Project title:	**Investigation into the non-destructive detection of Brown Heart in swedes (*Brassica napus*) using ultrasound**
Project number:	FV 444
Project leader:	M J Holmes. School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT.
Report:	Final report, 31-08-2015
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Key staff:	M J Holmes
Location of project:	School of Food Science and Nutrition, University of Leeds. LS2 9JT.
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Date project commenced:	01-05-2015
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The results and conclusions in this report are based on an investigation conducted over a four month *period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.*

A**U**THENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

This research investigated the non-destructive ultrasound technique to detect the internal defect Brown Heart in swedes, together with the texture and microstructure measurements, and suggests a viable proof of concept.

The Brown Heart condition influenced the ultrasound velocity on the swedes. The ultrasound technique showed a strong positive correlation with the Brown Heart severity as verified by subsequent visual inspection. Ultrasound velocity can be explained theoretically in terms of the volume ratio of air-water. This hypothesis is supported by microscopy of the internal cells of Brown Heart and healthy swedes and texture profile analysis.

Further research is required to fully explore different sample harvests and produce associated calibration data. In particular, to satisfy industry requirements, the techniques need to be able to evaluate swede samples radially without process 'cuts' and ideally with single transducers systems during in-line cleaning and weight grading processes. This would require a fully formulated research enquiry to fully parameterise the variation within the crop and post-harvest practices.

Headline

Ultrasound velocity measurements offer a potential method for the non-destructive detection of internal defects caused by Brown Heart deficiency in swedes (*Brassica napus* or Rutabaga).

Background

The aim of the research was to evaluate ultrasound techniques to identify interior defects with culinary swedes that currently may cause significant crop losses despite potentially low incidence within the entire harvest. This project primarily focused on Brown Heart detection in culinary swedes but the technology in principle may be exploited to detect other defect conditions and other vegetable formats enabling more efficient quality assessment.

The intended deliverable from the project was to establish a proof of concept in the use of ultrasound as a reliable methodology of internal defect detection in root vegetables. The research has shown that additional investigation and development is required to improve characterisation parameters and to design practical engineering solutions.

Summary

Preliminary assessment of the use of ultrasound to detect defects and to estimate the level of defect, indicated that ultrasound velocity measurements offer a potential method to

accurately identify Brown Heart defective swedes, although not to specifically identify the degree of the defect.

The aim of the research was to look at the development of an economical, non-destructive ultrasound method of internal defect determination within swedes. This will lay the foundations for later development of a production line system to identify defective individual roots on a conveyor belt during initial processing and provide a reliable quality assurance methodology. The project aimed to use ultrasound measurement techniques to evaluate swede crops non-destructively to accurately determine the presence of internal defects caused by stresses such as boron deficiency. This technique offers an objective method to rapidly evaluate the quality of individual swedes and minimize crop losses, offering the obvious financial benefits to growers and quality assurance to retailers.

Action Points and potential financial benefits

The work conducted in this project investigated the potential use of ultrasound measurements to non-destructively and non-invasively detect the presence of internal defects, specifically Brown Heart in swedes. The results suggest that this is a potentially viable technique which would benefit from a more detailed and extensive study to further explore the use of single transducer systems which do not require process cuts of samples (i.e. complete swedes) and test samples radially, as opposed to axial measurements which were conducted in this work.

Significant crop losses currently occur due to Brown Heart, resulting in entire crops being destroyed or wasted; this would be substantially reduced if a reliable detection method could be developed, offering the obvious financial benefits to growers and quality assurance to retailers.

SCIENCE SECTION

Introduction

Internal defects of tuber and root crops are a major problem to the horticultural industry. Brown Heart is a major defect occurring particularly in swede and turnip and is manifest by dark discolouration in the core region of the root. It is believed to be caused by deficiency of boron in the soil or is associated with rapid growth early in the season and sometimes heat stress in the crop. It leads to rejection of entire crops at the field stage if the presence has been detected even in essentially low samples. However, it is known that there is high variability in the presence of Brown Heart on very short length scales e.g. within the same field regions of Brown Heart can occur with major proportion being unaffected. Post harvest retail rejection and potentially customer complaints are also of significant concern. Thus accurate, efficient and rapid detection within individual roots is desirable.

While external defects are routinely detected either manually or using optical sensors, internal defects cannot be detected unless the crop is peeled or sliced open. Damaged or defected crops are therefore very difficult to identify on an inspection line; whole crop batches can be rejected even if only a few defective roots are present, leading to significant losses and waste. Therefore non-destructive technologies that can detect internal damage will be of significant economic benefit and reduce waste. Non-destructive techniques Near Infrared Spectroscopy (NIR), impact analysis, X-ray and computed tomography (CT), magnetic resonance imaging and ultrasound. These techniques have various benefits and problems in their implementation (Nicolaï et al. 2014). Ultrasound techniques, which have advanced significantly in recent years and are currently not employed in quality assurance, may be utilised and offer with great effect the opportunity to evaluate raw commodities and select batches in a robust statistical process controlled environment to minimise waste.

The texture of swedes is an important quality indicator of the internal flesh conditions. Brown Heart or 'water-core' symptom is a nutritional deficiency associated to boron imbalance during pre-harvest (Golob et al. 2002; Dermott & Trinder 1947). **Figure 1** shows examples of the Brown Heart symptom. Textural properties give an indication of the mechanical characteristics of the swedes, for example, elasticity relates to the compressibility level of fruits and vegetables which may be related to ripeness. The change of mechanical properties during growth and development, maturity and ripening corresponds to temporal changes in cell tissue structures (Abbott 2004; Szczesniak 1973; Bourne 2002). However, internal features cannot be detected by a visual sorting via external physical features. Therefore, failure to achieve standard quality criteria of swedes will potentially cause post-harvest losses, food waste, financial losses and increased consumers complaints.

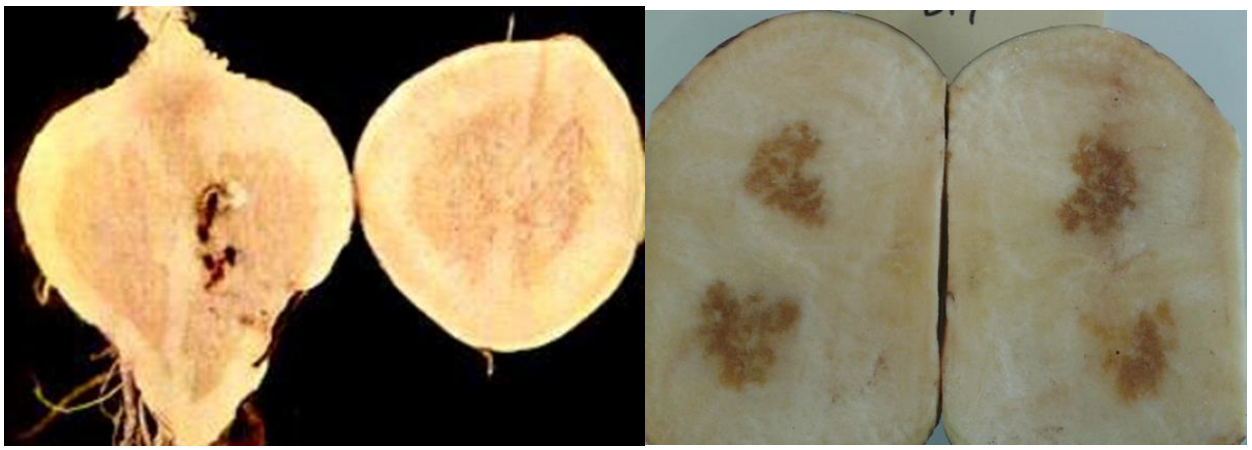
Brown Heart in swedes is commonly measured by a destructive technique by cutting through and assessing the flesh quality visually. Nevertheless, this technique is not effective and not objective because it is destructive and non-reproducible. In fact, it contributes to food waste. Therefore, to accurately determine the status of swede internal flesh quality postharvest to determine optimum quality on an individual basis prior to storage offers significant benefits. Therefore, non-destructive techniques are highly desirable techniques to detect the Brown Heart in swedes.

Ultrasound tests are a non-destructive method. Characteristics of the ultrasound wave, namely speed and amplitude, may be repeatedly and reliably measured in different materials (Povey 1998; Povey 1997; Povey 2007; Povey 2000; Zou & Zhao 2015). The behaviour of the low frequency sound wave propagated through the tested medium can provide information about the firmness of the medium and other geometrical features of the internal structure. This identification of mechanical properties may be associated with internal textural properties. Studies have shown a correlation between wave propagation and internal structure properties of fresh fruits and vegetables (Mizrach 2004; Mizrach 2007; Bechar et al. 2005; Mizrach 2008). Therefore this study investigated the hypothesis for an association between ultrasound propagation parameters and mechanical and biological properties of Brown Heart in the swedes.

Specifically, the aims of this experiment were to investigate ultrasound techniques as a non-destructive detection of the internal disorder Brown Heart in swedes. It was delivered by:

1. assessing different options of ultrasound devices and frequency ranges to verify the technique is a viable method of Brown Heart detection defects in Swede;
2. selecting appropriate sampling and testing criteria suitable for defect detection;
3. quantifying precision and accuracy of the selected technique for swede defect detection;
4. quantifying statistically, correct positive detection rates of Brown Heart swede samples;
5. evaluating potential use of x ray technology as a comparative measure;
6. use texture analysis to confirm mechanical properties of healthy and Brown Heart tissues;
7. use optical microscopy to confirm cellular structure of healthy and Brown Heart tissues.

Thus, it was to test if ultrasound is an alternative non-destructive technique for detection of internal Brown Heart disorder in swedes.



A B

Figure 1: Brown Heart symptoms visible in swedes (A and B): Upon cutting, they have brown, soft, watery areas, often called “brown-heart.”

Materials and methods

Materials and sampling procedures

Experimental work was conducted from May to June 2015 at the school of Food Science and Nutrition, University of Leeds.

Approximately 400 kg of swedes were supplied by K S Coles Ltd which were initially delivered at the end of January 2015 and stored at AHDB Sutton Bridge Crop Storage Research, East Bank, Sutton Bridge, Spalding, Lincs. PE12 9YD in cold storage facility at 1°C to ensure minimal deterioration of the samples. Samples were stored at Food Science cold storage facility at 6°C to reduce rate of deterioration of the samples. The sample set had a high prevalence of exhibiting the Brown Heart defect (**Figure 2**) therefore provided an excellent test sample. The date of harvest for the swedes was 19.01.15 and the variety for both was Emily. The non-Brown Heart swedes were grown at Gupworthy in Somerset and the affected swedes were grown at Preston Bowyer in Somerset.

The testing procedure was based upon the following strategy:

1. development of a 10-point Brown Heart index based on visual observation;
2. determination of Brown Heart condition for samples based on the developed Brown Heart index. This required internal inspection after specific tests;
3. Ultrasound velocity test in swedes using the PUNDIT Plus system;
4. texture profile analysis (TPA) via a penetration test by a Texture Analyser (TA);
5. optical microscopy of swede internal cell structures;
6. Ultrasound phase arrays-flaw detection using Olympus Omniscan systems;

7. X-ray analysis of samples.

A

B

C

Figure 2: Cross-sections taken from the swede samples: A. healthy swedes; B. Brown Heart swedes; and C. Brown Heart swedes – close up (provided by K S Coles) randomly selected from a consignment of Brown Heart deficient harvest. 23/01/2015. Note the variation in location of defective area within Swedes. Courtesy of Glyn Harper and Andrew Rutherford

Measurement procedures

Development of Brown Heart Index (as a reference)

A Brown Heart index was set up as a reference to determine the severity levels of the internal disorder in swedes for a visual sorting. The index was defined from 0 to 9 and it was based on the brown colour and the size of the disorder during a visual inspection under normal lighting conditions within the laboratory.

Determination of Brown Heart severity by visual sorting

The Brown Heart severities were inspected for 87 swedes based on the index in the section of Development of Brown Heart Index (as a reference). The coloured pictures of the index set were displayed during the visual categorising of the Brown Heart level within specific Swedes under test. The index number was recorded.

Ultrasound measurement by a PUNDIT transducers test

The velocity of the swedes was measured axially by using the Pundit transducers to investigate the signal intensity pattern correlated to the Brown Heart. The measurement was based on the time of flight and the path length of the travelled sound wave passed through the tested swedes. The experimental setup consisted of a PUNDIT and two transducers (transmitting and receiving transducers) (**Figure 3**). The PUNDIT Plus Transducers was an ultrasound pulse generator (CNS Farnell Electronics Ltd, 61-63 Holmes Road, London, NW5 3AL) and transducer system. The transmitting transducer produced 1kV pulse of a high voltage and it created a pulse pressure with a centre frequency of approximately 39 kHz. A pulse repetition rate was at 10 Hz. The PUNDIT displayed a digital numerical reading on its screen.

Sound is a travelling wave which is an oscillation of pressure transmitted through a solid, liquid or gas. The velocity c (ms^{-1}) of pressure wave is associated to the wavelength λ (m), frequency *f* (s^{-1}), distance of the wave propagated *d* (m) and time taken to propagated to the tested medium *t* (s) (**Figure 4**). Meanwhile, sound velocity is influenced by wavelength λ and known frequency f or known distance (d) and time (t) as the following formulas:

$$\text{Velocity } c = f\lambda \quad \text{(known frequency)} \qquad \text{Equation 1}$$

or

$$\text{Velocity } c = \frac{t}{d} \quad \text{(known distance)} \qquad \text{Equation 2}$$

(Povey 2000; Povey 2007; Mcclements & Gunasekaran 1997)

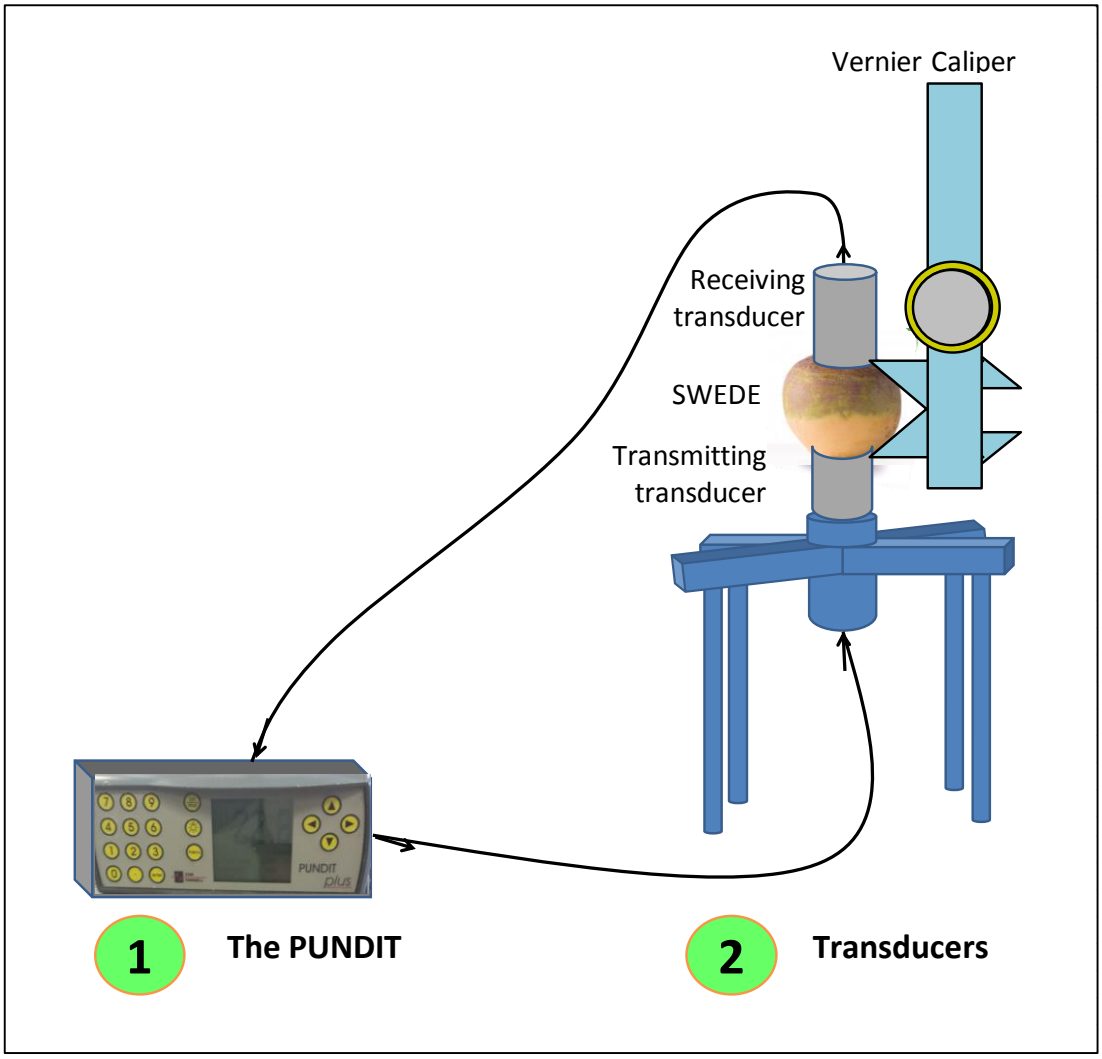


Figure 3: Schematic diagram of the experimental setup for PUNDIT transducer testing of a swede. Swede samples are measured in height and were placed between the transducers which initiated and received a pressure pulse whose time of flight is used to calculate the associated velocity.

A Pundit transducer test uses a low ultrasound frequency. As a result, the test is non-destructive because the pulse generated by the transducer will not alter the properties of the tested medium. Instead, the pulse is influenced by the properties of the tested medium.

The test uses an acoustic pulse-echo technique. A timer is chosen to start the ultrasound pulse at zero when the first pulse of sound is triggered by the transmitting transducer electrically. The pulse propagates through the wear plate of the transducer and the tested medium. Then, the returning pulse is captured by the receiving transducer showed as the first arrival of ultrasound wave. The ultrasound wave delay time is used for the determination of the velocity measurement (Povey 1997).

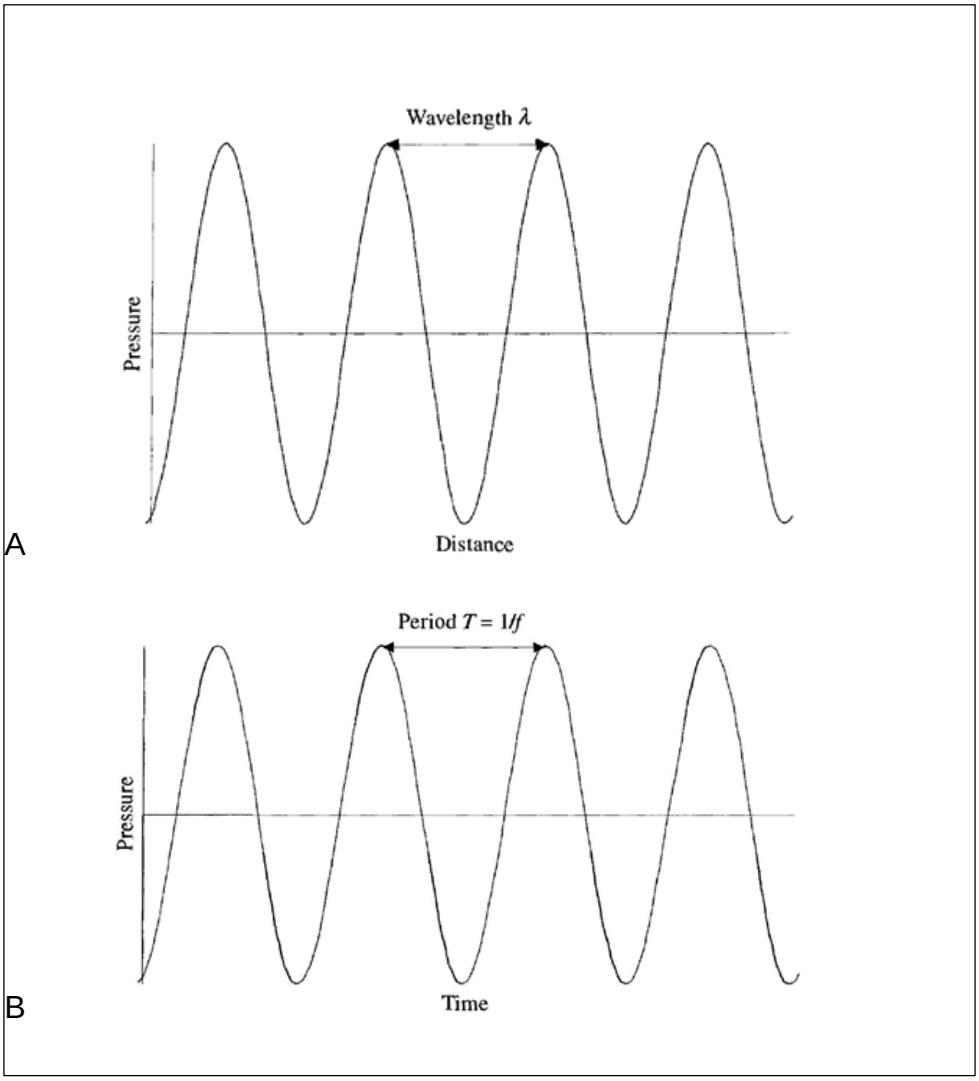


Figure 4: The pressure wave has a correlation among pressure, wavelength (λ), frequency (*f*), distance(*d*) and time (*t*) (Povey 1997).

The velocity of the ultrasound wave through swede samples were measured axially by placing it in between the pair of the transducers as in the position No.2 in **Figure 3**. The distance of an acoustical path of the vegetable between the transmitting and receiving transducers was measured with a Vernier caliper (±0.01 mm) see Figure 18 for height distribution of samples. It was important that clean and parallel cuts were made at the top and tail of the swede to ensure good contact between the transducers and the swede sample. The detected time of flight obtained from the screen of the PUNDIT was recorded (in the position No.1). Next, the velocity of the axial measurement was calculated by the measurement of the distance of ultrasound pathway divided by the time of flight (Equation 2). The measurement steps were then repeated for samples B1 – B87. n = 4 repeat measurements were taken in each swede and an average calculated. When velocity measurements had been obtained, the swede samples were cross-sectioned and examined for the presence of Brown Heart and if found a judgement assigned to the degree on browness based on the established index. In addition,

the geometric location of the index was also identified, examples are shown in Figures 2 and 12. Samples were tested immediately upon removal from cold storage.

The velocity measurement by a PUNDIT transducer test was used to analyse the following:

1. Measurement of velocity in sample of swedes which are healthy and have increasing level of Brown Heart as defined in the index
2. Produce calibration curve from measurements to provide predictive measure
3. Use velocity measurement in blind samples to estimate the detect or non-detection of Brown Heart condition in swedes
4. Prediction of degree of Brown Heart in a swede based on the velocity.

Texture profile analysis (TPA) curves by Texture Analyser (TA)

The texture (firmness) of the swedes was measured based on force-time measurement by using a Stable Microsystems Texture Analyser TA XT Plus (Stable Micro Systems, Surrey, U.K.) (**Figure 5**). The TA used a 2 mm diameter stainless steel punch cylindrical probe with 2.00 mm/s pre-test, 1.00 mm/s test, and 10.00 mm/s post-test speeds. First, the skin of the swede B1 was sliced radially by using a knife. Second, the vegetable was positioned between the lower base of the TA and the cylindrical probe (Figure 6). The probe punctured the vegetable until the puncture reached 10.000mm. Third, the measurement was repeated for all respective swedes B2 to B87. The graph of force versus time was generated and a maximum puncture force was recorded as the flesh firmness (measured property) expressed in a unit of gram.

Figure 5: A Stable Microsystems Texture Analyser TA XT Plus used for Force-time measurement to evaluate texture by using a puncture test

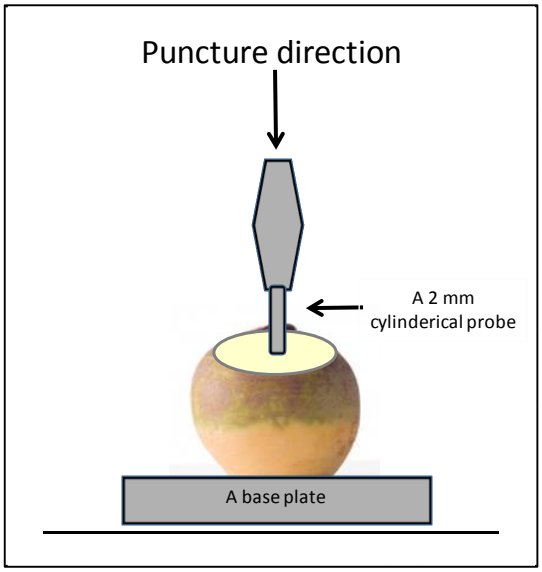


Figure 6: Schematic diagram of the experimental setup of a puncture test by using a Texture Analyser

The firmness measurement by a puncture test was used to analyse the followings:

1. texture profile analysis (TPA) curves;
2. a relationship between firmness against velocity of ultrasound in swede.

Swede internal cells measurement by a light microscope

Cell structures of the swedes were investigated to observe any differences in appearance between the Brown Heart and healthy vegetables. The swede flesh was cut into approximately 10 μm thickness using a blade and it was placed on a microscope slide. Next, the section was stained by toluidine (0.01% w/v Toluidine Blue O (T3260-5G, Sigma, UK) in Phosphate-buffered saline solution (Fisher chemical BPE9739-1, UK). Then, the section was observed under a light microscope (Olympus BH-2).

Cell internal structure measurement by ultrasonic phase arrays-flaw detector

The use of Olympus OmniScan MX2 (**Figure 7**) with the phased-array test head were attempted to evaluate the spatial swede internal structures. The test used the time of flight (TOF) and attenuation measurement to reconstruct internal structure. The pulser/ receiver had its aperture of 32 elements and 128 numbers of elements. Meanwhile, the gain of the receiver was between 0 and 74 dB with maximum input signal 1.25 Vp-p. The input impedance was 50 Ω and the system bandwidth was between 0.53 and 21 MHz (-3dB). The internal structure was reconstructed by the measurements of time of flight (TOF) and attenuation. The imaging was processed by using Corel PaintShop Pro X6.

Due to significant attenuation of the ultrasound at the operating frequencies this technique was not informative.

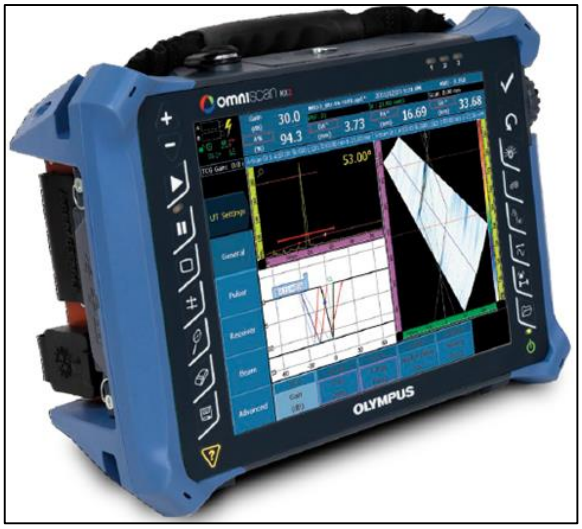


Figure 7: Olympus OmniScan MX2 used for Time of flight (TOF) and attenuation measurement to reconstruct internal structure of the swedes

Cell microstructure measurement by using an X- ray

The image of the internal cell structure of the swedes was scanned by Scanco XtremeCT, (Scanco Medical, Switzerland). The instrument was operated at a high-resolution peripheral quantitative CT scanner with resolutions up to 41 microns. The maximum field of view was 125mm and the maximum scan height (length) was 150mm.

Data analysis

The results were recorded and analysed by using Excel software (Version 2010, IBM Corporation, Chicago USA) and MATLAB software (Version R2013a. The Math Works Inc. MA, and USA). All statistical data analysis was based on p-value (one-tailed) < 0.05.

Results and discussion

Development of a reference for Brown Heart Index (BHI)

The brown index was developed as a reference in a sorting system for the Brown Heart in the experiment (Figure 8). The nine degrees of the Brown Heart severities were identified from 0 to 9. A sample of n = 10 healthy Swedes was used to identify index 0.

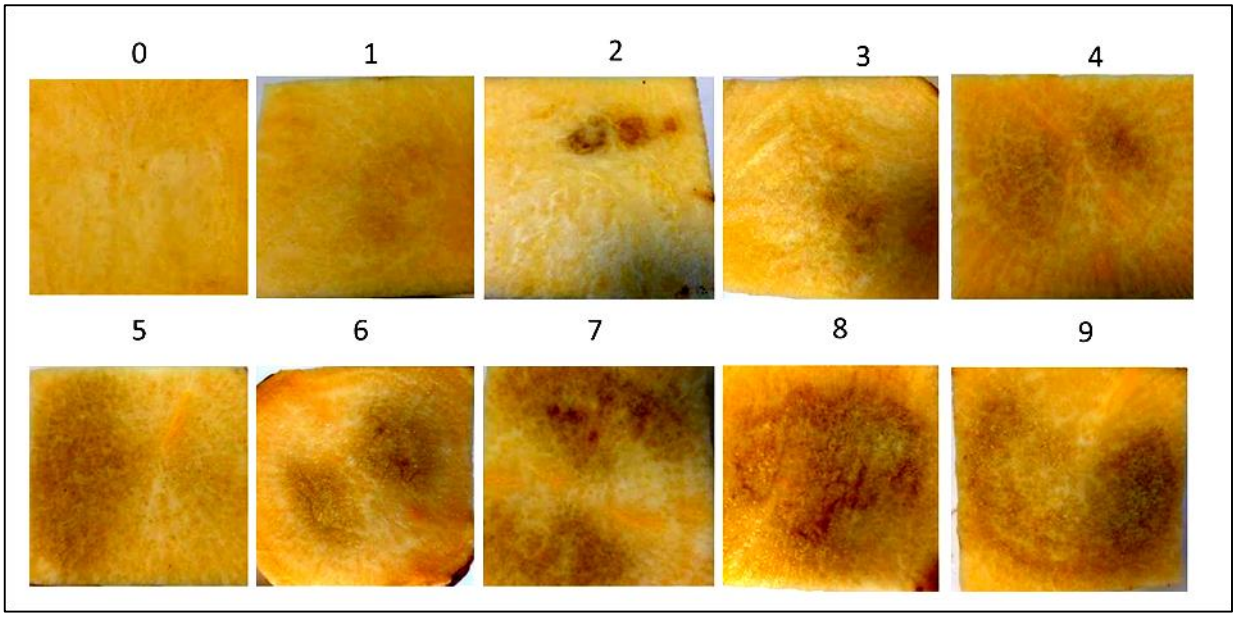


Figure 8: Characteristics of Brown Heart conditions of swede in a radial cut section. The scales from 0 to 9 (the least to the most severe Brown Heart observed with the samples received).

Determination of the Brown Heart conditions by the visual inspection and the Pundit test

Different Brown Heart categories from 0 to 9 were found in the 87 swedes (B1-B87) and the velocity showed directly a positive relation with the swede Brown Heart index (Figure 8). An independent sample of n = 10 healthy samples were used to calibrate velocity in healthy swede, These had a mean value of 235 ms^{-1} and standard deviation 17 ms^{-1}. Linear regression gave the best least square fitting with R^2 = 0.6693 and Pearson correlation coefficient was calculated to assess the association between the velocity and Brown Heart index.

There was a very strong positive correlation between the two variables, r = 0.82, n = 87, p < 0.0001. Overall, there was a strong, positive correlation between the prescribed optical browness index and measured velocity. Increases in the level of Brown Heart as defined by the level and extent of optical browness were correlated with an associated positive increase in the ultrasound velocity. The strength of the relationship suggests that velocity can be used to predict the severity of the Brown Heart of the Swedes with a linear regression providing a quantitative method to estimate browness based upon the measured velocity. Hence, there was an effect of Brown Heart on ultrasound velocity in swedes.

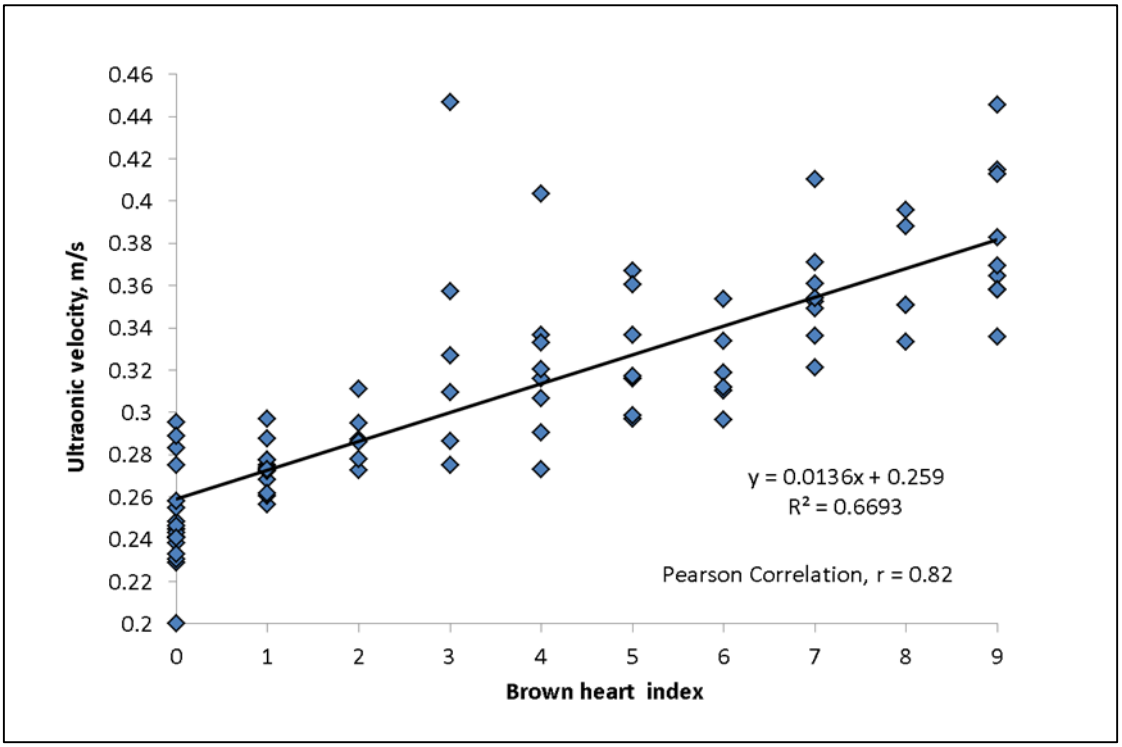


Figure 9: A strong positive relationship between ultrasound velocity and the degree of the Brown Heart of swedes for n = 87 swedes. Linear regression of the data is shown with a strong positive correlation r = 0.82, p < 0.0001. Each point represents the average of 4 repeat measurements.

Quality control chart of Brown Heart based on the velocity: Discrimination between the defected Brown Heart and healthy swedes

The velocity measurement within the swede can detect and differentiate between the defected Brown Heart and healthy swedes in the sample set provided. A quality control chart of ultrasound velocity was developed for healthy swedes and 39 suspected Brown Heart samples were evaluated. Table 1, Figure 10 and Figure 11 show results of this aspect. The values of Mean, 1 standard deviation (1SD) and 2 standard deviations (2SD) of the velocities taken from 10 healthy samples are represented by the long and short dashed and dotted lines respectively. The velocity of the defected Brown Heart swedes was higher compared to the healthy swedes. Only 4 (B2, B11, B26) out of 39 Brown Heart swedes (5%) fell into the 2SD quality control chart range of the healthy swedes and upon cross section these samples had no or low browness index with (0, 1, 0, 1 assessment respectivley). This finding showed that the non-destructive Pundit test could detect Brown Heart in swedes by 95% reproducibility and repeatability by using the ultrasound measurement. In terms of error in axial height measurement a 1% error in height measurement or time of flight translated into approximately 2.5 ms^{-1} providing acceptable tolerance. In addition, samples were allowed to equilibrate to

room temperature and repeat measurements were conducted to assess temperature effect. The variation due to temperature was within normal variation at the low storage temperature.

Table 1: Velocity measurements of B1 – B39 with corresponding images shown in figure 11. Cells highlighted in green indicate readings which lie within 2 standard deviations of the mean value of a healthy sample of n =10. Figure 10.

Sample	Velocity / $10^{-3}ms^{-1}$	Sample	Velocity / $10^{-3}ms^{-1}$
B1	0.336	B21	0.316
B2	0.229	B22	0.275
B3	0.334	B23	0.286
B4	0.357	B24	0.290
B5	0.351	B25	0.307
B6	0.364	B26	0.268
B7	0.316	B27	0.310
B8	0.286	B28	0.297
B9	0.297	B29	0.298
B10	0.367	B30	0.383
B11	0.256	B31	0.337
B12	0.273	B32	0.317
B13	0.321	B33	0.319
B14	0.287	B34	0.349
B15	0.321	B35	0.369
B16	0.245	B36	0.333
B17	0.273	B37	0.336
B18	0.410	B38	0.312
B19	0.352	B39	0.358
B20	0.327		

Prediction of Brown Heart index category based on the velocity: Blind predictive test

Table 2 shows a sample of the predictive capacity of 6 samples of blind tested swedes which were matched to the degree of the Brown Heart based on the regression model in **Error! Reference source not found.**. However, Brown Heart was successfully detected in all samples when based on the 'detect' or 'no-detect' criterion. Incorrectly assigned indices for the swedes based on velocity results were possibly due to the inherent variability of samples at each index of browness (see Figure 9) which on average are equivalent to the variability in healthy samples namely a standard deviation 17 ms^{-1} makes precise prediction of the browness index difficult. Additionally, subjectivity of the Brown Heart index under visual inspection and also the effect of localisation (Figure 12) of Brown Heart in the cross section of the sample introduces further uncertainties into the measurement and characterisation process. As examples, sample number 5 and 6 were categorised between 6 and 8 based on the Brown Heart index due to the regression. In contrast, the degree of the Brown Heart was found between 2 to 3 level based on the visual inspection/ grading. It suggests that sample an increase in sample sizes for calibration sets for the defined index scale in future experiment and/or that an objective colorimetry method be investigated so that the consistency of the blind test prediction could be improved.

Table 2: Test on a prediction of Brown Heart based on the velocity

Sample No.	Velocity (m/s)	Prediction of Brown Heart using the velocity regression model	Visual inspection using Brown Heart Index
1	265.1	1	1
2	281.1	2	2
3	272.1	1	1
4	275.5	1	2
5	340.1	6	2
6	374.1	8	3

Figure 11 shows some examples of the Brown Heart detected in the swedes based on the developed index. Different categories from 0 to 9 were found in the defected Brown Heart swedes. The Brown Heart category was recorded (red number in the middle of each picture). **Figure 12** shows examples of Brown Heart locations and orientations in the defected Swedes which reduces precision of the method.

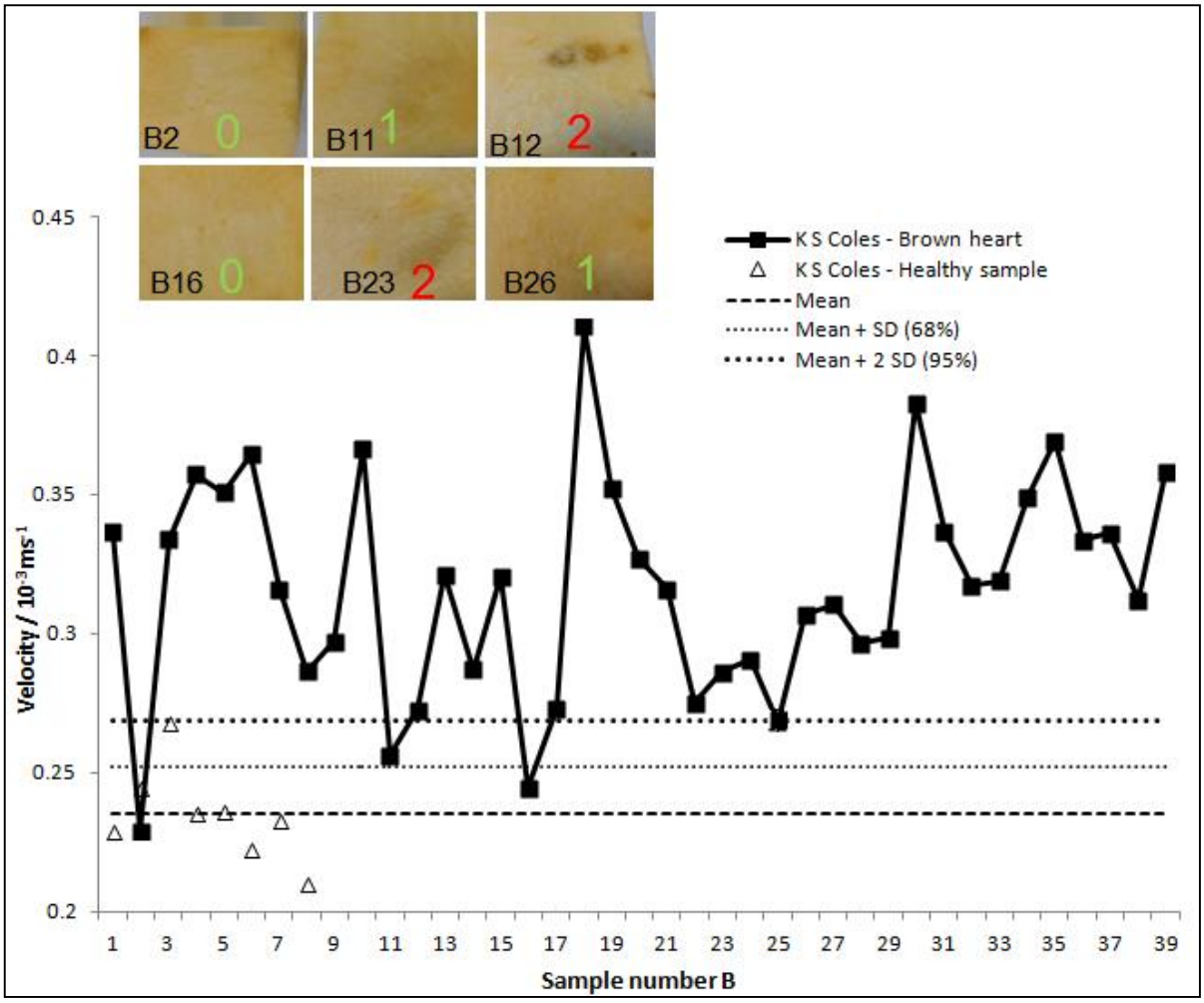


Figure 10: The ultrasound velocity of the Brown Heart swedes (■) and the healthy swedes (△). The quality control chart of the velocities for the healthy swedes was represented by by the long dashed line (— — —) for the mean value, short dashed line (----------) for the mean value + the 1 Standard Deviation (1SD) and dotted lines (·····) for the mean value + the 2 Standard Deviation (2SD). Two points of the swede detected Brown Heart fell within the quality control chart represented by number 1 and 2.

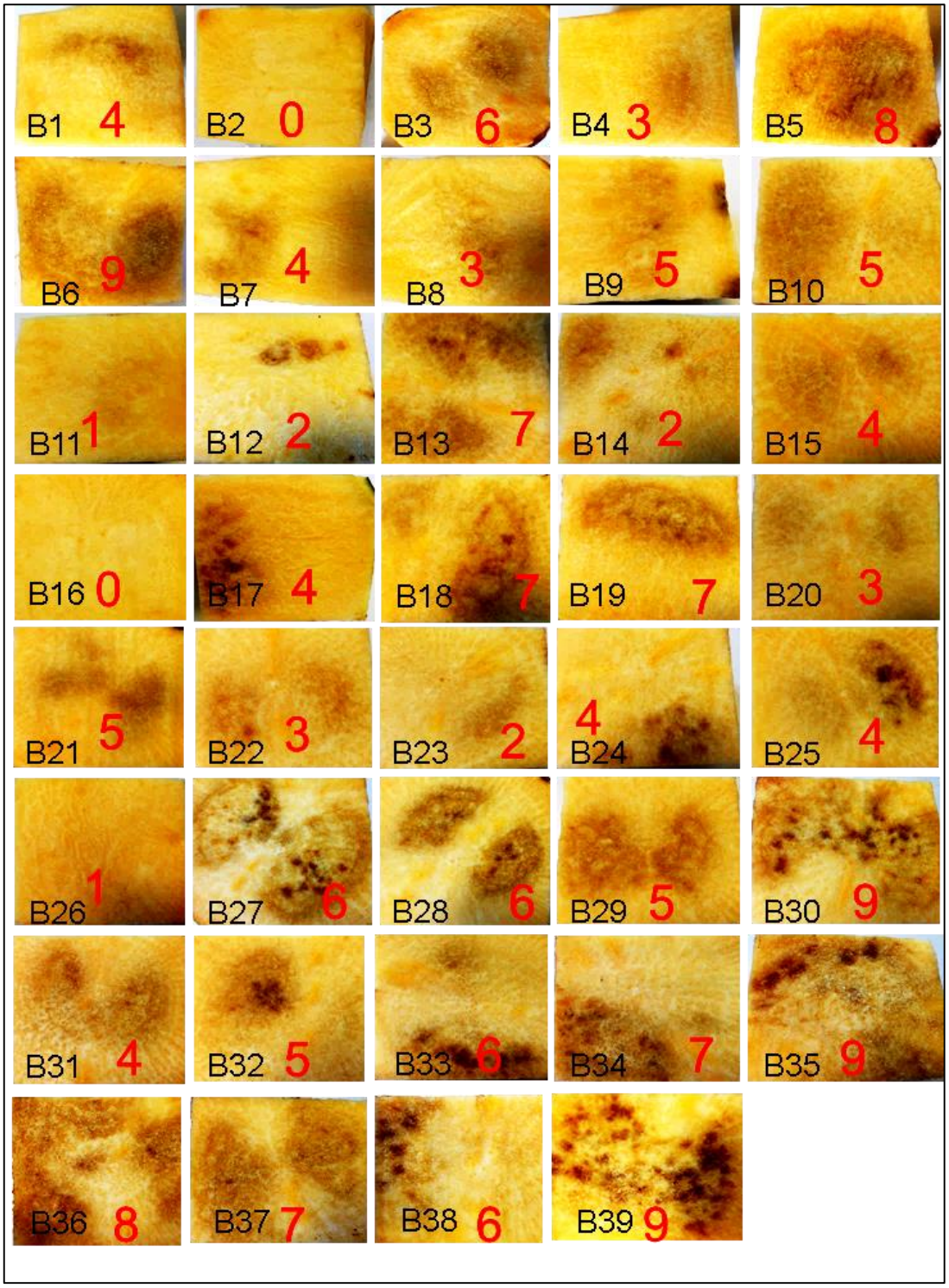


Figure 11: Examples of the visual inspection of the Brown Heart symptom of the measured swedes based on the developed Brown Heart index. The red value in the middle of each picture was the assigned a browness level as defined in the 0 – 9 scale..

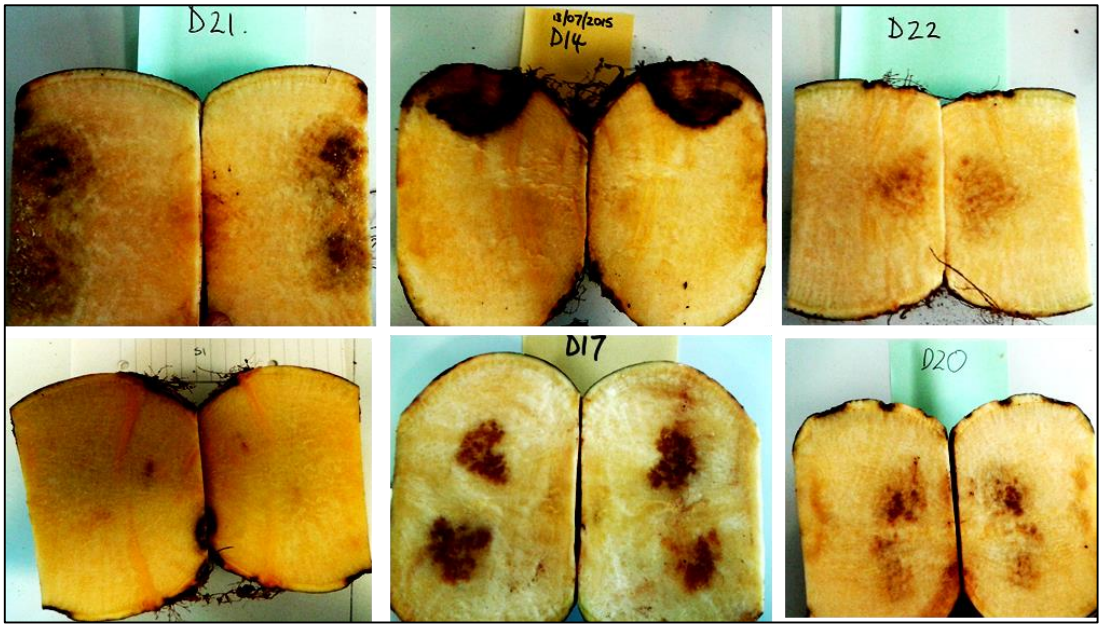


Figure 12: Examples of Brown Heart locations and orientations in swedes

Volume fraction of air in swedes

The presence of Brown Heart in swedes correlated with higher ultrasound velocity compared to the healthier swedes. A theoretical explanation of this observation is due to the changing air content in the internal vegetable cell tissues. In normal healthy cell membranes and in the intercellular spaces within the roots contain low volumes of air typically 2% volume fraction. Defective Brown Heart cells lose membrane integrity and cellular fluids flood membranes and inter-cellular spaces reducing the overall fraction of air to approximately 1%. This environment affects the characteristic of ultrasound velocity as can be seen in Figure 13. The result agreed with the experiment of the fraction by volume of air in water on velocity of sound in non-resonant air-water mixtures at 20°C (**Figure 13**) (Povey 1997). Based on the graph, the velocity of the Brown Heart swedes had the air in water around 1 %. Meanwhile, the velocity of healthy swedes had air in water around 2 %. These results indicated that velocity was higher when the Brown Heart was detected in swedes due to the air-water mixture level changes in the cells.

The experiment of the fraction by volume of air in water on velocity of sound in non-resonant air-water mixtures at 20°C is related to the Urick equation. The Urick equation (Equation 5) is an extension of the Wood equation (Equation 3 and Equation 4) and assumes no scattering. This equation shows the ultrasonic velocity in fluid mixtures is related to the properties of the tested material depend on adiabatic compressibility and density assuming no scattering. The Wood equation describes the equation in a pure material or a single phase material (Povey 1997).

The Wood Equation

Speed

$$c = \sqrt{\frac{B}{\rho}} = \sqrt{\frac{1}{\kappa\rho}}$$ Equation 3

Where B is the Bulk modulus, κ is the adiabatic compressibility and ρ is the density

1 phase system – continuous phase

$$k = \sum_j \emptyset_j k_j \qquad \rho = \sum_j \emptyset_j k \rho_j$$ Equation 4

Where $\emptyset$ is the dispersed phase volume fraction, $_j$ is the continuous number

The Urick Equation

2 phase system – dispersed phase

$$k = k_0 = \emptyset k_2 + (1-\emptyset)k_1 \qquad \rho = \rho_0 = \emptyset\rho_2 + (1-\emptyset)\,\rho_1$$ Equation 5

Where $\emptyset$ is the dispersed phase volume fraction, k is the effective adiabatic compressibility, ρ is the the density, $k_0\, and\, \rho_0$ are the volume average values and subscripts refer to constituent phases.

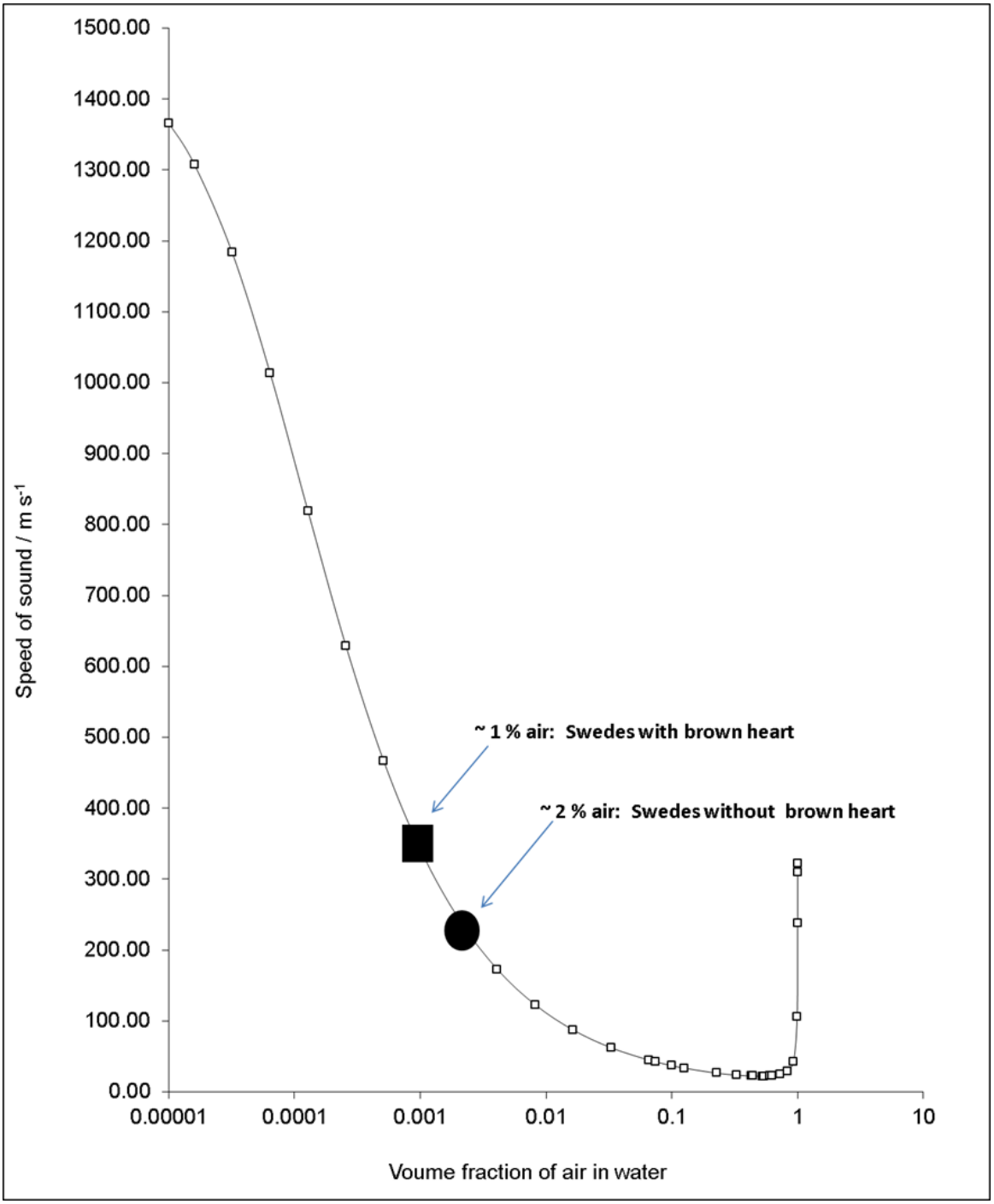


Figure 13: Effect of fraction by volume of air in water on velocity of sound in non-resonant air-water mixtures at 20°C (From Povey 1997, with permission). Volume fraction of air in water for swedes with Brown Heart was lower (■) (~1%) than those without Brown Heart (●) (~2%). The lower volume fraction of air in water in the Brown Heart swedes agreed with the internal cell tissue was flooded with water when Brown Heart was detected in swedes.

Swede internal cells measurement by a microscope

Evaluation using optical microscopy (40X magnification) further corroborated the hypothesis of cellular membrane flooding and indicated a clear differentiation between the cellular surfaces of Brown Heart cells and the healthy cells (Figure 14). The defective cells appeared in bluish green under the toluidine blue staining which indicates binding to OH groups. In contrast healthy swedes appeared in orange.

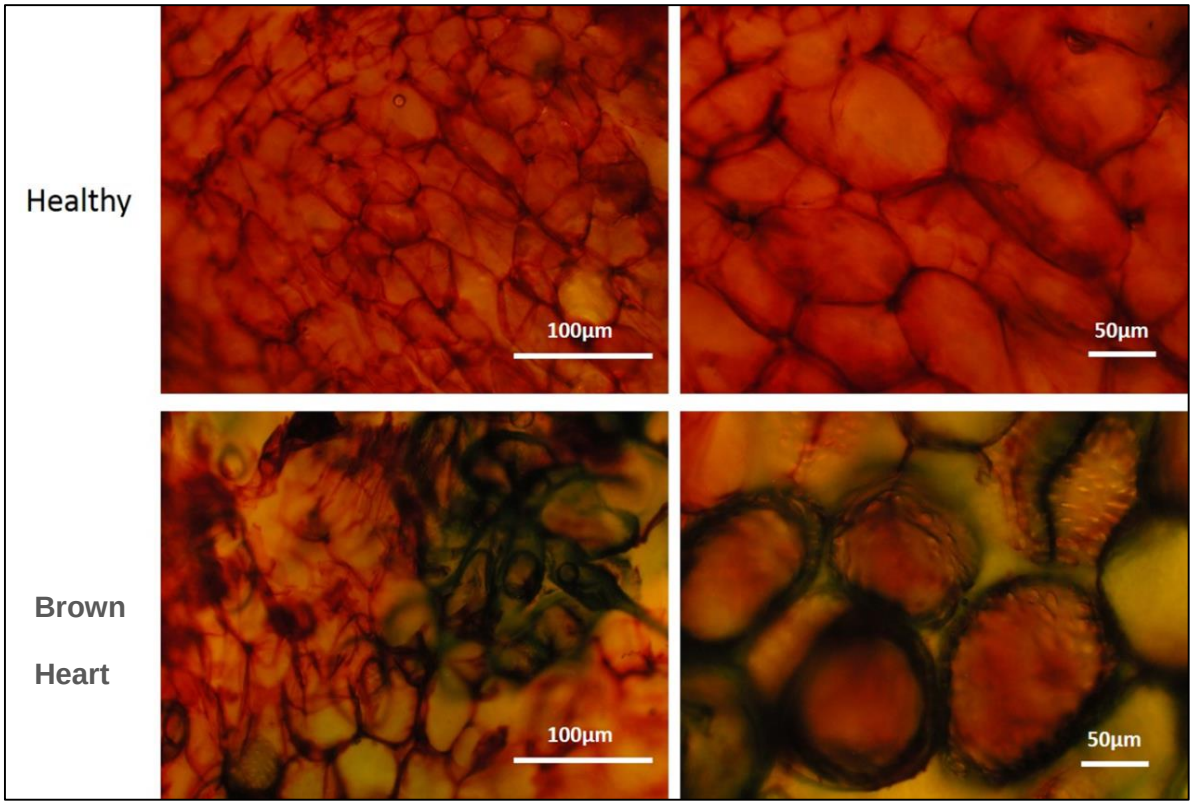


Figure 14: Cells of the Brown Heart swedes (bottom) with the blue green staining around the cell walls; and the healthy swedes (top) swedes without any staining observed under a microscope in 100µm and 50µm magnifications.

Texture profile analysis (TPA) curves

Texture profile analysis of puncture tests showed clear trends between Brown Heart and healthy swedes (

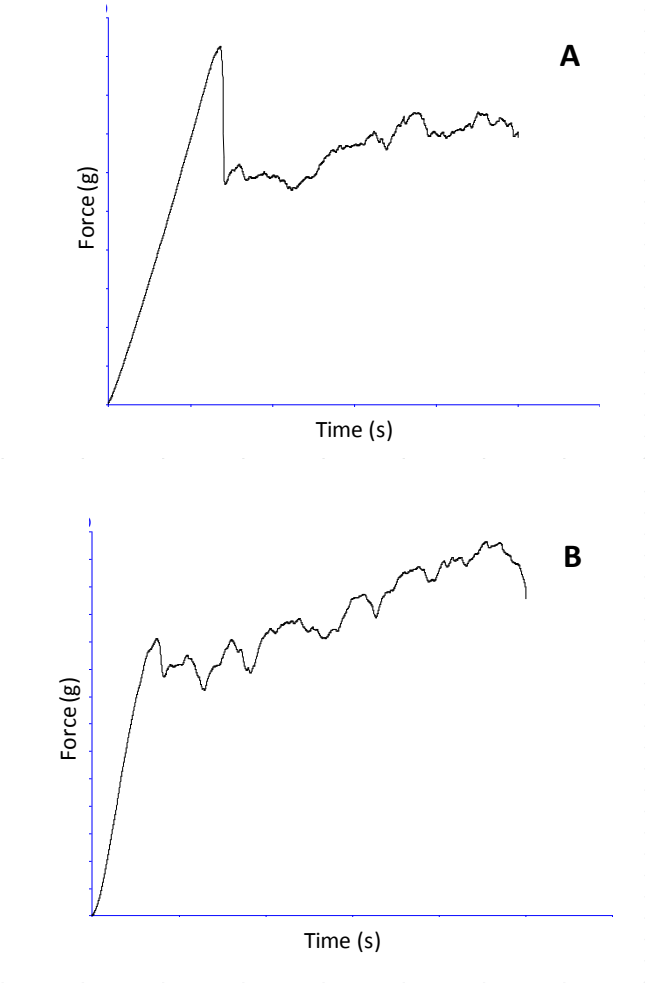


Figure 15). The bio-yield point or firmness maximum force of the healthy swede appeared sharply in A. Then, the curve diminished dramatically after the maximum force. In contrast, the bio yield point or the first puncture peak of the Brown Heart swede was rather less distinct compared to the healthy swede. In the next section of the curve, the force after the first peak was further increasing along the time of the experiment.

The TPA analysis of swede samples agree with the findings presented by a theory of puncture test (Bourne 2002). The healthy and Brown Heart provided qualitatively different TPA force-time curves which was due to the elasticity differences of the cell tissues. The cell tissue of the healthy swede contained less fluid and the more rigid cell membranes ensured that greater forces were required to compress and fracture the structure. However, upon yielding the steadily rise of the force was disrupted by the suddenly decreasing of the next phase as shown in Figure 15 (A). Further application of force compressed the structure due to the higher level of air within the cells. However, the cell tissues of the Brown Heart swede were flooded having a higher fluid environment. Compressibility is reduced as the fluid content of a medium increases. This characteristic can be traced from the Brown Heart curve (B) where there is a consistent increase of the force even after the yield peak.

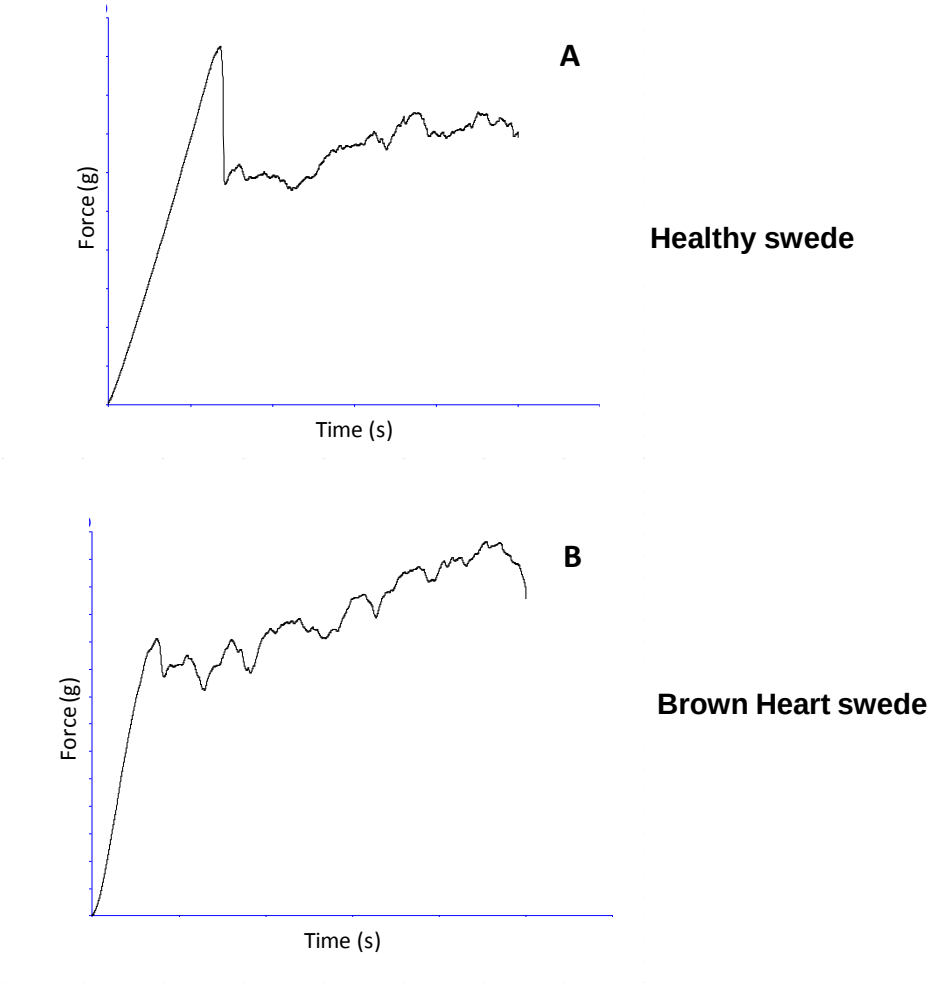


Figure 15: Example of texture profile analysis (TPA) force-time curves of a puncture test from the Stable Micro Texture Analyser for swede sample at pre-speed: 2 mm s^{-1}, test-speed: 1.00 mm/s and post-speed: 10.00 mm/s. A: Healthy swede; B: Brown Heart swede.

A relationship between firmness against velocity of swede

Despite the different curve patterns between the Brown Heart and healthy swedes from the texture profile analysis, no direct relationship was found between the velocity and firmness by the puncture test of Brown Heart of swedes (Figure 16). The correlation determination using the best least square fitting was weak (R^2 = 0.005).

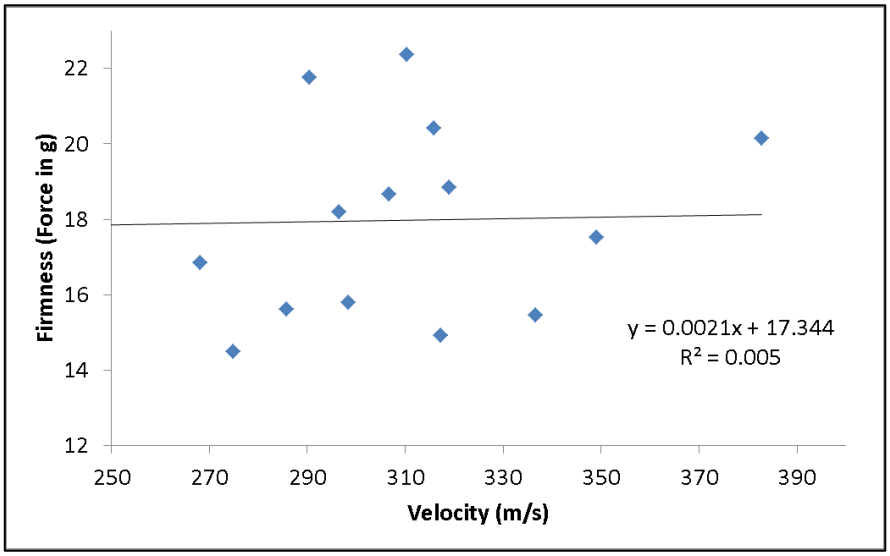


Figure 16: A relationship between firmness against ultrasound velocity of swede

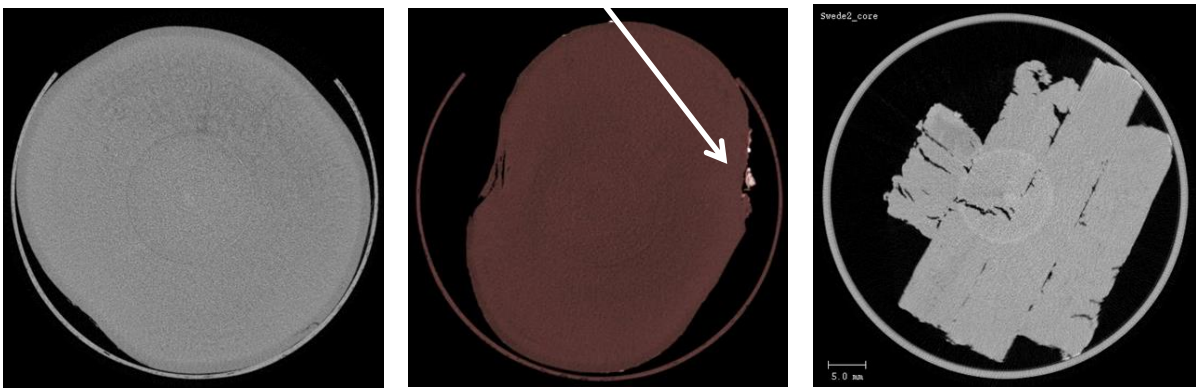
Cell internal structure measurement by ultrasonic phase arrays-flaw detector

The ultrasonic phase array-flaw detection was not feasible to detect the internal Brown Heart in swedes. It was discovered that the applied frequency suffered from significant attenuation and scattering and was not able to obtain data and only able to achieve penetration depth in mm range.

Cell microstructure measurement using X- ray

The differentiation in the microstructure of swede cells between healthy and Brown Heart was not successfully measured by using an X-ray. X-ray techniques rely upon density differences to construct interior images. Since we speculate that there are 1 % and 2% air volume fraction differences this was not sufficient in providing density differences and hence X-ray resolution (**Figure 17**). In addition, due to the nature of swede harvesting, significant sample cleaning would be required to remove external soil and debris which confounds the measurement process. Namely surface debris may have significantly higher density that the swede tissue as can be noted in Figure 17 (B). More samples are recommended to be conducted for more severe comparisons in a future experiment.

Soil/debris high contrast Higher resolution image



A B C

Figure 17: Samples of X-ray images A: a healthy swede; B: Brown Heart sample also showing soil/debris high contrast; and C: Higher resolution image of Brown Heart sample. X ray did not appear to be a viable method for Brown Heart detection.

General Discussion

The above experimental tests indicate Pundit velocity tests provide a feasible opportunity as a fast, economical, non-destructive method of Brown Heart detection in swedes. It was supported by the precision and accuracy of the method as well as able to provide statistically, correct positive detection rates of Brown Heart swede samples. The method presents a possible commercial scale detection system with the implementation of a suitable engineering solution. However, it is suggested that further research is required to evaluate several samples sets to establish calibration criteria on an individual sample set basis. Therefore, for a given harvest a calibration should be implemented which would then permit subsequent evaluation of individual swedes. It is recognised however that the tests implemented above relied upon the pre-process of samples to provide parallel cuts to the swedes which are generally not performed on the samples prior to cleaning and grading before storage. Process cuts are performed at the retail stage and not the storage stage therefore the methodology needs to be investigated to evaluate swede samples using radial measurements without the implementation of the axial process cut. This in theory is a possibility but would require further ultrasonic testing, probably involving a partial immersion of the swede in water and spatial measurement using a single transducer system and an independent optical method to determine spatial radial height of the sample as opposed the caliper system used in this study to determine sample size.

Conclusions

This research investigated the non-destructive ultrasound technique to detect an internal defect of Brown Heart in swedes together with the texture and microstructure measurements

and suggests a viable proof of concept. The Brown Heart condition influenced the ultrasound velocity on the vegetables. The ultrasound technique showed a strong positive correlation with the Brown Heart severity as verified by subsequent visual inspection. Ultrasound velocity can be explained theoretically in terms of the volume ratio of air-water. This hypothesis is supported by microscopy of the internal cells of Brown Heart and healthy swedes and texture profile analysis. Further research is required to fully explore different sample harvests and produce associated calibration data. In particular, to satisfy industry requirements the techniques need to be able to evaluate swede samples radially without process 'cuts' and ideally with single transducers systems in-line during cleaning and weight grading processes. This would require a fully formulated research enquiry to fully parameterise the variation within the crop and post-harvest practices.

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Appendix

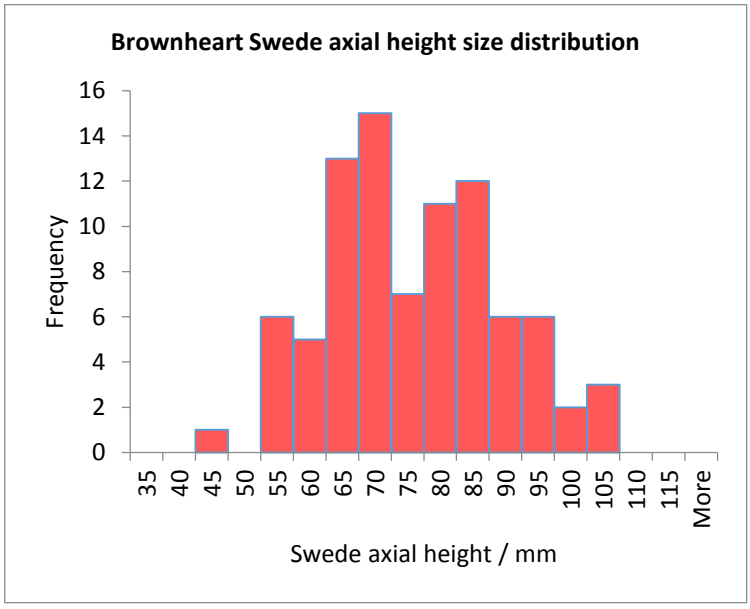

Figure 18: Axial test height distribution of B1 – B87 samples.

