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CHANGES OF BACKSCATTERING PARAMETERS DURING CHILLING INJURY IN BANANAS

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Abstract

The change in backscattering parameters during the appearance of chilling injury in bananas was investigated. Bananas were stored at a chilling temperature for two days and the degrees of the chilling injuries that appeared were measured before, during and after storage using backscattering imaging and visual assessment. Laser lights at 660 nm and 785 nm wavelengths were shot consecutively onto the samples in a dark room and a camera was used to capture the backscattered lights that appeared on the samples. The captured images were analysed and the changes of intensity against pixel count were plotted into graphs. The plotted graph provides useful information of backscattering parameters such as inflection point (IP), slope after inflection point (SA), and full width at half maximum (FWHM) and saturation radius (RSAT). Results of statistical analysis indicated that there were significant changes of these backscattering parameters as chilling injury developed.

Keywords: Banana, Chilling injury, Backscattering, Fruit quality, Imaging.

1. Introduction

Bananas are susceptible to chilling injury when exposed to chilling temperatures. Since the banana is the 4th most important food behind rice, wheat and maize in the world food ranking [1], chilling injury becomes a major problem in marketability in the banana industry. The shipment of the fruits from the producer countries to the importing countries requires the fruits to be stored in cold storage

to ensure that they will be in a good condition upon arriving at their destinations. However, bananas were reported to exhibit chilling injury symptoms when exposed to a temperature below 10°C which could degrade the quality of the fruits [2-5]. The injured fruit showed discoloration or browning, the symptom that the green or yellow colour of the fruit skin changed to brown and completely black, depending on the severity of the injury.

The appearance and severity of chilling injury was strongly influenced by temperature, time and ripening stage of the fruits [2, 6-7]. The severity increases and the quality deteriorate faster with lower storage temperatures and longer exposure to chilling temperature. The symptoms become clearer after the fruit is exposed to ambient temperature. The appearance of the symptoms will be exhibited immediately or takes several days in which case there is high possibility that the infected fruit could escape detection during the sorting process, which in turn will affect its marketability. In addition, the conventional method of chilling injury detection used i.e. visual assessment (VA) is easily exposed to human error due to the dependency of the technique on human visual skill. Thus, a more advance technology, non-destructive, inexpensive, faster and accurate method is needed to overcome these uncertainties.

Backscattering imaging is one of the advance non-destructive optical imaging methods that potentially could be used for quality detection in agricultural produce. The method physically applies a theory of interaction between light and fruit tissue. As light hits a fruit tissue, 4% of the light will be reflected back to the atmosphere while the rest will penetrate and being absorbed, transmitted or scattered back (diffuse reflectance) to the incident point [8]. The interaction of light during penetration in the fruit tissues carries useful information about the structure of the material which later could be used to measure the quality of the produce. As technology advance, the information of the interaction was reported can be extracted using optical imaging methods [9-13].

Image acquisition using backscattering imaging method provides backscattering images that consist of a circular illumination spot in which the intensity of the illumination decreases radially outwards. Tu et al. [9] reported that the outer part (low level intensity) of the illumination spot in the image showed high correlation with the change in the total number of pixel, thus contains useful information on the tissue characteristics. Qing et al. [13] found that a histogram of backscattering intensities is highly correlated with soluble solids content (SSC) of apples. Backscattering parameters obtained from the curve fitting of backscattering intensities such as inflection point (IP), full width at half maximum (FWHM), slope, etc., correlated well with the textural properties of peach [10], apples [11, 14], ripeness stages of tomatoes [12] and recently, the moisture content of bell pepper [15]. Since there were promising results obtained in the application of backscattering imaging in agricultural produce, the effect of chilling injury in bananas on the backscattering parameters was studied in order to evaluate the ability of the method to replace the conventional method of VA.

2. Materials and Methods

Musa cavendishii bananas from ripening stages two (R2), three (R3), four (R4) and five (R5) were obtained from a commercial banana ripening facility

(FruchtExpress Import Export GmbH, Germany). Samples were evenly divided into two groups, i.e. the chill-treated samples which were stored at a chilling temperature of 6°C and the control samples which were stored at 13°C. The experiment was spread over a period of 4 days. At day 1, both groups were stored at control temperature (13°C) for 24 hours. From day 2 to 4, the chill-treated samples were stored at chilling temperature. At day 4, all fruits were exposed to ambient temperatures. Data collection was carried out at before storage (day 1), during storage (day 3) and after storage (day 4) by using backscattering imaging method and visual assessment as reference data.

2.1. Image acquisition

Backscattering images of bananas were recorded using an in-house-developed laser-induced backscattering imaging system in the Department of Horticultural Engineering, Leibniz Institute for Agricultural Engineering, Potsdam-Bornim (ATB), Germany. The system was complete equipped with in-house-developed software that installed in the computer to assist the image acquisition process. Each banana was placed under a CCD camera (JVC KY-F50E) with zoom lens F2.5 and focal lengths of 18-108 mm. Laser diode of 1 mm beam size emitting at 660 and 785 nm with 45 mW maximal power was used as a light source. Backscattering images of sizes 720 x 576 pixels were acquired in a dark-room in order to obtain a good signal-to-noise ratio. A total of six images consisting of 3 images per side of a banana were taken to obtain the average value of the backscattering for each fruit. The Lambertian cosine law was applied to adjust the intensity values of the surface captured by the CCD camera.

The backscattering images were identified by the brightness of the light, Fig. 1(a) which decreased radially outwards as the distance from the illumination point increased, providing a symmetric backscattering profile, Fig. 1(b). From the backscattering profile, the backscattering parameters, i.e., the inflection point (IP), the slope after inflection point (SA), the full-width-half-maximum (FWHM) and the saturation radius (RSAT) were obtained. The IP was defined as the minimal value of the first derivative of the profile. The FWHM was given by the distance between two points on the curve at which the profile reached half its maximum value. The RSAT is the distance between the incident point and the IP of the backscattering profile. Values of the backscattering parameters obtained from the analysis were then transferred as text files to Matlab or MS-Excel for statistical analysis.

2.2. Visual assessment

The visual assessment method using a browning scale as described by Nguyen et al. [5] was performed immediately after the backscattering image acquisition. The browning scale was rated as follows: 1 = no chilling injury symptoms; 2 = mild chilling injury symptoms in which injury can be found in between the epidermal tissues; 3 = moderate chilling injury symptoms in which brown patches begin to become visible, larger and darker; 4 = severe chilling injury symptoms in which the brown patches are clearly visible and are larger and darker than at scale 3; 5 = very severe chilling injury symptoms in which the patches are relatively large on the surface.

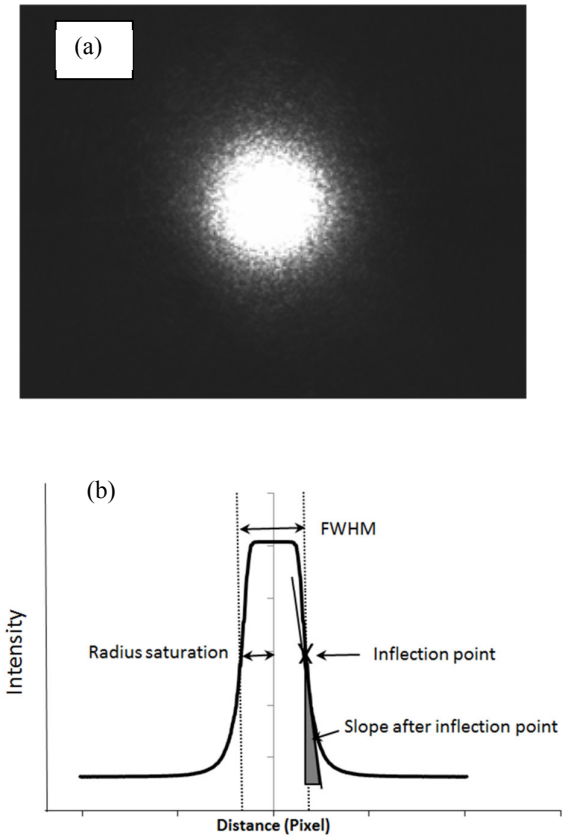

Fig. 1. (a) A Raw Backscattering Image Acquired Using a 785 nm Laser and (b) The Backscattering Profile and Parameters of the Raw Image.

2.3. Data analysis

The data obtained were subjected to descriptive statistics, error bar plots and analysis of variance (ANOVA). All statistical analyses were carried out using Matlab (Math Work Inc., USA) and SAS statistical software.

3. Results and Discussion

3.1. Visual assessment

All the bananas stored at the control temperature (13°C) were not affected by chilling injury after removal from storage and being exposed to ambient temperature; all had a VA value of 1 (Fig. 2). The fruits ripened normally and developed a golden yellow colour due to the breakdown of chlorophyll (green pigment) thereby unmasking the carotenoids (yellow-red pigment). The pulp became tender and soft thus making the fruit edible fresh which, as reported by Prasanna et al. [16] was due to the depolymerization and solubilisation of pectins

(carbohydarate molecules in the cell walls). This indicated that the temperature of 13°C is suitable for storage with no initiation of chilling injury to the samples. The present study together with the findings of [16] and [17] confirmed that the slowing down of normal ripening of bananas and the prolonging of the fruit shelf life can be achieved by storing at 13°C.

In contrast, the chill-treated bananas (6°C storage) did not ripen after being exposed to ambient temperature but exhibited discoloration, a phenomenon which is reported to be due to the accumulation of phenolic substances. This means that the ripening process of the chill-treated samples was halted and, due to the chilling injury, phenolic substances were being oxidized. The pulp also tended to be harder indicating that the process of disassembly of the cell wall and conversion of carbohydrates to sugar did not happen thus making the fruit to become off-flavours. Different parts of these observations were also observed by [5, 18, 19] using destructive methods.

Values of visual assessment for the chill-treated bananas at different ripening stages are as illustrated in Fig. 2. The mean values for bananas stored at 6°C increased from before to after storage indicating there was a change in the skin colour of the fruits upon exposure to chilling temperature. Ripening stage R2 obtained the lowest mean values compared to the more advanced ripening stages either during or after storage denoting that the skin colour was less affected by the chilling temperature. The degree of browning had a value of 2 which indicated that the browning symptom was at a mild stage. Although the browning was very slight, the injury was, nevertheless, severe. The bananas failed to ripen normally due to the failure of the fruit to produce ethylene. As a result, although the injured fruits maintained green the texture of the pulp became hard and were not fit for human consumption.

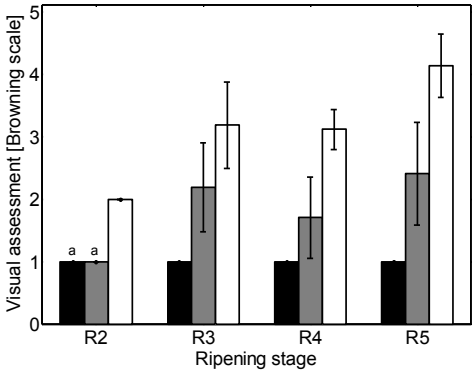

Fig. 2. Visual Assessment Values of Bananas at Different Ripening Stages Stored at 6°C (■: Before Storage, ■: During Storage, □: After Storage). Bars Represent Mean ± Standard Deviation. The Letter *a* Indicates Values that are not Significantly Different ($p > 0.05$).

For the more advanced ripening stages (R3, R4 and R5), the mean values changed from 1 (before storage) to a maximum of 4 (after storage) demonstrating that the browning symptom had developed and the severity increased as the treatment time progressed. The metabolism change was the same as in the R2

samples. However, as the colour of the more advanced stages before being stored in chilling temperature had already turned yellow due to the ripening process, the pulp for the advance stages already tender depending on their maturity level. Therefore, the symptoms of chilling injury were easily detected from the colour contrast between the browning and the light yellow colour of the skin. In addition, the disassembly of the cell walls and conversion of carbohydrates to sugar which contributed to the softening of the ripe fruit had exposed the fruits to diseases or fungal infections which could facilitate mechanical injury and decay. As a result, as the stage of ripening advanced, the severity of injury increased in tandem with the appearance of browning. This finding was in agreement with [2] and [20] who reported that the appearance of chilling injury symptoms becomes severe as the maturity level increases. The degree of browning also increased when the samples were exposed to ambient temperature. Thus, it can be inferred that time, temperature and maturity stage had strong influences in the appearance of chilling injury. This was supported by ANOVA as shown in Table 1.

Table 1. ANOVA of Visual Assessment (VA) in Bananas Subjected to Experimental Factors.

Factors	Mean squares	*F*-values	*p*-values
Temperature	178.673	15802.1	<.0001
Ripening stages	74.692	1001.37	<.0001
Treatment time	41.804	560.45	<.0001
Temperature* Ripening stages	74.692	1001.37	<.0001
Temperature* Treatment time	41.804	560.45	<.0001
Ripening stages* Treatment time	2.203	29.53	<.0001
Temperature* Ripening stages* Treatment time	2.203	29.53	<.0001

Results indicated that in all factors (temperature, ripening stage and treatment time) and factor combinations, the p-values were less than 0.0001, meaning that there was less than 0.01% probability that these factors had no effect on the development of chilling injury symptoms. This means that the null hypothesis could be rejected and it could be concluded that all the factors had significant effects on the change in the degree of browning as the chilling injury developed.

3.2. Backscattering parameters

The values of backscattering parameters measured using 660 nm laser for chill-treated bananas at before, during and after 6°C storage are shown in Fig. 3. Before storage, the values of the backscattering parameters IP, SA, FWHM and RSAT for 660 nm increased as ripening stages increased reflecting the increase in the backscattering areas. This phenomenon could be explained to be due to the interaction between the 660 nm wavelength and the chlorophyll which absorbed light at this wavelength. As the fruit ripened and maturity level increased, the chlorophyll pigment disappeared and carotenoids pigment appeared. This results in very little or no absorption of the light by chlorophyll and more photons were backscattered as maturity level increases. Parallel to this observation is that of [15] in which less scattering and a decrease in the total pixel number of green bell

peppers was reported and was explained to be due to the chlorophyll pigments absorbing light at approximately 670 nm bandpass.

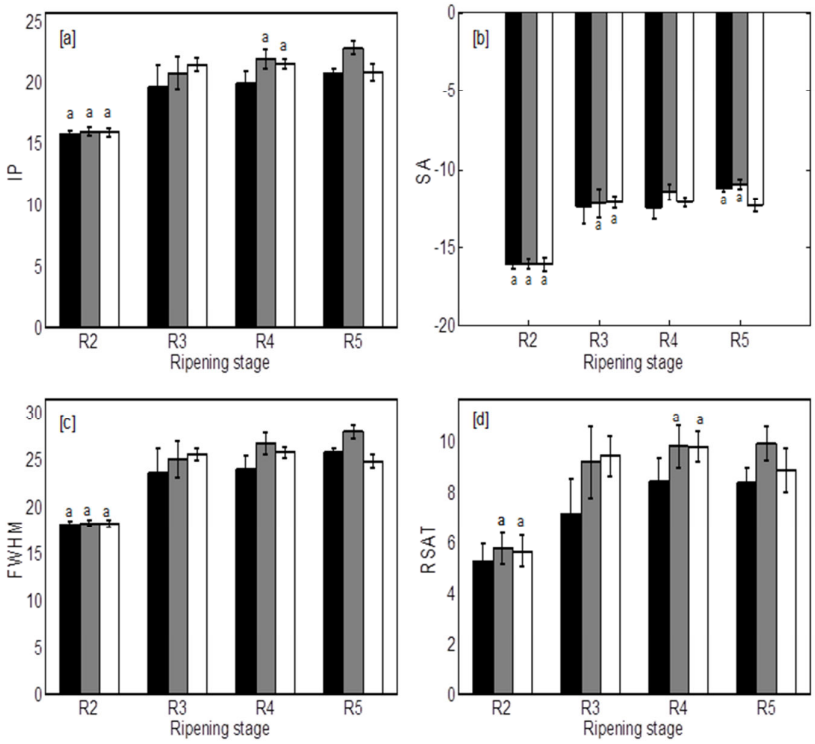

Fig. 3. Values of Various Backscattering Parameters at 660 nm of Bananas at Different Ripening Stages Stored at 6°C (■: Before Storage, ■: During Storage, □: After Storage). Bars Represent Mean ± Standard Deviation. The Letter *a* Indicates Values that are not Significantly Different ($p > 0.05$).

When stored in chilling temperature, the fruits resulted in lower levels of chlorophyll than did the control samples. This was due to the severe damage in the chloroplast membranes which later affect to acceleration of chlorophyll degradation during chilling injury. The lesser amount of chlorophyll present to absorb the incident light then resulted in an increased backscattering area. The changes can be seen clearly on IP, FWHM and RSAT. However, SA did not show any significant difference from during to after storage for all ripening stages. This was supported by ANOVA as shown in Table 2 which indicated that SA obtained the lowest F-value when subjected to treatment time factor.

For R3, the values of IP, FWHM and RSAT increased from during storage to after storage. For R4, these parameters did not change significantly in values and for R5 they decreased. However, each parameter had after-storage values that were about the same which could be due to the fruits being already severely injured and no chlorophyll was present anymore to effect any change in

parameters value. Statistically, all the backscattering parameters were significantly affected by all the experimental factors and their interactions (Table 2). However, results for R2 were different than the others in that the chlorophyll content was not affected from before to after storage.

Table 2. ANOVA of Backscattering Parameters (660 nm) in Bananas Subjected to Experimental Factors.

Factor	Parameter	Mean Squares	F-values	*p*-values
Temperature	*IP*	686.7440	1438.54	<.0001
	SA	436.6203	1754.85	<.0001
	FWHM	2015.5233	2007.93	<.0001
	RSAT	298.9686	519.70	<.0001
Ripening stage	*IP*	6527.1495	13726.9	<.0001
	SA	3751.1835	15129.2	<.0001
	FWHM	12911.5233	12850.7	<.0001
	RSAT	2826.6888	4913.69	<.0001
Treatment time	*IP*	45.7572	96.25	<.0001
	SA	7.0392	28.41	<.0001
	FWHM	74.0621	73.77	<.0001
	RSAT	50.2988	87.44	<.0001
Temperature*Ripening stage	*IP*	94.9278	199.37	<.0001
	SA	48.6192	195.97	<.0001
	FWHM	249.3856	248.41	<.0001
	RSAT	61.3597	106.66	<.0001
Temperature*Treatment time	*IP*	11.9360	25.12	<.0001
	SA	5.6086	22.63	<.0001
	FWHM	29.0285	28.91	<.0001
	RSAT	7.5563	13.14	<.0001
Ripening stage*Treatment time	*IP*	18.8082	39.55	<.0001
	SA	5.5930	22.56	<.0001
	FWHM	33.5190	33.39	<.0001
	RSAT	10.6624	18.53	<.0001
Temperature*Ripening stage*Treatment time	*IP*	2.0347	4.28	<.0001
	SA	1.3191	5.32	<.0001
	FWHM	4.6055	4.59	<.0001
	RSAT	4.2528	7.39	<.0001

While 660 nm is sensitive to pigment content, laser light at near-infrared wavelength (770-2500 nm) is sensitive to textural properties of the fruits [21]. The values of the collected backscattering parameters are as presented in Fig. 4.

In contrast to the changes showed by 660 nm, the IP and FWHM for 785 nm of R2, R3 and R4 decreased from before to after storage. The decreased could be related to the changes in the textural properties of the bananas as chilling injury developed. Exposure of bananas to chilling temperature resulted in a decrease in turgor pressure of the fruits which in turn enhanced water losses. Kasim and Kasim [22] explained that as chilling injury develops, water losses become greater due to cellular breakdown, deterioration of membrane integrity as well as loss of epicuticular wax which is important in controlling water exchange in the skin. Thus, the intercellular spaces become larger and filled with air promoting direct reflection or transmission of penetrated light instead of backscattering it. As a result, the

backscattering area decreased; hence the lower values of during storage IP and FWHM for the bananas. The injury became severe after storage and the backscattering area and backscattering parameter values decreased further.

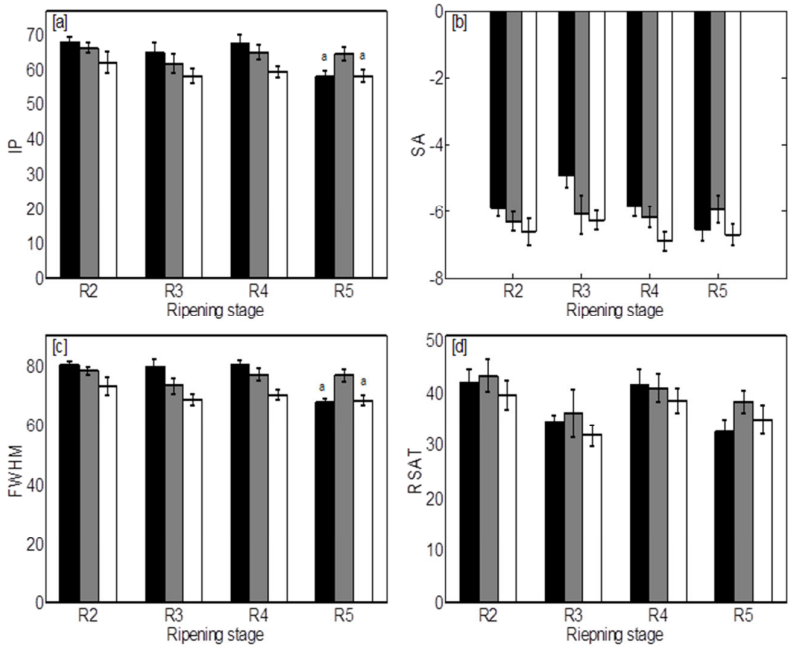

Fig. 4. Values of Various Backscattering Parameters at 785 nm of Bananas at Different Ripening Stages Stored at 6°C (■: Before Storage, ■: During Storage, □: After Storage). Bars Represent Mean ± Standard Deviation. The Letter *a* Indicates Values that are not Significantly Different ($p > 0.05$).

After exposed to chilling temperature, the bananas at R2, R3 and R4 became harder and gave about similar response to the penetrating laser light as reflected in the approximately same values of IP, Fig. 4(a) and FWHM, Fig. 4(c) between before and after storage. The values for R5 were insignificantly different from before to after storage. This could be due to the lesser solid component available at R5 as the fruits were fully ripened to scatter the penetrated light.

Results of ANOVA indicated that except for ripening stage and the three-factor interaction, FWHM showed the highest F-values for all the other experimental factors including the two-factor interactions (Table 3). For the three factors interaction, the highest was indicated by RSAT. The parameters SA and RSAT showed irregular changes which mean that SA and RSAT were apparently not significantly influenced by the changes in the texture of the bananas as chilling injury developed (Fig. 4). Nevertheless, all experimental factors and their interactions were statistically significant ($p < 0.05$). This means there is high potential that this method could be used as a non-destructive method for detecting chilling injury in bananas.

Table 3. ANOVA of Backscattering Parameters (785 nm) in Bananas Subjected to Experimental Factors.

Factor	Parameter	Mean Squares	F-values	*p*-values
Temperature	*IP*	10388.071	1898.84	<.0001
	SA	118.581	1057.40	<.0001
	FWHM	17703.868	3211.56	<.0001
	RSAT	4712.858	662.67	<.0001
Ripening stage	*IP*	1707.666	312.14	<.0001
	SA	14.636	130.51	<.0001
	FWHM	2013.094	365.18	<.0001
	RSAT	6064.957	852.79	<.0001
Treatment time	*IP*	601.241	109.90	<.0001
	SA	9.778	87.19	<.0001
	FWHM	1076.524	195.29	<.0001
	RSAT	572.982	80.57	<.0001
Temperature*Ripening stage	*IP*	53.278	9.74	<.0001
	SA	1.9390	17.29	<.0001
	FWHM	117.726	21.36	<.0001
	RSAT	88.837	12.49	<.0001
Temperature*Treatment time	*IP*	252.520	46.16	<.0001
	SA	2.861	25.51	<.0001
	FWHM	425.238	77.14	<.0001
	RSAT	115.280	16.21	<.0001
Ripening stage*Treatment time	*IP*	134.155	24.52	<.0001
	SA	5.226	46.60	<.0001
	FWHM	279.348	50.67	<.0001
	RSAT	160.510	22.57	<.0001
Temperature*Ripening stage*Treatment time	*IP*	8.780	1.60	0.0200
	SA	0.310	2.77	<.0001
	FWHM	14.123	2.56	<.0001
	RSAT	22.9149	3.22	<.0001

4. Conclusions

Laser-induced backscattering imaging using 660 and 785 nm wavelength lights have shown to be a good method for non-destructive detection of chilling injury in bananas. It can be concluded that:

- All parameters obtained from the backscattering profile were statistically significant subjected to temperature, ripening stage and treatment time. This means that backscattering imaging potentially could be used to detect the changes in colour and texture in bananas as chilling injury developed.
- As the backscattering parameters strongly influenced by pigment changes and textural properties, this method also potentially could be used not only for chilling injury detection but also maturity level and other fruit properties.

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