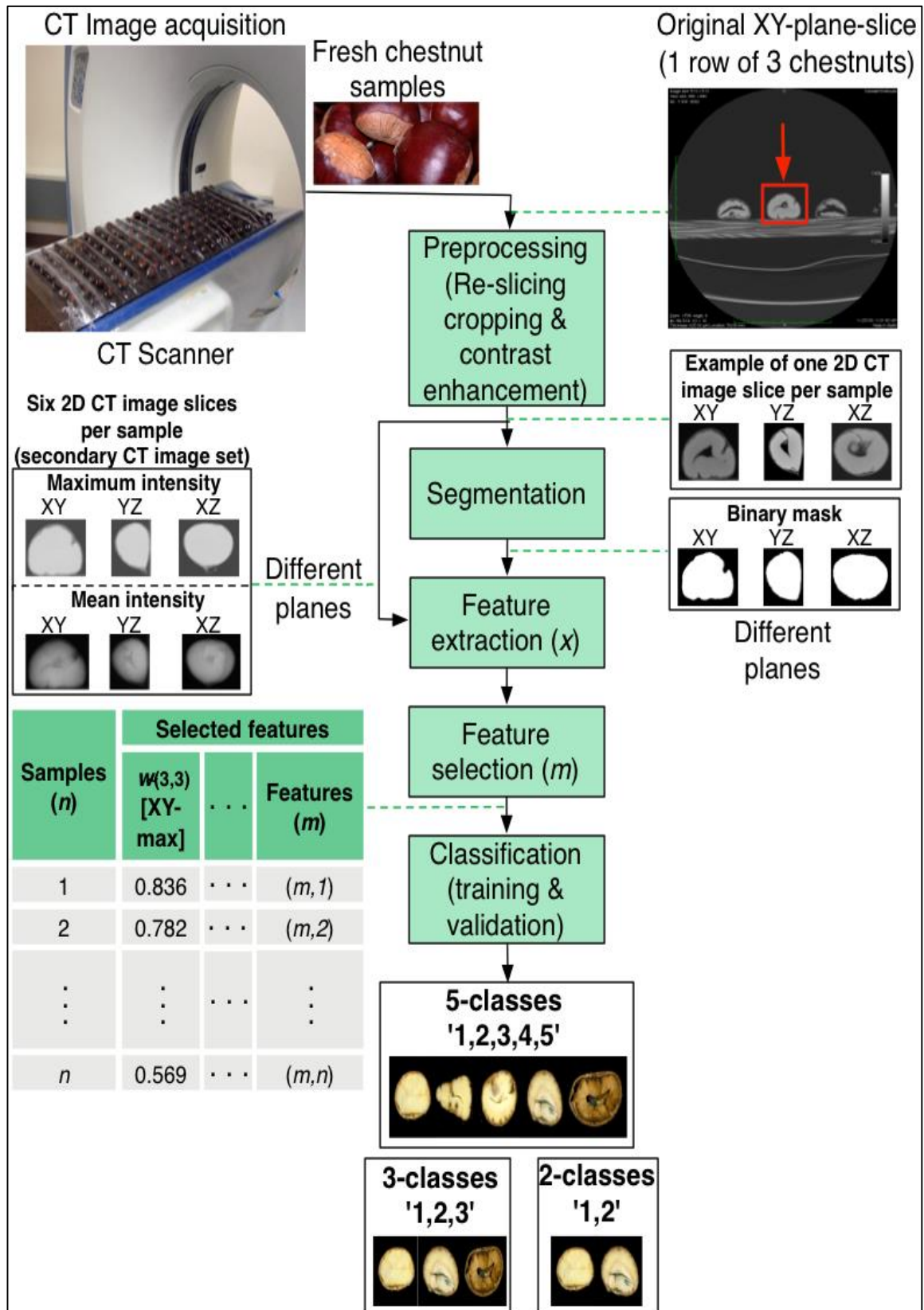


Figure 19. Procedure used to generate the pattern classification algorithm to categorize chestnut quality using CT images.

4.3 Materials and methods

4.3.1 Sample collection and preparation

A total of 1424 physiologically mature Chinese seedlings (*C. mollissima*) and 1424 (*C. sativa* x *C. crenata*) cv. 'Colossal' chestnuts (Total of 2848) were obtained from Chestnut Growers Inc. (CGI; Grand Haven, Michigan, USA). Chestnuts were collected from seven commercial farms in Michigan. Equal numbers of chestnuts were collected every year, from the 2009 through the 2012 harvesting season (September-October). Following common postharvest procedures samples were treated, after each collection year, as described in section 2.3.1. Samples were then handled as described in section 3.3.1, with the objective of attempting to generate a uniform distribution of a diverse range of internal characteristics (different classes of decay) needed for this study. Every year, after approximately 90 storage days (4 °C), chestnuts were similarly attached (facing toward the same direction) to rectangular polyethylene boards (915 mm x 335 mm x 2.8 mm), in 19 rows containing 7 chestnuts (133 total), as described in section 3.3.1 and seen in Fig. 3b. CT scans were then conducted on each board following the procedure described in section 3.3.2, only that in this case, instead of using the parameters corresponding to a slice thickness (d) of 0.625 mm (visualization purposes), a d equal to 2.5 mm was used, as concluded during the CT image optimization procedure in Chapter 1.

4.3.2 CT image preprocessing:

Image preprocessing (re-slicing, cropping and contrast enhancement), image visualization, segmentation, feature extraction, statistical analysis, and the automatic classification/validation for this study were done in MATLAB (2012a, The MathWorks, Natick, MA, USA) (<http://www.mathworks.com>), and in the language and environment for statistical computing software R (V2.10.0, R Development Core Team, Vienna, Austria) (<http://cran.r-project.org/>), using a Macintosh environment on a Lion operating system with 2.53 GHz Intel Core 2-Duo, 8 GB random access memory (RAM), 1067 MHz double data rate 3 (DDR3) (Apple Inc., Cupertino, California, USA). Feature extraction, feature reduction, and the automatic classification/validation for this study were performed by partially using the “Balú” free toolbox for pattern recognition (<http://dmery.ing.puc.cl/index.php/balu/>), developed by the Department of Computer Science at the Pontifical Catholic University of Chile (Santiago, Chile). This toolbox contains more than 200 functions for image processing, feature extraction, feature transformation, feature analysis, feature selection, classification, clustering, performance evaluation, image sequence processing, and more.

4.3.3 CT image re-slicing

Because of the intrinsic 3D characteristic of CT imaging, CT image slices were re-sliced following the steps described in section 1.5 (Fig. 5). Depending on chestnut physical size and d , each chestnut contains between 8 to 17 XY-, YZ-

and XZ-plane-slices representing virtual cross-sections of a chestnut along the Z, X and Y axes.

4.3.4 Individual chestnut CT image cropping

Chestnut rows and individual chestnuts were visually/manually cropped from the overall CT data set containing the scanning table, volume of air and other chestnuts, by determining their spatial location, as shown in Fig. 20. Pixels in Fig. 20a (entire board XZ-plane-slice) correspond to the mean intensity value of all pixels at the same planer location (x,z) in the XZ-plane-slices stack. In Fig. 20a, Z_1 - and Z_2 -spatial-location-values are manually determined to crop each chestnut row, generating Fig. 20b (row XZ-plane-slice), 20c (row XY-plane-slice) and 20d (row YZ-plane-slice). To crop each individual chestnut, X_1 -, X_2 -, Y_1 -, and Y_2 -spatial-location-values are manually inferred, as seen in Fig. 20a, 20b, 20c and 20d. Pixels in Figs. 20a-20d, correspond to the mean of all pixel values at the same planer location $((x,y), (y,z) \text{ or } (z,x))$ within each plane in the chestnut stack.

For each chestnut that is re-sliced making up the three different image stacks per plane, a total data set of approximately 50 raw CT image slices of about 50 x 50 pixels each (depending on chestnut physical size) are generated. For further analysis, data set dimensionality is then reduced from these original 50 raw CT images per chestnut sample to 6 resultant CT images per sample (secondary CT image set – Fig. 19). To accomplish this reduction, pixels in final cropped chestnuts, as seen in Fig. 20e (mean XZ-plane-slice), 20f (mean XY-plane-slice) and 20g (mean YZ-plane-slice) are generated from the mean

intensity value of all pixel values at the same planer location $((x,y), (y,z)$ or (z,x))
in all of the XZ-, XY- or YZ slices per chestnut.

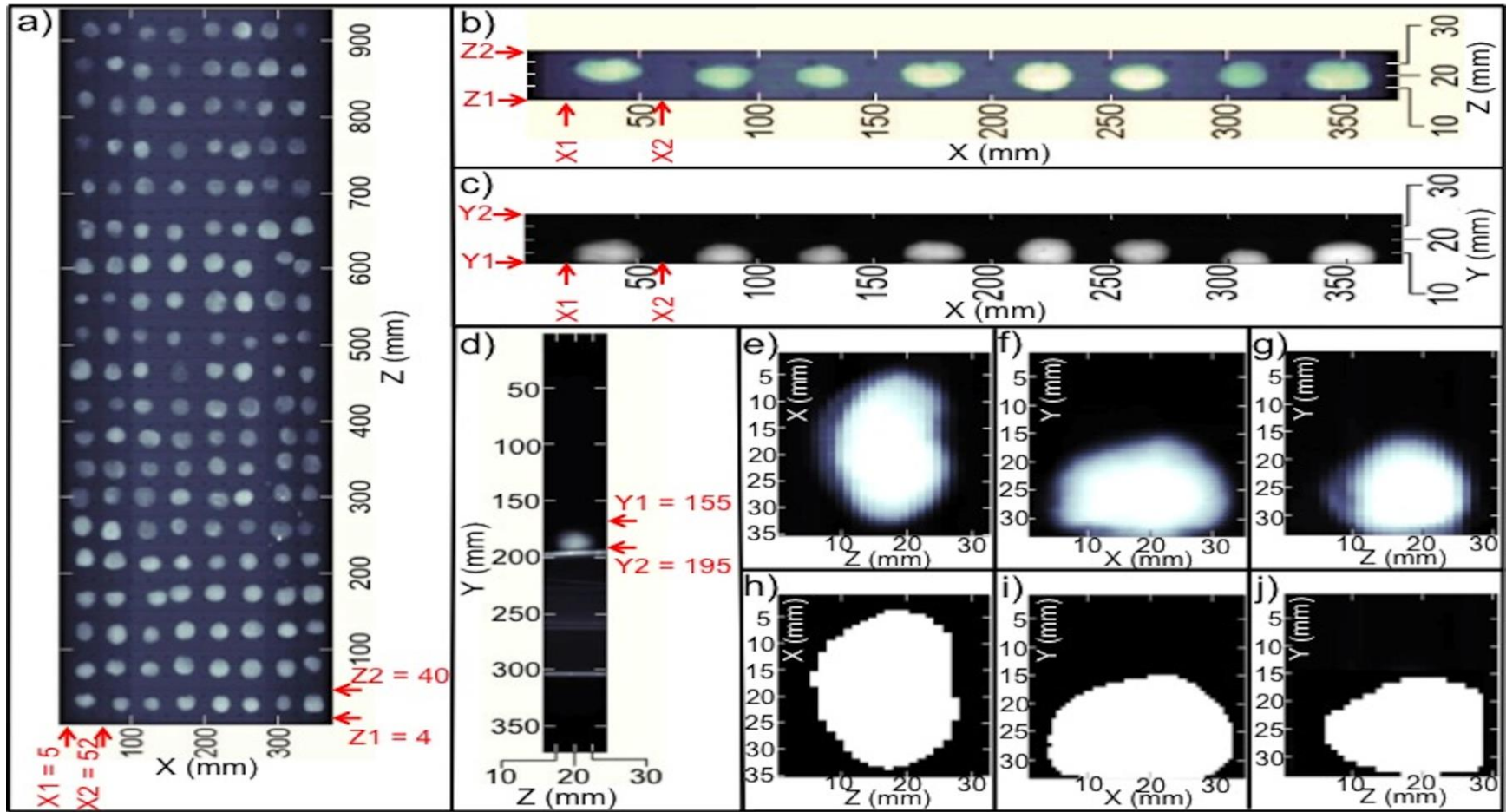


Figure 20. Mean intensity values of pixels in all of the: (a) YZ-plane-slice of the whole board containing chestnuts, (b) YZ-plane-slices for the first chestnut row, (c) XY-plane-slices for the first chestnut row, (d) XZ-plane-slices for the first chestnut row. Mean intensity values of pixels in final secondary CT cropped chestnut for the (e) YZ-, (f) XY- and (g) XZ-plane-slices. Maximum intensity values of pixels in final secondary CT cropped chestnut for the (h) YZ-, (i) XY- and (j) XZ-plane-slices. The beginning of the first (Z1-), the end (Z2-) of the row, the left (X1-), the right (X2-) side, the bottom (Y1-) and the top (Y2-) of the first chestnut -spatial-location-values are shown in red.

An additional example of a resultant mean CT image slice, viewed from the XZ-plane can also be seen in Fig. 21b. In addition, cropped chestnuts are also used to generate the second 3 resultant images based on the maximum intensity value of all pixel values at the same planer location $((x,y), (y,z) \text{ or } (z,x))$ in all of the reconditioned XZ-, XY- and YZ-plane-slices per chestnut, as shown in Figs. 20h (maximum XZ-plane-slice), 20i (maximum XY-plane-slice), and 20j (maximum YZ-plane-slice). An example of another resultant maximum CT image slice, viewed from the XZ-plane is also included in Fig. 21f. Therefore, three mean and three maximum intensity value CT images from the three different planes (XY, XZ and YZ) are obtained per chestnut sample (total of 6).

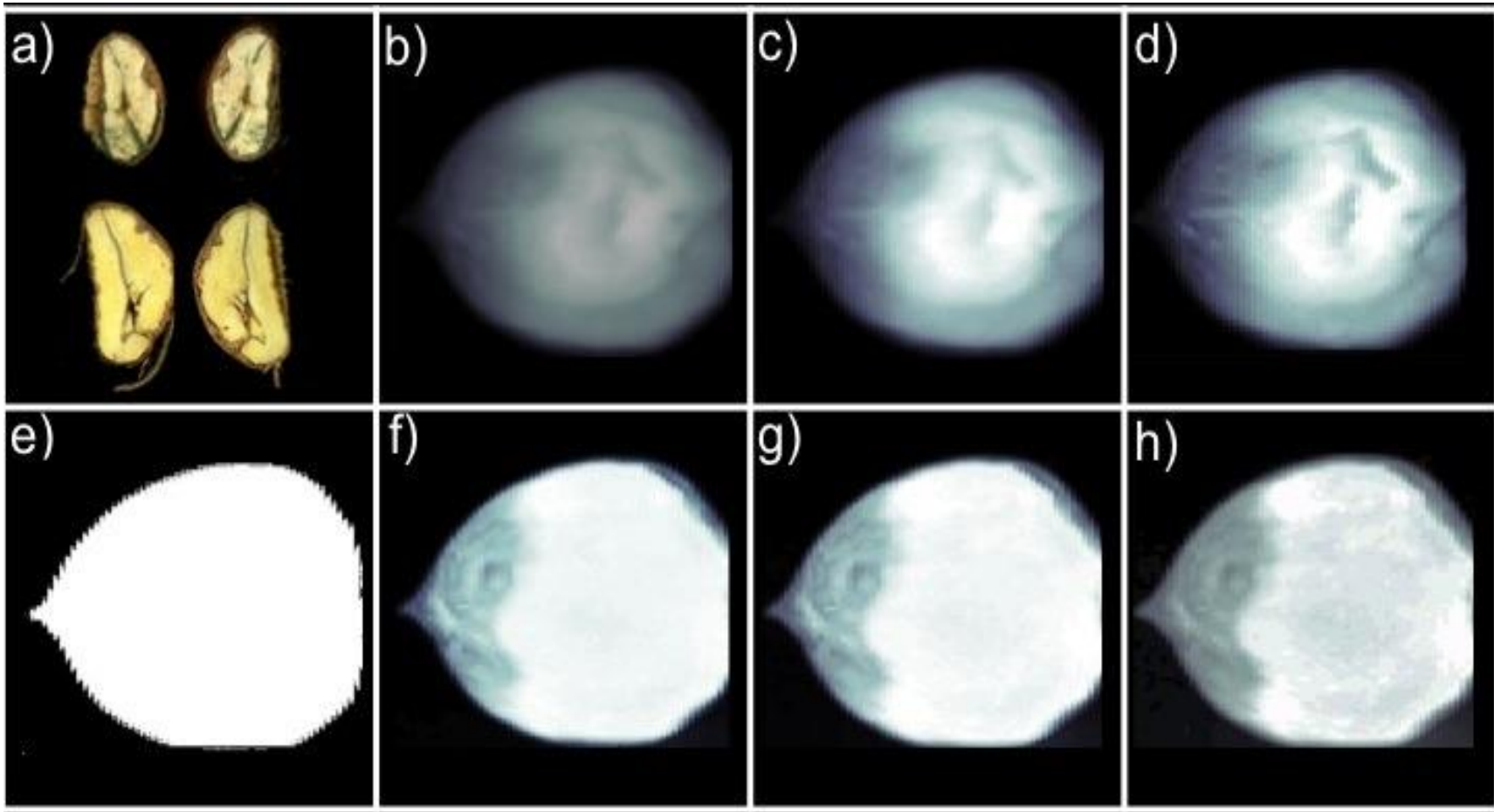


Figure 21. (a) Partially decayed fresh raw chestnut slices. (b) Original secondary mean CT image (YZ-plane-slice), (c) adjusted secondary mean CT image (YZ-plane-slice), (d) final contrast enhanced secondary mean CT image (YZ-plane-slice). (e) Segmented CT image from final contrast enhanced secondary mean CT image (YZ-plane-slice) (binary mask). (f) Original secondary maximum CT image (YZ-plane-slice), (g) adjusted maximum secondary maximum CT image (YZ-plane-slice), and (h) final contrast enhanced secondary maximum CT image (YZ-plane-slice).

4.3.5 Contrast enhancement

The most important characteristic in CT X-ray images is image contrast. Image contrast is the difference in luminance of the grayscale intensity values (separation between the darkest and brightest regions of an image) that makes the representation of different structures in a CT image discernable (Wang et al., 1983). This includes the different tissue types reflected in chestnut CT images (Donis-Gonzalez et al., 2012). Contrast enhancement is a vital step in image processing, and it is done to increase image quality (Zimmerman et al., 1998; Jagannath et al., 2012). Multiple techniques exist, which are used to improve digital image contrast, including morphological operations enhancement, as described in Sreedhar and Panlal (2012).

In this study, a set of image preprocessing steps were implemented to increase secondary CT image contrast, based on morphological enhancement as described in Wirth et al. (2004). First, image contrast is improved in each of the original secondary CT images (Fig. 21b and 21f) by creating an adjusted mean and maximum secondary CT image as exemplified in Fig. 21c and 21g, respectively. This first step is done by mapping the original outputted secondary CT image grayscale values (Fig. 21b and 21f), so that 1 % of their pixels at low and high intensities (2 % in total) are saturated (limited to lowest and maximum pixel values). Second, a top-hat operation (high grayscale intensity regions) using a 5-neighbors disk shaped flat structuring elements (SE) is applied to the adjusted secondary CT images (Fig. 21c and 21g), generating the top-hat image. Third, a bottom-hat operation (low grayscale intensity areas) using the same SE

as described in previous step is also applied to the secondary CT adjusted images (Fig. 21c and 21g), creating the bottom-hat image. The form and size of the SE is appropriate because of the natural shape of the chestnuts, reflected in the CT images. Finally, contrast enhanced secondary CT images are produced as illustrated in Fig. 21d (mean) and Fig. 21h (maximum) by adding the top-hat image to its corresponding adjusted image and subtracting its linked bottom-hat image (i.e. adjusted image + top-hat image – bottom-hat image). This stretches the high intensity areas toward increased intensity, while low intensity regions are stretched towards decreased intensity.

4.3.6 CT image segmentation (Binary mask):

Image segmentation is implemented to recognize the region of interest in an image, which in this case is the chestnut in each CT image. CT image segmentation was done by using the balanced histogram thresholding method, as described in Anjos and Shahbazkia (2008). This is a simple histogram based thresholding procedure broadly used for automatic image threshold. This methodology assumes that the CT image is divided in two main classes, (1) the foreground (chestnut CT image) and (2) the background (air). To do so, the methodology finds the optimum threshold level (first minimum grayscale value in mean CT image histogram) from a CT mean slice, which will divide the image into the two classes. Example of a segmented image for one plane, using this routine, can be found in Fig. 21f.

4.3.7 Visual based fresh chestnut quality and internal component assessment:

Raw color sliced internal faces were color scanned, as described in section 2.3.3, for record keeping. All chestnuts were then qualitatively assessed for disorders, void spaces, and embedded pellicle. All chestnuts were then categorized based on their internal disorder severity level into five, three and two categorical groups as described in section 4.1.8 and visualized in Figs. 23, 24, and 25, respectively. An additional representative example of cross-sectional raw color images in the fresh state is shown in Fig. 21a. In these images, fresh slices of a partially decayed chestnut can be viewed.

4.3.8 Feature extraction:

In general, feature extraction involves algorithms that detect and isolate desired information from segmented images, including CT images (Nixon and Aguado, 2008). The idea is to use the extracted features to decide to which quality class does a chestnut belong. The type of extracted features play an intrinsic and very important role in classifying images appropriately (Jain et al., 2000).

In this study, features were extracted from the six contrast enhanced 16-bit secondary CT intensity images per chestnut. A total of 199 features were extracted per secondary/resultant CT image, and then features from the six resultant CT images were concatenated to form a feature vector (x) with 1194 components, as partially illustrated in Fig. 19. Extracted features per CT image included: (1) 6 basic intensity features, (2) 26 Haralick textural (T_x) features, (3)

95 intensity local binary pattern (LBP) features, (4) 67 Gabor intensity textural features, and (5) 5 contrast features.

- Basic intensity features:

Six standard features, describing overall image intensity information, were derived from the segmented image region, for each of the contrast enhanced secondary CT grayscale images. Standard features included the mean (μ) – Eq. 4-1, standard deviation (σ) – Eq. 4-2, kurtosis (k) – Eq. 4-3, skewness (s) – Eq. 4-4, mean gradient [first-order derivative ($f'(x_{(i,j)})$)] – Eq. 4-5, and mean Laplacian [second-order derivative ($f''(x_{(i,j)})$)] – Eq. 4-6 (Shapiro and Stockman, 2001; Nixon and Aguado, 2008; Mery et al., 2011).

$$\mu = \frac{1}{n} \sum_{i=1}^n x_{(i,j)} \quad (4-1)$$

$$\sigma = \sqrt{\frac{1}{n} \sum_{i=1}^n (x_{(i,j)} - \bar{x})^2} \quad (4-2)$$

$$k = \frac{\frac{1}{n} \sum_{i=1}^n (x_{(i,j)} - \bar{x})^4}{\left(\frac{1}{n} \sum_{i=1}^n (x_{(i,j)} - \bar{x})^2 \right)^2} \quad (4-3)$$

$$s = \frac{\frac{1}{n} \sum_{i=1}^n (x_{(i,j)} - \bar{x})^3}{\left(\frac{1}{n} \sum_{i=1}^n (x_{(i,j)} - \bar{x})^2\right)^3} \quad (4-4)$$

$$f'(x_{ij}) = \frac{x_{(i,j)+1} - x_{(i,j)}}{((i,j) + 1) - (i,j)} \quad (4-5)$$

$$f''(x_{ij}) = \frac{f'(x_{(i,j)}) - f'(x_{(i,j)-1})}{((i,j) + 1) - (i,j)} \quad (4-6)$$

where, $x_{(i,j)}$ represents the grayscale value of pixels in each of the segmented CT images, and n the total number of evaluated pixels ($i \times j$).

- Haralick textural (Tx) features:

Twenty six Tx features were extracted from each of the contrast enhanced secondary CT grayscale images in order to obtain information about their intensity values distribution. To obtain these Tx features, first a co-occurrence matrix (C) (Eq. 4-7) is computed per CT image, which represents the joint probability distribution of intensity pairs of neighboring pixels.

$$C_{\Delta x, \Delta y}(n, m) = \sum_{i=1}^i \sum_{j=1}^j \begin{cases} 1, \text{ if } I(i, j) = Ng \text{ and } I(i + \Delta x, j + \Delta y) = Ng \\ 0, \text{ otherwise} \end{cases} \quad (4-7)$$

where, C is defined over an $i \times j$ CT image (I), parameterized by an offset ($\Delta x, \Delta y$).

This matrix is square with dimensions n (row) $\times$ m (column) (Eq. 4-8),

represented by the number of gray intensity level in the CT image (N_g), which is equal to 2^{16} (65536) in a 16-bit CT image. As it can also be seen in Eq. 4-7, an element (n,m) of C is generated by counting the number of times a N_g in the i -pixel is adjacent to an N_g in the j -pixel. Since, adjacency in a 2D image can occur in four directions or offsets - $(\Delta x, \Delta y)$ (horizontal, vertical, left and right diagonals) four C -matrices can be calculated.

$$C = \begin{bmatrix} c(1,1) & \cdots & c(1,N_g) \\ \vdots & \ddots & \vdots \\ c(N_g,1) & \cdots & c(N_g,N_g) \end{bmatrix} \quad (4-8)$$

Subsequently, basic T_x statistical features as described in Eqs. 4-9 – 4-23 were computed (Table 8). Both mean and range values for each of the T_x features, for a mask containing five different neighboring pixels, were processed. Since rotation invariance is an important criterion for features extracted from the CT images, invariance was accomplished for each of these statistics, by calculating the mean over the four directional C -matrices (4 offsets).

Table 8. Haralick textural (Tx) features (Haralick, 1979)

Tx feature	Formula
1. Angular Second Moment	$\sum_n \sum_m C(n, m)^2 \quad (4-9)$
2. Contrast	$\sum_{i=0}^{N_g-1} i^2 \left\{ \sum_{n=1}^{N_g} \sum_{m=1}^{N_g} C(n, m) \right\}, n - m = i \quad (4-10)$
3. Correlation	$\frac{\sum_n \sum_m (nm) C(n, m) - \mu_x \mu_y}{\sigma_x \sigma_y} \quad (4-11)$ where μ_x, μ_y, σ_x and σ_y are the means and standard deviation of C_x and C_y, the partial probability density functions.
4. Sum of Squares: Variance	$\sum_n \sum_m (n - \mu)^2 C(n, m) \quad (4-12)$
5. Inverse difference moment	$\sum_n \sum_m \frac{1}{1 + (n - m)^2} C(n, m) \quad (4-13)$
6. Average sum	$\sum_{n=2}^{2N_g} i C_{n+m}(n) \quad (4-14)$ where n (row) and m (column) are the coordinates of an entry in the C, as described in Section 2.6.2. $C_{n+m}(i)$ is the probability of C coordinates summing to $n + m$.
7. Entropy sum (f_7)	$- \sum_{n=2}^{2N_g} C_{n+m}(n) \log\{C_{n+m}(n)\} \quad (4-15)$
8. Variance sum	$\sum_{n=2}^{2N_g} (i - f_7)^2 C_{n+m}(n) \quad (4-16)$

Table 8. (cont'd)

Tx feature	Formula
9. Entropy (HXY)	$-\sum_n \sum_m C(n, m) \log(C(n, m)) \quad (4-17)$
10. Variance difference	$\sum_{n=0}^{N_g-1} n^2 C_{n-m}(i) \quad (4-18)$
11. Entropy difference	$-\sum_{n=0}^{N_g-1} p_{n-m}(n) \log\{p_{n-m}(n)\} \quad (4-19)$
12. Correlation meas. - 1	$\frac{HXY - HXY_1}{\max\{HX, HY\}} \quad (4-20)$
13. Correlation meas. - 2	$(1 - \exp[-2(HXY_2 - HXY)])^{\frac{1}{2}} \quad (4-21)$ where HX and HY are the entropies of C_n and C_m .
	$HXY_1 = -\sum_n \sum_m C(n, m) \log\{C_n(n)C_m(m)\} \quad (4-22)$
	$HXY_2 = -\sum_n \sum_m C_n(n)C_m(m) \log\{C_n(n)C_m(m)\} \quad (4-23)$

- Intensity local binary pattern (LBP) textural features:

Ninety-five LBP features (classic and semantic) were extracted from each of the contrast enhanced secondary grayscale CT images to compute the relationship between the intensity of each pixel with its eight neighboring pixels, using the occurrence histogram (classic – cLBP), and a histogram clustered by

the pixel intensity value (semantic – sLBP). LBP approach introduces an operator where a neighborhood of up to 36 pixels for the cLBP, and 56 pixels for the sLBP of the images are thresholded in relation to the center pixel value, forming a new binary sub-image (Ojala et al., 2002; Ahonen et al., 2009; Chai et al., 2013) (Eq. 4-24, Fig. 22). The rotations of the selected neighborhoods inside each image increase the number of LBP features, giving a rotation invariant reliable characterization of the image. The values of the pixels in the thresholded neighborhood are multiplied by the binomial weights given to the corresponding pixels. Finally, the values of the eight pixels are summed to obtain the LBP (Pietikäinen et al., 2000) (Eq. 4-25). LBP are defined as:

$$s(Ng_0 \cdot Ng_i) = \begin{cases} 1, & \text{if } Ng_i > Ng_0 \\ 0, & \text{if } Ng_i \leq Ng_0 \end{cases} \quad (4-24)$$

$$LBP_{(d,h)} = \sum_{i=1}^8 s(Ng_0 \cdot Ng_i) 2^{i-1} \quad (4-25)$$

where, Ng_0 is the gray value of the center pixel in the circularly symmetric neighborhood, and Ng_i takes the different eight pixels values from the neighborhood. LBPs have been found to be influential features for texture classification in images (Ojala et al., 2002; Ahonen et al., 2009). Example of cLBP images with different neighborhood size operations for a maximum intensity XY-plane-slice CT image of a partially decayed and a healthy chestnut can be found in Fig. 22.

- Intensity Gabor textural features:

Sixty-seven Gabor features were extracted per contrast enhanced secondary CT grayscale images. The general Gabor function is complex, exponential and modulated by a Gaussian envelope function (Kumar and Pang, 2002).

$$f(x, y) = \frac{1}{(2\pi\sigma_x\sigma_y)} \exp\left(-\frac{1}{2}\left(\frac{x^2}{\sigma_x^2} + \frac{y^2}{\sigma_y^2}\right)\right) \exp(2\pi j u_0 x) \quad (4-26)$$

where, σ_x and σ_y denote the Gaussian envelope along the x and y axes, and u_0 defines the radial frequency. In the frequency domain, the Gabor function acts as a multi-scale and multi-orientation band pass filter with a real and an imaginary component, using the Gaussian function. The self-similar filter banks can be obtained by dilations and rotation of $f(x, y)$ through the generating function, as seen in Eqs. 4-27 – 4-29:

$$f_{rs}(i, j) = \alpha^{-P} f(i', j') \quad (4-27)$$

where,

$$i' = \alpha^{-P} (i(\cos\theta_r) + j(\sin\theta_s)) \quad (4-28)$$

$$\alpha^{-P} > 1; r = 1, 2, \dots, S; s = 1, 2, \dots, L. \quad (4-29)$$

r and s represent the index for dilation (scale) and orientation, respectively. S is the total number of dilatations (scales) and L is the total number of orientations,

θ_r and θ_s are the angle for each r and s . In this study, $S = 8$ and $L = 8$ are used, as proposed by Ng et al. (2005b). By applying the Gabor filter (f_r) to an image $I(i, j)$, the magnitude information or magnitude filtered image ($I_{rs}(i, j)$) is obtained as follows:

$$I_{rs}(i, j) = \{ [f_{rs}(i, j)_e * I(i, j)]^2 + [f_{rs}(i, j)_o * I(i, j)]^2 \}^{\frac{1}{2}} \quad (4-30)$$

where “*” denotes a 2-dimensional convolution operation, and $f_{rs}(i, j)_e$ and $f_{rs}(i, j)_o$ represent the real (even), and imaginary (odd) parts of the Gabor filter, respectively.

After the feature extraction, the k extracted features were arranged in a k – vector: $W = [w_1 \dots w_K]^T$ and normalized (Mery and Soto, 2008):

$$\tilde{W}_{(a,b)} = \frac{w_{(a,b)} - \overline{w}_b}{\sigma_b} \quad (4-31)$$

a takes values from 1 to the number of samples (scales), and b takes values from 1 to the number of features (orientations). $\overline{w}_b$ and σ_b are the mean and standard deviation of the b -th. Gabor feature. Gabor features, extracted from the magnitude filtered images with different scales and orientations may be helpful for extracting useful textural features from an image and important for classification. Gabor features are useful in image processing applications such as

optical character identification, texture recognition in fruits/food, iris and fingerprint recognition (Zhang, 2002; Ng et al., 2005a; Zhu et al., 2007). Magnitude Gabor filtered maximum intensity XY-plane-slice CT images can be found for a healthy and a decayed chestnut in Figs. 22a and 22b, respectively.

- Contrast features:

Image contrast is defined in section 2.3.3. In addition to statistical textural techniques, which can be used to calculate contrast as described in Eq. 4-10, contrast was calculated in each of the contrast enhanced secondary CT grayscale images using additional techniques (5-methods) previously applied to detect defects in aluminum casting X-ray images (Kamm, 1998), as seen in Eqs. 4-32 to 4-34:

$$K_1 = \frac{G - G_N}{G_N} \quad (4-32)$$

$$K_2 = \frac{G - G_N}{G - G_N} \quad (4-33)$$

$$K_3 = \ln\left(\frac{G}{G_N}\right) \quad (4-34)$$

where, related to this study, G is the mean gray intensity value using an eroded segmented region of the originally segmented CT image. This eroded segmented region was obtained by applying an erosion algorithm (*imerode* in MATLAB) to the original segmented region (whole chestnut) using a 15 x 15 disk SE. As in contrast enhancement, this SE is appropriate because of the natural shape and

size of the original segmented chestnuts. GN is the mean gray intensity value of the whole segmented region (whole chestnut).

Other methods of measuring contrast as proposed in Mery (2001), and Mery and Filbert (2002) were also customized to this study. These other two contrast measurements are calculated through inputting values into Eqs. 4-37 and 4-38, obtained from three consecutive steps by: (1) calculating a gray intensity profile in the i -direction (P_1) and in the j -direction (P_2) in relationship to the centroid of the whole segmented image towards the border of the segmented image (background), (2) compute the first order function that contains the first and last points in P_1 and P_2 , therefore eliminating the bias ramp (approaching the background) for each of the profiles (R_1 and R_2), and (3) generate the new profiles (Q_1 and Q_2) without the background ramp, as seen in Eqs. 4-36 to 4-38:

$$Q_1 = P_1 - R_1 \quad (4-35)$$

$$Q_2 = P_2 - R_2 \quad (4-36)$$

$$K_\sigma = \sigma_Q \quad (4-37)$$

$$K = \ln(Q_{max} - Q_{min}) \quad (4-38)$$

where σ_Q , Q_{max} and Q_{min} are the standard deviation, the maximum and minimum value of Q , respectively. For a schematic representation and extended information of the steps required to obtain K_σ and K , refer to Mery and Filbert (2002).

4.3.9 Feature selection:

After feature extraction, it is necessary to select the best features to train the classifier (Mery and Soto, 2008). The purpose of the feature selection step, also known as feature reduction, is to obtain a smaller subset of features (m) from the original data set (x), which will yield the highest classification rate possible (Jain et al., 2000). High dimensionality increases time and space requirements for processing data. Also, in the presence of irrelevant and/or redundant features, classification methods tend to over-fit and become less interpretable, especially when the number of features is much larger than the number of samples. Feature selection algorithms usually involve maximizing or minimizing an objective function (f), whose output can be calculated for the generated m , therefore measuring their classification potential (effectiveness) and working as a feedback signal to select the best features.

In this study, the sequential forward selection (SFS) technique (algorithm), taking feature dependencies into account (eliminates features that are highly correlated $r \geq |0.95|$) (Silva et al., 2002), was selected as a search strategy (Jain et al., 2000). SFS is one of the most widely used techniques, it is fast and starts by selecting the best single feature in x ($m = 1$) then adds one feature at a time, while eliminating features that are not relevant, and constantly monitoring the classification effectiveness using different criterion functions. Three different objective functions were evaluated in this study: (1) the Fisher score ($J(W)$ – Eq. 4-39), (2) linear discriminant analysis (LDA – Eq. 4-40), and (3) quadratic discriminant analysis (QDA) objective functions (Jain et al., 2000; Bishop, 2007).

$$J(W) = \arg \max \frac{|\tilde{S}_B|}{|\tilde{S}_W|} \quad (4-39)$$

where *arg max* stands for the “argument of the maximum” or the points of the given argument for which $J(W)$ reaches its maximum value. $|\tilde{S}_B|$ and $|\tilde{S}_W|$ represent m -between-class (interclass) and m -within-class (intraclass) scatter (covariance), respectively (Duda et al., 2000; Jain et al., 2000).

LDA is a subspace feature selection method (i.e. feature transformation), allowing feature combination, which is based on $J(W)$. It finds a linear transformation ($W \in \mathbb{R}^{d \times m}$), projecting the x -data into an m -dimensional subspace in which the between-class ($|W^T \tilde{S}_B W|$) scatter is maximized while the within-class scatter ($|W^T \tilde{S}_W W|$) is minimized, as seen in Eq. 4-40. To calculate the between and within scatter (covariance) it is assumed that both have the same covariance matrix. (Duda et al., 2000; Quanquan et al., 2011).

$$LDA(W) = \arg \max \frac{|W^T \tilde{S}_B W|}{|W^T \tilde{S}_W W|} \quad (4-40)$$

Similar to LDA, QDA is a subspace feature selection method and can also be used for classification/validation (Section 4.1.8). The difference is for QDA, the between and within covariance matrices are assumed to be different between each other (arbitrary) (Duda et al., 2000).

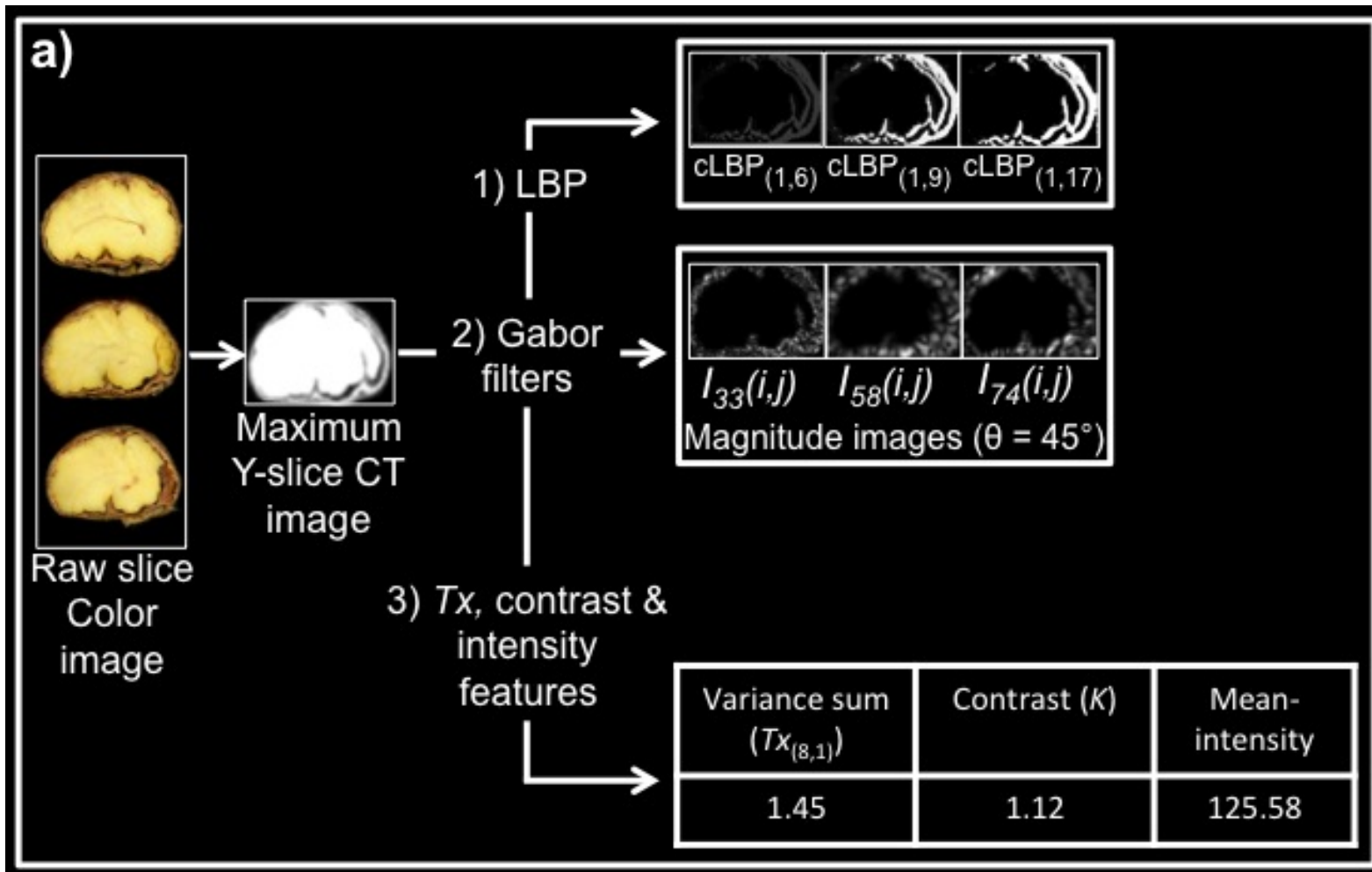
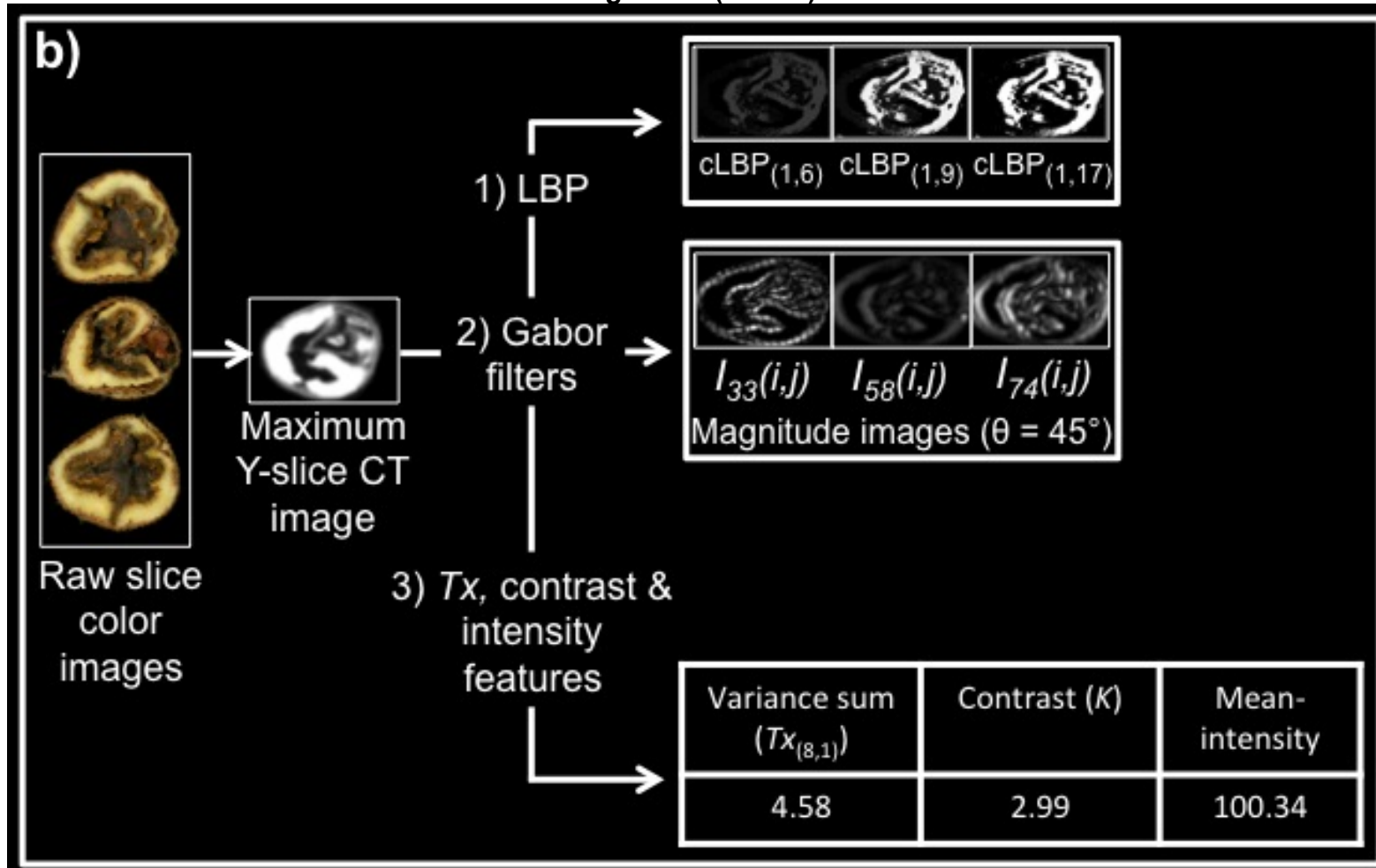


Figure 22. (a) Healthy and (b) partially decayed chestnuts with their corresponding secondary maximum CT image (XY-plane-slice) (55 pixels x 65 pixels). (1) Local Binary Pattern (LBP) transformations using different pixel comparison (d, h) was applied to the secondary maximum CT image. (2) Secondary maximum CT image Gabor filtered transformed images ($I_{rs}(i, j)$) at different scales (r) and orientation (s) for an $\theta = 45^\circ$ are included. (3)

Figure 22 (cont'd)



Example of Haralick textural (T_x) features, contrast and intensity features obtained from included secondary maximum CT image. For visual reference, three-color fresh raw image slices of the evaluated chestnut are included.

4.3.10 Classification (training and validation):

A supervised learning approach was used to train the pattern classification algorithm (Duda et al., 2000). Supervised classes, known as labels, were based on 5-, 3- and 2-categorical-groups, where each chestnut was invasively categorized into 5-, 3- or 2-quality-classes, based on their internal disorder (decay) severity level. An internally developed rating system was used, which clearly and accurately defines the classes, taking in account invasive standardized quality assessment methods, such as that described in UNECE (2010). The rater is experienced in detecting, identifying, and quantifying quality attributes (i.e. decay) in fresh chestnuts.

Quality classification per chestnut was expressed as the apparent percentage of decay tissue in relation to the total area of each chestnut. In the case of the 5-class-classification, a class-5 represents a chestnut that is completely decayed, class-2 through class-4 represent chestnuts that are partially to highly decayed, while class-1 is designated to chestnut with no decay (healthy), as seen in Fig. 19 and Fig. 23a. Sample distribution, used to train and validate the 5-class-classifier, can be observed in Fig. 23a. For the 3-class-classification, a class-3 represents a chestnut that is completely decayed, a class-2 denotes partial to high decay, and class-1 indicates that the chestnut does not contain decay, as seen in Fig. 19 and Fig. 24a. Sample distribution, used to train and validate the 3-class-classifier is included in Fig. 24a. For the 2-class-classification, a class-2 represents any chestnut with decay, while a class-1 shows that the chestnut does not contain decay, as seen in Fig. 19 and Fig. 25a.

Sample distribution, used to train and validate the 2-class-classifier, can be seen in Fig. 25a.

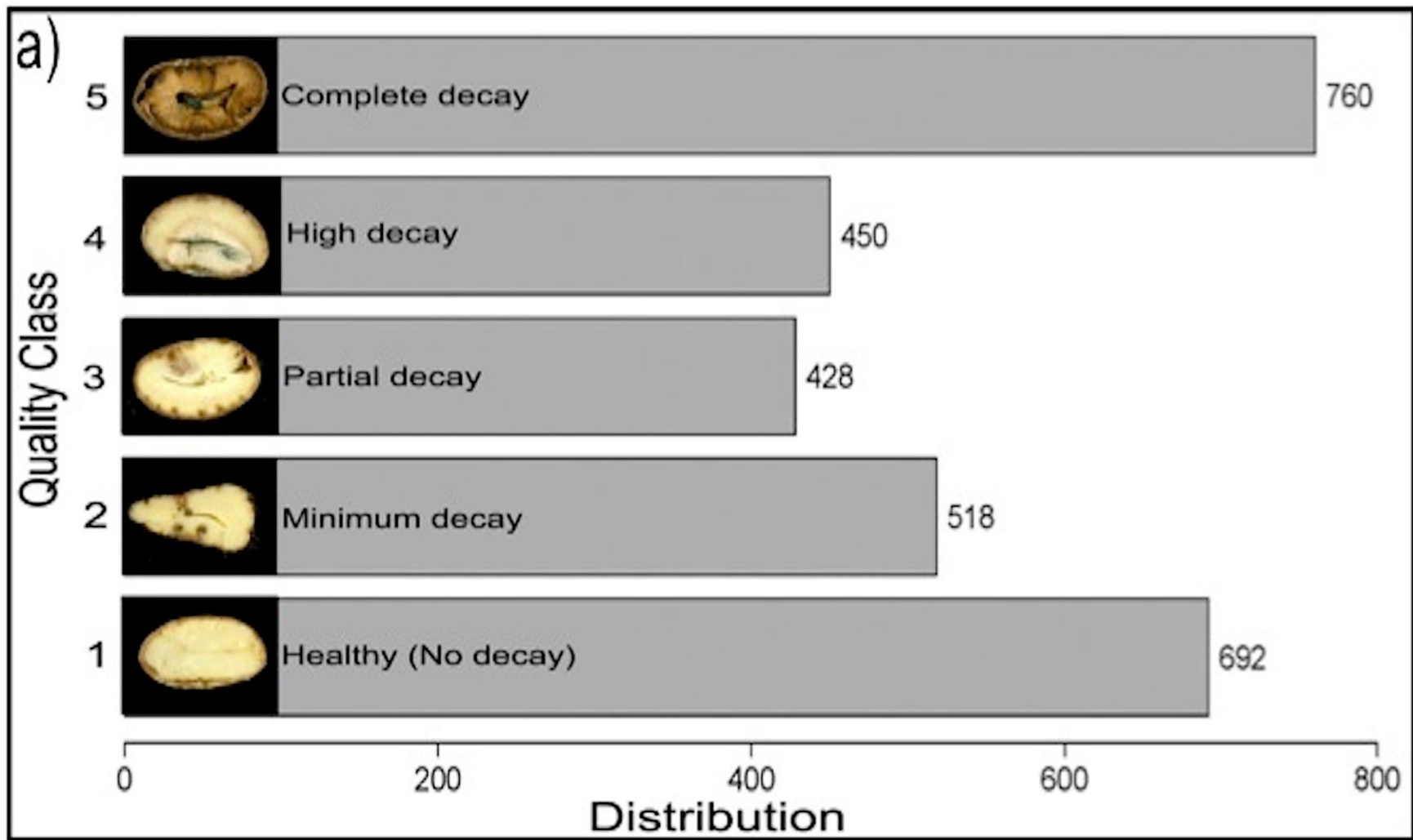
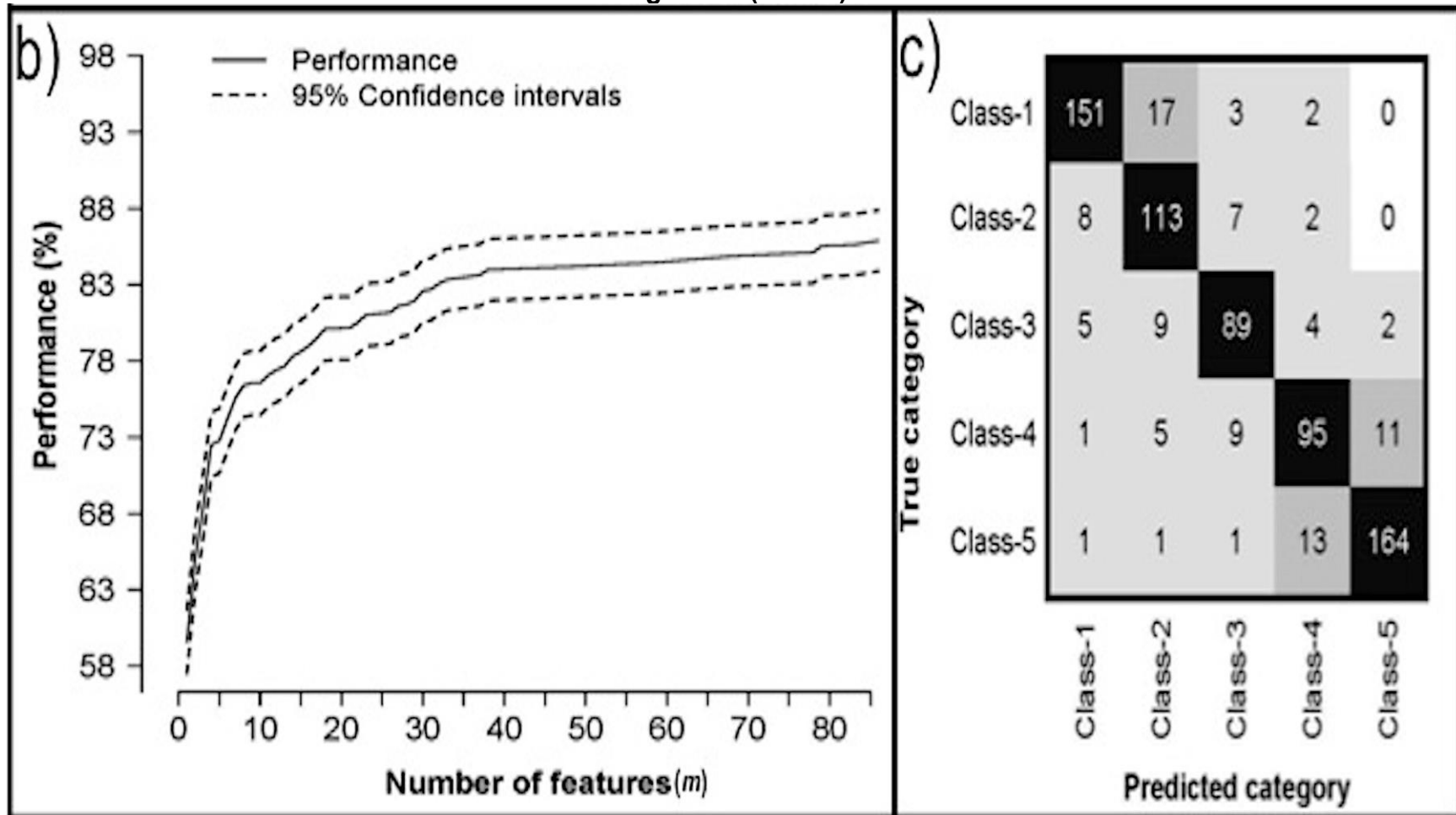


Figure 23. (a) Sample distribution used to train and validate the 5-class classifier. Figure also contains an example for each of the 5 categorical classes, representing the quality index levels.

Figure 23 (cont'd)



(b) Quadratic discriminant analysis (QDA) classifier performance using validation set with 4-folds, in relation to the number of selected features (m). Black line represents the classification mean performance, dotted black line (---) represent 95 % confidence intervals for the validation pool. (c) Validation confusion matrix corresponding to the chestnut quality class prediction using 25 % of samples with 86- m (overall accuracy rate equal to 85.9 %).

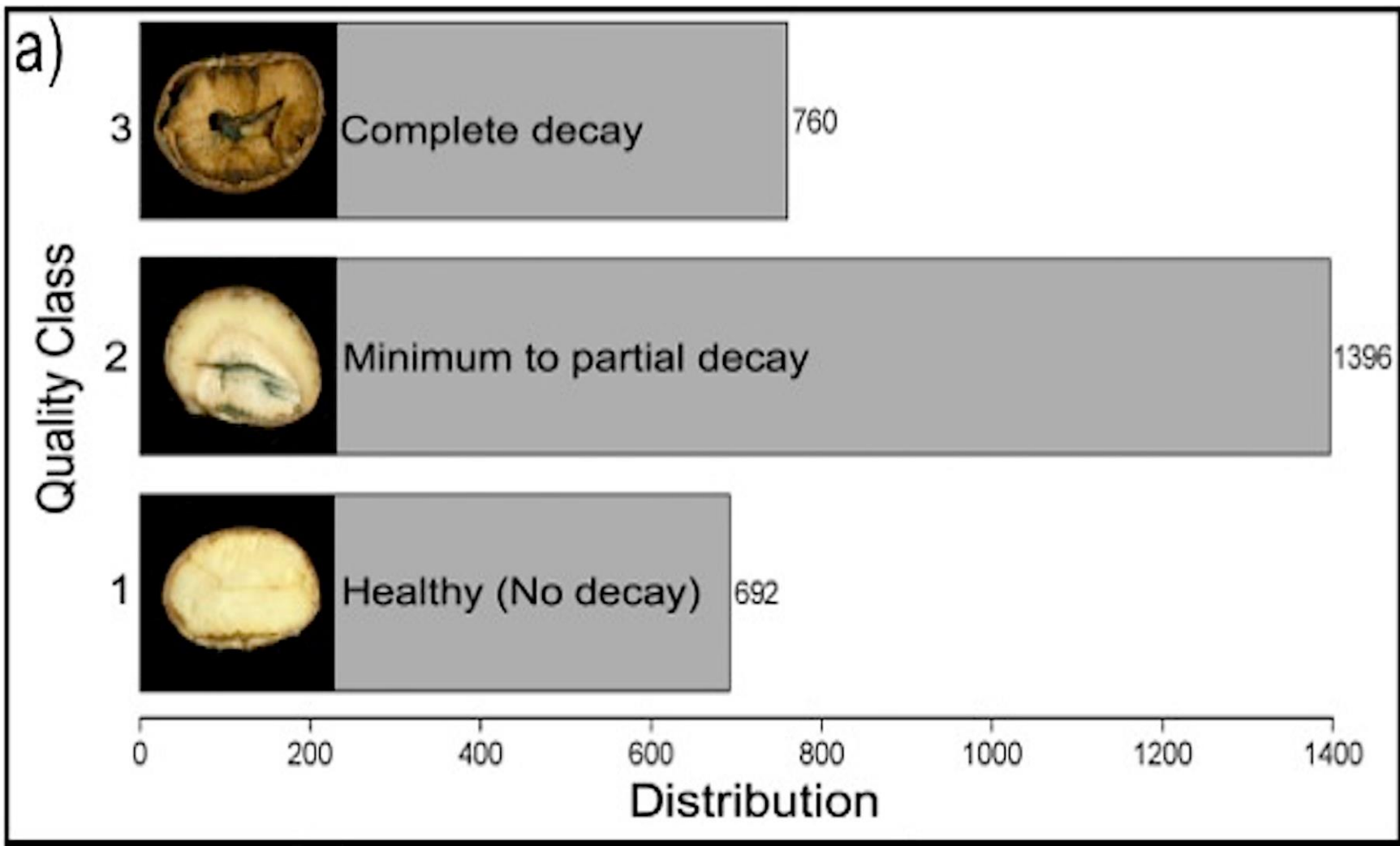
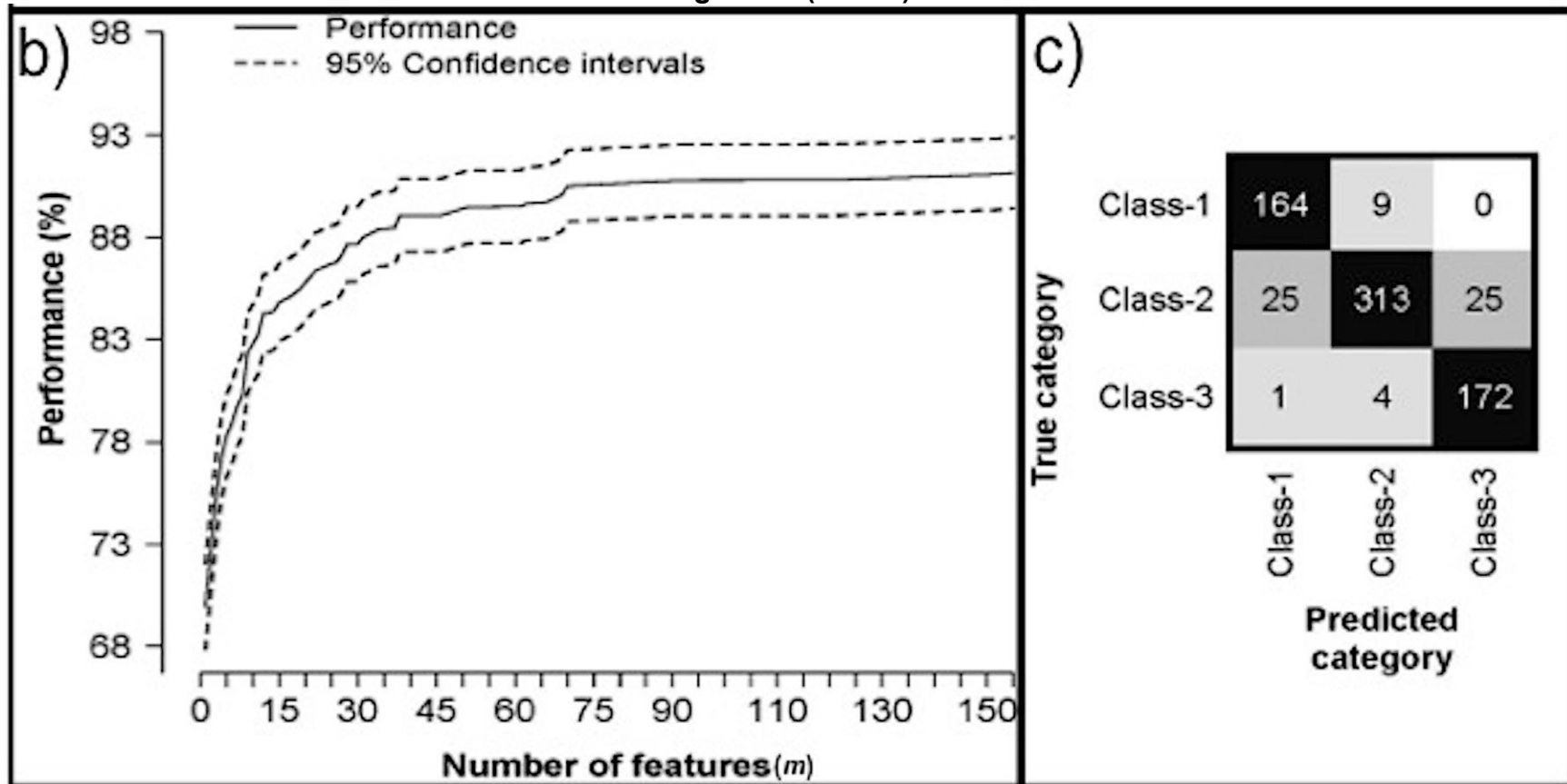


Figure 24. (a) Sample distribution used to train and validate the 3-class classifier. Figure also contains an example for each of the 3 categorical classes, representing the quality index levels.

Figure 24 (cont'd)



(b) Quadratic discriminant analysis (QDA) classifier performance using validation set with 4-folds, in relation to the number of m . Black line represents the classification mean performance, dotted black line (---) represent 95 % confidence intervals for the validation pool. (c) Validation confusion matrix corresponding to the chestnut quality class prediction using 25 % of samples with 155- m (overall accuracy rate equal to 91.2 %).

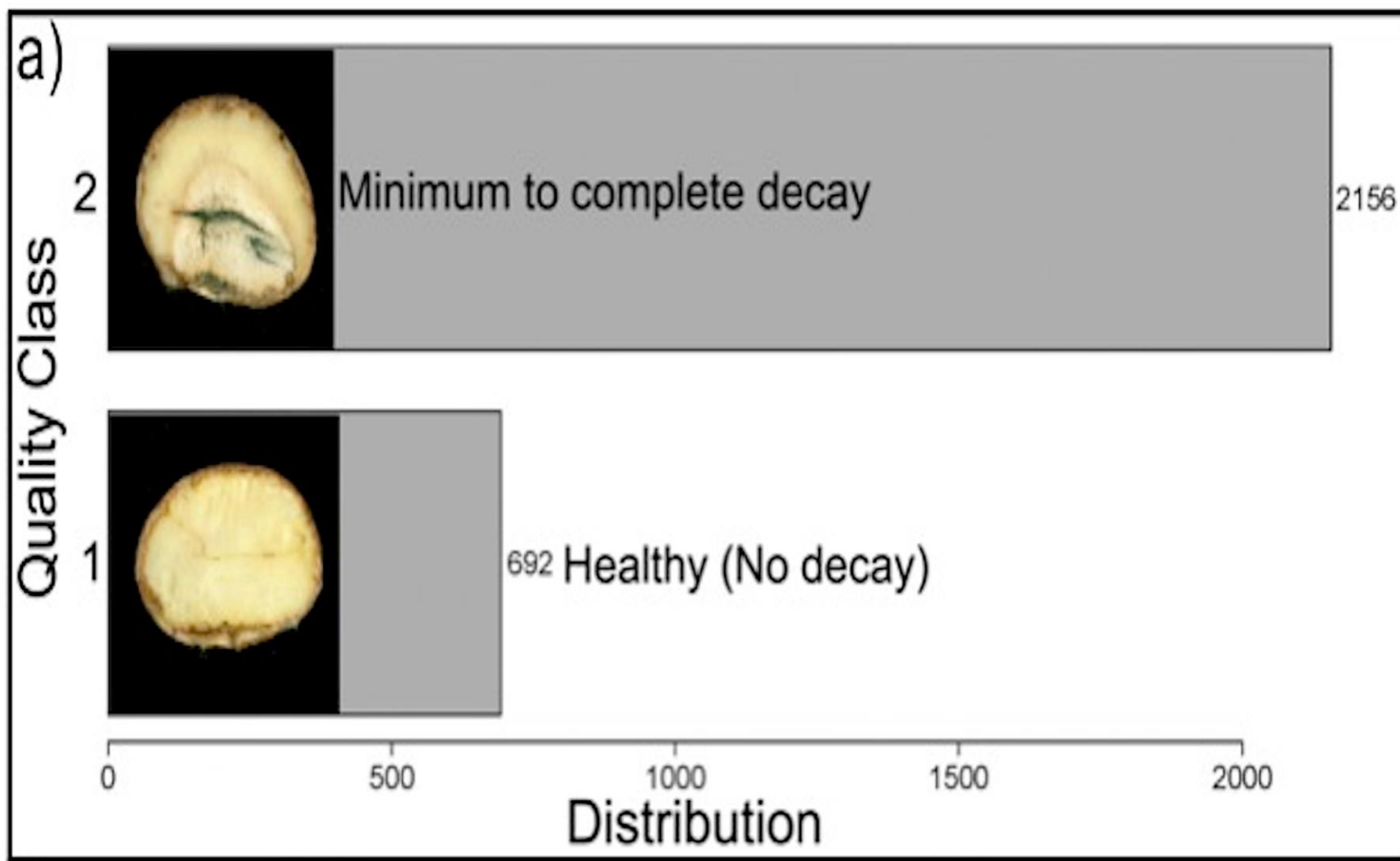
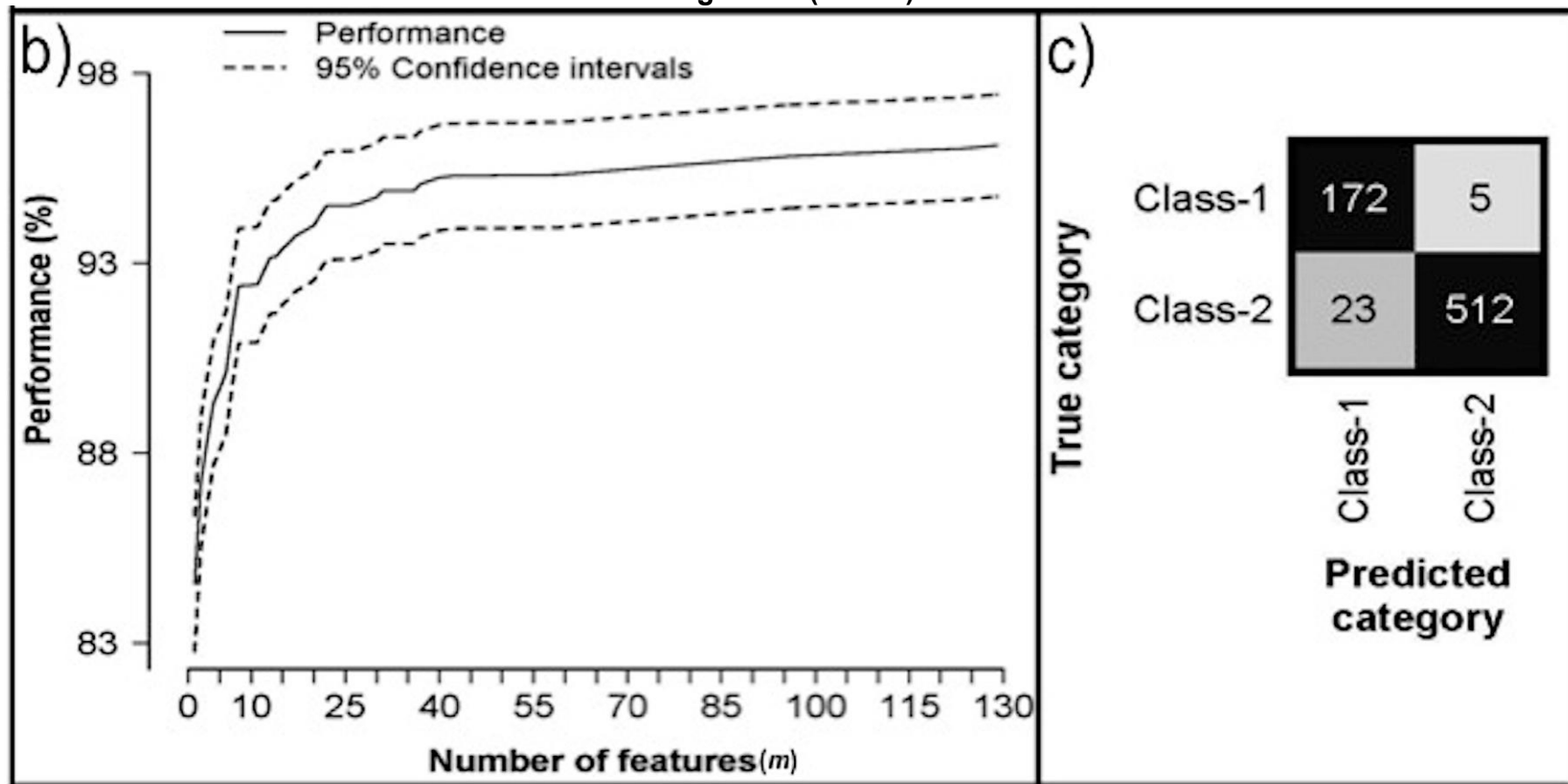


Figure 25. (a) Sample distribution used to train and validate the 2-class classifier. Figure also contains an example for each of the 2 categorical classes, representing the quality index levels.

Figure 25 (cont'd)



b) Quadratic discriminant analysis (QDA) classifier performance using validation set with 4-folds, in relation to the number of m . Black line represents the classification mean performance, dotted black line (---) represent 95 % confidence intervals for the validation pool. (c) Validation confusion matrix corresponding to the chestnut quality class prediction using 25 % of samples with 126- m (overall accuracy rate equal to 96.1 %). Figure is partially presented in color.

Using the optimized selected features obtained from section 4.3.9, decision boundary lines, planes, and hyper planes were implemented using LDA (Section 2.7, Eq. 41), QDA (Section 2.7), Mahalanobis distance (MD) (Eq. 4-41), a two-layer artificial neural network (ANN), a three-layer ANN using a logistic activation function, and a three-layer ANN using a Softmax activation function following the applied procedure in Ren et al. (2006; 2010), Mery et al. (2010), Leiva et al. (2011), and Donis-González et al. (2013). In general, this step assigns the object (i.e. set of 6 secondary CT images per chestnut) to a specific quality category (class).

LDA, and QDA were described in section 4.3.9. The MD measures the difference between two points in the space defined by two or more variables (features). The MD takes the correlations within a data set between the variable into consideration, meaning that it depends on the covariance matrix of the attribute and adequately accounts for the correlations. The MD is the distance between an observation and the mean for each group in m -dimensional space, defined by m -features and their covariance. A small value of MD increases the probability of a set of features (new sample) to be closer to the class's mean and the more likely it is to be assigned to that group-class. The MD between a set of features (m) and a class (μ) is defined as:

$$MD = \sqrt{(m - \mu)^t \sum^{-1} (m - \mu)} \quad (4-41)$$

where Σ^{-1} is the inversed covariance matrix. The MD does not depend upon the scale on which the variables are measured (Duda et al., 2000).

When used for classification ANN, is usually a collection of neuron-like processing units with weighted connections between the units. ANNs are computer algorithms, which are highly parameterized statistical models that can automatically find relations in the data without a predefined model. ANNs first linearly transform the input feature vector by multiplying it with a weight matrix (w_{ji}) then, the activation function ($f(\cdot)$) is applied to each coordinate of the resulting vector to produce a value. Training an ANN involves determining the w_{ji} that maximize the performance for a set of supervised training data, using a specific $f(\cdot)$ (Duda et al., 2000). In general, ANNs transform a feature vector in the input space (*input-i*) to a vector in the output space (*output-k*).

A two-layer ANN was evaluated in this study, this type of ANN can only implement a linear decision boundary or activation function ($f(\cdot)$). Multilayer networks can be built by subsequent application of additional w_{ji} and additional nonlinear $f(\cdot)$. In addition to the simple linear two-layer ANN, two three-layer ANNs were also evaluated in this study using two different $f(\cdot)$: (1) logistic and (2) Softmax. More information, in depth discussion, methodology description, and followed steps for each of the applied the different classifiers can be found in Duda et al. (2000).

Performance of each of the classifiers was measured as the correctly classified chestnuts, using the set of secondary CT images, in reference to its

supervised categorical class (label). Classifier validation was implemented using a 4-fold stratified technique, therefore yielding an average estimate of classifier performance with 95% CI (Confidence Intervals) for the validation pool (Jain et al., 2000). In the experiment, 75% of the samples were used for training and 25% were used for validation repeated four times.

4.4 Results

In this study, the features were reduced from 1194 to 86 (5-class classifier), 155 (3-class classifier) and 126 (2-class classifier) features, in order to avoid overtraining. This feature reduction was implemented using different algorithms. The SFS with the Fisher discriminant objective function ($J(W)$) method offered the most powerful features, yielding the best classifier performance. Reducing overall feature space diminishes the computational time, allowing for possible in-line implementation. The other feature selection methods, using addition objective functions, as described in section 4.3.9 performed poorly (Results not shown). The main selected features for all of the classifiers are enumerated in Table 9. Example of image transformation (cLBP and Gabor) and some of other selected features, obtained from a healthy and partially decayed chestnut can be seen in Figs. 22a and 22b, respectively. Validation of classification was carried out using a 4-fold stratified validation with 25% of the samples for each fold (repetition). The best overall classifier is the QDA classifier. Other classifiers were also tested, however the overall classification performance was lower in all cases, as can be seen in Table 10.

Table 9. Main selected features (seventy five) for the Quadratic discriminant analysis (QDA) classifier using sequential forward selection (SFS) in combination with the Fisher discriminant objective function ($J(W)$) for the 5-, 3- and 2-class classifiers.

<i>n</i>	Selected feature (<i>m</i>)	<i>n</i>	Selected feature (<i>m</i>)	<i>n</i>	Selected feature (<i>m</i>)
1	$\tilde{w}(3,3)$ [XY-max]	26	$\tilde{w}(1,1)$ [YZ-max]	51	Tx(13,1)-Mean [XY-max]
2	Tx(8,1)-Mean [XY-mean]	27	cLBP(1,6) [XY-max]	52	$\tilde{w}(3,3)$ [YZ-mean]
3	Tx(9,1)-Mean [XY-max]	28	cLBP(1,2) [XY-max]	53	$\tilde{w}(2,6)$ [XY-max]
4	Intensity-Std. Dev. [XY-max]	29	sLBP(1,48) [XY-max]	54	Contrast-Ks [XY-max]
5	$\tilde{w}(5,8)$ [XY-max]	30	cLBP(1,5) [XY-max]	55	cLBP(1,3) [XY-max]
6	Tx(1,1)-Mean [XY-max]	31	sLBP(1,40) [XY-max]	56	Contrast-K [XY-max]
7	Intensity-Skewness [XY-mean]	32	sLBP(1,5) [XY-max]	57	Tx(11,1)-Mean [XY-max]
8	cLBP(1,9) [XY-max]	33	sLBP(1,41) [XY-max]	58	Tx(6,1)-Range [XY-max]
9	$\tilde{w}(7,4)$ [XY-max]	34	sLBP(1,25) [XY-max]	59	Tx(10,1)-Mean [XY-max]
10	Tx(5,1)-Range [YZ-max]	35	$\tilde{w}(6,7)$ [xY-max]	60	Tx(7,1)-Range [XY-max]
11	Intensity-Mean [XY-max]	36	sLBP(1,40) [YZ-mean]	61	cLBP(1,36) [XY-max]
12	$\tilde{w}(4,7)$ [XY-max]	37	cLBP(1,16) [XY-max]	62	sLBP(1,20) [XY-max]
13	Mean Laplacian [XY-max]	38	cLBP(1,12) [XY-max]	63	Tx(6,1)-Mean [XY-max]
14	Tx(12,1)-Mean [XY-mean]	39	cLBP(1,26) [XY-max]	64	Tx(7,1)-Mean [XY-max]
15	Tx(13,1)-Mean [XY-max]	40	$\tilde{w}(4,4)$ [XY-max]	65	Tx(13,1)-Mean [XZ-mean]
16	Tx(3,1)-Mean [XY-max]	41	$\tilde{w}(4,6)$ [XY-max]	66	sLBP(1,54) [XY-max]
17	sLBP(1,50) [XY-max]	42	Tx(13,1)-Mean [XY-mean]	67	$\tilde{w}(5,7)$ [XY-max]

Table 9. (Cont'd)

18	$\tilde{w}(7,6)$ [XY-max]	43	$\tilde{w}(6,5)$ [XY-max]	68	sLBP(1,45) [XY-max]
19	sLBP(1,34) [XZ-max]	44	$\tilde{w}(2,3)$ [XY-max]	69	$\tilde{w}(1,7)$ [XY-max]
20	Tx(5,1)-Range [XY-mean]	45	$\tilde{w}(1,3)$ [XY-max]	70	Tx(9,1)-Mean [XZ-max]
21	Intensity-Skewness [XY-mean]	46	$\tilde{w}(1,4)$ [XY-max]	71	sLBP(1,47) [XZ-mean]
22	Intensity-Skewness [XY-max]	47	Tx(4,1)-Mean [XY-max]	72	Tx(5,1)-Mean [XZ-mean]
23	Tx(12,1)-Mean [XY-max]	48	LBP(1,13) [XY-max]	73	Mean Laplacian [XY-mean]
24	Tx(3,1)-Mean [XY-mean]	49	sLBP(1,13) [XY-max]	74	LBP(1,32) [XY-max]
25	LBP(1,1) [XY-max]	50	LBP(1,24) [XY-max]	75	Tx(1,1)-Mean [XZ-mean]

$Tx(k,p)$ -(Mean, Range): Haralick texture features, where k is the texture type, as seen in section 2.6.2 – Table 8, and p is the number of neighbor pixels.

LBP(d,h): Classic local binary patterns semantic. sLBP(d,h): Semantic local binary patterns. Where d is the number of compared pixels with h – neighboring pixels. See section 2.6.3.

$\tilde{w}(a,b)$: Gabor filters, where a is the frequency number, and b is the number of orientations.

Between brackets [] are the different CT images used to extract features (XY-mean = XY-plane-slice mean images, YZ-mean = YZ-plane-slice mean image, XZ-mean = XZ-plane-slice mean image, XY-max = XY-plane-slice maximum image, YZ-max = YZ-plane-slice maximum image, and XZ-max = XZ-plane-slice maximum image). For reference, see sections 1.5 and 4.3.3.

Table 10. Classifier performance using selected features (*m*) with a 4-folds validation

Classifier	5-classes			3-classes			2-classes		
	Mean	UCI ¹	LCI ²	Mean	UCI	LCI	Mean	UCI	LCI
QDA	85.9	87.2	84.6	91.2	92.1	90.3	96.1	96.9	95.3
ANN-Logistic	85.9	87.3	84.5	88.9	89.7	88.1	95.6	96.5	84.7
ANN-Softmax	85.6	87.0	84.2	86.7	87.5	85.9	95.9	96.6	95.2
LDA	84.6	86.3	82.9	91.0	91.9	90.1	96.5	97.4	95.6
MD	83.9	85.4	82.4	87.6	88.5	86.7	93.2	94.1	92.3
ANN-linear	81.6	82.9	80.2	86.6	87.5	85.7	93.3	94.4	92.2
<i>m</i>	86			155			126		

¹Upper Confidence interval

²Lower Confidence interval

Performance results for 5-, 3- and 2-classes, using the selected QDA classifier, for increasing number of features (m), are included in Figs. 7b, 8b and 9b, respectively. It can be seen how performance increases in relation to an increase in m and that with the 86-, 126- and 155- m the classifiers had a high overall performance accuracy classification rate of 85.9 % (5-classes), 91.2 % (3-classes) and 96.1 % (2-classes). Classifier performance slightly increases after the reduced number of selected features (m), but not notably (> 0.5 %), so additional features are not required to classify internal chestnut quality. In addition, it is not recommended to use a higher number of features to avoid classifier over-training, due to overall sample size, as recommended by Duda et al. (2000).

Figs. 23c, 24c and 25c include the confusion matrix corresponding to the overall QDA classifier performance. This matrix is beneficial, because it visibly documents the misclassified classes (Shapiro and Stockman, 2001).

4.5 Discussion

By observing Table 9, it can be seen that the most important features for classification mainly include textural features (approximately 88 %) including: (1) LBP features, (2) T_x and contrast features, and (3) Gabor features at different scale and orientation, acquired from the different CT images. Less influential, about 12 % of the utmost important features involve the basic intensity features. Examples of some of the extracted features, and image transformations to XY-plane-slice CT images can be seen in Fig. 22.

Textural features are important because they cannot be attributed to a single pixel value, but rather to several pixels in the image and their relationship. It can be postulated that LBP textural features are among the most influential features, because they are invariant to monotonic grayscale changes; therefore CT image noise and imaging variability do not play an imperative role (Ojala et al., 2002). LBP grayscale textural features captured the local structure and textural variations between decayed, healthy and partially decayed chestnuts clearly reflected in the CT images and the generated LBP images, as reflected in Fig. 22. In general, T_x and contrast features, which were also highly important, described the pixel spatial variation and their relationship in the segmented image. For example, CT images that contain pixels which are similar between each other have a low variance sum ($T_{x(8,p)}$), while images that contain a high variability of pixel intensity values have a high contrast (K) and high variance sum ($T_{x(8,p)}$) (Haralick, 1979). Therefore, in this study, a uniform healthy or decayed chestnut will represent pixels that are highly correlated (low variance sum - $T_{x(8,p)}$), but a low overall image contrast (K) as observed in Fig. 22a. On the other hand, CT images of chestnut that have a high variation between the pixels (e.g. decayed tissue embedded in healthy tissue from partially decayed chestnuts) will have a higher variance sum ($T_{x(8,p)}$) and contrast values (K) as exemplified in Fig. 22b. Gabor textural features captured different image information by means of combining different scaling and orientation factors. Gabor features, extracted from magnitude Gabor filtered images ($I_{rs}(i,j)$), have

been found to be appropriate for texture representation and discrimination (Zhu et al., 2007), thus it is hypothesized that Gabor features offer information regarding the development of chestnut decay, yielding a different feature value to chestnuts with partial decay in comparison to chestnuts that are either completely decayed or healthy. Examples, of several magnitude Gabor images ($I_{rs}(i,j)$) generated by applying 3 different Gabor filters to a maximum secondary CT images (XY-plane-slice) of a healthy and partially decayed chestnut can be observed in Figs. 6a and 6b, respectively. These $I_{rs}(i,j)$ are useful to visualize how Gabor features can differentiate between chestnuts that contain internal decay in comparison to a healthy chestnut. It is clear that Gabor filtered secondary CT images of a decayed chestnut present a higher level of textural attributes in comparison with a healthy chestnut.

Even though simple image intensity features only account for roughly 12 % of the most important features, these features represented and summarize the overall quality appearance of the chestnuts in the CT images. It could be quantified that decayed tissue has a lower intensity in comparison with healthy tissue (See Fig. 22). However, possibly because of the high variation between the CT images of the same class and CT image noise, simple intensity features by themselves do not provide enough information and are not as sensitive to accurately classify chestnuts, as it was initially hypothesized and briefly discussed in Donis-González et al. (2012).

It was observed that the secondary contrast enhanced maximum intensity value CT images (Figs. 20h-20j, Fig. 21f and Fig. 22), which summarized the

overall pixel values of all of the cropped chestnut CT image slices, offered better features than the secondary contrast enhanced mean intensity value CT images (Figs. 20e-20g and Fig. 21d). After feature selection, 80 % of the features were extracted from secondary maximum intensity CT images, while only 20 % are extracted from secondary mean intensity CT images. This might be the case, because maximum images are less variable, have a higher contrast and can easily discern between healthy and decayed tissue, as visually observed in Fig. 21. Fig. 21 shows that it is visually easier to distinguish the decay tissue in the secondary maximum intensity CT image in comparison to the secondary mean intensity CT image. In addition, secondary XY-plane-slices offer better features in comparison to reconditioned secondary YZ-plane-slices and XZ-plane-slices. The reason behind this is that image spatial resolution is higher in the XY-plane in comparison to the images in the YZ- and XZ-planes (anisotropic voxels), as was summarized in Table 1. Loss in image resolution in both planes comes from the fact that images are re-sliced onto the Z-axis using CT images that have been acquired every 2.5 mm (d), while pixel size in both the X and Y planes is equal to 0.73 mm, considerably smaller than d . It is hypothesized that this loss in resolution could be emended by evaluating different CT scanning techniques and CT image reconstruction algorithms, but more research is required to accurately address this issue.

A confusion matrix is a specific table layout that allows visualization of the performance of a classification algorithm. Each column of the matrix represents the instances in a predicted class, while each row represents the instances in an actual class. The name stems from the fact that the matrix makes it easy to see if

the system is confusing two classes (i.e. commonly mislabeling one as another). It also reports the number of false positives, false negatives, true positives, and true negatives. The diagonal of the matrix represents the true positives and true negatives of each class (correct classification), while the other values represent the false positives and false negatives (classification error) (Duda et al., 2000). In this study, false negatives designate chestnuts that are classified as decayed, while being healthy. In this scenario, healthy chestnuts might be unnecessarily discarded creating direct profit losses. On the other hand, false positives reflect chestnuts that are decayed and classified as healthy. This means that potentially a client will obtain chestnuts that are decayed while expecting to receive a healthy product. False negatives might generate customer complaints and might create an indirect economic loss, which is difficult to directly calculate. By analyzing the confusion matrix in Fig. 23c, it could be seen that classification errors occur evenly across the different classes. This means that in general, healthy chestnuts (class-1) and minimally decayed chestnuts (class-2) present similar classification accuracy when trying to discriminate between chestnuts that contain medium to high decay (class 3,4 and 5). In other words, chestnut quality is similarly discerned regardless of the quality level. Similar results are seen in the confusion matrices found in Figs. 24c and 25c for the 3- and the 2-class classifiers, respectively.

Results from this study show that CT images, acquired using a medical grade CT scanner, can be used as a technique that will be able to classify chestnuts based on their internal quality. This technique objectively, rapidly, and automatically classifies chestnut slices into up to 5-classes by measuring different

textural, and basic intensity features from six CT images. Most importantly, chestnuts that are healthy can be accurately classified from decayed chestnuts with a 96 % accuracy rate (2-class-classifier). This high classification rate can be accomplished with a relatively low number of features in relation to the available number of images, with appropriate feature selection (i.e. = SFS), and classification techniques (i.e. = QDA). Therefore this study offers a tool, to objectively forecast the overall quality of fresh chestnuts. It helps the research community to understand which are the features that play an important role in the ideal classification of CT images. In addition, it creates a general structure that could be used as a reference tool in the development of an in-line noninvasive quality sorter of fresh chestnut, and similar products, like peanuts, almonds, and other fresh commodities (e.g. apples, cucumbers, cherries, pineapples) using a fast CT systems (i.e. using digital cameras). Transferring this tool to different commodity industries will enable them to control and promote the quality of their products. In addition, for the chestnut and other fruits, vegetables, and nuts, this application will provide the opportunity to select products according to their final use.

4.6 Conclusions

The CT imaging system provides high-resolution and high-contrast images of the internal structure and components of fresh chestnuts. After scanning and cropping, approximately 50 original CT image slices (stack) were obtained per chestnut, from three different planes (angular orientations) across the longitudinal (Z) (XY-plane-slice), horizontal (YZ-plane-slice) and vertical (XZ-plane-slice)

axes. From this image stack, 6 secondary CT images per chestnut sample, including mean and a maximum intensity value images for each of the planes were extracted. Secondary extracted CT images were then preprocessed (contrast enhancement) and segmented, using a balanced histogram thresholding method. Thereafter, a total of 1194 grayscale intensity, and textural features were extracted from the segmented region in the 6 secondary CT images per sample. SFS was carried out to reduce the dimensionality of the total image-extracted features. Ultimately, 86, 155 and 126 features were found to be effective in designing a QDA classifier with a 4-fold cross-validated overall performance accuracy of 85.9 %, 91.2 % and 96.1 % for 5, 3 and 2 classes, respectively.

Although the specific developed classification procedure might not be applicable to other foods, agricultural commodities or other applications directly, the methodology is broadly valid. Before applying the classification procedure to other commodities or other applications (e.g. industrial), it will be necessary to train it appropriately. These results showed that this method is an accurate, reliable, and objective innovative tool to determine chestnut internal quality, and would be applicable to an automated noninvasive in-line CT sorting system.

CHAPTER 5. DISCUSSION AND FINAL REMARKS

In response to the need for postharvest internal quality sorting equipment for fresh chestnuts, and the economical benefits of appropriate produce quality assessment, the long-term goal of this research is to aid in the design of commercial-scale fast in-line CT sorting equipment, thus enabling the chestnut industry to quickly sort produce, based on its internal quality attributes (i.e. internal decay). Nonetheless, before developing commercially available in-line CT equipment to sort chestnuts and other commodities, some concerns need to be addressed, including the potential sorting speed of the CT equipment, cost, and client acceptability (reaction to the potential of food irradiation).

The first CT scanner developed by Godfrey Hounsfield in 1971, took several hours to acquire the projection images and took several days to reconstruct them into a single 2D slice. After the CT system's 42 years of applied history, current CT systems can scan a whole adult human body and create a 3D reconstruction within seconds (Bushberg et al., 2002). In non-medical fields (e.g. agriculture), scanning can be made several times faster by sacrificing image resolution and quality, reducing the number of projection images to reconstruct 2D slices, decreasing number of slices acquired per sample, changing the energy and current required for scanning, implementing new technical and hardware scanning concepts, among several other parameters. In the agriculture and food industries, the sorting throughput requirements vary among commodities, but range between one to forty items per second (Chen and Sun, 1991), with chestnuts falling within this range. The intention would be to eventually develop a

sorting machine that can fulfill these throughput needs. In this study, image acquisition and reconstruction is equal to approximately 0.6 s per chestnut. Feature extraction, re-slicing and classification all together total 1.8 s per chestnut. This would yield a classification throughput rate of approximately 1 chestnut every 2 s. This time is only ideal for the lower end of the agricultural application speed sorting range, resulting in equipment that could sort around 500 kg of chestnuts on an 8 h shift day. I am confident that future research, including equipment parameter and hardware modifications for in-line sorting, could significantly increase this throughput. Development of such sorting equipment to fulfill these high throughput requirements, using computed tomography, is a challenge. Yet, based on observed changes over the life span of CT, and especially recent advances that have significantly increased image acquisition rate, reconstruction speed, image quality, resolution, and reduced X-ray exposure, I think that soon it will be possible to use this technique for sorting purposes.

Such technology is already being developed and tested for a fast in-line CT quality inspection system for mass production in the car industry (Stuke and Brunke, 2010). Other scientists, such as Hampel et al. (2005) and Bierberle et al. (2009) are evaluating the use of an ultra-fast CT X-ray system to study gas-liquid two-phase pipe flow. As another example, with the purpose of preliminary evaluation of a method that can significantly increase traditional CT scanning speed and can be developed further to in-line sorting purposes, a small set of chestnut samples ($n = 400$, year = 2011) were scanned using the ultrafast ROFEX-scanner (Fraunhofer Institute of Electron Beam and Plasma Technology,

Dresden, Germany – Fig. 26a). This scanner was operated at its fixed settings equal to 150 keV maximum X-ray energy with a maximum electron beam power of 10 kW. The system can provide a temporal resolution of up to 7000 frames s⁻¹ at a spatial resolution of roughly 1 mm, depending on attenuation behavior of the object. To achieve similar results in objects with higher attenuation behavior than water, temporal resolution has to be reduced, therefore increasing scanning time as was required in chestnuts. Chestnuts were labeled and packed in a thin plastic hose. The hose was pulled through the scanning plane using a stepper motor, at a constant speed of 1 m s⁻¹, as seen in Fig. 26b. From this, sets of cross sectional 2D CT images for every chestnut were acquired. The frame rate was chosen to 2000 frames s⁻¹ (10-20 times faster than the traditional CT imaging systems), which is a good compromise between image quality and temporal resolution. Fig. 27 offers preliminary visual results of what can be inferred about fresh quality using ultrafast ROFEX CT-images. It can be seen that differences in the gray scale intensity values (HU-values) between different tissues types are better observed when distinctive tissue types coexist within the same chestnut, such as in the images in Fig. 27b. It can also be observed from these CT images it is difficult to visually distinguish between extremely decayed (Fig. 27c) and healthy chestnuts (Fig. 27a). On the other hand, when decay tissue is embedded between healthy tissue (Fig. 27b), a slight visual difference in grayscale values can be visually observed. Nonetheless, the presence of void spaces and pellicle were easily discerned. Preliminary results indicated that the scanning conditions - using the ROFEX scanner were not optimal to detect decay because the

maximum X-ray energy of 150 keV was too high, resulting in diminishing the ability to differentiate tissue differences. Therefore, future studies will focus in optimizing image quality using different scanning parameters (e.g 60 keV X-ray energy); with the objective of rapidly and effectively detecting properties in different fresh agricultural produce, for example chestnut decay. Technology, such as ROFEX CT technology, if appropriately applied with its fast scanning capabilities, could easily be potentially installed in-line to automatically and rapidly sort agricultural commodities, including chestnuts.

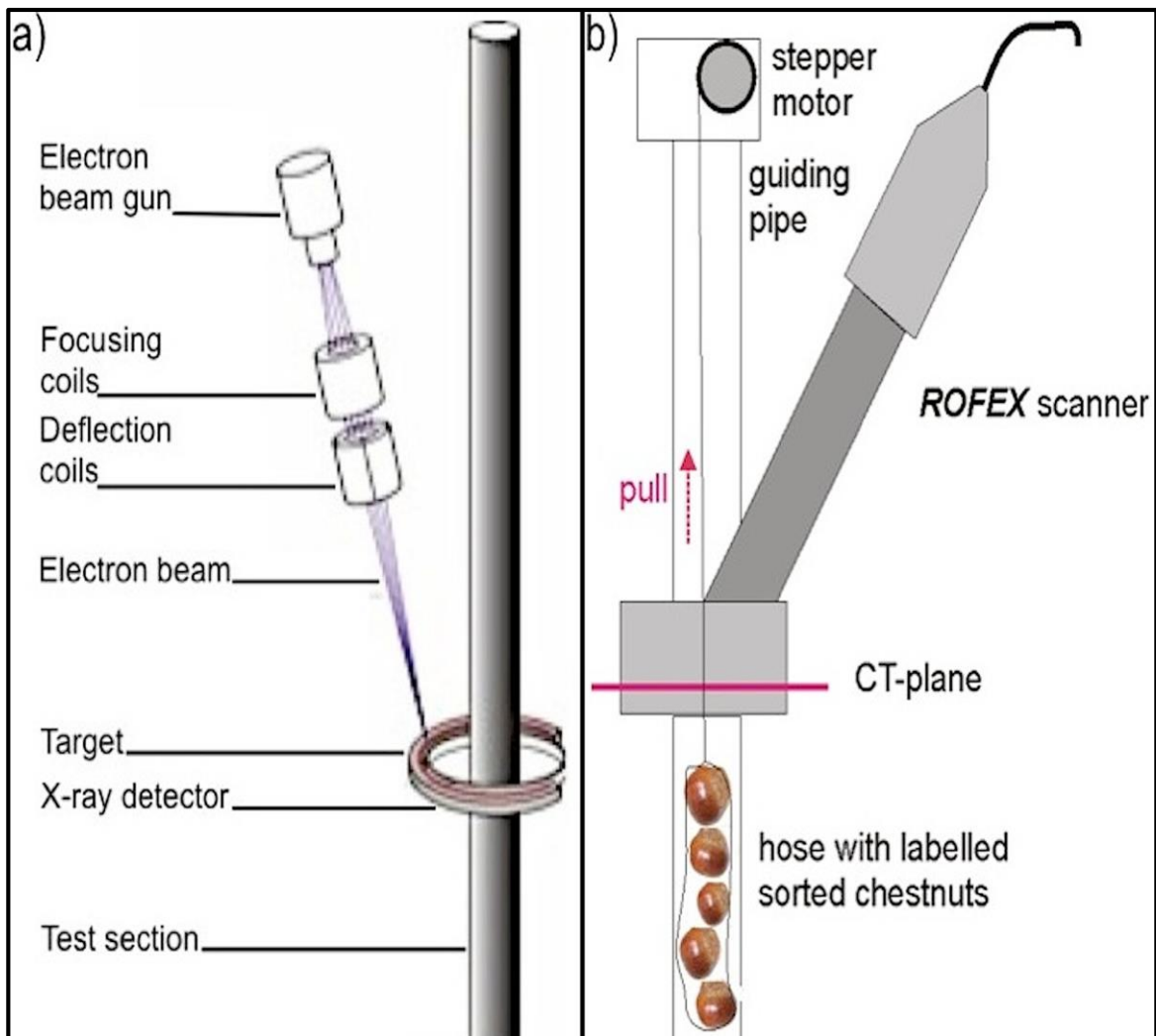


Figure 26. (a) Ultrafast Rossendorf electron beam X-ray tomograph (ROFEX) scanner working principle. (b) Experimental setup of the ROFEX-scanner.

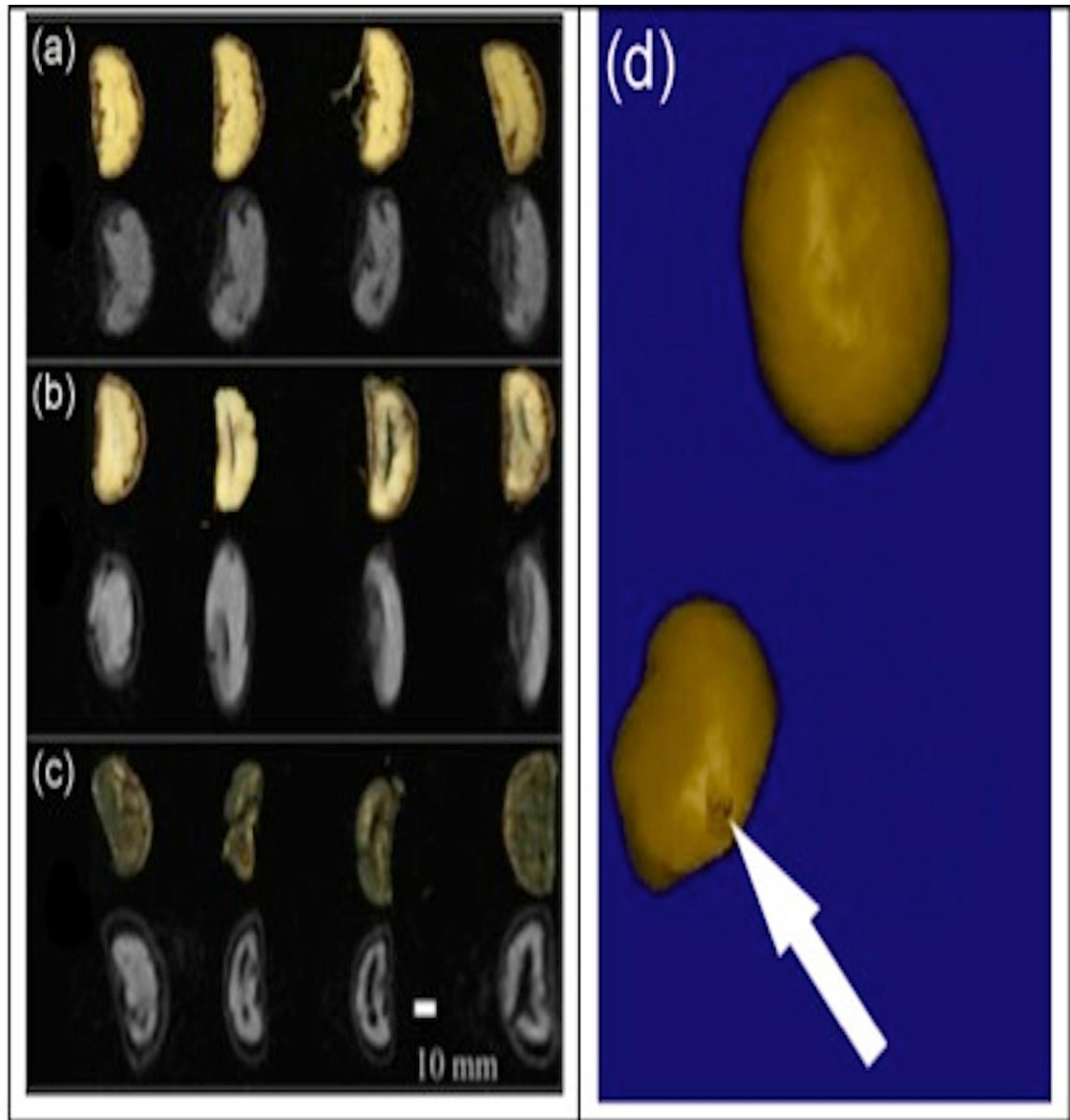


Figure 27. Visual preliminary results of CT images (lower row) obtained using the ROFEX-scanner with its corresponding color raw image slices (upper row). (a) Healthy, (b) partially decayed (rotten) and (c) completely decayed chestnuts. (d) 3D reconstruction of two chestnuts, showing a rotten section in one of the chestnuts (white arrow).

Currently, an accurate estimation of the cost and cost effectiveness of using CT sorting systems is not available and is difficult to predict or even speculate because the equipment is not commercially available. More studies are

required to accurately measure these. Prices depend on the type of machine, but in general, a new CT scanning machine costs between 75,000 US\$ to 1,000,000 US\$, without considering operating and maintenance costs (Abrams and McNeil, 1978; Cenegage and Krapp, 2002). This is considerably less expensive than a homologous MRI device, but more expensive than 2D X-ray equipment (Alanen et al., 2004). Prices are especially high and significantly inflated because currently CT is only used in the medical industry and the economics of scale and Moore's law have not applied (Goetz, 2010). Nevertheless, it can be expected that in the near future, changes in the technology, efforts to develop new concepts, and different applications, should significantly reduce the price of CT equipment, enabling the technology to be used in agriculture, food, and other in-line sorting applications.

Differently to X-ray for food quality diagnostic purposes, as described in this dissertation, food treatment using X-ray radiation to improve microbial safety has been extensively studied. The safety of consumption of irradiated food has also been comprehensively studied. Several international specialist groups in collaboration with the World Health Organization (WHO), the FAO, the International Atomic Energy Agency (IAEA), and the Scientific Committee on Food (SCF) of the European Commission concluded that irradiated foods, with appropriate technologies, are safe and nutritionally suitable. Specific applications of food irradiation are approved in over 55 countries, including the United States of America. In general, food irradiation is described as a process where food is exposed to ionized energy, utilizing X-rays of 5 MeV maximum kinetic energy,

gamma photons emitted by ^{60}Co and ^{137}Cs radioisotopes, and 10 MeV accelerated electrons. None of these sources have induced radioactivity in the food or its packaging. In general, the maximum allowed absorbed dose (measured in grays - Gy) in food applications is 60 kGy, which occurs with meat, poultry and fish. To extend shelf-life of fresh fruits/vegetables and dry spices dosages ranging between 0.1-3.0 kGy are recommended (Farkas and Mohácsi-Farkas, 2011). In the case of the CT scanner used in this study, applying the settings optimized in Chapter 2, the CT equipment emits up to 0.1 MeV kinetic energy, and a maximum of 8×10^{-9} kGy per cm^3 scanned. These values are considerably lower than the amount permitted in food irradiation. Because of this, I am confident that CT sorting of agricultural products should not cause any substantial safety concerns. Consumer acceptance is a matter of education, and good communication leads to lessening the unfair image that irradiated food is malicious (Teisi et al., 2009). Misrepresentation of food irradiation prevents the utilization of a safe and beneficial process. The advantage is that marketing trials have shown that an increasing amount of consumers are willing to buy irradiated foods, if properly informed about the technology (Eustice and Bruhn, 2006).

Up to now, work related to CT for internal quality attributes in food and agriculture has been limited to visualization. In addition to the optimum visualization of internal attributes of chestnuts, which was described in this study, this research also provides a novel technique to automatically classify chestnuts using CT images. The research conducted to develop this dissertation provides important tools and scientific information to appropriately develop, and hopefully

move forward in the incorporation of CT equipment technology into a specific agro-food industry application (chestnuts quality inference), and possibly others. Information obtained from this dissertation, even though specific to chestnuts, offers a blue print to be applied to other commodities (see appendices C), it is likely to be adaptable to measure and classify a wide range of internal quality attributes in other commodities. In addition, if practically applied, the sorting equipment will enable the chestnut industry to: Efficiently use by-products; lessen the negative environmental and health effects of field agro-chemical usage because of better postharvest monitoring; offer safer products (e.g. early detection of mycotoxins producing microorganism); avoid loss of produce quantity; detect postharvest chestnut pathogens, with the primary objective of taking immediate actions to avoid negative effects of microorganisms colonization (e.g. reduction of nutritional quality), thus increasing industry revenue and sustainability.

This study demonstrated that CT is a reliable technique for chestnut internal quality detection and classification, capable of providing unique quality information that is not obtainable by any other commercially available equipment, and is appropriate to apply as an in-line sorting system. It indicated that CT technology has the ability to effectively detect common and economically important internal quality attributes from fresh chestnuts, with better and higher spatial and contrast resolution than commercially available 2D X-ray sorters (Fig. 28a). As a demonstration and evaluation, a preliminary study with whole fresh intact chestnuts, conducted in the years 2009 and 2010 with a commercially available 2D X-ray sorter, yielded slight, almost undetectable, differences

between chestnut quality levels (Fig. 28b). On the other hand, as demonstrated in this study and dissertation, when chestnuts were imaged using a medical grade CT scanner, excellent visual chestnut tissue characterization, accurate quantification of internal defects, and high non-invasive classification rates were observed.

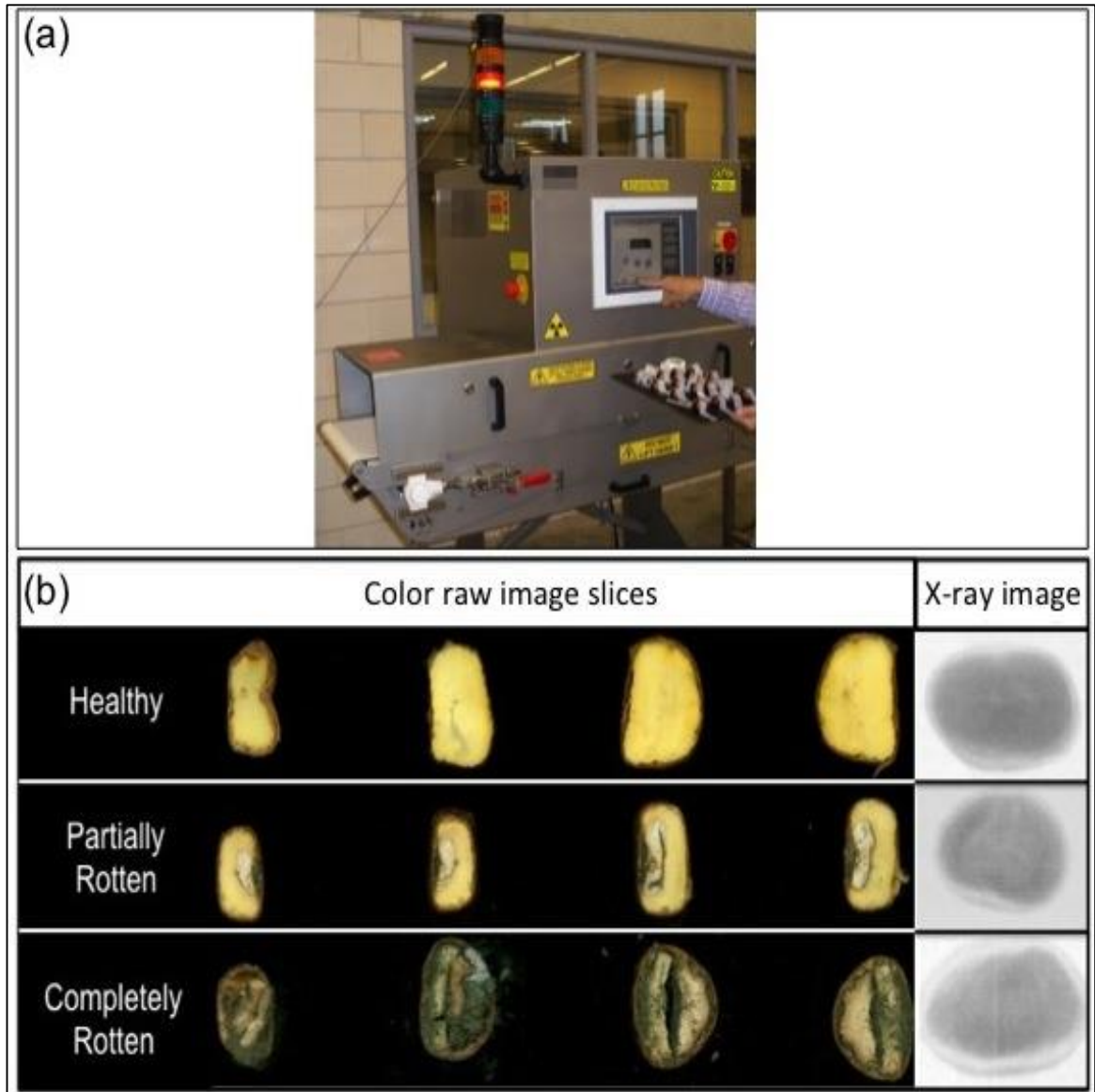


Figure 28. (a) Commercially available in-line traditional X-ray sorter, and (b) its corresponding 2D X-ray images.

Additional to the previously mentioned benefits, the possible integration of this postharvest technology sorting system into the traditional packinghouse setup, which includes chestnuts, as sketched in Fig. 29, will lead to reducing the industry reliance on hand labor, decreasing steps during the packaging process, possibly minimizing waste, reducing water usage and, lessen product losses.

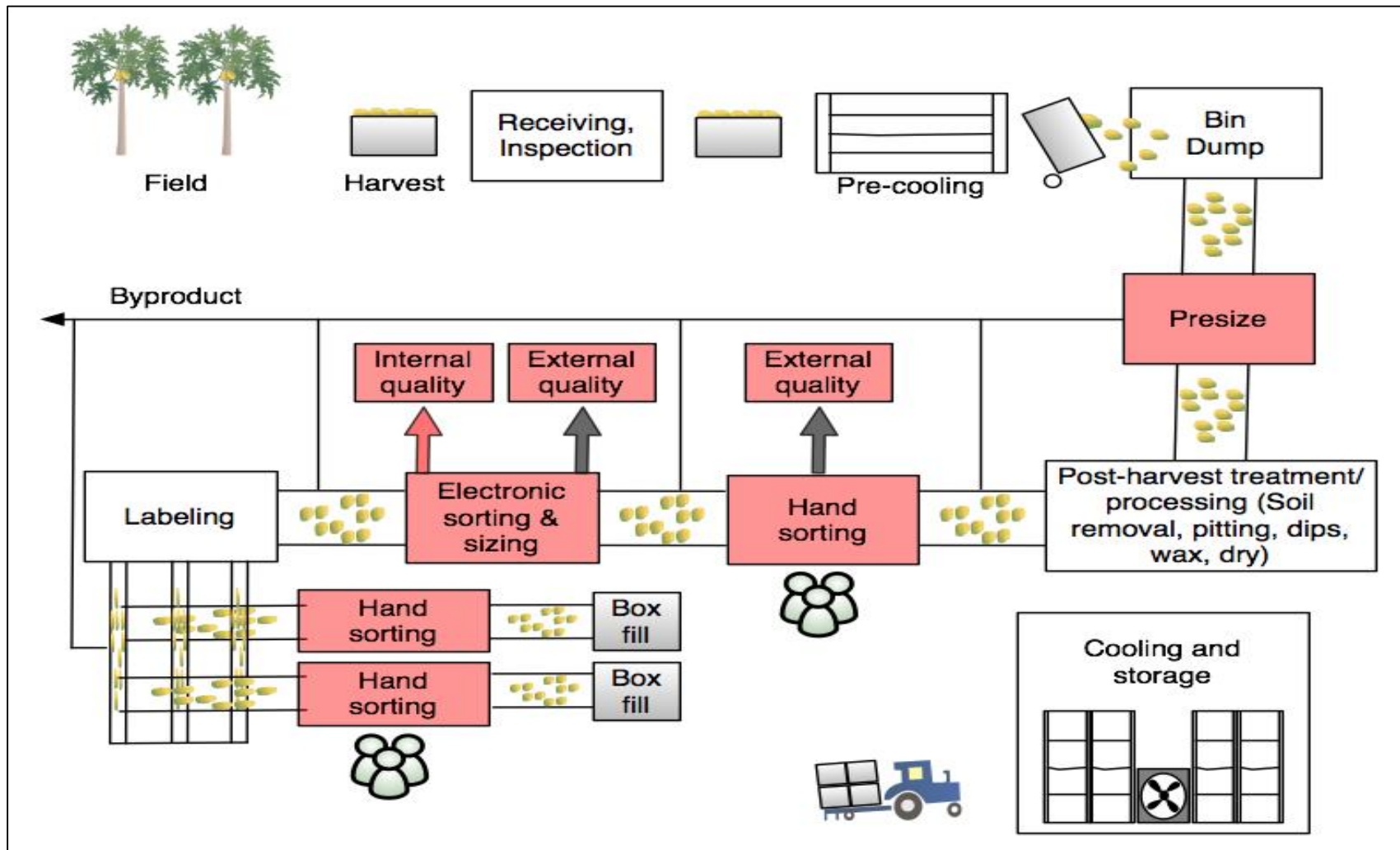


Figure 29. Typical unit operations in mechanized packinghouse (Thompson et al., 2002). Vision of future integration of in-line Computed Tomography (CT) for sorting fresh agricultural products*. *: Flowchart in red are the proposed sections that will be potentially improved.

APPENDICES

APPENDIX A

Estimation of SNR, Volume accuracy, High contrast spatial resolution (HCSR), Low Contrast Detectability (LCD), and digital quality assessment (DQA)

A. 1: Estimating SNR. The mean value of 10 repeated ROI measurements from different 16-bit CT images (Fig. 7a), containing healthy chestnut tissue was used as the final *SNR* value within each run.

$$SNR = \frac{\sum_{i=1}^{\#ROI} \left(\frac{\left(\frac{\sum_{px=1}^x HU_{px}}{x} \right)}{\left(\frac{\sqrt{\sum_{px=1}^x (HU_{px} - \overline{HU})^2}}{x-1} \right)} \right)}{\#ROI} \quad (A. 1)$$

where,

HU_{px} = Hounsfield unit value of each pixel (px).

$\overline{HU}$ = Mean intensity of all pixels in each ROI.

x = Number of pixels for each ROIs.

A. 2: Estimating Volume Accuracy. Teflon® reference cylinder estimated volume was digitally estimated by calculating the area of each XY-plane 2D CT image as seen in Fig. 7b, using corresponding binary images (after applying a simple global threshold of 135) to count the number of pixels in the segmented region, multiplying this value by the slice thickness and then summing the total number of acquired images per cylinder. Therefore, total volume accuracy is defined in Eq. (A. 2).

$$VA = \sum_{j=1}^{\#RC} \left(\frac{1}{\#RC * |TV_j - EV_j|} \right) \quad (A. 2)$$

where,

TV = Teflon® reference cylinders true volume ($\sim 14,743 \text{ mm}^3$).

EV = Digitally estimated Teflon® reference volume

RC = Number of reference cylinders (3).

A. 3 and A. 6: *Estimating HCSR and LCD*. $HCSR$ was calculated using a replication of three 8-bit CT images (e.g. Fig. 7c), without the aid of a human observer and adapted from the specified protocol in General Electric Company (2007), by deriving and applying Eq. (A. 3).

$HCSR$

$$HCSR = \frac{\sum_{h=1}^{im} \left(\sum_{lp=1}^N \left(\frac{\sqrt{\sum_{px=1}^x (I_{px} - \bar{I})^2}}{x-1} \right)_{lp} - \left| c - \frac{\sqrt{\sum_{px=1}^x (I_{px} - \bar{I})^2}}{x-1} \right|_{lp=1} + \frac{CC}{TCC} \right)_h}{im} \quad (A. 3)$$

where,

im is the number of analyzed images (3).

N each box sized to fit into each pattern (lp) as observed in Fig. 7c, representing a quantitative assessment of changes in image resolution.

Values of N range from 1 to 5, where N equal to 1, 2, 3, 4, 5 are boxes in the patterns with a lp equivalent to 1.6 mm, 1.3 mm, 1.0 mm, 0.6 mm, and 0.5 mm, respectively.

I_{px} is the intensity value of each px .

$\bar{I}$ is the mean intensity of all px in each box (N).

x is the total number of pixels in each box (N).

c = Constat equal to 40 I , indicating the optimum SD intensity for an lp of 1.6 mm (baseline), as specified by General Electric Company (2007).

CC = Number of Connected Components (objects or blobs) in the binary image (Shapiro and Stockman, 2001) after applying a simple global threshold of 134 to its corresponding XY-plane 2D CT image (Fig. 7d).

TCC = Total Connected Components (CC) in one image (30).

The LCR was consistently calculated for each run; using a replication of three CT images as exemplified in Fig. 7d. Combined sets of criteria (Eq. (A. 4) and (A. 5)) were used to develop Eq. (A. 6), which characterizes LCD.

$$MC = \frac{\sum_{h=1}^{im} \left(\sum_{k=1}^H \left(\frac{2 * \sqrt{A}}{P} \right)_k \right)_h}{im} \quad (A. 4)$$

where,

MC = Mean Circularity (Shapiro and Stockman, 2001) from three binary image repetitions after applying a simple global threshold of 94 to its

corresponding XY-plane 2D CT image (e.g. Fig. 7d) for each set of holes (H) - k (total of 5). Values of k range from 1 to 5, where k equal to 1, 2, 3, 4, 5 represents the circular holes in the membrane with a diameter equivalent to 10.0 mm, 7.5 mm, 5.0 mm, 3.0 mm, and 1.0 mm, respectively.

im = Number of analyzed images (3).

A = Area of each k .

P = Perimeter of each k

In theory, MC increases as each k becomes more circular (Shapiro and Stockman, 2001).

$$MAE = \frac{\sum_{i=1}^{im} \left(\sum_{j=1}^H \left(\left| \frac{TA - EA}{TA} \right| \right)_j \right)_i}{im} \quad (A. 5)$$

where,

MAE = Mean Area Error from a triplicate of binary images after applying a simple global threshold of 94 to its corresponding XY-plane 2D CT image (e.g. Fig. 7d) for each hole (H).

im = Number of analyzed images (3).

TA = True Area (known from each H true diameter).

EA = Estimated Area (counting number of pixels for each H in binary image).

MAE decreases, as each hole (H) is closer to its true area (TA).

$$LCD = \frac{MC}{|MAE + SDH|} \quad (A. 6)$$

where,

MC = Mean Circularity using Eq. (A. 4)

MAE = Mean Area Error using Eq. (A. 5).

SDH = Mean area value of the Smallest Detected hole (H).

A. 7: Estimating Digital Quality Assessment (DQA). To perform the *DQA*, each 16-bit CT gray scale image (e.g. Fig. 8b) corresponding to the subjectively evaluated fresh-raw color image (e.g. Fig. 8a), was first segmented into a binary image containing the area (total number of pixels) of whole chestnut tissue per slice, by applying a simple global threshold of 400 HU (e.g. Fig. 8c). Second, transition points, Shell, and Pellicle area was determined after applying a Sobel filtering method to detect edges and tissue transition points (Shapiro and Stockman, 2001), as seen in Fig. 8d. Third, healthy tissue was determined by calculating the binary image area after segmentation, using a simple global threshold of 1050 HU (Fig. 8e). A simple global threshold can effectively be used to segment any of the CT images because images are not sensitive to changes in lighting conditions, scanned object color, and potential seasonal changes, as it is the case when processing real color images (Blasco et al., 2007). Ultimately, CT digital *DQA* was expressed as seen in Eq. (A. 7).

$$DQA = \left(1 - \frac{HT}{(WT - SP)} \right) * 100 \quad (\text{A. 7})$$

where,

HT = Area of healthy chestnut tissue.

WT = Area of whole chestnut tissue.

SP = Area of shell, pellicle and transitions points.

APPENDIX B

CT imaging in other fresh agricultural commodities

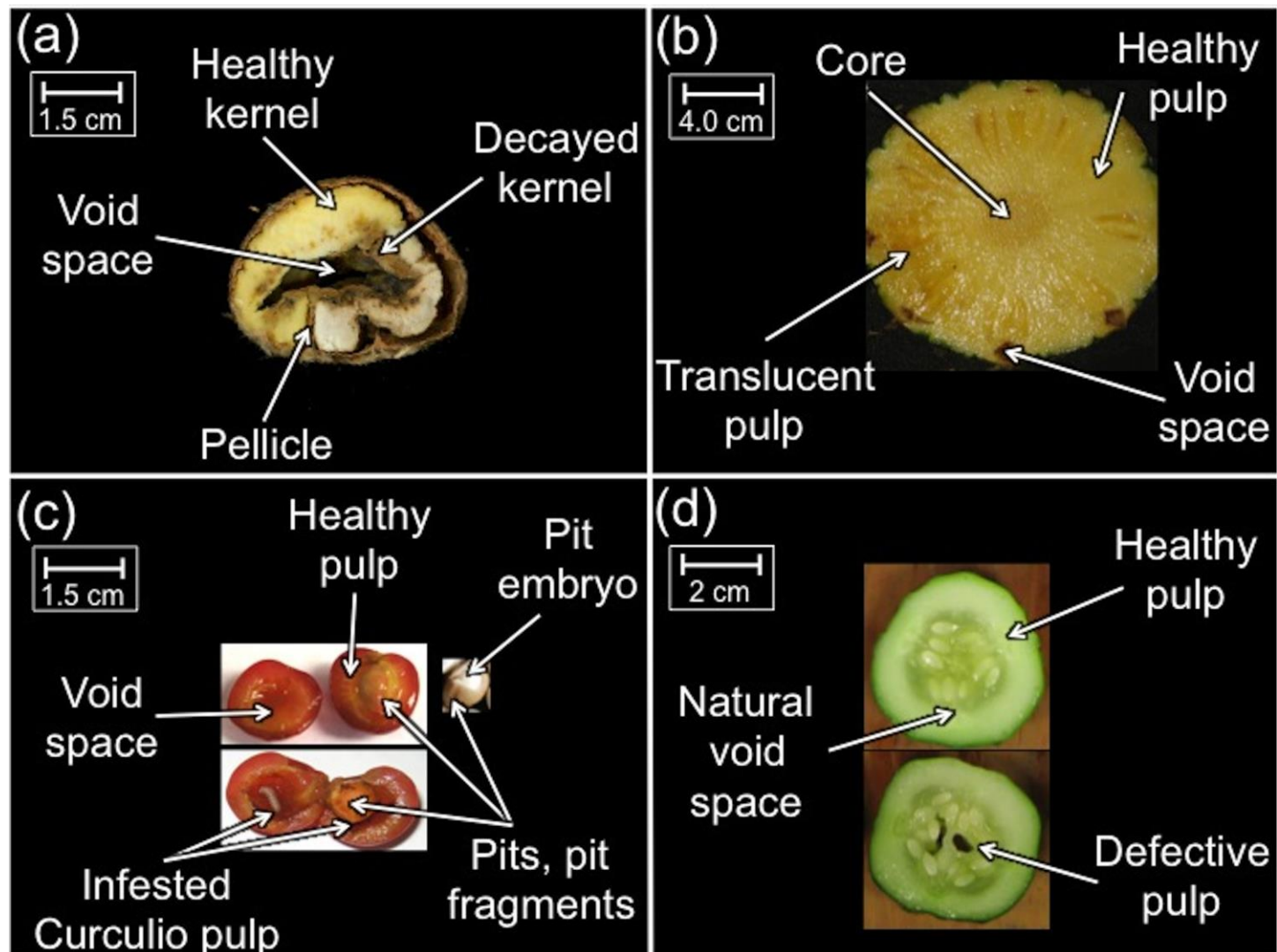


Figure 30. Color raw images of (a) chestnuts, (b) pineapples, (c) tart cherries and (d) pickling cucumbers showing the regions of interest (ROIs) for each fresh commodity.

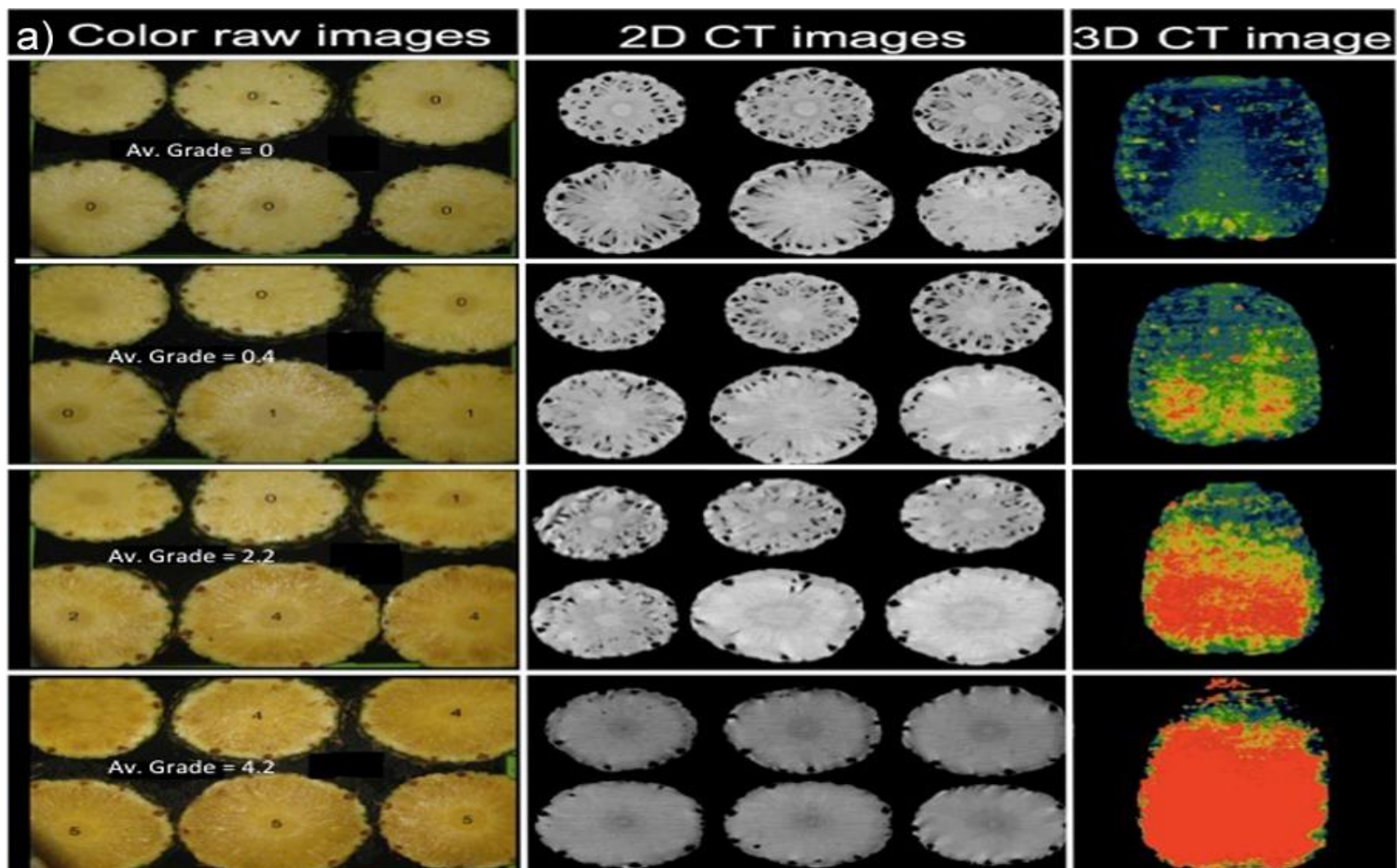
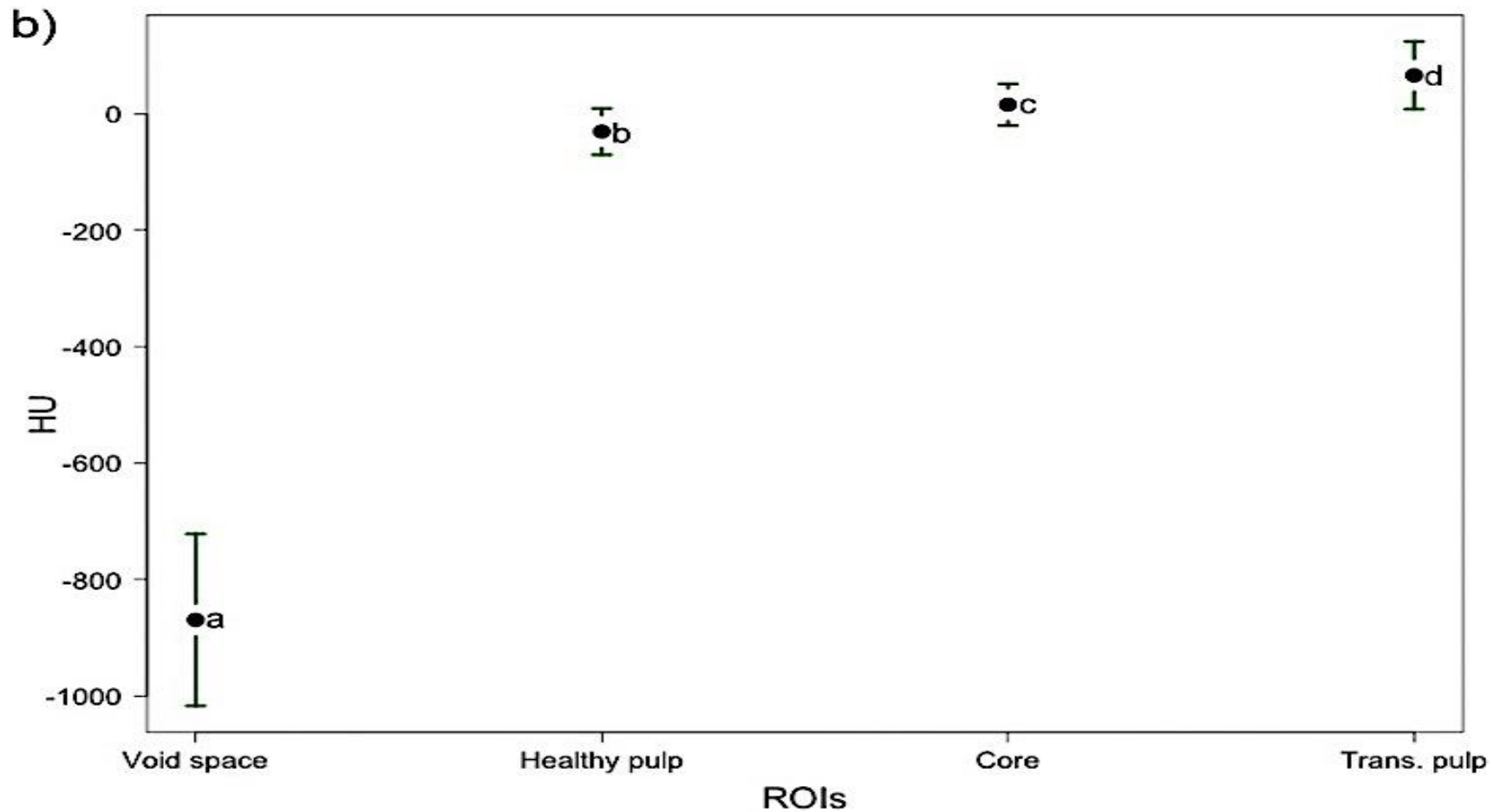


Figure 31. (a) Color raw image slices, cross-sectional 2D CT images acquired using the GE BrightSpeed™ RT 16 Elite CT scanner, and 3D reconstruction of pineapples.

Figure 31 (cont'd)



(b) Black dots showing the HU-values for the mean of 100 data points per each pineapple ROI ($n = 100$). Values followed by the same letter are not significantly different between each other at $P = 0.05$ (ANOVA) (Tukey multiple comparison of means). Vertical bars represent the standard deviation (SD) of each ROI.

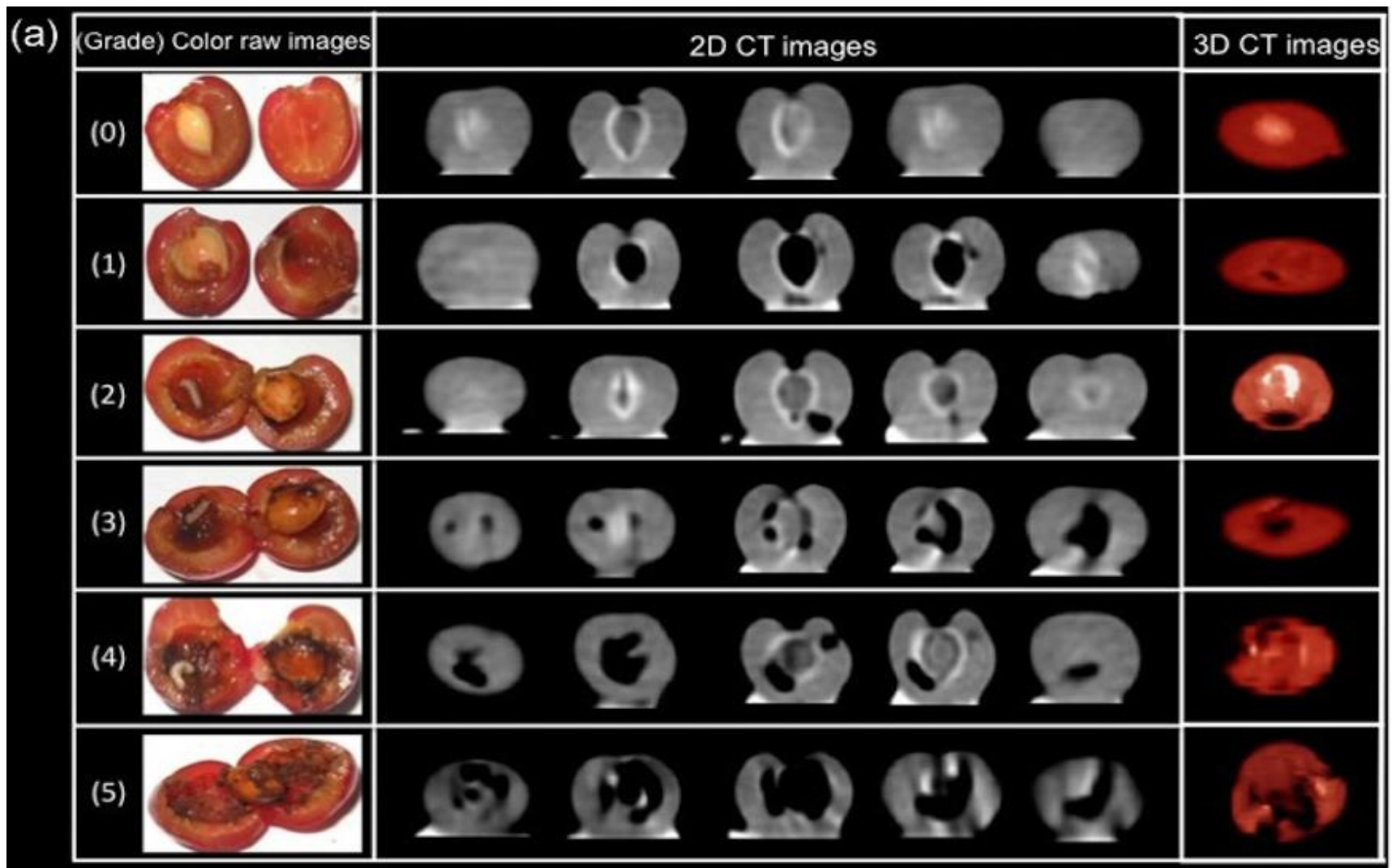
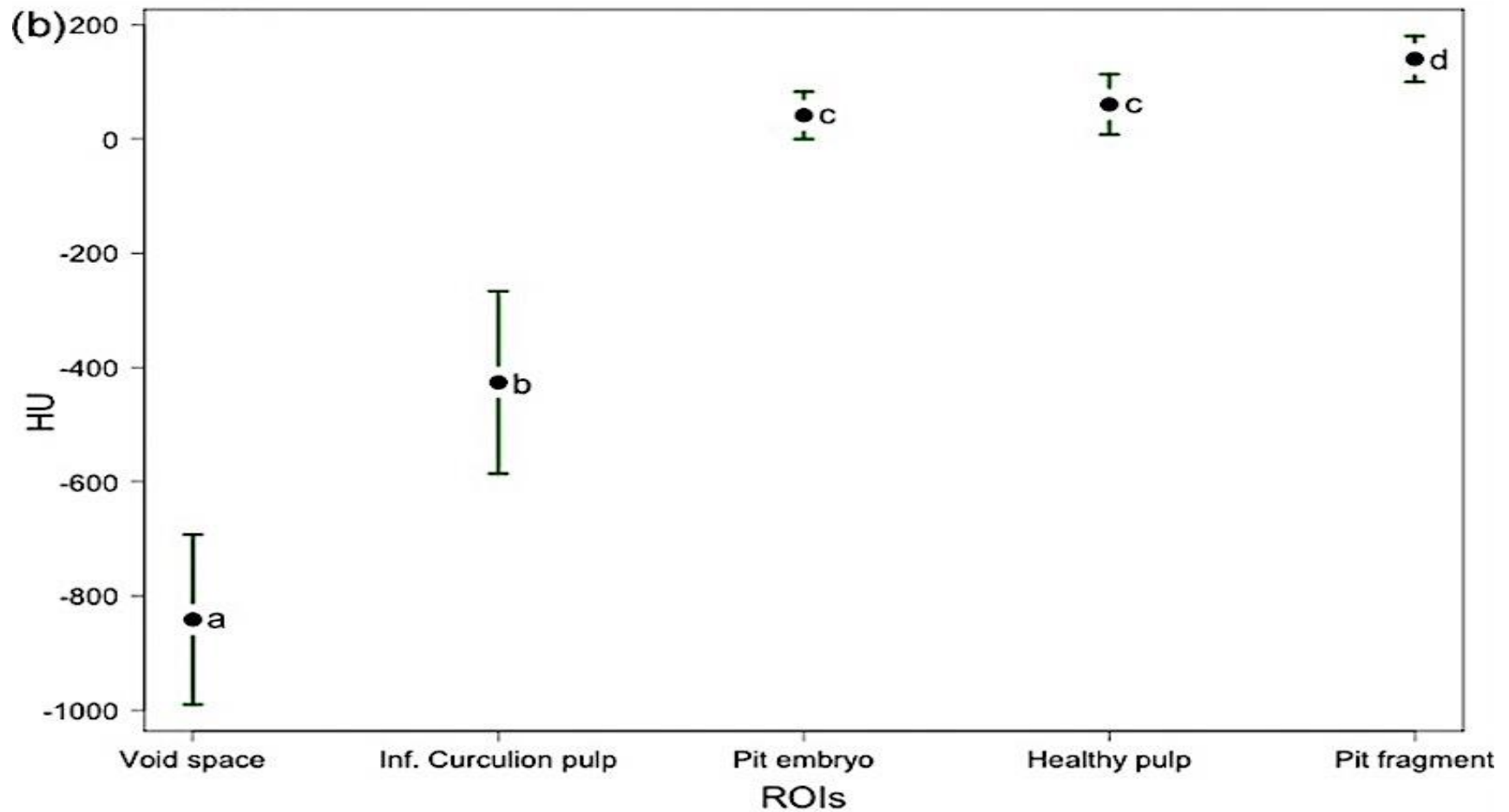


Figure 32. (a) Color raw image slices, cross-sectional 2D CT images acquired using the GE BrightSpeed™ RT 16 Elite CT scanner, and 3D reconstruction of tart cherries.

Figure 32 (cont'd)



(b) Black dots showing the HU-values for the mean of 100 data points per each tart cherry ROI (n = 100). Values followed by the same letter are not significantly different between each other at P = 0.05 (ANOVA) (Tukey multiple comparison of means). Vertical bars represent the SD of each ROI.

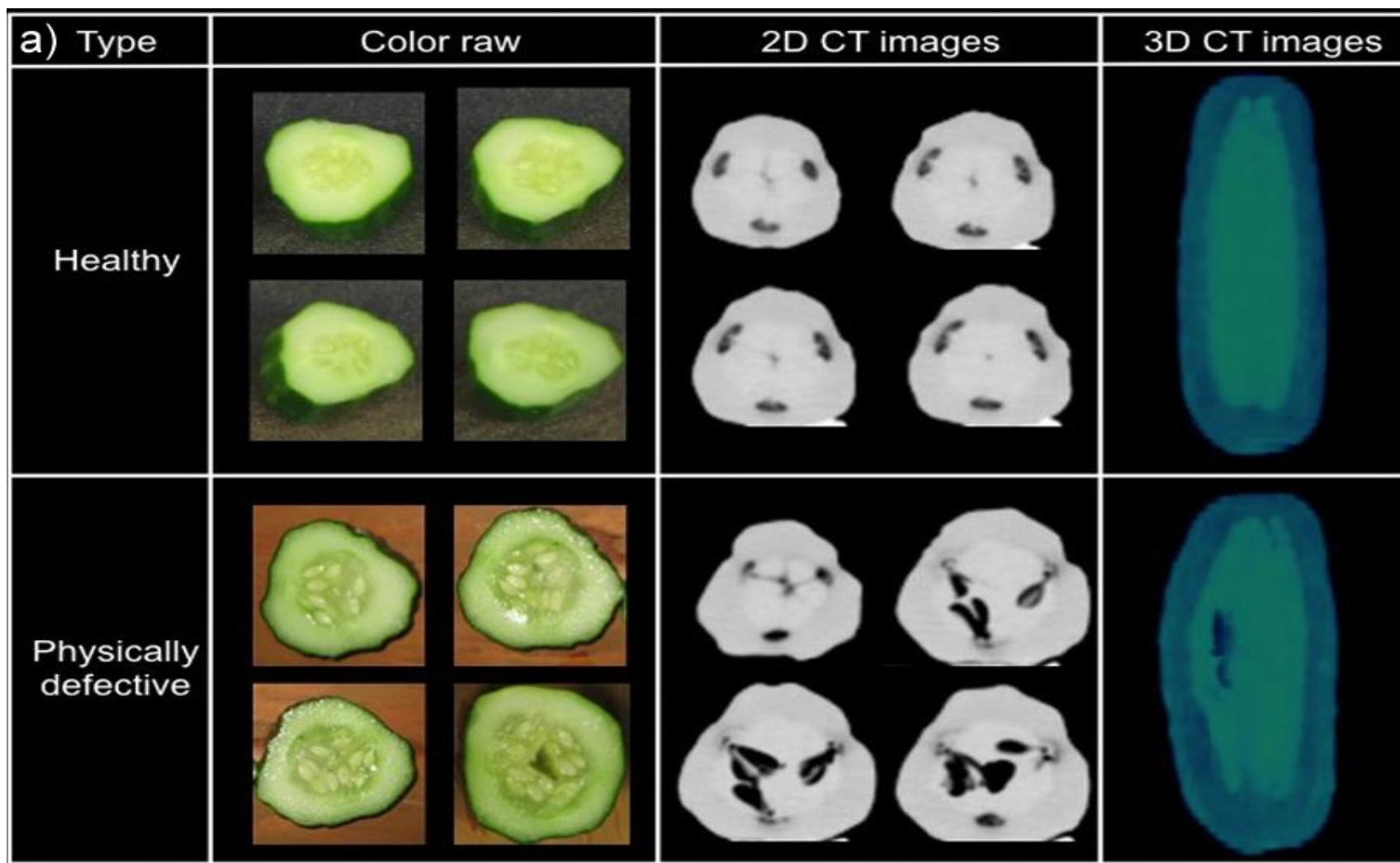
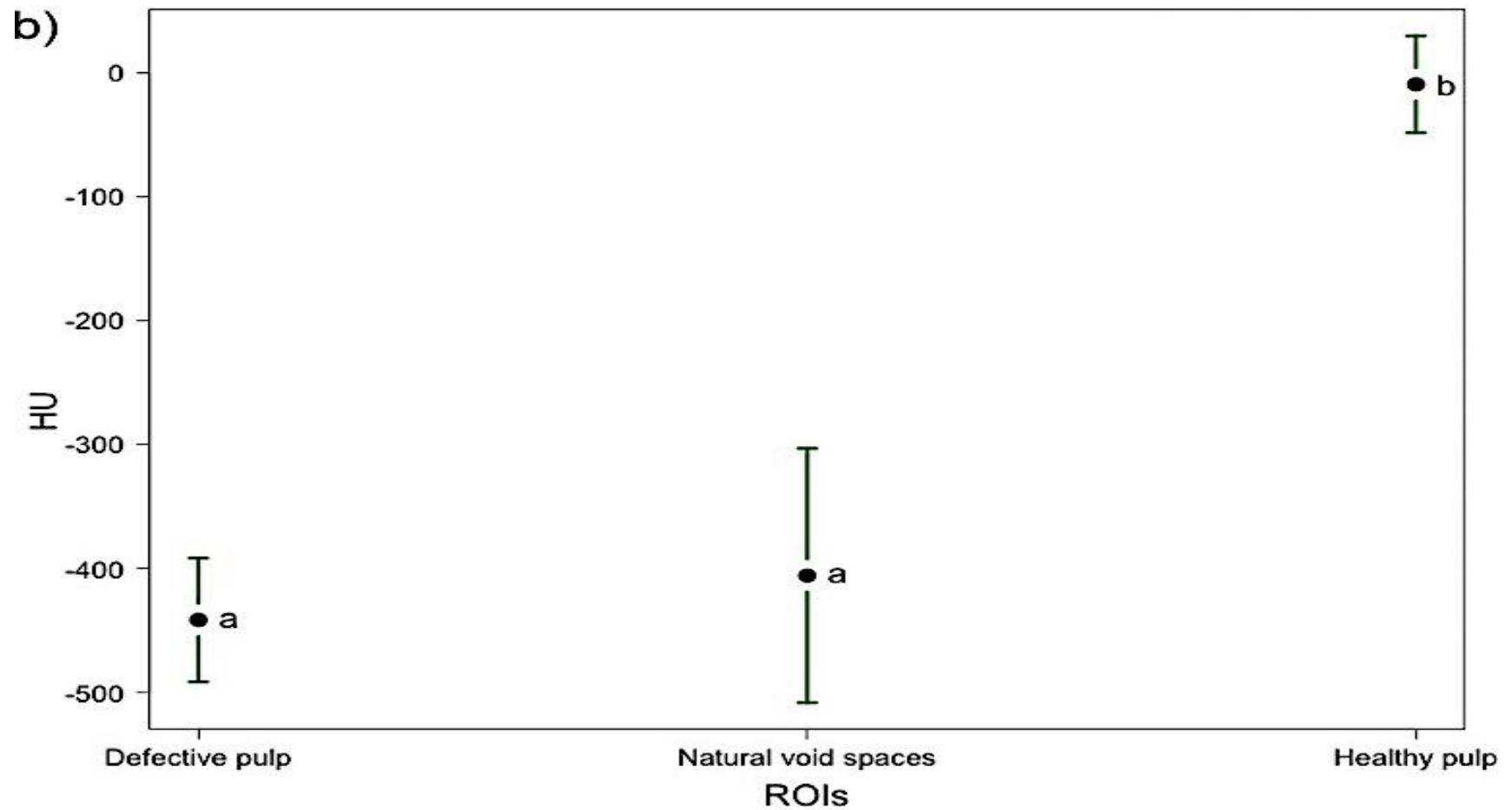


Figure 33. (a) Color raw image slices, cross-sectional 2D CT images acquired using the GE BrightSpeed™ RT 16 Elite CT scanner, and 3D reconstruction of pickling cucumbers.

Figure 33 (cont'd)



(b) Black dots showing the HU-values for the mean of 100 data points per each pickling cucumber ROI (n = 100). Values followed by the same letter are not significantly different between each other at $P = 0.05$ (ANOVA) (Tukey multiple comparison of means). Vertical bars represent the SD of each ROI.

REFERENCES

REFERENCES

- Abbott, J.A., 1999. Quality measurement of fruits and vegetables. *Postharvest Biol. and Technol.* 15, 207-225.
- Abrams, H.L., McNeil, B.J., 1978. Computed Tomography: Cost and Efficacy Implications. *Am J Roentgenol* 131, 81-97.
- Abrams, H.L., McNeil, B.J., 1978. Computed Tomography: Cost and Efficacy Implications. *Am J Roentgenol* 131, 81-97.
- Adams, M.R., Moss, M., 2000. *Food Microbiology*. Royal Society of Chemistry, Guildford, UK.
- Ahonen, T., Matas, J., He, C., Pietikäinen, M., 2009. Rotation invariant image description with local binary pattern histogram fourier features. In: Salberg, A.-B., Hardeberg, J.Y., Jenssen, R. (Eds.), *In Proceedings of SCIA*. 61-70.
- Alanen, J., Keski-Nisula, L., Blanco-Sequeiros, R., Tervonen, O., 2004. Cost comparison analysis of low-field (0.23 T) MRI- and CT-guided bone biopsies. *European Radiology* 14, 123-128.
- Anagnostakis, S.L., Gordon, P., Hebard, F.V., 1998. Identification of chestnut trees. *Annu. Rpt. Northern Nut Grower Assn.* 89, 1-4.
- Anagnostakis, S.L., Devin, P., 1999. Nutrients in chestnuts. *Annu. Rpt. Northern Nut Grower Assn.* 89, 1-4.
- Anjos, A.D., Shahbazkia, H., 2008. Bi-level image thresholding - A Fast Method, *In Proceedings of BIOSIGNALS*. 70-76.
- Ariana, D.P., Lu, R., 2010. Evaluation of internal defect and surface color of whole pikles using hyperspectral imaging. *J of Food Eng* 96, 583-590.
- Arnold, B.A., Xiang, P., Mao, S.S., Budoff, M.J., 2010. Peak SNR in automated coronary calcium scoring: Selecting CT scan parameters and statistically defined scoring thresholds. *Medical Physics* 37, 3621.
- Badr, I., Thomas, S.M., Cotterill, A.D., Oduko, J.M., Fitzgerald, M., Adam, E.J., 1997. X-ray pelvimetry - Which is the best technique? *Clinical Radiology* 52, 136-141.
- Barcelon, E.G., Tojo, S., Watanabe, K., 1999a. X-ray computer tomography for internal quality evaluation of peaches. *J. Agric. Eng. Res.* 73, 323-330.

- Barcelon, E.G., Tojo, S., Watanabe, K., 1999b. Relating X-ray absorption and some quality characteristics of mango fruit (*Mangifera indica* L.). J. Agric. Food Chem. 47, 3822-3825.
- Bierberle, M., Fischer, F., Schleicher, E., Koch, D., Menz, H.-J., Mayer, H.-G., Hampel, U., 2009. Experimental two-phase flow measurement using ultra fast limited-angle-type electron beam X-ray computed tomography. Exp. Fluids 47.
- Biomhoff, R., Carlsen, M.H., Andersen, L.F., Jacobs, D.R., 2006. Health benefits of nuts: potential role of antioxidants. British J. Nutr. 96, S52-S60.
- Bishop, C., 2007. Pattern Recognition and Machine Learning (Information Science and Statistics). Springer.
- Blackwell, R., 2006. Chestnut Farms, Inc. Business plan. Chestnut Farms, Inc. Allendale, Michigan: CGI.
- Blasco, J., Aleixos, N., Molto, E., 2007. Computer vision detection of peel defects in citrus by means of a region oriented segmentation algorithm. Journal of Food Engineering 81, 535-543.
- Bolliger, S.A., Oesterhelweg, L., Spendlove, D., Ross, S., Thali, M.J., 2009. Is differentiation of frequently encountered foreign bodies in corpses possible by hounsfield density measurement. J. Forensic Sci. 54, 1119-1122.
- Boone, J.M., Geraghty, E.M., Siebert, J.A., Wootton-Gorges, S.L., 2003. Dose reduction in pediatric CT: A rational approach. Radiology 228, 352-360.
- Borges, O., Goncalves, B., Soeiro de Carvalho, J.L., Correia, O., Silva, A.P., 2008. Nutritional quality of chestnut (*Castanea sativa* Mill.) cultivars from Portugal. Food Chem. 106, 976-984.
- Braga, F.R., Araujo, J.V., Soares, F.E., Araujo, J.M., Genier, H.L., Silva, A.R., Carvalho, R.O., Queiroz, J.H., Ferreira, S.R., 2010. Optimizing protease production from an isolate of the nematophagous fungus *Duddingtonia flagrans* using response surface methodology and its larvicidal activity on horse cyathostomins. J Helminthol, 1-7.
- Brosnan, T., Sun, D.-W., 2004. Improving quality inspection of food products by computer vision—a review. Journal of Food Engineering 61, 3-16.
- Bullerman, L.B., Bianchini, A., 2007. Stability of mycotoxins during food processing. Int. J. of Food Microb. 119, 140-146.

- Bushberg, J.T., Seibert, J.A., Leidholdt, E.M., Boone, J.M., 2002. The essential physics of medical imaging, 2. Lippincott Williams & Wilkins, Philadelphia, Pa.
- Butz, P., Hofmann, C., tauscher, B., 2005. Recent developments in noninvasive techniques for fresh fruit and vegetable internal quality analysis. J. of Food Sci. 70, R131-R141.
- Cenegage, G., Krapp, K., 2002. CT Imagin Equipment. In: Cenegage, G., Krapp, K. (Eds.), eNotes.com. 2010. Encyclopedia of Nursing & Allied Health, <http://www.enotes.com/nursing-encyclopedia/ct-imaging-equipment>.
- Chai, Z., Mendez-Vazquez, H., He, R., Sun, Z., Tan, T., 2013. Semantic pixel sets based local binary patterns for face recognition. Lecture Notes in Computer Science 7725, 639-651.
- Chen, P., Sun, Z., 1991. A review of non-destructive methods for quality evaluation and sorting of agricultural products. J of Agr. Eng. Res. 49, 85-98.
- Cubero, S., Aleixos, N., Moltó, E., Gómez-Sanchis, J., Blasco, J., 2010. Advances in Machine Vision Applications for Automatic Inspection and Quality Evaluation of Fruits and Vegetables. Food and Bioprocess Technology 4, 487-504.
- De la Montaña Miguelez, J., Miguez Bernández, M., García Queijero, J.M., 2004. Composition of varieties of chestnuts from Galicia (Spain). Food Chem. 84, 401-404.
- Deglint, H.J., Rangayyan, R.M., Ayres, F.J., Boag, G.S., Zuffo, M.K., 2007. Three-dimensional segmentation of the tumor in computer tomographic images of neuroblastoma. J. of Digital Imaging 20, 72-87.
- Donis-Gonzalez, I.R., Ryser, E.T., Guyer, D., Fulbright, D.W., 2010. Shell mold and kernel decay of fresh chestnuts in Michigan. Acta Hort. 866, 353-358.
- Donis-Gonzalez, I.R., Guyer, D.E., Pease, A., Fulbright, D.W., 2012. Relation of computerized tomography Hounsfield unit measurements and internal components of fresh chestnuts (*Castanea* spp.). Postharvest Biology and Technology 64, 74-82.
- Donis-González, I.R. 2008. Management of microbial decay of fresh and peeled chestnuts in Michigan. Michigan State University East Lansing, MI, USA.
- Donis-González, I.R., Mandujano, M., Medina-Mora, C., Fulbright, D.W., 2010a. Presence of mycotoxins after 90 days of storage in fresh chestnuts. 4th

- International Chestnut Symposium, Beijing, China. *Acta Horticulturae* 844, 69-74.
- Donis-González, I.R., Ryser, E.T., Guyer, D., Fulbright, D.W., 2010b. Efficacy of postharvest treatments for reduction of molds and decay in fresh Michigan chestnuts. First European Chestnut Conference. *Acta Horticulturae* 866, 563-569.
- Donis-González, I.R., Guyer, D.E., Leiva-Valenzuela, G.A., Burns, J., 2013. Assessment of chestnut (*Castanea* spp.) slice quality using color images. *Journal of Food Engineering* 115, 407-414.
- Du, L.Y., Umoh, J., Nikolov, H.N., Pollmann, S.I., Lee, T.Y., Holdsworth, D.W., 2007. A quality assurance phantom for the performance evaluation of volumetric micro-CT systems. *Phys Med Biol* 52, 7087-7108.
- Duda, R.O., Hart, P.E., Stork, D.G., 2000. *Pattern Classification*. Wiley-Interscience.
- Ellis, M.B., Ellis, P.J., 1985. *Microfungi on land plants, an identification handbook*, 2. Macmillan Publishing company, New York, NY, USA.
- Ertan, E., 2007. Variability in leaf and fruit morphology and in fruit composition of chestnuts (*Castanea sativa* Mill.) in the Nazilli region of Turkey. *Genet. Resour. Crop. Evol.* 54, 691-699.
- Eustice, R.E., Bruhn, C.M., 2006. *Consumer acceptance and marketing of irradiated foods*. Oxford: Blackwell Publ., Ltd.
- Farkas, J., Mohácsi-Farkas, C., 2011. History and future of food irradiation. *Food Science & technology* 22, 121-126.
- Food and Agriculture Organization Statistics Division (FAOSTAT), 2013. Chestnut harvested area, yield and production quantity. Jun. 20, 2013. <http://faostat.fao.org/>
- Ford, N.L., Thornton, M.M., Holdsworth, D.W., 2003. Fundamental image quality limits for microcomputed tomography in small animals. *Medical Physics* 30, 2869.
- Fromm, J.H., Sautter, I., Matthies, D., Kremer, J., Schumacher, P., Ganter, C., 2001. Xylem water content and wood density in spruce and oak trees detected by high-resolution computed tomography. *Plant Physiology* 127, 416-425.
- Fulbright, D.W., Mandujano, M., 2000. Establishing chestnut orchards in Michigan. *Annu. Rpt. Northern Nut Grower Assn.* 91, 1-5.

- Furnols, M.F.I., Teran, M.F., Gispert, M., 2009. Estimation of lean meat content in pig carcasses using C-ray Computed Tomography and PLS regression. *Chemometrics and Intelligent Lab. Syst.* 98, 31-37.
- Gao, Z.-j., Lin, H., Xiao, H., 2008. Air-impingement de-shelling of chestnuts (*C. mollissima*): Process parameter optimization. *Internatl. J. Food Engineering* 4, 1-15.
- Garvin, D., 1984. What does product quality really mean. *Sloan Management Review* 26, 25-45.
- General Electric Company, 2007. BrightSpeed Series: Technical Reference Manual. Tokyo, Japan. 192.
- Goetz, T., 2010. From success of the Fab Four, a key driver of health-care costs arose. *The Washington Post*, <http://www.washingtonpost.com/wp-dyn/content/article/2010/02/19/AR2010021905963.html>.
- Gold, M., Cernusca, M.M., Godsey, L., 2004. Consumer preferences for chestnuts, eastern black walnuts, and pecans. *Hort. Tech.* 14, 583-589.
- Goldman, L.W., 2007. Principles of CT: Radiation dose and image quality. *J of Nuclear Medicine Technology* 35, 213-225.
- Goldman, L.W., 2008. Principles of CT: Multislice CT*. *J Nucl Med Technol* 36, 57-68.
- Gomes, J.F.S., Leta, F.R., 2012. Applications of computer vision techniques in the agriculture and food industry: a review. *European Food Research and Technology* 235, 989-1000.
- Grau, P., France, A., 1999. Chestnut production in Chile: Some steps toward its improvement. *Acta Hort.* 494, 37-42.
- Gravatt, G.F., Marshall, R.P., 1926. Chestnut blight in the Southern Appalachians. U.S.D.A. circular 370, Washington, D.C.
- Guyer, D., Fulbright, D.W., Mandujano, M., 2003. A perspective on chestnut handling and peeling. *Annu. Rpt. Northern Nut Grower Assn.* 94.
- Guyer, D., Fulbright, D., Mandujano, M., 2005. A perspective on Chestnut handling and peeling. *Chestnut Growers of America* 7, 1-5.
- Hampel, U., Speck, M., Koch, D., Menz, H.-J., Mayer, H.-G., Fietz, J., Hoppe, D., Schliecher, E., Zippe, C., Prasser, H.-M., 2005. Experimental ultra fast X-

- ray computed tomography with a linearly scanned electron beam source. *Flow Measur. & Instr.*, 65-72.
- Hanke, R., Fuchs, T., Uhlmann, N., 2008. X-ray based methods for non-destructive testing and material characterization. *Nuclear Instr. and Meth. in Physics Research A* 591, 14-18.
- Haralick, R.M., 1979. Statistical and structural approaches to texture. In *Proceedings of the IEEE* 67, 786-804.
- Harte, J.B., Dolan, K., Camaron, A., Fulbright, D.W., 2003. Effects of curing and storage on fresh Chinese chestnuts quality. *Annu. Rpt. Northern Nut Grower Assn.* 94, 22-31.
- Holmström, L., Koistinen, P., 2010. Pattern recognition. *Wiley Interdisciplinary Reviews: Computational Statistics* 9999, n/a.
- Huda, W., Scalzetti, E.M., Levin, G., 2000. Techniques factors and image quality as functions of patient weight at abdomina CT. *Radiology* 217, 430-435.
- Jacobs, D.F., 2007. Toward development of silvical strategies for forest restoration of American chestnut (*Castanea dentata*) using blight-resistant hybrids. *Biolog. conservation* 137, 497-506.
- Jagannath, H.S., Virmani, J., Kumar, V., 2012. Morphological enhancement of microcalcifications in digital mammograms. *J of The Intitution of Enginners (India): Series B* 93, 163-172.
- Jain, A.K., Duin, R.P.W., Jianchang, M., 2000. Statistical pattern recognition: a review. *Pattern Analysis and Machine Intelligence, IEEE Transactions on* 22, 4-37.
- Jerimini, M., Conedera, M., Sieber, M., T.N., S., Scharer, A., Scharer, H., Jelmini, G., 2006. Influence of fruit treatments on perishability during cold storage of sweet chestnuts. *J. Sci. of Food and Agri.* 86, 877-885.
- Jha, S.N., Narsaiah, K., Sharma, A.D., Singh, M., Bansal, S., Kumar, R., 2010. Quality parameters of mango and potential of non-destructive techniques for their measurement - a Review. *J. of Food Sci. and Techn.* 47, 1-14.
- Kader, A.A., 2002. Postharvest technology of horticultural crops. Regents of the University of California Division of Agriculture and Natural Resources. UC-Davis, California, USA.
- Kamm, K.-F., 1998. Grundlagen der Röntgenabbildung. In: Ewen, K. (Ed.), *Moderne Bildgebung: Physik, Gerätetechnik, Bildbearbeitung und -*

kommunikation, Strahlenschutz, Qualitätskontrolle. Georg Thieme Verlag, Stuttgart & New York. pp. 45-62.

- Kavdir, I., Guyer, D.E., 2007. Evaluation of different pattern recognition techniques for apple sorting. *Postharvest Biol. and Technol.* 99, 211-219.
- Kelley, K.M., Behe, B.K., 2002. Chefs' perceptions and uses of 'Colossal' chestnuts. *Hort. Tech.* 12, 172.
- Kelly, D.M., Hasegawa, I., Borders, R., Hatabu, H., Boisselle, P.M., 2003. High-Resolution CT using MDCT: Comparison of Degree of Motion Artifacts Between Columetric and Axial Methods. *American J. of Roentgenology* 182, 757-759.
- Kim, S., Schatzki, T.F., 2000. Apple watercore sorting system using X-ray imagery: I. Algorithm development. *Trans. of the ASABE* 43, 1695-1702.
- Kincaid, R., Krol, A., Fourmaux, S., Kieffer, J.C., Serbanescu, C., Servol, M., Vogelsang, L., Wilkins, S., Stevenson, A., Nesterets, Y., Lipson, E., Ye, H., Pogany, A., 2008. Development of ultrafast laser-based X-ray in-vivo phase-contrast micro-CT beamline for biomedical applications at Advanced Laser Light Source (ALLS). *Proc Soc Photo Opt Instrum Eng* 7078, 707818 707811-707818 707812.
- Klinac, D., Seelye, R., Nguyen, H., 1999. Chestnut production and research in New Zealand. *Acta Hort.* 494, 43-48.
- Kotwaliwale, N., Weckler, P.R., Brusewitz, G.H., 2006. X-ray attenuation coefficient using polychromatic X-ray Imaging of pecan components. *Biosystems Eng.* 94, 199-206.
- Kumar, A., Pang, G.K.H., 2002. Defect detection in textured materials using Gabor filters. *Industry Applications, IEEE Transactions on* 38, 425-440.
- Lammertyn, J., Dresselaers, T., Hecke, P.V., Jacsók, P., Wever, M., Nicolai, B., 2003. MRI and X-ray CT study of spatial distribution of core breakdown in 'Conference' pears. *Reson. Imaging* 21, 805-815.
- Ledenius, K., Gustavsson, M., Johansson, S., Stalhammar, F., Wiklund, L.M., Thilander-Klang, A., 2009. Effect of tube current on diagnostic image quality in paediatric cerebral multidetector CT images. *Br J Radiol* 82, 313-320.
- Leiva, G., Mondragón, G., Mery, D., Aguilera, J., 2011. The automatic sorting using image processing improves postharvest blueberries storage quality. In *Proceedings of 11th International Congress on Engineering and Food*.

- Lillard, H.Z., Hanlin, R.T., Lillard, D.A., 1970. Aflatoxigenic isolates of *Aspergillus flavus* from pecans. *Applied Microb.* 19, 128-130.
- Long, S., 2012. Internal kernel breakdown threatens the Michigan chestnut industry - Interview with Dr. Dennis Fulbright. *Midwest Nut Producers Council Journal* 1, 3.
- Lorente, D., Aleixos, N., Gómez-Sanchis, J., Cubero, S., García-Navarrete, O.L., Blasco, J., 2011. Recent Advances and Applications of Hyperspectral Imaging for Fruit and Vegetable Quality Assessment. *Food and Bioprocess Technology* 5, 1121-1142.
- Lu, R., Peng, Y., 2006. Hyperspectral scattering for assessing peach fruit firmness. *Biosystems Eng.* 93, 161-171.
- Mandujano, M., Nash, B., Fulbright, D.W., 1998. Ethephon treatment of chestnuts for harvest management. *Annu. Rpt. Northern Nut Grower Assn.* 89, 36-41.
- Mateo, R., Medina, A., Mateo, E.M., Mateo, F., Jiménez, M., 2007. An overview of Ochratoxin A in beer and wine *Int. J. of Food Microb.* 119, 471-475.
- Mayo, J.R., Hartman, T.E., Lee, K.S., Primack, S.L., Vedal, S., Müller, N.L., 1994. CT of the Chest: Minimal tube current required for good image quality with the least radiation dose. *AJR* 164, 603-607.
- McCollough, C.H., Brueswitz, M.R., Kofler, J.M., 2006. CT Dose reduction and dose management tools: Overviews of available options. *RadioGraphics* 26, 503-512.
- Mencarelli, F., 2001. Postharvest handling and storage of chestnuts (English). *FAO-AG-TCP/CPR/8925*, Job No: AC645. Agricultural Support Systems Div. 2003. Project: Integrated Pest Management and Storage of Chestnuts in Xinxian County, Henan Province, China, CPR/8925, Rome, Italy.
- Mendoza, F., Verboven, P., Mebatsion, H.K., Kerckhofs, G., Wevers, M., Nicolai, B., 2007. Three-dimensional pore space quantification of apple tissue using X-ray computed microtomography. *Planta* 226, 559-570.
- Merkel, H.W., 1905. A deadly fungus on the American chestnut. *N.Y. Zoological Society* 10, 97-103.
- Mery, D. 2001. Automated Flaw Detection in Castings from Digital Radioscopic Image Sequences - Thesis in German. Technical University of Berlin, Berlin.

- Mery, D., Filbert, D., 2002. Classification of Potential Defects in Automated Inspection of Aluminium Castings Using Statistical Pattern Recognition, In Proceedings of 8th European Conference on Non-Destructive Testing (ECNDT 2002), Barcelona, Spain.
- Mery, D., Pedreschi, F., 2005. Segmentation of colour food images using a robust algorithm. *Journal of Food Engineering* 66, 353-360.
- Mery, D., Soto, A., 2008. Features: the more the better, In Proceedings of the 8th conference on Signal processing, computational geometry and artificial vision. World Scientific and Engineering Academy and Society (WSEAS), Rhodes, Greece.
- Mery, D., Chanona-Perez, J.J., Soto, A., Aguilera, J.M., Cipriano, A., Velez-Rivera, N., Arzate-Vazquez, I., Gutierrez-Lopez, G.F., 2010. Quality classification of corn tortillaz using computer vision. *Journal of Food Engineering* 101, 357-364.
- Mery, D., Lillo, I., Loebel, H., Rizzo, V., Soto, A., Cipriano, A., Aguilera, J.M., 2011. Automated fish bone detection using X-ray imaging. *Journal of Food Engineering* 105, 485-492.
- Metcalf, H., Collins, J.F., 1909. The present status of the chestnut bark disease. Volume 1. G.P.O., Washington.
- Milczarek, R.R., Saltveit, M.E., Garvey, T.C., McCarthy, M.J., 2009. Assessment of tomato pericarp mechanical damage using multivariate analysis of magnetic resonance images. *Postharvest Biology and Technology* 52, 189-195.
- Miller, G., 2003. Chestnuts. In: D.W., F. (Ed.), *A Guide to Nut Tree Culture in North America*¹. Northern Nut Growers Association, Inc., East Lansing, Michigan. pp. 167-181.
- Monarca, D., Cecchini, M., Antonelli, D., Mordacchini Alfani, M.L., Salcini, M.C., Massantini, R., 2005. Mechanical harvesting and quality of 'Marroni' chestnut. *Acta Hort.* 682, 1193-1198.
- Montealegre, J., Gonzalez, S., 1986. Hongos causantes de pudriciones en fruto de *Castanea sativa* (Mill). *Simiente* 56, 166-169.
- Moreda, G.P., Muñoz, M.A., Ruiz-Altisent, M., Perdigones, A., 2012. Shape determination of horticultural produce using two-dimensional computer vision – A review. *Journal of Food Engineering* 108, 245-261.
- Myers, R.H., Montgomery, D.C., Anderson-Cook, C.M., 2008. Response surface methodology: Process and product optimization using designed experiments. John Wiley & Sons, Inc. , Hoboken, New Jersey.

- Ng, C.B.R., Guojun, L., Dengsheng, Z., 2005a. Performance Study of Gabor Filters and Rotation Invariant Gabor Filters, In Proceedings of the 11th International Multimedia Modelling Conference. 158-162.
- Ng, C.B.R., Guojun, L., Dengsheng, Z., 2005b. Performance Study of Gabor Filters and Rotation Invariant Gabor Filters, Multimedia Modelling Conference, 2005. MMM 2005. Proceedings of the 11th International. 158-162.
- Nixon, M.S., Aguado, A.S., 2008. Feature extraction and image processing. Elsevier London, UK.
- Ojala, T., Pietikainen, M., Maenpaa, T., 2002. Multiresolution gray-scale and rotation invariant texture classification with local binary patterns. Pattern Analysis and Machine Intelligence, IEEE Transactions on 24, 971-987.
- Overy, D.P., Seifert, K.A., Savard, M.E., Frisvad, J.C., 2003. Spoilage fungi and their mycotoxins in commercially marketed chestnuts. Int. J. of Food Microb. 88, 69-77.
- Paglietta, R., Bonous, G., 1979. Il castagno da frutto. Frutticoltura Moderna, Collana di Manuali Tecnici Dell'Edagricole, 2. Italy
- Patil, M.S., Mathew, J., Rajendrakumar, P.K., Karade, S., 2010. Experimental studies using response surface methodology for condition monitoring of ball bearings. J. of Tribology 132, 044505.
- Peng, Y., Lu, R., 2008. Analysis of spatially resolved hyperspectral scattering images for assessing apple fruit firmness and soluble solids content. Postharvest Biology and Technology 48, 52-56.
- Perkins-Veasie, P., 2004. Commodity summary, raspberries, the commercial storage of fruits, vegetables and florist nursery stock. In: Gross, K.C., Wang, C.Y., Saltveit, M. (Eds.), United States: US Department of Agriculture Handbook. Volume: 66.
- Pietikäinen, M., Ojala, T., Xu, Z., 2000. Rotation-invariant texture classification using feature distributions. Pattern Recognition 33, 43-52.
- Pratx, G., Xing, L., 2011. GPU computing in medical physics: A review. Medical Physics 38, 2685.
- Prokop, M., 2003. General principles of MDCT. European Journal of Radiology 45, S4-S10.

- Qin, J., Lu, R., 2005. Detection of pits in tart cherries by hyperspectral transmission imaging Trans. of the ASAE 48, 1963-1970.
- Quanquan, G., Zhenhui, L., Jiawei, H., 2011. Linear Discriminant Dimensionality Reduction, In Proceedings of the 21st European Conference on Machine Learning (ECML), Athens, Greece.
- Ren, Y., Liu, H., Xue, C., Yao, X., Liu, M., Fan, B., 2006. Classification study of skin sensitizers based on support vector machine and linear discriminant analysis. *Analytica Chimica Acta* 572, 272-282.
- Renaudin, C., Magnin, I.E., Picard, C., Troussset, Y., Sergent, M., Amiel, M., 1993. Image quality study in 3D X-ray angiography: a first approach using the experimental design strategy. *Comput. Med. Imaging Graph* 17, 309-322.
- Ridé, M., Gudin, C., 1960. Sur la biologie de quelques champignons parasites de la châtaigne et plus particulièrement de *Phoma endogena* (Speg.). *Compte Rendu de l'Academie d'Agriculture France* 46, 536-543.
- Ridley, J.D., 1999. Market development opportunities in the Australian chestnut industry. *Acta Hort.* 494, 55-60.
- Sande, E.P., Martinsen, A.C., Hole, E.O., Olerud, H.M., 2010. Interphantom and interscanner variations for Hounsfield units-establishment of reference values for HU in a commercial QA phantom. *Phys Med Biol* 55, 5123-5135.
- Schollenberger, M., Muller, H.-M., Ruffle, M., Drochner, W., 2008. Natural occurrence of 16 Fusarium toxins in edible oil marketed in Germany. *Food Control* 19, 475-482.
- Sellakumar, P., James-Jebaseelan-Samuel, E., Supe, S., 2007. Investigation of optimal scanning protocol for X-ray computed tomography polymer gel dosimetry. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 264, 395-399.
- Shapiro, L., Stockman, G., 2001. *Computer Vision* Prentice Hall, Inc. , Upper Saddle River, NJ, USA.
- Sharma, D.S., Sharma, S.D., Sanu, K.K., Saju, S., Deshpande, D.D., Kannan, S., 2006. Performance evaluation of a dedicated computed tomography scanner used for virtual simulation using in-house fabricated CT phantoms. *J Med Phys* 31, 28-35.
- Sieber, T.N., Conedera, M., Jerimini, M., 2007. Effects of the harvest method on the infestation of chestnuts (*Castanea sativa*) by Insects and Moulds. *Phytopathology* 155, 497-504.

- Silva, R.R., Siqueira, M.H.S., Calôba, L.P., 2002. Contribution to the Development of a Radiographic Inspection Automated System, In Proceedings of 8th European Conference on Non-Destructive Testing (ECNDT 2002), Barcelona, Spain.
- Smyth, A.B., Cameron, A.C., 1998. Modified-atmosphere packaging of cut iceberg lettuce: Effect of temperature and oxygen partial pressure on respiration and quality. *J. of Agriculture and Food Chem.* 46, 4556-4592.
- Sornsrivichai, J., Yantarasri, T., Kalayanamitra, K., 2000. Nondestructive techniques for quality evaluation of pineapple fruits. *Acta Hort.* 529, 337-341.
- Sreedhar, K., Panlal, B., 2012. Enhancement of Images Using Morphological Transformations. *International Journal of Computer Science and Information Technology* 4, 33-50.
- Stadler, A., Schima, W., Prager, G., Homolka, P., Heinz, G., Saini, S., Eisenhuber, E., Niederle, B., 2004. CT density measurement for characterization of adrenasal tumors *ex vivo*. *AJR* 182, 671-675.
- Stuke, I., Brunke, O., 2010. Inline-CT: A new concept for the fast CT inspection in mass production. In: Brunke, O., Neuber, D. (Eds.), *High-Resolution X-ray CT Symposium*. GE Sensing & Inspection Technologies, Dresden, Germany. 23.
- Talasila, P.C., Cameron, A.C., Joles, D.W., 1994. Distribution of steady-state Oxygen partial pressures in modified-atmosphere packages of cut broccoli. *J. of American Society Hort. and Scie.* 119, 556-562.
- Tanaka, K., Sago, Y., Zheng, Y., Nakagawa, H., Kushiro, M., 2007. Mycotoxins in rice. *Int. J. of Food Microb.* 119, 59-66.
- Teisi, M.F., Fein, S.B., Levy, A.S., 2009. Information effects on consumer attitudes toward three food technologies: organic production, biotechnology and irradiation. *Food Quality and Preference* 20, 586-596.
- Thompson, J.F., Mitchan, E.J., Mitchell, F.G., 2002. Preparation for fresh market. In: Kader, A.A. (Ed.), *Postharvest technology of horticultural crops*. University of California Agriculture and Natural Resources, Oakland, CA. pp. 67-79.
- Tsukagoshi, S., Ota, T., Fujii, M., Kazama, M., Okumura, M., Johkoh, T., 2007. Improvement of spatial resolution in the longitudinal direction for isotropic imaging in helical CT. *Phys. Med. Biol.* 52, 791-801.

- Upchurch, B.L., Affeldt, H.A., Aneshansley, D.J., Birth, G.S., Cavalieri, R.P., Chen, P., W.M. Miller, Sagig, Y., Schmilovitch, Z., Throop, J.A., Tollner, W.W., 1993. Detection of Internal Disorders, Proceedings of the International Workshop funded by the United States-Israel Binational Agricultural Research and Development Fund (BARD). American Society of Agricultural Engineers, Sponkane, Washington 5, 80-85.
- van-Daatselaar, A.N., van-der-Stelt, P.F., Weenen, J., 2004. Effect of number of projections on image quality of local CT. *Dentomaxillofacial Radiology* 33, 361-369.
- Varga, L., Koncz, Z., Kocsube, S., Matrai, T., Teren, J., Ostry, V., 2007. Mycobiota of grapes collected in Hungarian and Czech vineyards in 2004. *Acta Alimentaria* 36, 329-341.
- Verboven, P., Kerckhofs, G., Mebatsion, H.K., Ho, Q.T., Temst, K., Wevers, M., Cloetens, P., Nicolai, B.M., 2008. Three-dimensional gas exchange pathways in pome fruit characterized by synchrotron X-ray computed tomography. *Plant Physiology* 147, 518-527.
- Vettraino, A.M., Paolacci, A., Vannini, A., 2005. Endophytism of *Sclerotinia pseudotuberosa*: PCR assay for specific detection in chestnut tissue. *Micol. Res.* 109, 96-102.
- Vossen, P., 2000. Chestnut culture in California. UCDAVIS-Publication 8010, 16 California.
- Wang, D.C.C., Vagnucci, A.H., Li, C.C., 1983. Digital image enhancement: A Survey Computer vision, graphics, and image processing 24, 363-381.
- Wang, T., Liang, H., Yuan, Q., 2011. Optimization of ultrasonic-stimulated solvent extraction of sinigrin from Indian mustard seed (*Brassica Juncea* L.) using response surface methodology. *Phytochem Anal* 22, 205-213.
- Wells, J.M., 1980. Mycoflora and Market quality of chestnuts treated with hot water to control the chestnut weevil. *Plant Dis. Rep.* 64, 999-1001.
- Willis, R., McGlasson, B., Joyce, D., 2007. Postharvest. An introduction to the physiology and handling of fruit, vegetable and ornamentals. CABI, South Wales, Australia.
- Wirth, M., Fraschini, M., Lyon, J., 2004. Contrast enhancement of microcalcifications in mammograms using morphological enhancement

- and non-flat structuring elements In Proceedings of 17th IEEE Symposium on Computer-Based Medical Systems (CBMS'04). 134-139.
- Woodford, C., Yartsev, S., Van Dyk, J., 2007. Optimization of megavoltage CT scan registration settings for brain cancer treatments on tomotherapy. *Phys Med Biol* 52, N185-193.
- Wulfshohn, D., Sarig, Y., Algazi, R.V., 1993. Defect sorting of dry dates by image-analysis. *Canadian Agr. Eng.* 35, 133-139.
- Zhang, J., 2002. Invariant Texture Segmentation Via Circular Gabor Filters. In: Tieniu, T., Li, M. (Eds.). 20901-20901.
- Zhu, B., Jiang, L., Luo, Y., Tao, Y., 2007. Gabor feature-based apple quality inspection using kernel principal component analysis. *Journal of Food Engineering* 81, 741-749.
- Zimmerman, J.B., Pizer, S.M., Staab, E.V., Perry, J.R., McCartney, W., Brenton, B.C., 1998. An evaluation of the effectiveness of adaptive histogram equalization for contrast enhancement *IEEE Trans of Medical Imaging* 7, 304-312.