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REFLECTANCE SPECTROPHOTOMETRY OF BRUISING IN POTATOES. II. ACQUISITION OF SPECTRA IN THE VISIBLE SPECTRUM WITH AN INTEGRATING SPHERE*

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A b s t r a c t. Diffuse reflectance spectra from 400 to 700 nm were measured with an integrating sphere from bruised and unbruised, unpeeled and peeled tubers *cvs.* Desiree, Pentland Dell and Record. Stepwise discriminant analysis was used to determine wavelengths that were sensitive to bruising and to formulate classification algorithms to determine whether a tuber from an unknown sample was bruised. Wavelengths selected from unpeeled tubers were dependent on cultivar, while spectra from peeled tubers were independent of cultivar. The accuracy of classifying tubers as bruised or unbruised was between 76 and 91% for unpeeled tubers and 89 and 100% for peeled tubers. When the identity of tubers was unknown to the discriminant program, 70 to 82% of unpeeled and 73 to 83% of peeled tubers were correctly classified as bruised or unbruised. The integrating sphere increased the percentage of spectra correctly classified compared to spectra collected with a bifurcated fibre optic light guide. The increased accuracy was due to a smaller experimental error, greater sampling area and the acquisition of more truly diffuse reflectance.

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

INTRODUCTION

Reflectance spectrophotometry was used in a previous study [4] to determine wavelengths from the ultraviolet (UV) to near infrared (NIR) that were associated with bruising in potato tubers *cv.* Record. If the discolouration associated with bruising could be non-invasively detected with this method, it could perhaps give the potential for on-line grading of tubers. The previous study [4] demonstrated that detecting bruising in peeled tubers was very successful, but in unpeeled tubers it was not so promising. The reasons for the low detection rate of bruising in unpeeled tubers might have been the result of using a bifurcated fibre optic light guide. While this type of fibre optic light guide is commonly used to quantify reflectance, it has the disadvantage of a small sample area, and/or, not all the reflected light is captured.

One method for increasing the sampling area and reducing the specular component of reflected light is to use an integrating sphere which collects all the diffusely reflected radiation. An integrating sphere is composed of two hemispheres that are mated together to form a spherical cavity. The sphere is machined from, or coated with, a highly reflective material. Holes or ports in the sphere are used to transmit a collimated beam of light onto a sample at another port (Figs 1 and 2).

The radiation reflected from the sample consists of a specular and a body reflectance component. The reflected light undergoes multiple reflections on the wall of the sphere,

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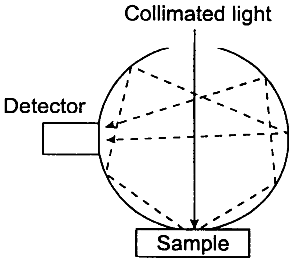

Fig. 1. Pathways of light within an integrating sphere.

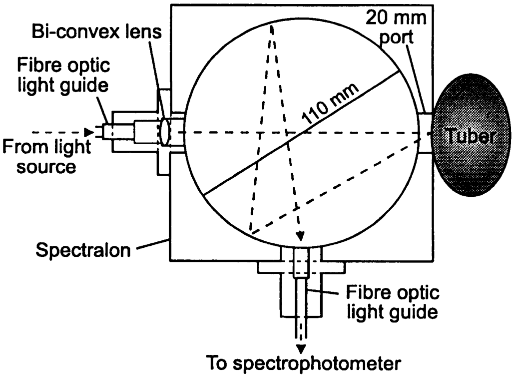

Fig. 2. Cross section of integrating sphere designed at the Scottish Centre of Agricultural Engineering.

which results in a uniform, spatially integrated radiation distribution within the sphere. A detector placed in a port in the sphere wall measures an output that is completely independent of the angular properties of the input light. The independence of the output of a sphere from the angular properties of the input light, and the uniform internal distribution of light make integrating spheres ideal for measuring diffuse reflectance [15]. In addition to evaluating the performance of an integrating sphere, the effect of potato cultivar on reflectance spectra was also investigated.

METHODS

An integrating sphere, internal diameter 110 mm, was machined 'in-house' from a solid block of Spectralon (Labsphere Inc., North Sutton, East Anglia, UK). Spectralon is a thermoplastic resin that gives the highest diffuse reflectance of any known material; the reflectance is generally greater than 99 % over a range from 400 to 1500 nm, and greater than 95% from 250 to 2500 nm. Light was transmitted from a 150W halogen light source (FOT 150; All Inspection NDT Ltd., UK) along a randomly oriented fibre optic light guide (4 mm diameter; Melles Griot Ltd., Waterbeach, UK) to a port in the sphere. The fibre optic light guide was held in place by a plug, painted matt black inside to reduce any stray reflections (Fig. 2). The plug contained a 15 mm bi-convex lens (focal length 20 mm) made from BK-7 glass (Melles Griot Ltd.). A light source located at the focal length of a positive lens gave a collimated light beam which illuminated an area of 255 mm^2 on the sample held at the opposite sampling port. Reflected light from the sample was reflected off the walls of the sphere until it passed along a randomly oriented fibre optic light guide (4 mm diameter; Melles Griot Ltd.) mounted in plug at 90° to the sample port (Fig. 2). The fibre optic light guide passed the reflected light to a Monolight monochromator (6118) and photomultiplier tube (Rees Instruments Ltd., Godalming, Surrey, UK) which analysed light from 400 to 700 nm.

Although the previous study indicated that wavelengths above 700 nm in the NIR might be useful in the discrimination of bruised and unbruised tubers, the method used here was limited to the spectral range of 400 to 700 nm. The main reason for the restricted range was that a photo multiplier tube was needed to detect the relatively low light signal from the sphere. The photo multiplier was sensitive to light from 200 nm to 800 nm, but had a useful sensitivity from about 250 to 750 nm. The sensitivity was further restricted by having a lens that was manufactured from BK 7 glass that attenuated light below 380 nm [9]. The lower than expected light signal received by the photomultiplier tube was due to the following factors. Firstly, light from the transmitting fibre optic light guide had a transmission angle of approximately 70° and therefore the 15 mm diameter lens did not capture all the transmitted light. Secondly, the area of the detecting fibre optic was approximately 12 mm^2, which was a small proportion (0.032%) of the total internal area of the sphere. Thirdly, the collimated beam of light was not perfectly collimated; there was a 'halo' of light around a bright central 'core'. The core is focused to provide an illuminated area of 18 mm diameter, but *c.a.* 5% of the collimated light is reflected off the wall of the sphere.

One hundred tubers of each of the cultivars Desiree, Pentland Dell and Record were hand dug, washed and stored at 4°C. Tubers were bruised using the same method described in the previous study [4,8] and placed in a hotbox for 16 h. All reflectance spectra (both relative and first derivative spectra) were collected following the procedure detailed previously [4]. Spectra were taken of the bruised and unbruised sites on unpeeled and peeled tubers and classified as bruised if there was any visible discolouration after peeling. Bruised tubers were further classified as blackspot or shatter bruised [12].

The spectra from unbruised and bruised tubers were divided into two groups; 'known' and 'trial' spectra. Stepwise Discriminant Analysis (BMDP Statistical Software Inc., California, USA) was used to find the combination of wavelengths from the 'known' spectra which best gave the best separation of bruised and unbruised tubers. The combination of wavelengths were used to create a linear classification function which can be used in the classification of new cases whose group membership is not known [6,13]. A successful classification was one which obtained a $p<0.001$ for Wilk's Lambda [2,4,6].

RESULTS AND DISCUSSION

All varieties had a high percentage of bruised tubers (52 to 80%). Record was the most bruise susceptible (80% tubers bruised) and had the highest level of blackspot bruising (96% of bruised tubers). Desiree was the least bruise susceptible (52% of tubers were bruised), and Pentland Dell had the highest percentage of shatter bruising (29% of bruised tubers).

For both unpeeled and peeled tubers, there was a decrease in reflectance in bruised tubers for all cultivars (Figs 3 and 4) with a greater decrease for peeled tubers. The decrease is thought to be due to the effect of melanin which has a broad range of absorption bands in this spectral range [1]. Error bars are not shown in these figures to enable the effect of bruising on the averaged spectra to be seen more clearly. However, it is important to consider the amount of spectral variation and it was found that there was less variation compared to results from the previous study [4]. The reduction in variation is probably due to an increased sampling area and the collection of more diffuse reflectance from tissue below the skin with the integrating sphere. A two-tailed t-test did not reveal any significant differences ($p<0.001$) between the spectra of unbruised and bruised unpeeled tubers, while for peeled tubers there was a significant difference ($p<0.001$) from 500 to 700 nm in the reflectance spectra and at 510 nm in the first derivative spectra for all cultivars.

When the first derivative spectra of unpeeled tubers were examined, it was revealed that cultivar had an effect on the spectra, which was not apparent in peeled tubers (Figs 3 and 4).The effect of cultivar was particularly marked in Desiree, which has a red skin compared to the brown skin of *cv.* Pentland Dell and *cv.* Record; this was evident from the difference between unbruised and bruised spectra between *c.a.* 600 and 650 nm for *cv.* Desiree. The effect of cultivaron unpeeled tubers was also demonstrated by the wavelengths selected by discriminant analysis, particularly from the reflectance relative spectra (Table 1). No wavelengths were selected to discriminate between reflectance spectra of *cv.* Pentland Dell, but there were differences between the first derivative spectra which enabled the discrimination of bruised and unbruised tubers.

Peeled tubers showed a similar difference between unbruised and bruised spectra for all cultivars, particularly in the first derivative spectra (Fig. 4). The similarity between cultivars was also demonstrated by the wavelengths selected by discriminant analysis (Table 1) from both reflectance and first derivative spectra. The change in reflectance at *c.a.* 500 to 550 nm is probably attributed to the pigments dopachrome and 5.6 dihydroxy

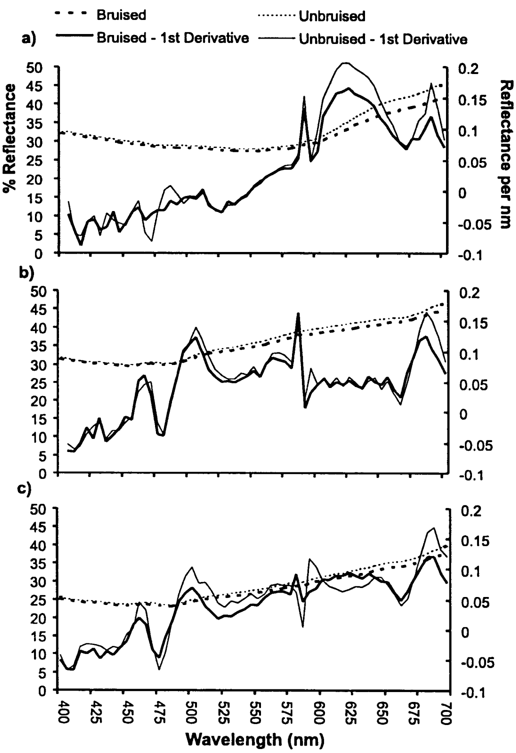

Fig. 3. Mean reflectance relative from bruised tubers (a: n = 52, min. SD 1.0% at λ_{400}, max. SD 3.9% at λ_{700}; b: n = 71, min. SD 1.0% at λ_{450}, max. SD 3.5% at λ_{700}; c: n = 80, min. SD 1.3% at λ_{500}, max. SD 2.9% at λ_{700}) and unbruised tubers (a: n = 148, min. SD 1.0% at λ_{400}, max. SD 2.6% at λ_{700}; b: n = 129, min. SD 1.0% at λ_{450}, max. SD 3.6% at λ_{700}; c: n = 120, min SD 1.3% at λ_{500}, max. SD 3% at λ_{700}) and first derivative spectra from bruised tubers (a: n = 52, min. SD 0.01 at λ_{550}, max. SD 0.07 at λ_{700}; b: n = 71, min. SD 0.01 at λ_{550}, max. SD 0.04 at λ_{500}; c: n = 80, min. SD 0.02 at λ_{550}; max. SD 0.08 at λ_{700}) and unbruised tubers (a: n = 148, min. SD 0.01 at λ_{550}, max. SD 0.05 at λ_{700}; b: n = 129, min. SD 0.01 at λ_{550}, max. SD 0.04 at λ_{500}; c: n = 120, min SD 0.01 at λ_{550}, max. SD 0.06 at λ_{700}). All tubers were unpeeled, *cv.* Desiree (a), Pentland Dell (b) and Record (c). Units for SD are in % reflectance relative for untransformed spectra and reflectance relative per nm for first derivative spectra (c).

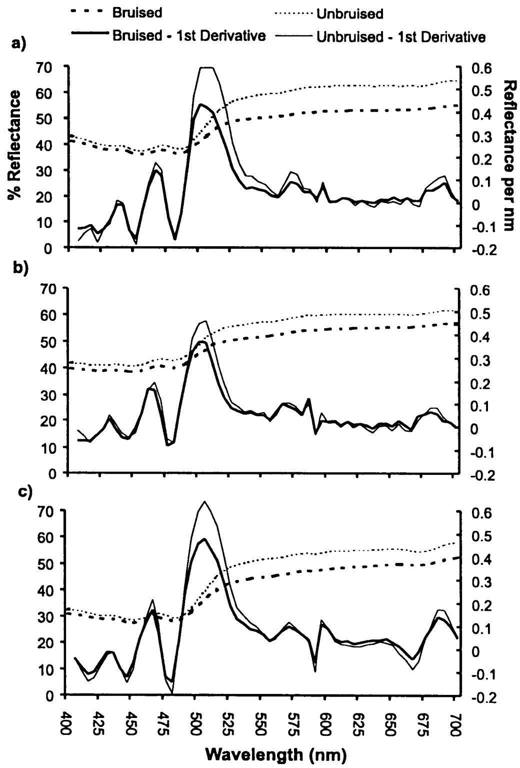

Fig. 4. Mean reflectance relative from bruised tubers (a: n = 52, min. SD 2.3% at λ_{450}, max. SD 6.5% at λ_{700}; b: n = 71, min. SD 1.9% at λ_{450}, max. SD 4.2% at λ_{700}; c: n = 80, min. SD 2.7% at λ_{500}, max. SD 4.9% at λ_{700}) and unbruised tubers (a: n = 148, min. SD 1.9% at λ_{450}, max. SD 2.3% at λ_{700}; b: n = 129, min. SD 1.9% at λ_{450}, max. SD 3.0% at λ_{700}; c: n = 120, min. SD 2.3% at λ_{500}, max. SD 5.3% at λ_{700}) and first derivative specra from bruised tubers (a: n = 52, min. SD 0.01 at λ_{550}, max. SD 0.11% at λ_{500}; b: n = 71, min. SD 0.01% at λ_{550}, SD 0.08 at λ_{500}; c: n = 80, min. SD 0.01 at λ_{550}, max. SD 0.08 at λ_{500}) and unbruised tubers (a: n = 148, min. SD 0.01 at λ_{550}, max. SD 0.06 at λ_{500}; b: n = 129, min. SD 0.01 at λ_{550}, max. SD 0.06 at λ_{500}; c: n = 120, min SD 0.01 at λ_{550}, max. SD 0.08 at λ_{500}). All tubers were peeled, *cv.* Desiree (a), Pentland Dell (b) and Record (c). Units for SD are in % reflectance relative for untransformed spectra and reflectance relative per nm for first derivative spectra (c).

-indole which have similar absorption wavelengths to this when they are analysed *in vitro* [7,11].

Discriminant analysis gave the highest percentage classification when wavelengths from the first derivative spectra of 'known' unpeeled and peeled tubers were used (Tables 2 and 3). Conversely, when these classification functions were used on the 'trial' spectra, the classification was consistently better when the reflectance spectra were analysed (Tables 2 and 3). The percentage of 'known' reflectance spectra from peeled tubers correctly classified by discriminant analysis was greater than 80%

Table 1. Wavelengths selected by discriminant analysis to separate spectra from unbruised and bruised, unpeeled and peeled tubers

'Know' Spectra	Desiree	Pentland Dell	Record
		Unpeeled Tubers	
Reflectance spectra	695	none	465, 435
First derivative spectra	490, 610, 625, 540, 510, 530, 410, 520	590, 440, 650, 655	655, 490, 425, 630, 585, 515, 435
		Peeled Tubers	
Reflectance spectra	590, 665, 500	550, 680	550, 700, 500
First derivative spectra	515, 680, 420	520, 480, 620, 420, 645	615, 665, 465, 605, 505, 405, 660, 415

*Wavelengths (nm) shown in order that discriminant analysis selected variables on basis that F value was greater than 4.

Table 2. Percentage of 'Known' and 'Trial' spectra from unpeeled tubers correctly classified as bruised or unbruised

Unpeeled Tubers	Desiree	Pentland Dell	Record
		'Known' Spectra	
Reflectance spectra	67*	none	82
First derivative spectra	91	76	90
		'Trial' Spectra	
Reflectance spectra	82	none	72
First derivative spectra	61*	36*	65*

Table 3. Percentage of 'Known' and 'Trial' spectra from peeled tubrts correctly classified as bruised or unbruised

Peeled tubers	Desiree	Pentland Dell	Record
		'Known' Spectra	
Reflectance spectra	87	82	90
First derivative spectra	91	89	100
		'Trial' Spectra	
Reflectance spectra	73	84	83
First derivative spectra	80	69*	67*

(Table 3) for all cultivars, and this increased when first derivative spectra were analysed (up to 100%). The poor detection of bruising in unpeeled *cv.* Pentland Dell may be due to the higher percentage of shatter bruise (29% of bruised tubers) compared to the other cultivars studied. Shatter bruising caused more disruption to the tissues than blackspot bruise [12] and may have resulted in a loss of water in addition to the production of melanin [14]. Therefore, wavelengths in the NIR which are more sensitive to water status [10] may be more suitable for detecting this type of bruising in unpeeled tubers than the visible spectrum [4].

A comparison of the discriminant analysis of spectra obtained with a bifurcated fibre optic

Table 4. Comparison of the percentage of 'Trial' first derivative spectra collected with bifurcated fibre optic cable and integrating sphere correctly classified as bruised or unbruised from unpeeled and peeled tubers

	'Trial' spectra	Desiree	Pentland Dell	Record
Bifurcated cable	Reflectance spectra	49*	40*	55*
	First derivative spectra	55*	52*	35*
Integrating sphere	Reflectance spectra	82	none	70
	First derivative spectra	61*	36*	65.2*
			Peeled Tubers	
Bifurcated cable	Reflectance spectra	78	69*	57*
	First derivative spectra	74	57*	57*
Integrating sphere	Reflectance spectra	73	84	83
	First derivative spectra	80	69*	67

*not significant: $p>0.0001$ for Wilk's Lambda.

light guide [4] and the integrating sphere, showed that the integrating sphere gave a consistently higher percentage of tubers correctly classified for all cases (Table 4). An increase in accuracy would be expected as the integrating sphere is a more appropriate tool for collecting the diffuse reflectance and increasing the sampling area.

CONCLUSIONS

In unpeeled tubers, the wavelengths selected to discriminate between unbruised and bruised tubers appear to be dependent on variety; this was probably due to the effect of skin colour. If this method has future potential for automatic bruise detection in unpeeled tubers, then algorithms will have to be tailored for individual varieties. In contrast to unpeeled tubers, the spectra from peeled tubers did not appear to be affected by cultivar. The absence of skin made the reflectance spectra more consistent and the difference between spectra from unbruised and bruised tubers was greater. However, this may be more of a theoretical than practical interest as it is bruising in unpeeled tubers that go unnoticed through quality control. A possible application for reflectance spectrophotometry would be where tubers are peeled just prior to processing. Sensors could be designed to rapidly monitor wavelengths associated with bruising and machinery designed to automatically reject bruised tubers.

Discriminant analysis of both unpeeled and peeled tubers was best when first derivative spectra were used from the 'known' group. However, when the classification functions were used on the 'trial' spectra, the discrimination was best when reflectance relative spectra were analysed for both unpeeled and peeled tubers. One of the disadvantages of using discriminant analysis is that it generates linear functions which may not be the best method for modelling the data. A more appropriate tool for modelling the data may be neural networks which are good at dealing with non-linear and noisy data [5]. Indeed, it has been found that neural networks consistently increased the accuracy of identifying 'trial' spectra for all samples studied, but the success of identifying bruising in unpeeled tubers never reached 100% for 'trial' spectra [3].

A comparison of data obtained with the integrating sphere and the bifurcated cable used in the first study [4] revealed that the integrating sphere was consistently better for reducing experimental error and increasing the accuracy of bruise detection, especially for peeled tubers. The detection of bruising in unpeeled tubers might have been further improved if the integrating sphere could have collected wavelengths above 700 nm, particularly for detecting shatter bruise in *cv.* Pentland Dell.

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