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Bioactivity evaluation and freshness identification of cantaloupe fruit (*Cucumis melo L. var. cantaloupensis*) using biospeckle method

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Abstract

Biospeckle can be used as a method for analysis the activity of biological materials illuminated with laser beam. Physically, biospeckles are the result of scattering of coherent light on moving particles inside living tissue. Therefore, the aim of this study was to investigate biospeckle activity and determine the degree of ripeness of cantaloupe fruit using observation of its dynamic speckle pattern. The speckle pattern of laser light scattered in cantaloupe fruits were measured through their quantification. For the quantification of the variation by biospeckle, two different methods of image analysis were used: moment of inertia and profiles of time history speckle pattern (THSP) which contains data of time information of dynamic speckle. Furthermore, the result of measurement of the dynamic speckle varies for fruits as their quality decrease, and the values change with the position of images.

Keywords: Speckle, bioactivity, cantaloupe, freshness

1. Introduction

Cantaloupe melons are one of the most traded melons around the world. Cantaloupe has a unique odor, appearance and taste, which are distinctive characteristics, therefore, it is more demanding among consumers (Vargas *et al.*, 2010), especially during the summer. Watermelon or cantaloupe is one of the refreshing snacks and could be eaten for prevention of dehydrate because of high water content. In addition, cantaloupe also contains the nutrients that are beneficial to our health i.e. vitamins, minerals and antioxidant compounds (Vargas *et al.*, 2010). Therefore, cantaloupe is very popular among children and adults alike groups. Freshness, specific shelf life and good quanlity of fresh cantaloupe are important for consumer buying decision. However, selection process related to fresh cantaloupe preparation may have an impact on the shelf life, eating quality and consumer acceptance. Therefore, the rapid and accuracy methods for quality, freshness and texture determination are important for evaluation in the markets.

The main important problems of cantaloupe distribution and marketing are freshness and degree of ripeness that are difficult to observe and determine with physical appearance of fruit.

Although the chemical and sensory methods are mostly used to determine these qualities, the samples have to be destroyed. Therefore, non-destructive methods for quality determination of whole fruit are beneficial and enhance the values of cantaloupe in the market. Study on harvesting and postharvest processes have been focused (Rabelo *et al.*, 2005). Moreover, the development of assessment methods for fruit quality determination based on mechanical behavior by studying the mechanical resistance of tissues such as elasticity test, compression, stress strain, impact test, continuity test, isotope and homogeneity were reported (Cornuault *et al.*, 2018, Shahbazi and Rahmati, 2014, Figueira *et al.*, 2013).

Speckle formation is a phenomenon inherent to both classical and quantum waves. Characterized by a random granular structure, a speckle pattern arises when a coherent wave undergoes a disorder-inducing scattering process. The statistical properties of a speckle pattern are generally universal-commonly referred to as Rayleigh statistics featuring a circular-gaussian distribution for the complex-field joint probability density function, and a negative-exponential intensity probability density function. Dynamic laser speckle (DLS) or Biospeckle laser (BSL) is a non-destructive technique used to monitor the biological and non-biological activities (Postnov *et al.*, 2020). These techniques can be applied to different materials with different behaviors. So the laser technique consists the study of movement patterns of speckle dynamic on bio-sample. The phenomenon occurs when the laser shines along a sample surface indicating that the certain activity is taking place (Romero *et al.*, 2009). The nature of this phenomenon is similar to that of a surface that is boiling liquid and moving dynamical or boiling point (biospeckle). Recent research have been used to identify the different point patterns of biological materials indicating the dynamic speckle correlated to the freshness properties of fruits and vegetables (Abou Nader *et al.*, 2019), which also could be used with different surface pigments (Ansari and Al-Shaeri, 2019). Therefore, the objective of this study was to assess the freshness of cantaloupe with a non-destructive method in each post-harvest period by measuring the physical activity of cantaloupe fruit surface using the speckle phenomenon.

2. Materials and Methods

2.1 Raw materials

Cantaloupes from Sa Kaeo Province, Thailand were used in this experiment. In Sa Kaeo, cantaloupe has been cultivated for 50 years and beame to a popular fruit because of its good-taste and ordor. There are two types of cantaloupe with main characteristics that are smooth and mesh surface types. In this research, the sun sweet d 25 cantaloupe (smooth surface type) was selected for experiments due to it is one of the most popular cultivars in Sa Kaeo Province. Cantaloupes were obtained from Rai DEE TOR JAI farm, Aranyaprathet District, Sa Kaeo Province, Thailand. The freshness levels were determined by monitoring its elasticity index (EI) with a non-destructive acoustic vibration method (Taniwaki *et al.*, 2010). This method is complex, highly capitalized and resource intensive. In addition, the evaluation time and cost of this biospeckle method are low, therefore, this biospeckle method can be used as an alternative method for bioactivity evaluation.

2.2 Experimental setup

In this experiment, 50 mW diode laser with a wavelength of 400-700 nm was used through a diffuser to shoot a sample using a 2560 × 1920 pixels CCD camera with a frame rate of 32 fps. Samples were recorded every 0.05 s. The samples were photographed at the terminal, end and surface areas, where the area was taken 1 time per day, 500 images per day, for 4 days. After the images were obtained, the images were then converted to gray scale. The THSP technique (Rabelo *et al.*, 2005) was used to indicate the changes or biological activity that occurred, or to indicate the freshness or ripeness of the sample. Relevant parameters are aperture size. Beam diameter and the position of the observer in the movement of the spectral point both size and position are altered, resulting in the different patterns on the sample. If the sample has a lot of biological activity, for example pigment drying the occurrence of surface traces or other related activities, the spectral points or motion will be high. On the other hand, less biological activity will produce spectral points with little motion. The experimental tool model is shown in figure 1.

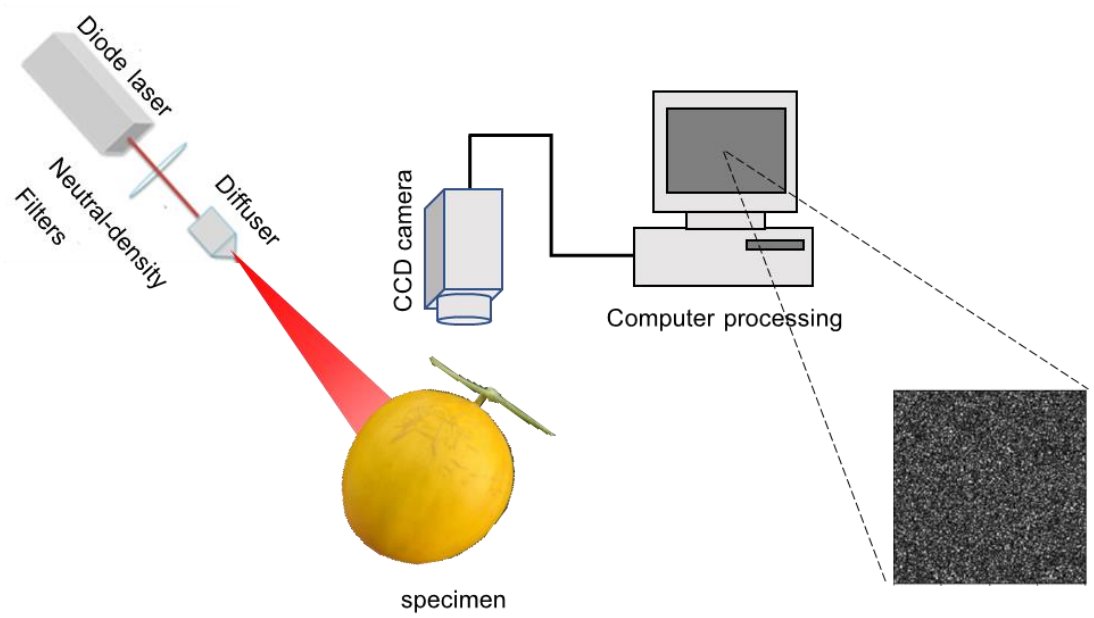


Figure 1 Experimental setup of cantaloupe biospeckle laser A, B - equator, C - peduncle insertion and D – apex

Four points were chosen over the cantaloupe surface to test their biospeckle activity. Points A and B were located in the equatorial region, point C was in the peduncle insertion, and point D was placed in the apex region of the fruit. Figure 2 shows the points over the cantaloupe surface (A, B - equatorial region; C - peduncle insertion and D - apex).

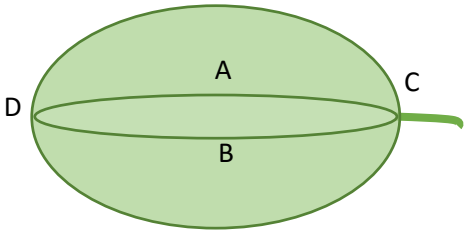


Figure 2 Schematic representation of the positions where images were taken on the surface of cantaloupe

2.3 Inertia moment (IM)

Arizaga *et al*. (1999) developed a process following the intensity and continuity changes occurring in images by selecting images at the same column at different intervals. These columns are then put together to form a THSP image. This process relies on the repeated gray scale correlation matrix analysis in the outline of the image. The resulting matrix is called as a common matrix (Co-occurrence Matrix, COM) is defined by Equation 1.

$$com = [N_{ij}] \quad (1)$$

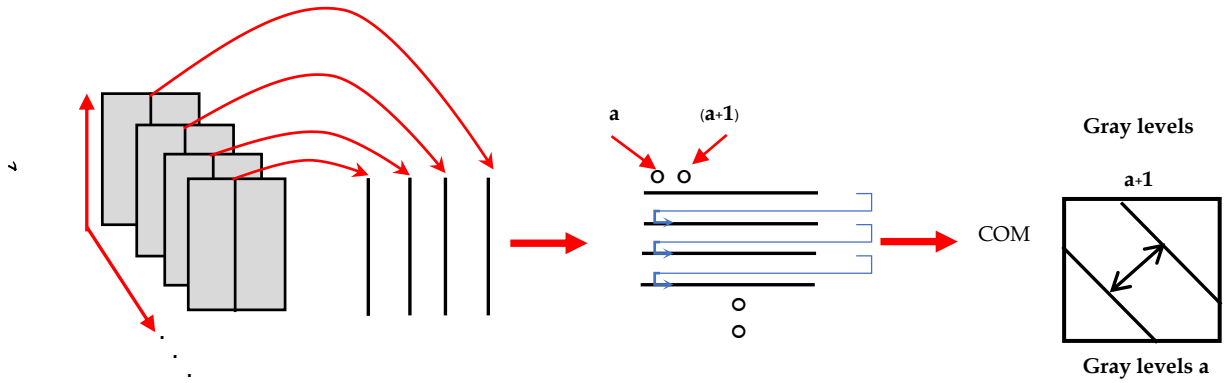


Figure 3 The data combination for the temporal history speckle pattern (THSP) and co-occurrence matrix

The entries N_{ij} of the co-occurrence matrix is the number of occurrences of a certain intensity value i followed by an intensity value j, which according to Rabelo *et al.* (2005) gray level dependence matrix, is employed to characterize the image texture. Figure 3 presents the construction of THSP and its corresponding co-occurrence matrix.

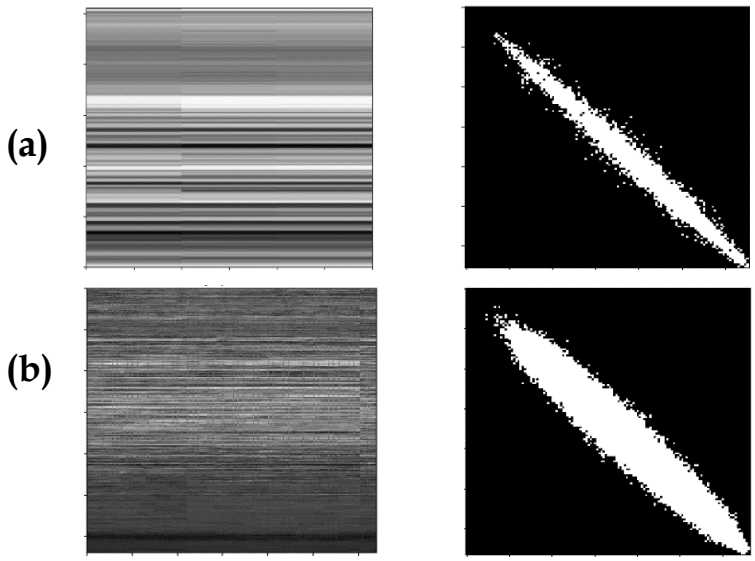


Figure 4 Results of biospeckle technique from cantaloupe sample. (a) THSP and COM values of low bioactivity, (b) THSP and COM values of high bioactivity.

COM is a useful tool for estimating the resulting biological and non-biological activities. Figure 4 shows the occurred differences. If the calculated sample images had small differences, the result was a small contradiction distribution of the matrix values. The diagonal data was very valuable due to the amount of data distribution could be used to measure the biological activity taking place on a sample. The measure of the spread of a matrix around the diagonal was the sum of the matrix values multiplied by the square's row spacing. The result showed that the matrix moment of inertia (IM) was calculated using equation 2, which could be applied for another way to measure the activity occurring in a biological sample.

$$IM = \sum_{ij} M_{ij} (i - j)^2 \quad (2)$$

$$\text{Where , } M_{ij} = \frac{N_{ij}}{\sum_j N_{ij}} \quad (3)$$

3. Results and Discussion

3.1 Spatial-Temporal speckle cross-correlation analysis

The correlation can analyse two or more speckle patterns, where one was considered as an image of a reference state. The reference pattern and the patterns of subsequent object states were separated into an equal number of regions and then cross-correlation of each pair of fragments were calculated. The calculation of the cross-correlation coefficients for a series of speckle pattern sub-images write in the given temporal order permit the temporal dependencies of these coefficients obtained the functions of the biospeckle pattern movement (Zdunek *et al.*, 2007). The analysis, biospeckle activity (BA) was evaluated using a correlation $C^{k\tau}$ coefficient where k is the frame number and τ is the delay time (1/0.7 s) (Zdunek *et al.*, 2007). Matrix data consist of the pixel intensity of the first frame containing the following frame data matrix from the biospeckle data collection. In this study, C was analyzed by using the correlation coefficient between the first frame and frame at kτ = 0.3 s. and 0.7 s. So, BA = 1-$C^{0.3}$ was a parameter that showed the biospeckle activity for the sample. The high biospeckle activity corresponded to the high 1-$C^{0.3}$ value. The correlation coefficient C was calculated using module in the anaconda software, python language. At the first frame, kτ = 0, so, C = 1, when the speckle pattern did not show the temporal fluctuation on τ time i.e. k = 0 = constant and $C^{k\tau}$ = 1. The value of $C^{k\tau}$ decreased rapidly below constant value 1 when the speckle pattern changes in time, as shown in Figure 5. Hence, the biospeckle activity BA was a C value when speckle showed no temporal change of C. For temporal change over τ, bioactivity could be determined as follows

$$BA = 1 - C^{k\tau} \quad (4)$$

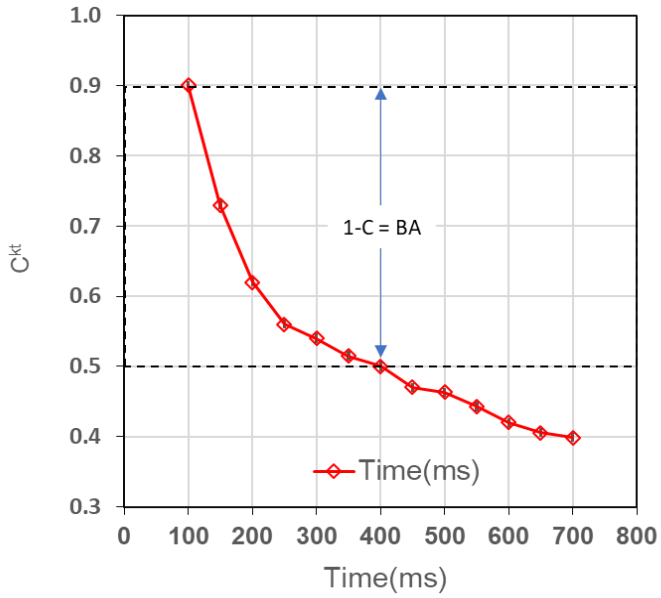
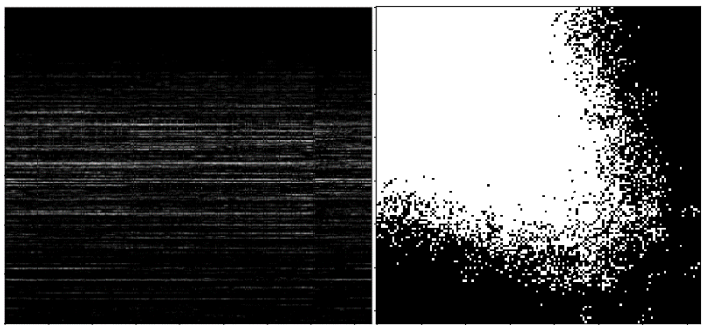
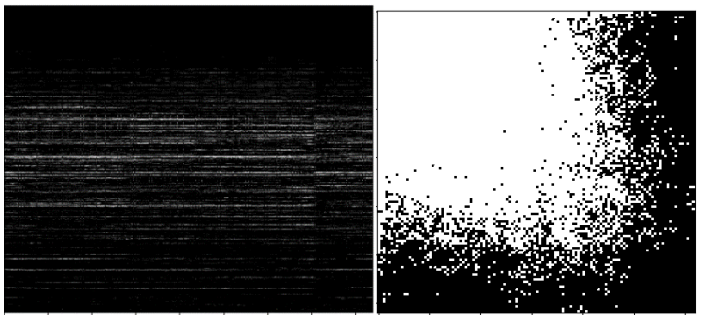


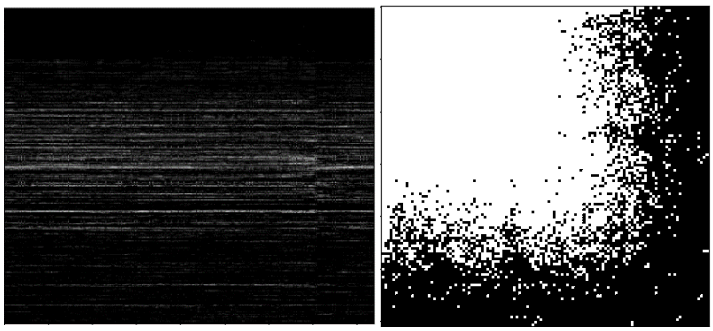
Figure 5 Calculation of biospeckle activity (BA)



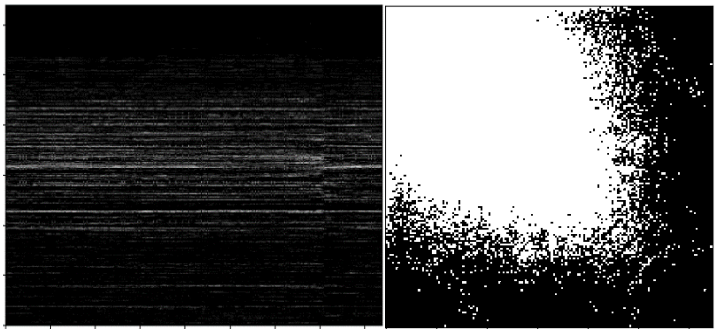
(a)



(b)



(c)



(d)

Figure 6 THSP and MCOM for images taken on positions: (a),(b) – equator, (c) -peduncle insertion and (d) - apex (two days)

Table 1 the average values of inertia moments

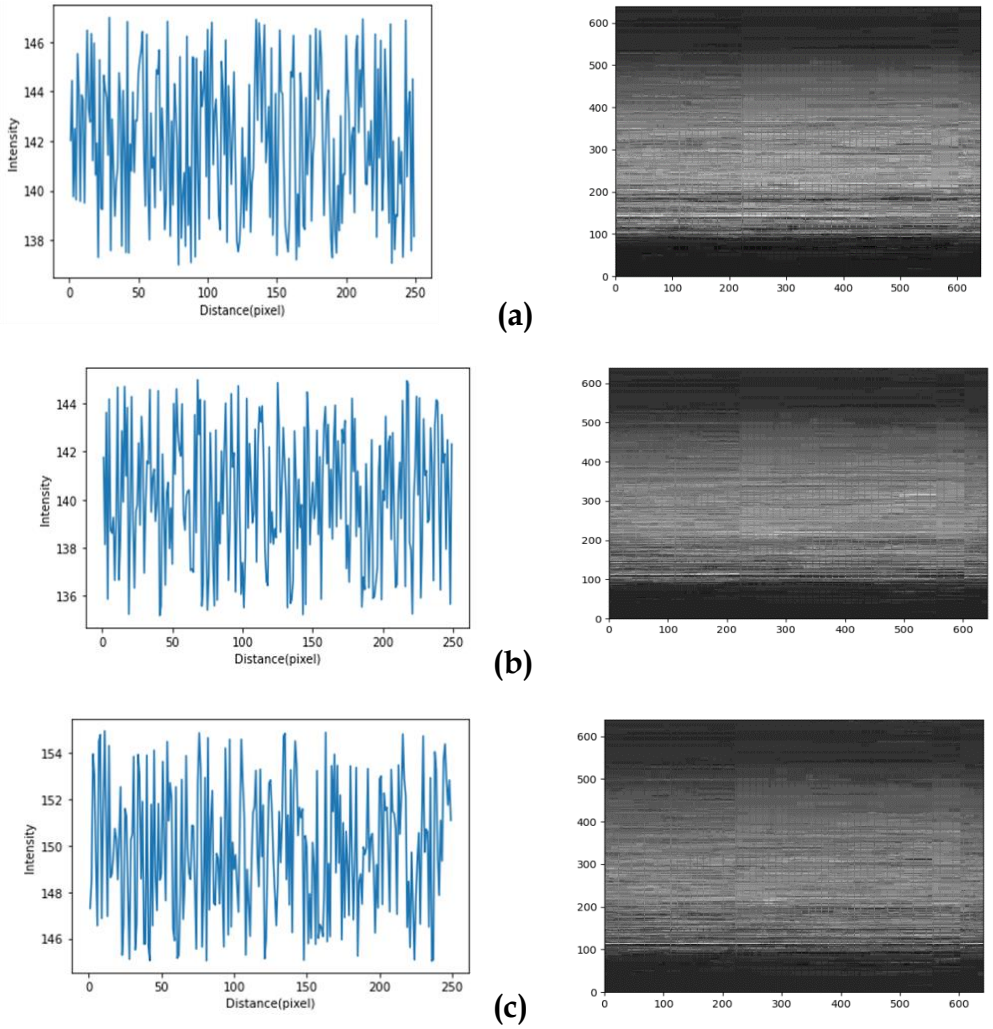
Day of storage		Point of illumination of the cantaloupe			
	Inertia moment values	A	B	C	D
1	Mean	2110.38	2080.65	2892.64	2471.14
	SD	196.7	180.36	258.21	208.21
	SE	40.2	35.32	57.48	47.48
2	Mean	2002.52	1820.63	2341.78	2182.78
	SD	110.36	84.68	126.57	117.57
	SE	33.2	26.51	40.89	34.89
5	Mean	1001.25	988.87	1280.54	1010.54
	SD	67.54	61.58	82.7	70.7
	SE	17.6	16.8	21.41	18.45
8	Mean	457.21	584.89	596.78	566.45
	SD	42.45	51.25	53.98	48.24
	SE	9.64	10.12	12.1	10.01

Figure 6 shows the daily variation of IM values for one specimen, where it is possible to compare the evolution of the process by IM. IM approach presents the ability to separate fresh highly active and less active fruits. Inertia Moment (IM) values were estimated at different points of illuminations (A, B – equator, C – peduncle insertion and D – apex) on Calculation surface (during different days of storage). As the calculation ages, IM values was decreased as shown in Table 1. Point C presents high activity where the peduncle is inserted. The IM values for points A and B did not show significant differences. Therefore, the points C and D were the points that allowed identifying the activity differences between the maturation stages of the fruit. When the cantaloupe was fresh at 2nd day of storage, the $C^{k\tau}$ value decreased rapidly and the decay was more rapid at the insertion region C. The insertion point C presented relatively high biospeckle activity (BA) as calculated in Table 2 for one specimen.

Table 2 Biospeckle activity (BA) calculation at different points of illumination

Point of illumination	BA at 0.3 s	BA at 0.7 s
A	0.3747	0.5460
B	0.3540	0.4674
C	0.5919	0.6425
D	0.5136	0.5914

The experiments were shown that the biospeckle laser technique was an ideal method for analyzing the biological processes occurring during the freshness of cantaloupe. The biospeckle activity measurement was varied according to the post-harvest period. It is also a good opportunity to obtain useful information from the THSP method of the fruit by comparing how the frequency generating the signal varies over time, considering the practical application of the biospeckle method. The biological activity evaluation of the fruit using the moment of inertia method and the correlation coefficient showed the significant results. These results indicated that this non-destructive test was effective in predicting the shelf life of fruits and vegetables. This method may be used as a new commercial technique for a fruit quality assessment.



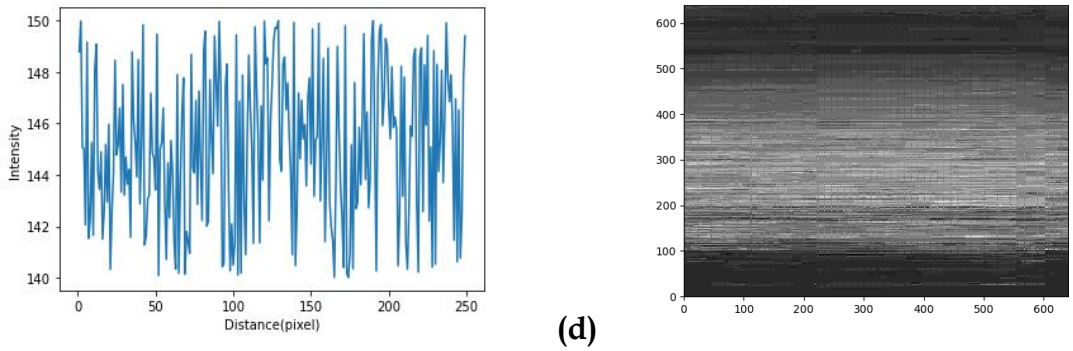


Figure 7 The graphic of THSP at different points of illumination and respective line profiles (gray level) with different IM values. Note the major influence of high frequencies on IM values: (a) IM = 2098, (b) IM = 1898.46, (c) IM = 2305.85, and (d) IM = 2188.89

From Figure 7, THSP, which showed different intensity patterns at each point of illumination. Equation 2 stated that the main data in IM calculations was the instantaneous difference between two pixels. Therefore, if the time history showed low-frequency characteristics, then the low-frequency behavior curve modulation was not shown in the IM calculations. Therefore, the results of the IM could be directly related to the respective THSP image profiles. Three different activities were involved with a contour pattern of the THSP imagery. The fast pattern was responsible for increasing the value of the IM as an example, with the respective IM values showing the main influence of the high frequency on the IM value (Figure 7).

4. Conclusion

The development of the THSP technique allowed identifying the activity of the differences that occurred during the freshness periods of the fruit. The IM method offers both active and less active fruit sorting capabilities. Influence of the taller, the change in activity is expressed suddenly. The rapid variation of the THSP pixel is an example increment of the IM value, with the respective IM values indicating the influence of high frequencies on the IM value. The inertia and correlation coefficients were decreased when increasing of the post-harvest period of cantaloupe melon. Therefore, the measurement of the dynamic point can be varied for the different ripen stages of fruits. The prominent profile line of the THSP, which contains the time information of the dynamic point from the biospeckle activity, it can show the moment of inertia value depending on the frequency of the line detail of the THSP. A fast form of signal change, profile line was responsible for increasing the value of IM. Therefore, measuring the dynamic point change with illumination to the fruit can represent the complex and dynamic environments. Depending on the different points of illumination, it represents different environments.

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