Assessment of fruits during shelf-life storage using biospeckle laser

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Abstract: Biospeckle is a phenomenon that interprets an optical pattern formed by illuminating a surface under changes with coherent light. These patterns are usually analyzed by numerical as well as graphical methods. Present work evaluated the application of different numerical methods to analyze biospeckle for the assessment of fresh mature fruits. Quantitative first and second order moments namely Inertia moment (IM) and Absolute Values of Differences (AVD), based on the recording of time history of speckle pattern were used. Results showed that during shelf-life storage of ten days of different fruits, the IM and AVD values were found to decrease. In addition, to assess the impact of ripening of fruits on their optical properties, speckle grain size was measured and the variation of its dimensions evaluated. This implied that speckle grain size evaluation could be a non-destructive evaluation of their maturity.

Keywords: biospeckle, fruit, speckle grain, inertia moment, absolute value of the differences

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1 Introduction

The use of non-invasive and non-destructive methods to follow the ripening of fruits and vegetables is a subject of interest in agriculture. Research have been done to follow the ripening of climacteric fruits when they ripen off tree and eventually to predict the optimum storage life (Nassif R. et al. 2012). Several methods are currently developed and used. Some are destructive, like the measurement of the sugar content or the use of the penetrometer (Harker et al. 1996) and some are not, such as acoustic method and infrared spectrometry (Maurizio et al. 1998).

Biospeckle is a novel non-invasive method recently applied to this field. Speckle is a phenomenon produced by laser illumination of a medium, resulting from the temporally non-stationary interference of light scattered by diffusing objects. This method proved its ability to monitor fruits ripening and to assess bruising in fruits using correlation between images (Zdunek et al. 2007), and Time History of a Speckle Pattern method (THSP) (Rabelo et al. 2005) or the Weighted Generalized Differences method (WGD) (Pajuelo et al. 2003). Scientists have also tested a non-destructive and non-invasive optical method to analyse fresh fruits as well as fresh cut fruits using (Alvarenga et al. 2013).

The apparent activity of biospeckles is the result of physical movement of particles inside cells and affected by the variation of the absorption of light by tissue pigments. Therefore, the activity of biospeckles can provide information about various living processes occurring inside a cell. Moreover, the method is considered to be a non-destructive one because no visible results of tissue interaction with light have been reported up to now when low power lasers have been used. A typical setup for biospeckle measurement is very simple (Figure 2). It requires an expanded laser light, which might be a He-Ne laser, a detector such as a Charge Coupled Device camera with a lens and a PC with a frame grabber that is able to record a set of images with a constant time lag. From the time series images, the biospeckle activity is estimated using various mathematical methods. These features make this method attractive to many applications, which require fast and non-destructive sampling.

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Fruits, even hard peel ones, are naturally active materials and show speckle activity (Oulamara et al. 1989). The use of speckle activity measurements for the determination of shelf life and maturity has already been proposed (Bergkvist et al. 1997). In addition, the drying of other botanical specimens was assessed using these techniques (Rabal et al. 1996).

In the case of plant tissue, biospeckle fluctuations are usually not a result of well-defined fluxes of matter, such as those observed during blood flow. Therefore these case studies were carried out to show the correlation between biospeckle activity and physiological processes.

This paper reports this non-destructive tests based on laser techniques, which have been studied for the assessment of fruits and vegetables at matured stage. The laser technique consists in studying the temporal variations of the dynamic speckle on different fruits namely apple, pear and tomato. Recent researches based on the identification of the speckle pattern for different biological materials indicate the application of the dynamical speckle in correlation with mechanical properties, seed viability, staining, drying, etc. Despite the complexity of the phenomenon, some approaches have been developed to quantify the temporal variation of the speckle pattern to characterize biological changes. In order to quantify temporal variations of the biospeckle, two approaches have been employed: (a) the first one, based on the calculation of the moment of inertia of the co-occurrence matrix of the temporal history of speckle pattern (THSP) and (b) the second one, based on the calculation of the AVD the co-occurrence matrix of the THSP. The construction of THSP was proposed by Xu et al. 1995.

Further, we considered fruits as scattering media presenting variable sizes of scatterers along maturation. Thus, our issue is to characterize these diffusers, their relative size and their variation on the speckle image. This approach allows us to link speckle image variation and diffusion properties evolution to fruits inner physiology.

2 Materials and methods

2.1 Dynamic speckle patterns

Dynamic speckle pattern also known as biospeckle is a phenomenon that interprets an optical pattern formed by illuminating a surface under changes with coherent light. Therefore, the dynamic change of the speckle patterns caused by biological material is also known as biospeckle.

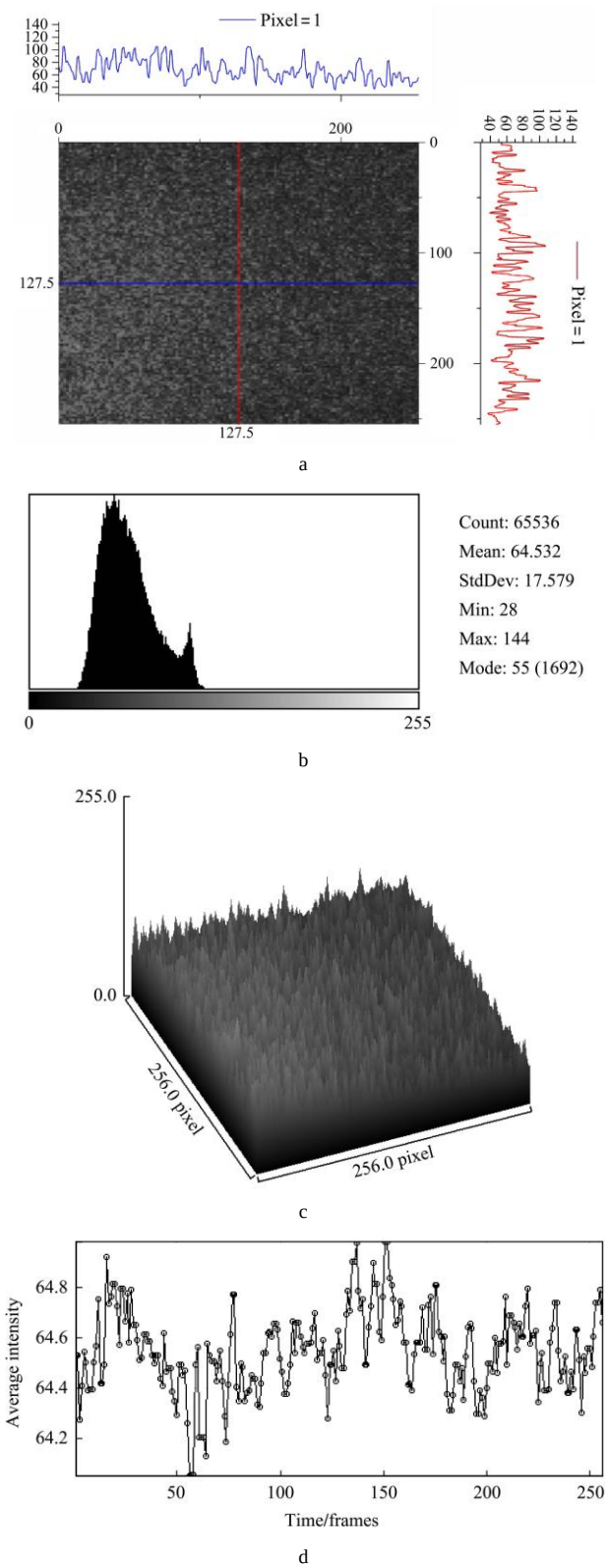

Figure 1 Characteristics of (a) horizontal and vertical profile,(b) thehistogram, (c) surface profileofspeckle pattern and (d) Mean Intensity profilefrom abiospeckle movie over 250 seconds

Figure 1 shows (a) a biospeckle pattern along with horizontal and vertical profiles, (b) the histogram, (c) surface profile of speckle pattern and (d) Average Intensity over 250 seconds of the dynamic speckle scattered from the fruits surface.

2.2 Inertia Moment based on THSP

The time history of speckle patterns (THSP) was proposed to analyze the dynamic speckle patterns generated from active media. If each speckle pattern size is 256×256 pixel, a new image is composed by setting side by side, the chosen column of the first image, the same column of the second image, and so on, 256 times. As a result, a new 256×256 pixel composite image is then constructed, namely, the THSP. Its rows represent different points on the object and the columns their intensity state in every sampled instant.

When a phenomenon shows low activity, time variations of the speckle pattern are slow and the THSP shows a horizontally elongated shape. Conversely, when the phenomenon is very active, the THSP shows fast intensity variations that resembled an ordinary spatial speckle pattern.

The THSP images could be analyzed using the inertia moment (IM) of the gray level co-occurrence matrix (GLCM), which is defined as (Arizaga et al. 1999):

$$P(i, j) = [N_{ij}] \tag{1}$$

The entries are the number N of occurrences of a certain intensity value i that is immediately followed by an intensity value j. In the spatial case, its principal diagonal is related to homogeneous regions and the nonzero elements far from it represent high contrast occurrences. As the sample shows activity, intensity values change in time and the number N outside the diagonal increases and the matrix resembles a cloud.

For normalization purposes, it is convenient to divide each row of this matrix by the number of times that the first gray level appeared. There are $2(N–1)$ points horizontally adjacent in each row of the GLCM, and by multiplying them by the number of rows N we obtain the total number of the adjacent points of $2N(N–1)$ in the GLCM. Finally, the normalized matrix of GLCM can be written as,

$$p_{ij} = \frac{P(i, j)}{2N(N-1)} \tag{2}$$

Then, using the inertia moment (IM) of the matrix with respect to that diagonal in the row direction, the spread of the GLCM is measured, and the calculated equation is given as,

$$IM = \sum_i \sum_j (i - j)^2 p_{ij} \tag{3}$$

The occurrences in the diagonal do not contribute to increases in the IM-value while far away p entries add their more heavily weighted values.

2.3 Absolute value of difference (AVD)

The AVD is a first order statistic moment. It was proposed as an alternative to the routine IM method (Braga et al. 2011, Ansari & Nirala 2013). This new approach calculates of the absolute value of the difference from the principal diagonal. Analysis carried out by the AVD showed better results in some cases of biospeckle activity, especially when the THSP matrix contained no data at high frequencies. It is based on the principle that the summation of differences is the main information searched (Braga et al. 2011):

$$IM = \sum_i \sum_j (i - j) \mid p_{ij} \mid \tag{4}$$

2.4 Speckle grain size

Fruits are complex environment presenting different molecular size distributions depending on the maturation level. Green fruits are rich in starch chains $(C_6H_{12}O_5)_n$ which are relatively large (0.1 to 200 microns), as well as pectin, organic acids and amino acids. During fruit maturation process, starch and amino acids are hydrolyzed to carbohydrates such as glucose $C_6H_{12}O_6$ (Cordenunsi & Lajolo, 1995). Unlike starch, these are small molecules, of the order of few nm. Any modification of the fruit inner constituents affects its optical properties, specially the absorption and the scattering coefficients. Thus, one can monitor different fruit maturation stages using speckle images and parameters, namely the speckle grain size.

The speckle grain size is estimated using the normalized autocovariance function of the speckle intensity pattern $I(x, y)$ obtained in the observation plane (x, y). This function corresponds to the normalized autocorrelation function of the intensity. It has a zero base and its width provides a reasonable measurement of the average width of a speckle grain. If $I(x_1, y_1)$ and

$I(x_2, y_2)$ are the intensities of two points in the observation plane (x, y), the intensity autocorrelation function is defined by Equation (5) (Goodman 1984):

$$R_I(\Delta x, \Delta y) = \langle I(x_1, y_1) I(x_2, y_2) \rangle \quad (5)$$

where, $\Delta x = x_1 - x_2$ & $\Delta y = y_1 - y_2$. $\langle\ \rangle$ Corresponds to a spatial average. If x_2=0, y_2=0, x_1=x & y_1=y, we can write:

$$R_I(\Delta x, \Delta y) = R_I(x, y) \quad (6)$$

The normalized autocovariance function of the intensity, $c_I(x, y)$, is given by Eq. (7):

$$c_I(x, y) = \frac{R_I(x, y) - \langle I(x, y) \rangle^2}{\langle I(x, y)^2 \rangle - \langle I(x, y) \rangle^2} \quad (7)$$

As implied by the Wiener-Khintchine theorem, the autocorrelation function of the intensity is given by the inverse Fourier transform (FT^{-1}) of the power spectral density (PSD) of the intensity:

$$PSD_I(v_x, v_y) = |FT[I(x, y)]|^2 \quad (8)$$

FT is the Fourier transform.

$c_I(x, y)$ calculated from the intensity distribution measured of the speckle is:

$$c_I(x, y) = \frac{FT^{-1}[|\, FT[I(x, y)]\,|^2] - \langle I(x, y) \rangle^2}{\langle I(x, y)^2 \rangle - \langle I(x, y) \rangle^2} \quad (9)$$

$c_I(x, 0)$ and $c_I(0, y)$ are the horizontal and the vertical profile of $c_I(x, y)$, respectively. Let us term dx the width of $c_I(x, 0)$ so that $c_I(dx/2, 0)$=0.5 and dy the width of $c_I(0, y)$ such as $c_I(0, dy/2)$=0.5.

2.5 Samples

Fresh matured fruits were brought from a local supplier and sorted so that they are as homogeneous as possible and almost identical in terms of mass and maturity. Fruits at different maturation stages were taken as follows:

(a) Apple- mature radish-blue stage

(b) Pear- mature yellow stage

(c) Tomato- mature red stage

They were stored for 10 days at normal room temperature and humidity. Three selected fruits apple, pear and tomato were studied by biospeckle measurements once per day. Every day, five samples from each fruit (apple, pear and tomato) were sacrificed for speckle measurements. Each fruit was placed in a fixed support so that acquisitions can be repeatable and the same area of the fruit targeted from the same angle, day after day. We have tested biospeckle laser to measure the physiological activity in fruits stored at room temperature for 10 days. The images were analyzed by the calculation of inertia moment (IM) and AVD to quantify the physiological activities of illuminated samples.

2.6 Experimental configuration

All the experiments were developed in Biomedical optics Laboratory of Indian School Mines, Department of Applied Physics, Dhanbad, India.

Biospeckle measurement device consists of a low power He-Ne laser (2 mW; 632.8 nm wavelength), with a microscopic objective (10x) as the beam expander to illuminate the sample, a digital CMOS camera (PixeLink, Canada) and a personal computer with an image processor. The camera-object distance was maintained at a suitable distance to obtain the uncompressed images (TIF, 8 bit). The speckle pixel size was larger compared to each pixel dimensions 4.5 μm × 4.5 μm of the camera. Gain and brightness of the camera were optimized to avoid overexposed pixels on an image histogram. The image acquisition settings were kept unchanged during the whole experiment. The acquired images (256 × 256 pixels) were digitalized to 8 bits (256 gray levels) and stored in the memory of the computer and the problem to be solved was the isolation of vibration.

Successive 256 biospeckle images of the fruits were taken at an interval of 1 sec to find the IM and AVD values. For the co-occurrence matrix analysis, a column of the subjective speckle pattern was read every second and then, a composite image of 256 by 256 pixels was formed by stacking consecutive columns.

Finally, this image was retrieved and respectively the first and second-order moments of its co-occurrence matrix were calculated.

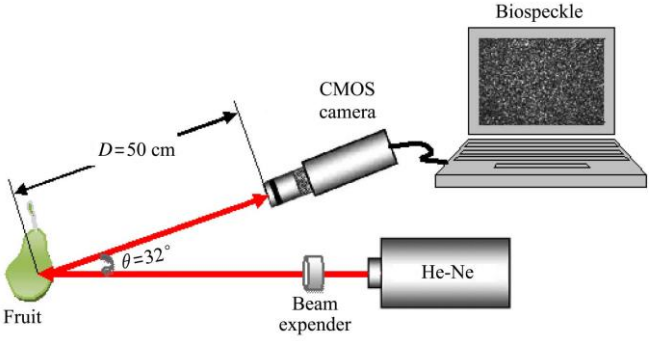

Figure 2 Schematic arrangement of data acquisition system

3 Results and discussions

3.1 Inertia Moment based on THSP calculation

As shown in Figure 3, Inertia moment values of the fruits were found to decrease over time. This reflected diminishing activity of the fruits during storage period. The same phenomena have been quantified using the AVD calculation as shown in Figure 4. We therefore show the efficiency of biospeckle technique for monitoring ripening of fruit.

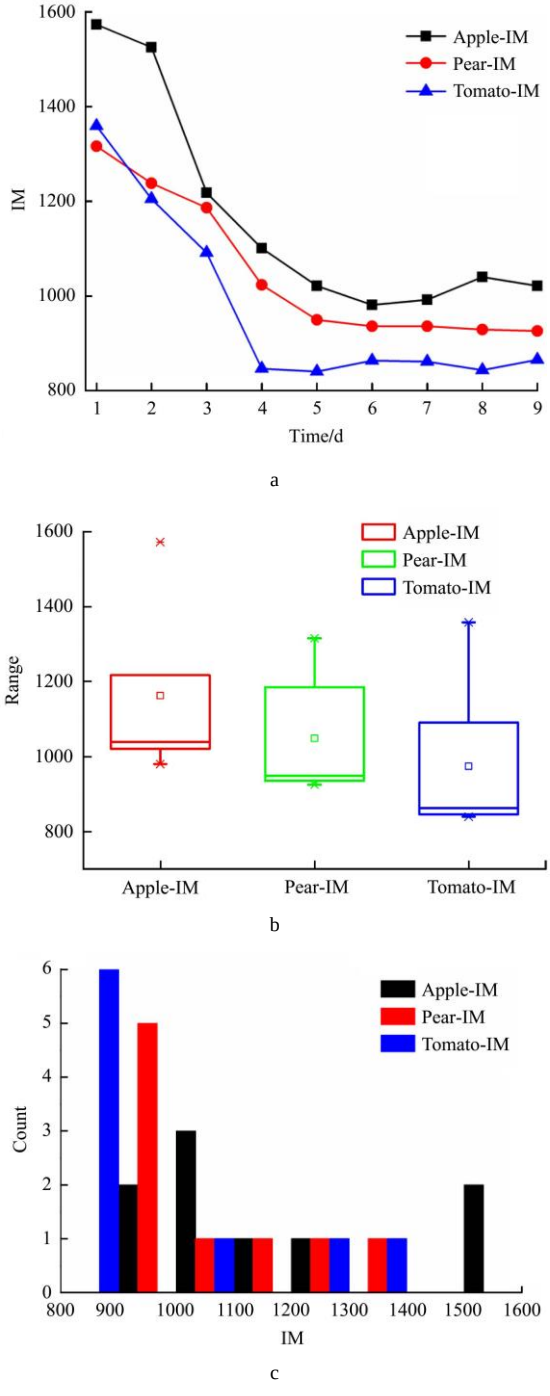

Figure 3 (a) Temporal variation of IM, (b) Range of IM of the three fruits, (c) Histogram of IM over 10 days

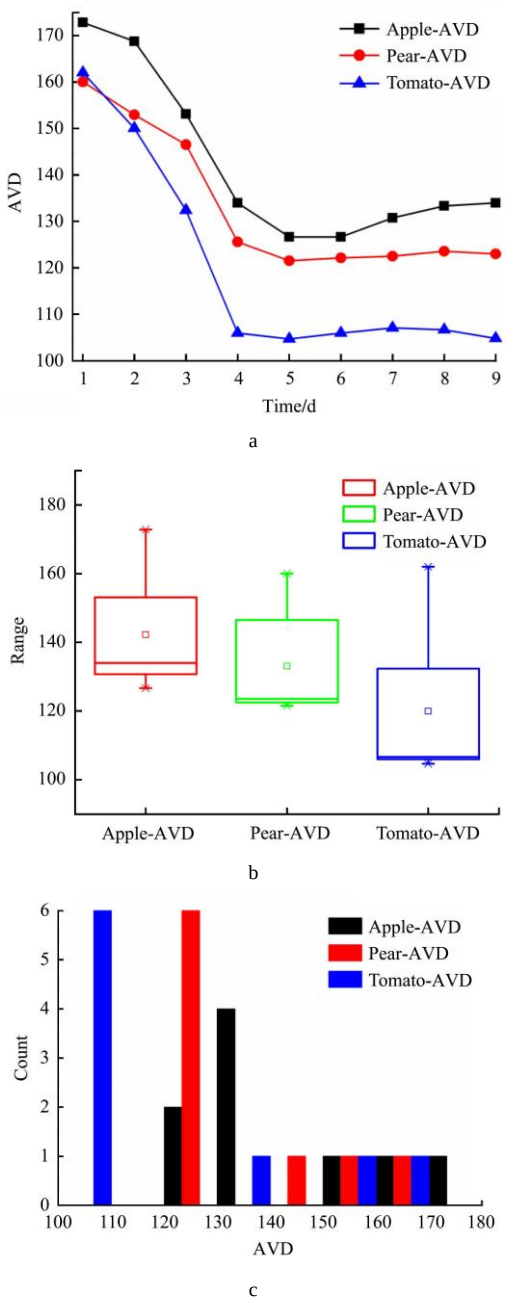

Figure 4 (a) Temporal variation of AVD, (b) Range of AVD of the fruits and (c) Histogram over 10 days

3.2 AVD based on THSP calculation

3.3 Speckle grain size

3.3.1 Speckle size as per FWHM calculation

To assess the impact of ripening on their optical properties, speckle grain size was measured and the variation of its dimensions has been evaluated. Speckle grain size have been calculated using the widths at which the Gaussians fits to two arrays generated by the sums of the normalized auto-covariance of 1) all rows in the image and 2) all columns in the image fall to values of .5 and $1/e^2$.

In Figure 5 evolution of the speckle grain as a

function of shelf-life days are presented. Note the continuous decreasing in their size.

3.3.2 Speckle size as per $1/e^2$ calculation

In the case of apple and pear, average speckle size of 'dy' is comparatively bigger than that of horizontal speckle size of 'dx'.

Next the decrease in the size of speckle grain is considered related to the illuminated volume. It has been reported that decrease in speckle grain size is related to the degradation of chlorophyll (Nassif R.et al. 2012) during fruit ripening. Thereby it is resulting in a decrease in the absorption. When absorption decreases, the illuminated spot size on the fruit will increase. In other terms, the illuminated volume becomes greater and the size of speckle grains, which is inversely proportional to the diffusion spot, will therefore, be smaller.

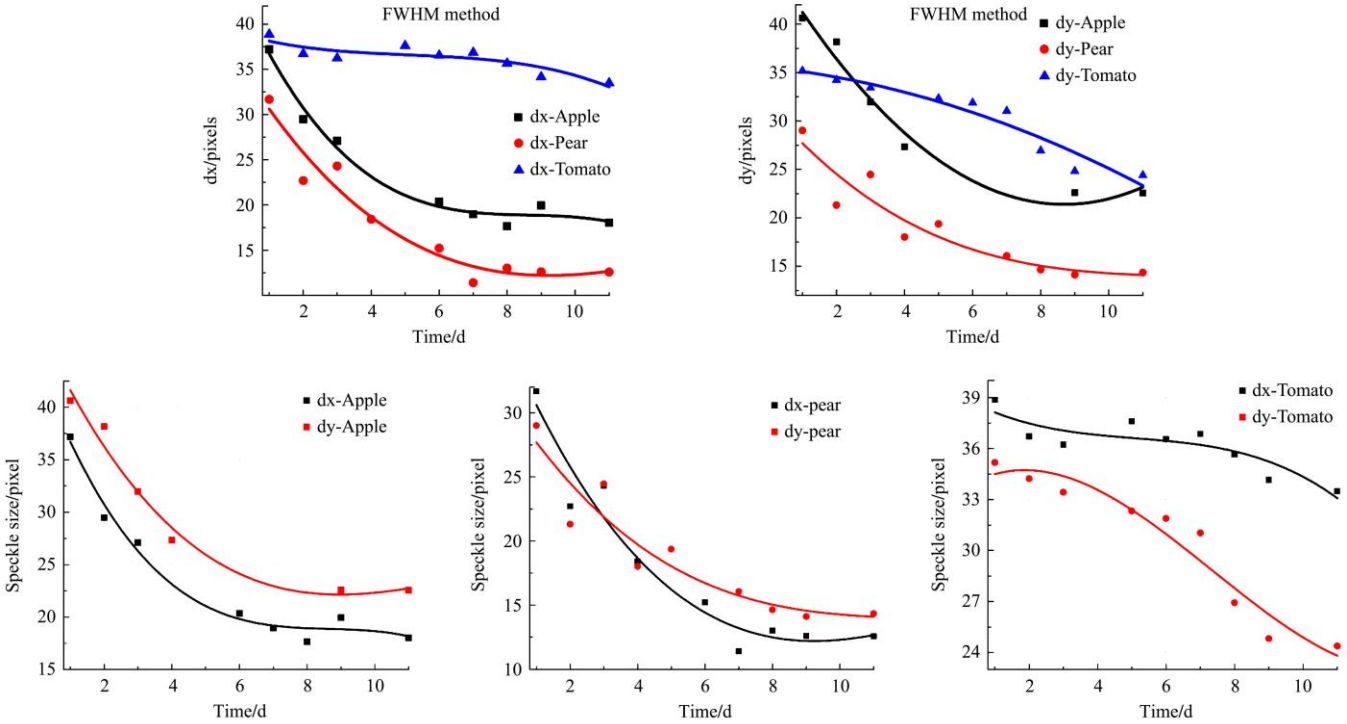

Figure 5 Evolution of the horizontal and vertical speckle grain size as per FWHM calculation during storage

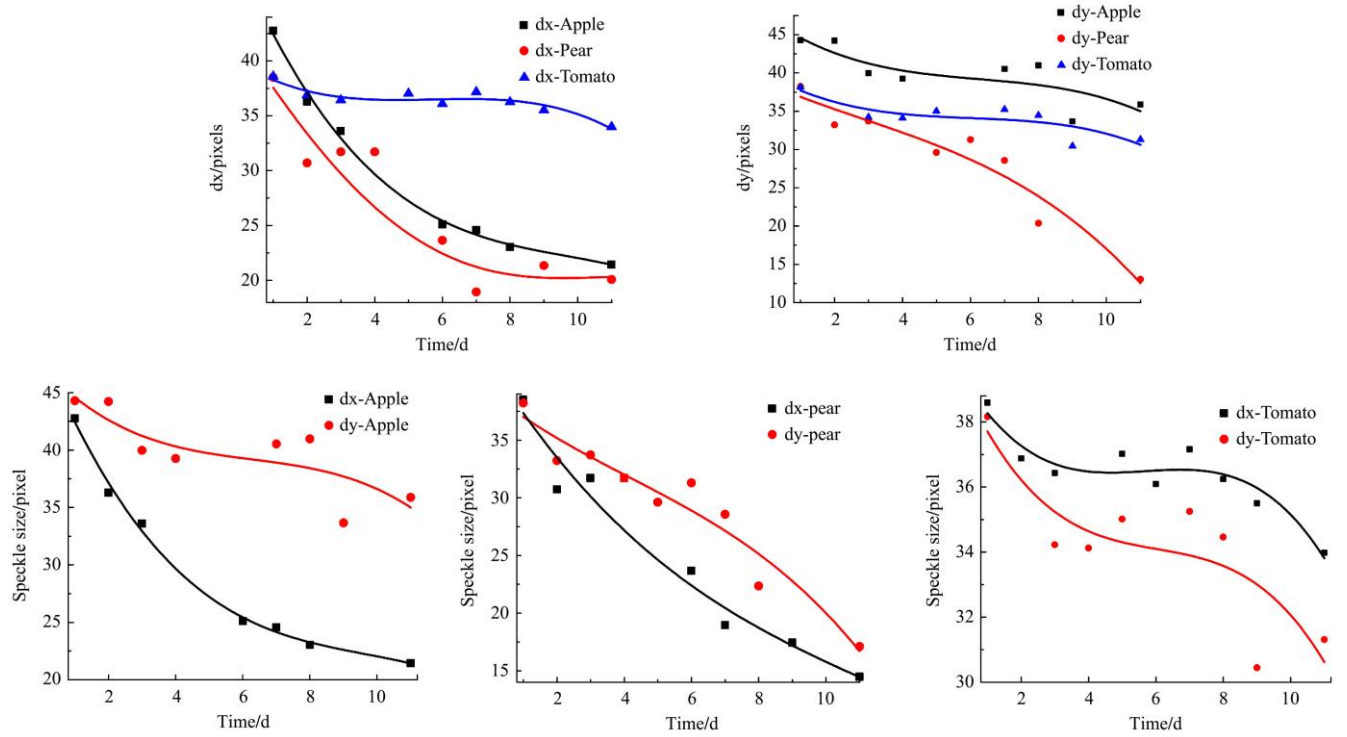

Figure 6 Evolution of horizontal and vertical speckle grain size as per $1/e^2$ calculation during storage of different fruits

4 Conclusions

1) Moment of inertia and AVD values decrease as the post-harvest period of the fruits increases.

2) The measure of the dynamic speckle varies for fruits at different stages of ripening.

3) Speckle grain size decrease during shelf-life storage of fruits. Speckle grain size evaluation could thus be a non-destructive evaluation of their maturities.

4) The temporal speckle evaluation among different commodities, i.e., an apple, a pear and a tomato are different.

5) Aging and decrease in vitality causes a decrease in biospeckle activity.

6) IM and AVD calculations can be used as a parameter to indicate maturation stages of fruits and vegetables.

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