*Int. Agrophysics, 1999, 13, 203-209*

# REFLECTANCE SPECTROPHOTOMETRY OF BRUISING IN POTATOES. I. ULTRAVIOLET TO NEAR INFRARED*

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*Accepted July 13, 1998*

A b s t r a c t. The reflectance spectra of bruised and unbruised tubers was measured with a scanning monochromator and bifurcated fibre optic light guide from 250 to 1750 nm. The spectra were converted into reflectance relative and first derivative format and divided into two groups: known and trial spectra. Discriminant analysis selected wavelengths from the known spectra that were most sensitive to differences between unbruised and bruised tubers and to generate linear classification functions to predict whether a tuber was bruised or unbruised. It was found that 75 to 95% of tubers were correctly identified as bruised, with wavelengths selected in the visible and NIR for unpeeled and peeled tubers. When the classification functions were used on the 'trial' spectra to determine the robustness of the method, none of the unpeeled tubers were correctly classified, while 68 to 85% of peeled tubers were.

K e y w o r d s: *Solanum tuberosum* L., quality, damage, internal

## INTRODUCTION

Bruising is the production of melanin, a blue-black pigment, in the sub-surface tissue of a potato tuber. The internal discolouration is initiated when a potato tuber receives an impact that leads to disruption of cell membranes. The damaged membranes then allow the enzymatic oxidation of phenols which forms the blue-black pigment melanin [14]. Bruising is thought to be one of the biggest problems in the potato industry with an annual loss of many millions of pounds [22]. One of the reasons for the financial loss is that the discolouration of bruised tissue is often only visible when a tuber is peeled so tubers can pass unnoticed through quality control. Bruised tubers reaching the processor and consumer can then result in potato wastage or in a loss of confidence in the producer. Quality assessment is also time consuming and costly because of the need to peel or slice a tuber first to detect bruising [24]. If potatoes could be non-invasively and automatically scanned for bruising during grading then this may help to offset some of the losses currently incurred by the potato industry.

Transmission spectrophotometry has been used to determine with 81% certainty the discolouration associated with bruising and greening [2]. An alternative approach is to use reflectance spectrophotometry to measure light that has passed into a sample and been reflected from the internal tissue; the diffuse or body reflectance [3]. It has been demonstrated [7] that the diffuse reflectance can be measured from tissue up to *c.a.* 5 mm deep in unpeeled tubers and up to *c.a.* 8 mm deep in peeled tubers; the region where bruising is most likely to be found [25]. In theory, reflectance spectrophotometry could improve the accuracy of bruise detection because it

*Paper presented at 6ICA

is sampling from a more defined volume of tissue. Transmission spectrophotometry has the disadvantage of trying to detect bruising in a much larger volume of healthy tissue, and where the proportion of light scattering in the tissues is much greater [3]. Reflectance spectrophotometry has been used to study bruising in apples [4,11,26,29] but to date, reflectance spectrophotometry has only been used to detect surface defects on tubers [17,23] and not sub-surface defects such as bruising. Therefore, the aim of this study was to investigate reflectance spectrophotometry as a possible method for non-invasively detecting the discolouration associated with bruising.

## METHODS

Tubers of *cv.* Record were hand dug, washed and stored at 4°C (the *cv.* Record was chosen as it is widely recognised as being susceptible to bruising). Prior to impact, a temperature probe was used to ensure the temperature of each tuber was below 10°C [21]. The unpeeled tubers were given a consistent impact by using a drop tester [15] to drop the tubers from 200 mm onto a steel cylinder (10 mm diameter); this gave sufficient force to initiate bruising, but not enough to cause splitting or cracking of the skin. The site of bruising could be clearly felt immediately following an impact and its location was indicated with a marker containing waterproof ink.

The damaged tubers were then placed in a hot 'box' at 40°C, relative humidity 95%, for 16 h. The 'hot box' reduced the time for bruise development; it would typically take a few days in ambient conditions [12]. Spectra were then taken from bruised and unbruised regions before peeling with a hand held peeler. Immediately after peeling, spectra were taken from the bruised and an unbruised site. A sample was classified as bruised if there was any visible discoloration after peeling. Bruised tubers were then classified as blackspot or shatter bruise, depending on whether the internal tissue had fissures or cracks [19]. One hundred tubers per wavelength region were used. For three wavelength regions, this gave a total of 300 tubers and with four different types of tuber tissue analysed (bruised and unbruised regions from unpeeled and peeled tubers) a total of 1200 spectra.

The wavelengths at which melanin might be detected in whole tubers was unknown, so in this study, reflectance spectra were taken from 250 nm (Ultraviolet - UV) to 1750 nm (Near Infra Red - NIR). Wavelengths from 250 to 400 nm (UV) were collected by transmitting light from a deuterium lamp (Rees Instruments Ltd., Godalming, Surrey, UK) along a fibre optic light guide with UV grade synthetic fused silica (Melles Griot Ltd., Waterbeach, UK) to the sample. The reflected light from the sample then passed back along the bifurcated fibre optic cable to a 6112 Monolight monochromator and 6118 photo multiplier tube (Rees Instruments Ltd.). Wavelengths from 400 to 700 nm were collected with a 150W halogen light source (FOT 150; All Tech Inspection NDT Ltd., Corpus Christi, Texas, USA), BK-7 glass bifurcated fibre optic light guide (Melles Griot Ltd.), 6102 Monolight monochromator and 6118 photo multiplier tube (Rees Instruments Ltd.). Wavelengths from 700 to 1750 nm were collected using a 150W halogen light source source (All Tech Inspection NDT Ltd.), BK-7 glass bifurcated fibre optic light guide (Melles Griot Ltd.), 6102 Monolight monochromator and 6111 germanium detector (Rees Instruments Ltd.). The fibre optic light guide had to be 1 mm from the surface of all the samples. If the fibre optic light guide was in direct contact with the sample, the reflected light was attenuated too strongly. Therefore, an aluminium holder built in-house was placed on the measuring end of the fibre optic cable to ensure that for each sample the incident radiation was at 90° and 1 mm from to the surface of the sample.

A spectrum was created by taking a reference reading from a highly reflecting white target surface of Spectralon (Spectralon; Labsphere, North Sutton, East Anglia, UK). The 'normalised' or relative reflectance spectrum

was than created by calculating the ratio of reflectance measurements from the sample and Spectralon by using Eq. (1):

$$R = (\lambda_s - \lambda_n)/(\lambda_r - \lambda_n) \quad (1)$$

where $\lambda_s$ - reflectance sample, $\lambda_r$ - reflectance of Spectralon reference, $\lambda_n$ - background noise or 0% reflectance. A reference reading was taken for every 30 samples. Due to the need for a high signal to noise ratio, an average of 200 consecutive measurements was taken; this took approximately 15 s to acquire each sample spectrum. Spectral data were then converted into first derivative spectra using a program written in QBASIC (Microsoft Corporation, Santa Rosa, California, USA). The first derivative is a measurement of the slope of the spectrum, or the rate of reflectance change per nm;

$$\frac{R_{\lambda 1} - R_{\lambda 2}}{\lambda_1 - \lambda_2} \quad (2)$$

where $R_{\lambda 1}$ and $R_{\lambda 2}$ denote the percentage relative reflectance values at two adjacent wavelengths; and $\lambda_1$ and $\lambda_2$ are the respective wavelength values. Since derivative spectra measure spectral slope they are uniquely insensitive to uncontrollable baseline shifts in the untransformed spectrum. Shifts in the baseline can occur due to varying angles of incident light and surface defects [4].

The reflectance and first derivative spectra were analysed using Stepwise Discriminant Analysis (BMDP Statistical Software Inc., California, USA) [9]. Half of the spectra from bruised and unbruised tubers were used to determine the classification function (denoted as known tubers) and the remainder (denoted as trial tubers) used to determine the success of classifying a tuber as bruised or unbruised when the classification of a tuber was unknown to the discriminant program. The success of the classification function was quantified by determining the probability of this classification occurring by chance using Wilk's Lambda, the multivariate extension of R-squared [10]. A 'successful' classification is one in which a large percentage of tubers are correctly classified as bruised or unbruised. The meaning of large is evaluated by obtaining a probability (p) less than 0.001 for Wilk's Lambda [6].

## RESULTS AND DISCUSSION

There was a large amount of variation in the spectra and it was found that a two-tailed t-test did not show any significant differences ($p < 0.001$) between spectra from unbruised and bruised spectra, except for reflectance data from peeled tubers between 480 and 700 nm. Despite the variation in sample spectra there were some interesting observations to be made when the averaged spectra were examined. It could be seen that the reflectance spectra of bruised tubers have a lower reflectance than unbruised tubers between 250 and 800 nm for both unpeeled and peeled tubers (Figs 1a and 2a). A reduction in reflectance between these wavelengths would be expected in bruised tubers because of the production of dark coloured melanin. From 800 to 1750 nm, bruised tubers have a higher reflectance than

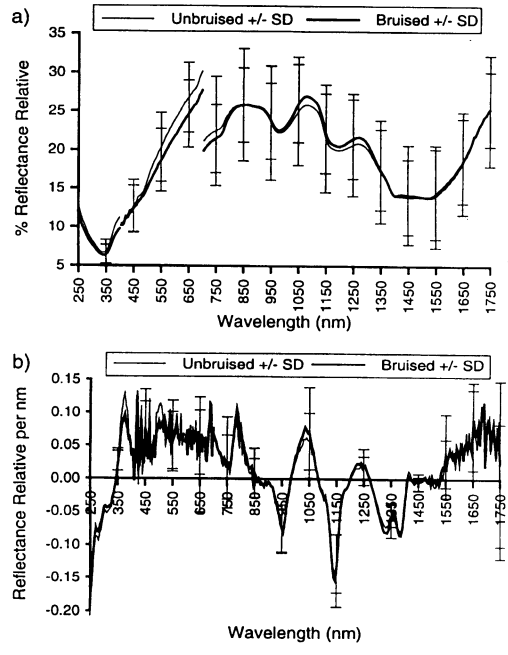


**Fig. 1.** Mean +/- SD of: a) % reflectance relative and b) first derivative spectra from unbruised (n = 149) and bruised (n = 51) unpeeled tubers.

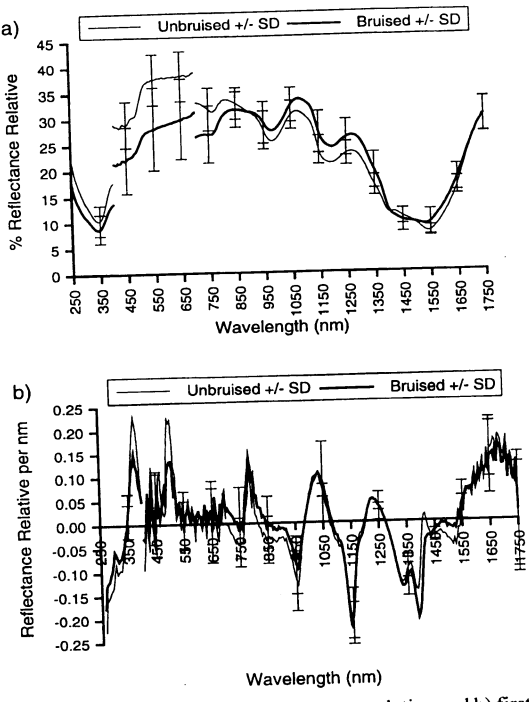


**Fig. 2.** Mean +/- SD of: a) % reflectance relative and b) first derivative spectra from unbruised (n = 149) and bruised (n = 51) peeled tubers.

unbruised tubers (Fig. 1a). The reason for the higher reflectance in the NIR is unclear, but it may be due to the accumulation of secondary metabolites associated with the production of melanin [28].

However, while there appeared to be differences in the reflectance spectra of bruised and unbruised tubers from 250 to 1750 nm, particularly in peeled tubers, the first derivative spectra revealed few parts of the spectrum where there was a change in spectral slope. For unpeeled tubers, the differences in slope between bruised and unbruised were very small, occurring between 450 and 650 nm (Fig. 1b). In peeled tubers the differences in spectral slope were more obvious than unpeeled tubers, with changes at about 380 to 400 nm, 475 to 500 nm, 700 to 850 nm and 1350 to 1550 nm (Fig. 2b).

The large amount of variation in the spectra meant that absolute values of reflectance at a single wavelength could not be used as a reliable method for identifying a tuber as bruised or unbruised. Discriminant analysis can overcome this problem to an extent by selecting several wavelengths to separate the spectra of unbruised and bruised tubers. The classification functions generated with several wavelengths may be more robust than relying on a single wavelength. In general, discriminant analysis selected wavelengths from reflectance and first derivative spectra in the regions *c.a.* 700 nm, 1500 to 1600 nm for unpeeled tubers and *c.a.* 300 to 400 nm, 500 to 800 nm, 1000 nm for peeled tubers (Table 1).

The effect of bruising on the reflectance spectra may correspond in some instances to absorption bands associated with pigments formed in the bruise reaction. The chemical dopachrome is an orange-reddish pre-cursor pigment to melanin with absorption bands *in vitro* of 300 and 475 nm [18]. Non-enzymatic oxidation then rearranges dopachrome to form the purple pigment 5.6 dihydroxy-indole with maximum light absorption in vitro at 305 and 475 nm [13]. The 5,6 dihydroxy-indole then undergoes repeated condensation, polymerisation and interactions with proteins to give brown, and finally black melanin pigments [5]. Absorption bands at 1670 to 1690 nm and 1250 nm are normally associated with carboxyl groups in melanin *in vitro* [1], but discriminant analysis of NIR spectra did not accord any particular significance to these wavebands and did not select them. Changes in NIR spectra would appear to reflect changes in bruised tissue that are due to cellular processes either indirectly associated with or other than melanin production. Indeed, the whole band from 1300 nm upwards is greatly affected by the water absorption band at 1450 nm [16]. It is likely therefore that changes in this region would be due to alterations in the water status of the bruised tissue [27]. The change in water status could also have been enhanced by the large proportion of bruised tubers with shatter bruise, which disrupts the tissue more severely than blackspot bruising (of the 51% bruised tubers, 80% had blackspot bruise, 20% had shatter bruise). Using the wavelengths from the untransformed reflectance spectra gave a higher percentage of known tubers correctly identified

**Table 1.** Wavelengths selected by discriminant analysis to separate untransformed and first derivative spectra from unbruised and bruised unpeeled and peeled tubers. Wavelengths are shown in the order that were selected by the discriminant analysis when F value was >4

| | UV | Visible | NIR |
|---|---|---|---|
| | | Unpeeled tubers | |
| Reflectance spectra | none | 700 | 710, 1780, 990 |
| First derivative spectra | 290 | 670, 605 | 820, 1610, 1480, 1650 |
| | | Peeled tubers | |
| Reflectance spectra | 275, 355 | 545, 640, 470 | 710, 790, 1440 |
| First derivative spectra | 345, 350, 375, 330, 400 | 505, 590, 595, 645, 625, 585 | 800, 740, 1110, 1050 |

as bruised or unbruised, than wavelengths from first derivative spectra for both unpeeled and peeled tubers (Table 2). For both unpeeled and peeled tubers, the highest percentage of tubers correctly classified was in the NIR, followed by the visible then the UV spectrum. For unpeeled tubers the overall percentage classification in each spectral region was less than peeled tubers, with no significant classifications in the UV (Table 2).

When the linear discriminant functions were used on the' trial' spectra, the classification was only significant for peeled tubers in the UV spectrum (Table 3). The reduction in classification accuracy of trial spectra, particularly unpeeled tubers, is probably due to the following three factors. Firstly, the bifurcated fibre optic cable has a small detecting area of about 12 $mm^2$. In unpeeled tubers, the exact location of a bruise was only known after peeling. Therefore, it is

**Table 2.** Percentage of 'known' unpeeled and peeled tubers correctly classified as bruised or unbruised

| | UV | Visible | NIR |
|---|---|---|---|
| | | Unpeeled tubers | |
| Reflectance spectra | none | 78.4 | 93 |
| First derivative spectra | 56.5* | 72.5 | 86 |
| | | Peeled tubers | |
| Reflectance spectra | 76.1 | 90.2 | 95.2 |
| First derivative spectra | 82.6 | 57.1* | 95.2 |

*The classification is not significant: p>0.001 for Wilk's Lambda.

**Table 3.** Percentage of 'trial' unpeeled and peeled tubers correctly classified as bruised or unbruised

| | UV | Visible | NIR |
|---|---|---|---|
| | | Unpeeled tubers | |
| Reflectance spectra | none | 55.1* | 65.9* |
| First derivative spectra | 45.5* | 34.7* | 54.5* |
| | | Peeled tubers | |
| Reflectance spectra | 79.5 | 57.1* | 55.8* |
| First derivative spectra | 68.2 | 57.1* | 51.1* |

*Explanation as in Table 2.

likely that even with the greatest care, both bruised and adjacent healthy tissues were sampled simultaneously. Thus, the signature from the bruised area would be diluted. Secondly, the geometry of the contact between the optical light guide and the tuber surface might have increased the specular component of the reflected light; reflectance readings were of the surface rather than the of sub-surface tissue. Thirdly, despite care being taken in selection of clear potato surfaces, the presence of incipient disease, soil or reticulated skin may have affected the nature of the reflected spectrum.

## CONCLUSIONS

Reflectance spectra from unpeeled bruised tubers had a lower reflectance in the UV to visible wavelengths, and a higher reflectance in the NIR than unbruised tubers. In peeled tubers, the differences were bigger in these regions and the changes in spectral slope in the first derivative spectra were more noticeable than in unpeeled tubers. It is suggested that these changes correspond to absorption bands associated with pigments formed in the bruise reaction *in vitro*. However, there were also structural changes which occur in the cells of bruised tissue which may have altered the light scattering properties, particularly if shatter bruising was present.

If reflectance spectrophotometry is to be a useful method for non-invasively detecting bruising it has to be capable of detecting bruising in an unknown sample. The results shown here indicate that bruise detection may be possible in unpeeled tubers, and almost certainly in peeled tubers. However, the method requires to be improved if it is to be a reliable technique. A possible improvement may be the use of an integrating sphere which enables true diffuse reflectance measurements to be made with little or no effect from the specular component [30]. Tuber cultivar is another variable with the potential to affect reflectance characteristics; this will be the subject of a subsequent paper [8].

## ACKNOWLEDGEMENTS

The authors would like to thank the Potato Marketing Board for funding this work as part of a PhD studentship work; and Douglas McRae, David Ross and Calum Dewar for helpful advice and discussions.

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