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Optical Coherence Tomography imaging of potato skin to understand variability in response to preharvest and postharvest factors

S. Landahl[1], S. Foukaraki[1,3], S. McWilliam[2] and L. Terry [a,1]

[1] Plant Science Laboratory, Cranfield University, Cranfield, United Kingdom; [2] PepsiCo International Ltd., Leicester, United Kingdom; [3] University of Crete, Heraklion, Greece

Abstract

In order to be able to assess the effect of pre- and postharvest treatments on different potato varieties and tissues, information is needed on the skin architecture of tubers. Optical Coherence Tomography (OCT) was utilised as an appropriate non-destructive technique due to its high spatial resolution. It uses near-infra-red optical radiation to map the internal structures of semi-transparent samples. Samples in this study were potatoes with known provenance under selected pre- or postharvest treatment. The study herein aimed to examine the effect of growing environment and a desiccant chemical on skin layer development of several potato varieties and to increase understanding of the variability in skin thickness after application of sprout suppressant. Different thicknesses of the skin layer were found between different farming locations, varieties and spatial positions on individual tubers (60 to 100 µm). In detail, the spatial difference of skin layer thicknesses developed over time. Duration of desiccation and duration of curing did not cause significant differences in skin thickness. However, skin thickness changes were observed during storage and were variety dependant. Chlorpropham treatment did not significantly influence skin layer thickness. Still images recorded by means of OCT were a convenient and non-destructive tool to quantitatively evaluate skin thickness of potatoes. In conclusion, the high resolution of the acquired still images allowed confirmation of tissue identification. It appears that tissue position on the tuber may have a higher influence on phellem thickness than harvest date or curing.

Keywords: *Solanum tuberosum*, OCT, phellem, eye movement, desiccant

INTRODUCTION

Potato losses from storage continue to be a serious problem for the potato industry with poor skin-set an important factor in reducing tuber storability (Sabba and Bussan, 2012; Heltoft et al., 2017). Skinning injury of potatoes is a widespread problem that can lead to costly disease, physiological defects, and shrinkage during storage (Lulai, 2002). Anecdotal information has led to incorrectly attributing skin thickness to development of resistance to skinning, but instead the phellogen (cork cambium) integrity should be attributed; fracture causes skinning, since the phellogen holds the phellem (cork) of the potato tuber to the underlying phelloderm (Lulai, 2002). Resistance to skinning has been linked to the thickening of phellogen cell radial walls during periderm maturation: the phellogen cell layer serves as a lateral meristem for the periderm and as it becomes inactive during maturation, the periderm becomes resistant to skinning. During periderm maturation, tuber phellogen radial walls thickened and became resistant to fracture (Sabba and Bussan, 2012). In this study, optical coherence tomography (OCT) was attested for its suitability to examine skin architecture as well as phellem thickness. The suberised phellem cells are filled with air providing thermal insulation and suppress disease and desiccation (Vulavala et al., 2017).

Optical coherence tomography (OCT) produces a time-of-flight measurement of the scattered signal returned from biological tissue structures. OCT is used primarily for

[a] l.a.terry@cranfield.ac.uk

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mapping the internal structure of semi-transparent biological samples in a region just below the sample surface. The technique, which uses near-infra-red optical radiation at wavelengths in the range 800-1550 nm, detects the positions of refractive-index discontinuities corresponding to boundaries between different types of tissue (Fercher et al., 2003; Fujimoto, 2003; Ford et al., 2012). In turbid biological tissue, scattering and absorption effects limit the maximum imaging depth of OCT to about 1 mm below the surface. However, a spatial resolution of 3-20 μm can be achieved, depending on the source and optical configuration used, which is at least an order of magnitude higher than that offered by ultrasound, magnetic resonance or X-ray imaging. OCT has been reported as a diagnostic tool for examination of structural changes within fruit and vegetable tissues, viz. onion bulbs and mandarins (Meglinski et al., 2010; Ford et al., 2012; Magwaza et al., 2013). There is slight uncertainty of the boundaries of cell-like features in the images due to human error and instrument limitation: in onions (Hrebesh et al., 2009) and apples (Verboven et al., 2013), lateral resolution of OCT images has been previously reported to be lower than those obtained with confocal or transmission microscopy. However, OCT offers a considerably larger field of view in both lateral and axial dimensions of an object, while providing similar information to confocal microscopy (Verboven et al., 2013).

The aim of the presented study was to use OCT to better understand the variability in tuber skin thickness and skin architecture as affected by both preharvest and postharvest factors.

MATERIALS AND METHODS

Preharvest plant materials

In part A, cvs. Saturna and VR808 potatoes (n = 3 and 3 size grades per cv. per timing) were supplied by PepsiCo International Ltd. The tubers were sent to Cranfield University prior to desiccation of above ground haulm with Reglone (active ingredient diquat) and at three additional sampling dates after Reglone treatment (measurement 06/09/13, 19/09/13, 25/09/13, after curing in a potato store 08/10/13 (commercial conditions); i.e. pre desiccation, desiccation + 2 weeks, desiccation + 3 weeks, desiccation + 4 weeks and post curing. Skin imaging of each intact tuber (at three points: one apical and two opposing lateral) was carried out on tubers (n=72) using OCT (Thorlabs Ltd., 2010) and data were analysed manually using 'image tool' in Matlab© software (v. 9, The Mathworks Inc.™, USA). On the Thorlabs system a 'live' display of the sample is observed, updating at 8 frames per second, and a selected image is stored on request, via a key press. Each two-dimensional (2D) image comprises 500 pixels laterally and 512 pixels in depth. Two-dimensional images represent a plane perpendicular to the surface of the sample, defined by the illumination beam as it sweeps out a line 1-8 mm in length across the surface. The intensity scale used for display is logarithmic, as the signal intensity variation between different regions of the image can be several orders of magnitude.

In part B, potato tubers (3 x sites, 2 x cultivars (Saturna and VR808) and 10 x reps) supplied by PepsiCo International Ltd were sent to Cranfield University for non-invasive assessment and analysis of skin thickness using OCT imaging (measurement 20/09/13, 27/09/13, 11/10/13). Potatoes were stabilised on a motorised stage with blue tack® and the stage was initiated at the same time as the image recording by key press. The speed of the stage was synchronised with the recording of 256 frames in 32 s, so that a distance of 2 mm was travelled (62.5 μm/ s). Potatoes (n=60) were provided from Shropshire, Yorkshire and Norfolk. Skin imaging of intact tubers (one apical and two opposing lateral) was carried out using OCT and the data automatically analysed using LabVIEW© 3D imaging software (v. 13, National Instruments corp., USA) and Matlab© software. Imaging and analysis was only carried out on freshly harvested tubers such that storage was not a factor.

Postharvest plant materials

Tubers (1 x site, 2 x Chlorpropham (CIPC) [untreated, treated], 5 x cultivars (Saturna, VR808, Hermes, Lady Rosetta, Lady Claire), 3 x reps) were supplied by PepsiCo International Ltd. In all cases ca. 60 mm diameter sized tubers were selected (n=30). Tubers were cured and CIPC-treated (or not treated) at Sutton Bridge Crop Storage Research (SBCSR) and immediately transferred to Cranfield University and stored under simulated commercial conditions (10°C). Non-destructive skin thickness measurements of each intact tuber (at multiple points: one apical and two opposing lateral) were carried out repeatedly and automatically by means of OCT, and data were analysed as described below. Repeated measurements were done 30/09/13, 10/10/13, ca. 04/12/13, ca. 18/01/14, ca. 04/02/14, 20/02/14 (6 timings).

Automatic image analysis

In part B, skin imaging of each intact tuber was carried out using OCT (Ford et al. 2012) and data were analysed using imaging tools in Matlab© software. Information was analysed from ".bmp" (still images) or ".txt" (scans) files. These contained information on intensity of every single pixel measured. Original still images were saved in grey scale. Batch processing algorithms were developed to obtain objective measurements of skin thickness. Details of the algorithms are as follows:

1. Skin thickness from still images – Still images have superior resolution per image compared to scans (500 * 512 pixels, [w x d]) and were more readily analysed automatically than scans. Data were loaded into the workspace as batch per measurement date. Each image was cropped to half depth (potato features did not cover the full depth of the image). Then the contrast was maximised to have an intensity range of 0 to 255 in each image. A median filter was applied to remove noise ("salt and pepper noise", which would make an image appear grainy). The image was subsequently converted into a black and white image in order to do 'region analysis'. The threshold value (0.7) to convert the grey images to black and white was used throughout the experiment. Small areas below 100 pixels were omitted. An envelope was drawn around the remaining large area and the short axis of this envelope recorded as result for skin thickness (Figs. 1 and 2 right: The line represents the thickness measured by the program). The images covered a real width (x-axis) of 2 mm and theoretically the vertical resolution (y-axis) was 3 µm (Thorlabs). The refractive index of suberin is not known, therefore the refractive index of sunflower oil was used instead (1.44) to estimate phellem thickness (each pixel 3 µm/ 1.44 = 2.08 µm, therefore the max. depth that could be measured is 1.07 mm).

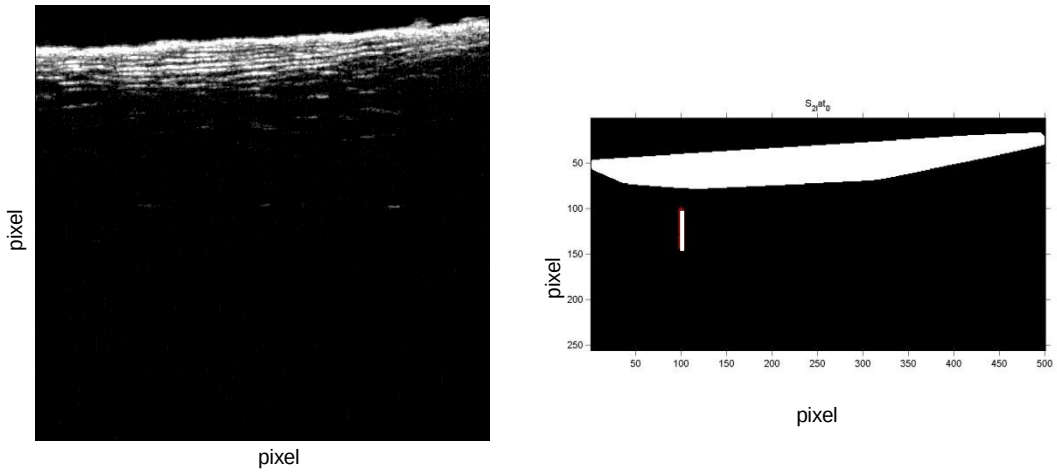


Figure 1. Left: Example of full size OCT image of cv. Saturna potato skin after contrast adjustment and filtering. Right: Cropped image with the envelope drawn for the same sample by the program to measure skin thickness. The line represents the maximum skin thickness measured.

2. Skin thickness from scanned images – A scan consists of 256 consecutive equally-distanced images of 256 * 512 pixels (Fig. 2 left). Firstly the 256 separate images had to be extracted from the recorded raw data file with the LabVIEW 2010 Thorlabs Data Reader. The image data were saved as 256 '.txt' files. These were batch processed with the algorithm above. Then the median of all 256 results was recorded as skin thickness value per location on a tuber. By using the median rather than the mean value, outliers were avoided.

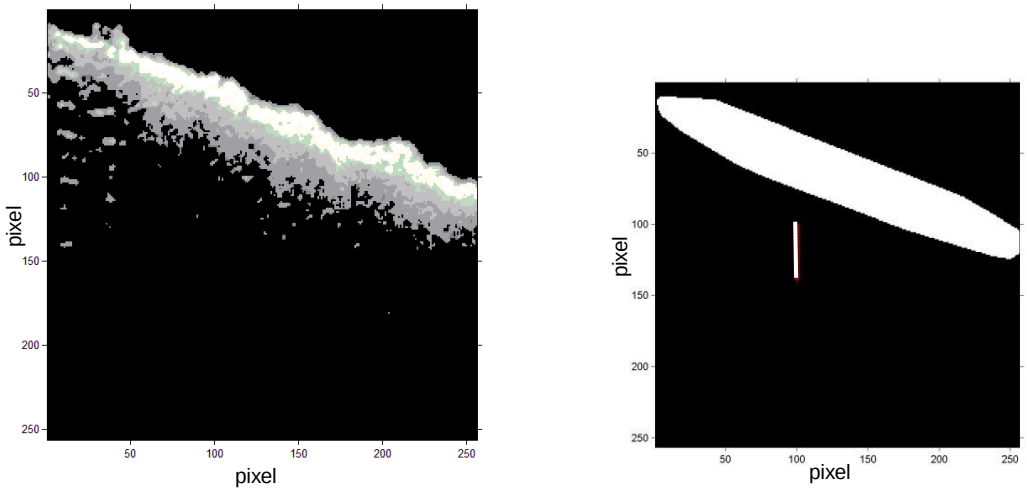


Figure 2. Left: Example of an OCT image of cv. VR808 potato skin, CIPC-treated, after contrast adjustment and filtering from scan. Right: Image of the same sample with envelope drawn to measure skin thickness. The line represents the maximum skin thickness measured.

An ANOVA was performed on these values according to sampling day, variety, treatment and apical plus lateral locations (Genstat© v.16, VSN Int. Ltd., UK). The skin thickness of the potatoes were measured in terms of pixels and then transferred to µm. Preharvest factors: The two lateral measurements from one tuber were averaged before performing ANOVA to obtain a balanced design. Postharvest factors: The two lateral measurements were fed into the statistics software to allow it to find outliers. An ANOVA for repeated measurements was used.

RESULTS AND DISCUSSION

It was clearly visible in Figs. 1 and 2 (left) that the resolution of still images was far superior to scanned images, where cell structure was visible (Fig. 1) with a horizontal resolution of 4 µm. However, without better resolution it was difficult to discern the cell wall thickness of the phellogen, even if the location could be detected (Fig. 3). Other authors found that method of haulm destruction affected skin adhesion strength with skins adhering more rapidly and more strongly to the tuber following haulm pulling compared with either desiccation with diquat or mechanical flailing (Bowen et al., 1996).

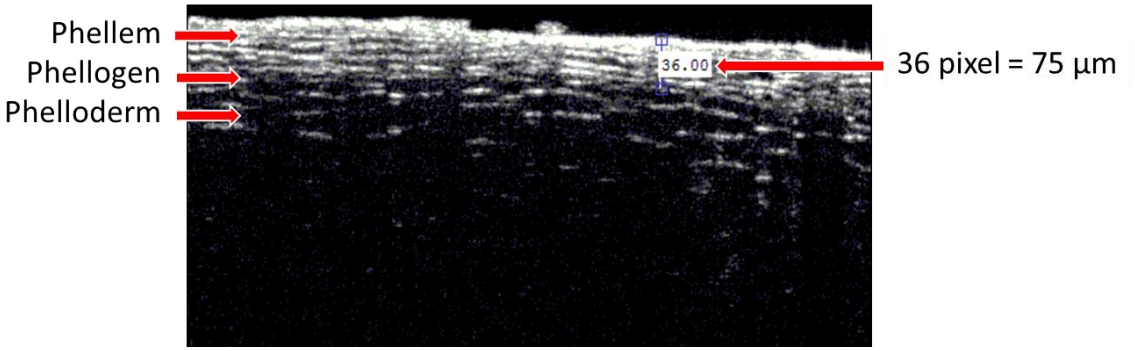


Figure 3. Original bitmap still image produced by commercial OCT software (Thorlabs). Periderm image collected from cv. 'Saturna' 06/09/13 (bottom half cropped). Sample: S2a, medium size tuber, lateral position.

Preharvest skin thickness

Significant differences were found between measurement location on the tuber and variety (Table 1). In general the skin of cv. VR808 tubers was thicker than that of cv. Saturna (part A). In addition, the skin was thicker at the apical end than in lateral positions. Potato crops are typically flailed or chemically desiccated several weeks prior to harvest to promote tuber maturity and facilitate harvest operations (Waterer, 2007). However, desiccation and curing period did not have a significant effect on skin thickness and also tuber size had no significant effect on skin thickness.

As seen in table 1, significantly lower values for lateral than apical tissue were found for cv. VR808 in tubers, pre desiccation for medium-sized tubers and also in desiccated tubers (2 to 3 weeks) for large tubers (6%). In cv. Saturna significantly thinner phellem appeared in lateral tissue in large tubers pre desiccation and after desiccation (2 weeks) in medium-sized tubers. The apical skin of cv. Saturna was significantly thinner than cv. VR808 for medium-sized tubers pre desiccation and 4 weeks after desiccation and also for small tubers 4 weeks after desiccation (12%). Lateral skin thickness was lower in cv. Saturna for large tubers pre desiccation and medium-sized tubers 2 weeks after desiccation (Table 1). In cv. VR808 the lowest skin thickness of the apical end by 15% was measured in small potatoes pre desiccation. Again pre desiccation thin apical skin was found in medium-sized potatoes of cv. Saturna. Lateral skin thickness in cv. VR808 and apical skin thickness in cv. Saturna showed a trend to be thicker at later harvest dates. This was not as clear as observed for another recent study into the role of physical maturity of potato tubers during storage; cv. Saturna had higher torque values [mN m], a measure for skin-set, than cv. Asterix and torque increased in the three weeks before harvest (Heltoft et al., 2017). In that study, skin-set was the only predictor that contributed significantly to the model predicting weight loss during storage.

Table 1 Mean values of potato phellem thickness, part A (μm). Difference in letters indicates a significant difference. The bold letters indicate significance across the whole table, but were mainly considered along the vertical axis. Cursive letters indicate significance only along the same row in the same variety. The underlined values are the minimum and maximum of the entire table. The F probability was F=0.025.

		Saturna						VR808					
harvest	size	apical			lateral			apical			lateral		
pre desiccation	large	67.3	**b**	*a*	<u>55.2</u>	**bc**	*b*	76.7	**ab**	*a*	74.4	**ab**	*a*
	medium	60.9	**bc**	*bc*	66.3	**b**	*b*	87.8	**a**	*a*	74.8	**ab**	*b*
	small	71.0	**ab**	*a*	71.3	**ab**	*a*	66.2	**b**	*a*	68.0	**ab**	*a*
2 weeks after desiccation	large	73.5	**ab**	*bc*	74.2	**ab**	*b*	<u>91.5</u>	**a**	*a*	77.4	**ab**	*b*
	medium	85.9	**a**	*a*	64.0	**bc**	*b*	83.7	**a**	*a*	76.8	**ab**	*a*
	small	71.6	**ab**	*a*	72.4	**ab**	*a*	80.4	**a**	*a*	79.9	**a**	*a*
3 weeks after desiccation	large	73.8	**ab**	*ab*	77.0	**ab**	*ab*	88.9	**a**	*a*	85.1	**a**	*a*
	medium	75.1	**ab**	*a*	79.5	**a**	*a*	84.4	**a**	*a*	78.6	**ab**	*a*
	small	82.3	**a**	*a*	70.5	**ab**	*ab*	83.3	**a**	*a*	79.4	**a**	*a*
post curing, desiccation +4 weeks	large	76.7	**ab**	*b*	69.2	**ab**	*b*	89.4	**a**	*a*	75.9	**ab**	*b*
	medium	69.0	**b**	*b*	67.3	**ab**	*b*	81.0	**a**	*a*	77.1	**ab**	*a*
	small	68.9	**b**	*ab*	75.6	**ab**	*a*	83.4	**a**	*a*	76.6	**ab**	*a*

* In the statistical calculations four outlier tubers were removed and one was missing since it was damaged during transport.

In part B, the magnitude of skin thickness was the same in the automatically assessed images as in the manually measured images above (Fig. 4.1). Significant differences were found for the combined effect of site, measurement locations on the tuber and variety (F probability was F=0.046). The skin of cv. VR808 tubers was about 9% thicker than that of cv. Saturna in agreement with the results above. In cv. Saturna, the skin at the lateral region of the tuber was 14.3% thicker than the apical skin region, but lateral skin was not thicker on VR808 from Yorkshire and Shropshire (Fig. 4.1). The thinnest skin was found on potatoes originating from the Yorkshire site, therefore in order to explain the reason for this result, the agronomical data needs to be investigated in the future. In the past, Sabba and Bussan (2012) did not find a consistent effect of soil type on skin-set even when they found significant variation dependant on year and soil type.

Postharvest skin thickness

The potatoes originating from the Yorkshire site, which were stored for several weeks showed significant differences in skin thickness. These differences were found either over time*variety (F=<.001), or time*CIPC (F=0.006), or position (F=<.001). For better presentation in Figure 4 bottom right, the position was plotted versus time as well (F=0.077). Skin thickness appeared to increase for the first three months of storage, and there was a difference between the lateral and apical positions (Fig. 4.4). It needs to be investigated if the phellogen is still active. Skin at the apical end of the tubers was thickest. The course of change was different according to variety (Fig. 4.3). Cv. L. Rosetta showed the highest values and cv. Saturna the lowest. However, after four months the skin appeared to shrink especially in the apical region for almost all varieties examined. It needs to be

investigated if this is related to sprout growth. Chlorpropham treatment did not significantly influence skin thickness (Fig. 4.2). However, Ravichandran et al. (2012) found that after studying storage behaviour of seed tubers with treatments of chemicals on standing crop and resultant storage under normal open rack storage condition, the transverse section of tuber skin showed differences in thickness of phellem and arrangement of starch granules between control, maleic hydrazide and chlorocholine chloride treatments. This is in agreement with the notion that chlorpropham acts in a different manner than those sprout suppressants.

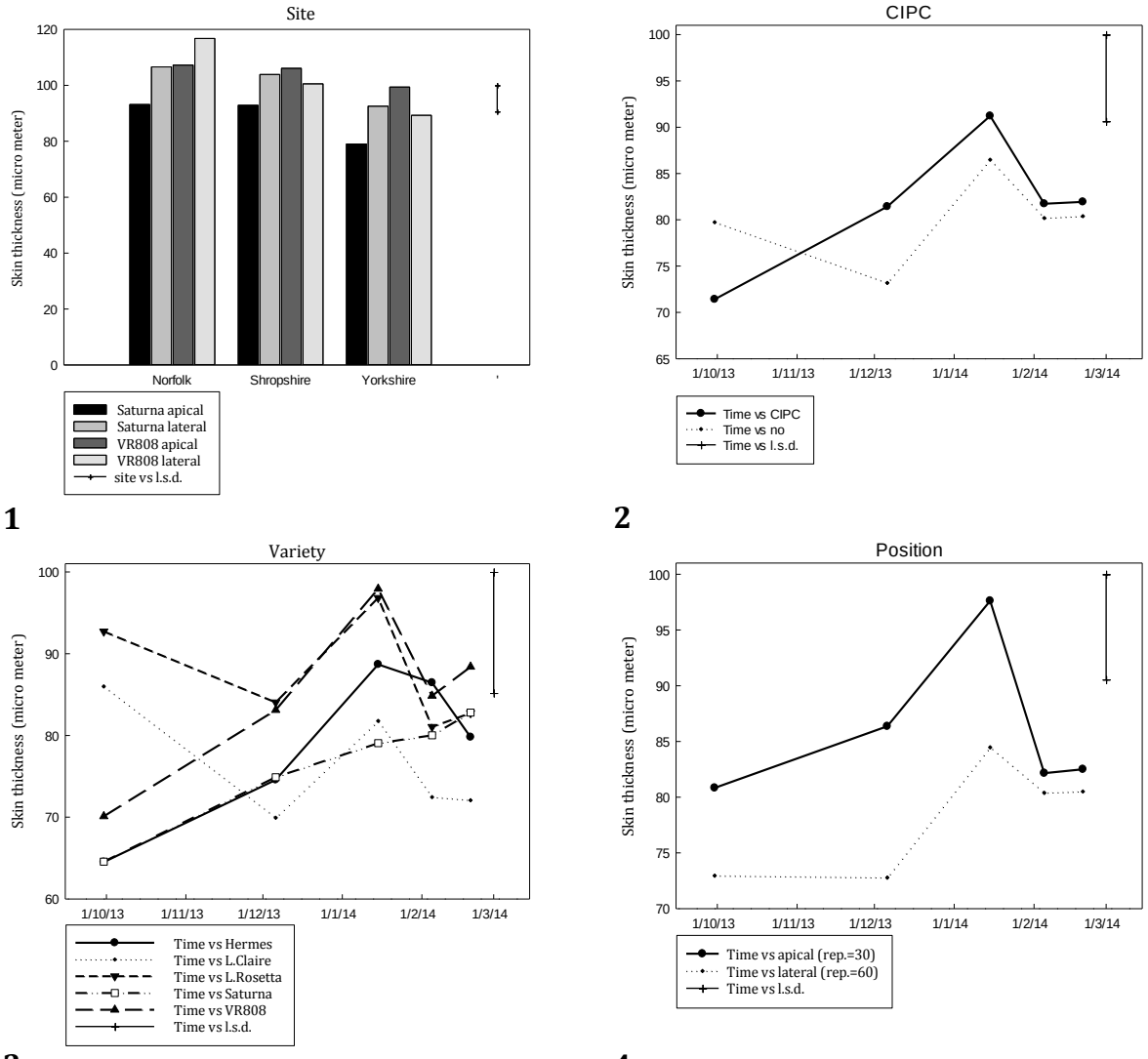


Figure 4. Skin thickness (μm) of potato tubers. 1: Preharvest part B fresh tubers measured only. 2, 3, 4: Tubers measured repeatedly during storage after harvest from Yorkshire site. (All values that were recorded on 10/10/13 were smaller than the other days, therefore an instrument misalignment was suspected and they were ignored.)

CONCLUSIONS

Still images recorded by means of OCT were shown to be a convenient and non-destructive tool to automatically evaluate skin thickness of potatoes. The high resolution of the acquired still images allowed confirmation of tissue identification. However, the

resolution was not sufficient to assess cell wall thickness of phellogen. It appears that tissue position on the tuber may have a higher influence on phellem thickness than harvest date or curing, although various preharvest factors and storage had had an effect on skin thickness.

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