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Biospeckle Techniques in Quality Evaluation of Indian Fruits

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***Abstract*—**In this study spatial-temporal speckle correlation techniques have been applied for the quality evaluation of three different Indian fruits namely apple, pear and tomato for the first time. The method is based on the analysis of variations of laser light scattered from biological samples. The results showed that cross-correlation coefficients of biospeckle patterns change subject to their freshness and the storage conditions. The biospeckle activity was determined by means of the cross-correlation functions of the intensity fluctuations. Significant changes in biospeckle activity were observed during their shelf lives. From the study, it is found that the biospeckle activity decreases with the shelf-life storage time. Further it has been shown that biospeckle activity changes according to their respiration rates.

***Keywords*—**Biospeckle, cross-correlation, respiration, shelf-life.

I. Introduction

BIOSPECKLE is an optical technique for nondestructive evaluation of biological materials. In this method, coherent laser light illuminates an object of interest. The backscattered light interferes and a speckle pattern is created in an observation plane. If the sample does not show activity, the speckle pattern is stable in time. However in the case of biological samples, the speckle pattern consists of two components: the static one from stationary elements of the tissue and the variable one from moving particles of the tissue. The variable in time speckle pattern is characteristic for biological tissue and has been called as the biospeckle.

The observation of biospeckles indicates that they generally fluctuate in a space time fashion. This is due to the complicated structure and activity of living objects. Thus, the dynamic behavior of biospeckles should be analyzed statistically. Although the accurate mathematical description of different dynamic speckle patterns is very difficult, a statistical analysis can be established. Some statistical approaches have been taken for the intensity fluctuation of biospeckles, which is being discussed in the present work.

The senescence process of fruits depends on the postharvest time, and because the biological activity changes as the postharvest time increases, one can expect that the biospeckle follows the biological variations. The monitoring of such a feature using dynamic laser speckle is being reported in this work. There is need to evaluate fruits quality at different stages of pre and post harvest technology in order to provide product of the best quality to consumers [1]. Recently, a few interesting optical techniques and devices have been developed and successfully used for nondestructive evaluation of fruit and vegetables: Vis/NIR spectrophotometry [2], time-resolved reflectance spectroscopy [3], hyperspectral backscattering imaging [4]-[5], laser-induced light backscattering [6,7] or chlorophyll fluorescence [8]-[10]. Nikolai et al. [11] have reviewed most of the above techniques, collectively naming them NIR spectroscopy.

Next Bragga et al. [12] have shown that processes related with movement of the scattering centers in the tissue, such as cytoplasmic streaming, organelle movement, cell growth and division during fruits maturation and biochemical reactions are responsible for a certain biospeckle activity. Brownian motions should be considered as a source of biospeckle activity too.

It has also been shown that biospeckle activity changes with an age or with some surface properties, for example an infection of a biological object. It is also found that biospeckle activity changes with water [13], chlorophyll and starch contents [14]-[15]. Less chlorophyll content causes higher apparent biospeckle activity [15] due to light absorption by this pigment and in consequence shallower light penetration through a tissue.

Biospeckles carry useful information about the biological and physiological activity of biological samples. With the extended use of laser technology in the medical and biologic fields, the applications of biospeckle techniques have been increasing in the last years, for example, measurements of blood flow in blood vessels [16], viability of seeds [17]-[18], activity of parasites in living tissues [19]-[20], analysis of maturation and bruising of fruits and vegetables [21]-[22]. These studies showed that decaying of a tissue conditions caused by age, illness/infection or damage, relates with lower biospeckle activity.

The overall objective of this work is to study the biospeckle phenomenon as a measuring procedure of different Indian fruits quality as it changes during the post-harvest period.

II. Theory

Digital speckle correlation (DSC) technique is a measuring process based on the correlation analysis of a reference speckle pattern of the specimen in its initial state with sequential speckle patterns while changing the surface or subsurface of the specimen [23].

In order to obtain the temporal dependencies of biospeckle pattern movement speed, each pattern is separated on M by N

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sub images and each m,nth sub image is correlated with the respective subimage belonging to any other pattern of the same studied area. The cross-correlation function of respective fragment pairs with identical indexing can be expressed as [24]:.

$$C_{m,n}(k,l)=\frac{1}{IJ}\sum_{i}^{I}\sum_{j}^{J} r_{m,n}(i,j)s_{m,n}(i+k,j+l) \quad (1)$$

where $r_{m,n}$ is the m,nth fragment of the pattern 1, $s_{m,n}$ is the m,nth fragment of the pattern 2, m = 1,..., M and n = 1,..., N are the numbers of fragments, i = 1,..., I and j = 1,..., J are the numbers of fragment pixels, k = 1,..., K and l = 1,..., L are the discrete samples of the cross-correlation function.

To obtain the temporal dependencies of biospeckle pattern movement speed, each pattern was separated on M by N subimages and each m,nth subimage was correlated with respective subimage belonging to any other pattern of the same studied area. As a result, cross-correlation coefficients were obtained using the equation:

$$C_{m,n}^{k\tau}=\left|\frac{\left\langle\left(S_{i,j}^{t_0}-\left\langle S_{i,j}^{t_0}\right\rangle\right)\left(S_{i,j}^{t_0+k\tau}-\left\langle S_{i,j}^{t_0+k\tau}\right\rangle\right)\right\rangle}{\sigma_{i,j}^{t_0}\sigma_{i,j}^{t_0+k\tau}}\right| \quad (2)$$

where i, j is the pixel number in the m,nth sub image of the digital biospeckle pattern, i = 1,…, I, j = 1,…, J, m = 1,…, M, n = 1,…, N, $S_{i,j}$ is the i, jth pixel intensity, k is the number of biospeckle patterns, τ is the interval between two adjacent frames containing recorded biospeckle patterns, $\sigma_{i,j}=\sqrt{\left\langle\left(S_{i,j}-\left\langle S_{i,j}\right\rangle\right)^2\right\rangle}$ is the variance. Calculation of the cross-correlation coefficients for series of speckle pattern's subimages recorded in the given temporal order allows receiving the temporal dependencies of these coefficients as functions of the biospeckle pattern movement speed. Due to homogeneity of biospeckle properties of each surface fragment, intensity of which is calculated as a mean value of intensities of all correlation peaks and the correlation coefficient can be expressed as:

$$C^{k\tau}=\left|\frac{\left\langle\left(S_{im,jn}^{t_0}-\left\langle S_{im,jn}^{t_0}\right\rangle\right)\left(S_{im,jn}^{t_0+k\tau}-\left\langle S_{im,jn}^{t_0+k\tau}\right\rangle\right)\right\rangle}{\sigma_{im,jn}^{t_0}\sigma_{im,jn}^{t_0+k\tau}}\right| \quad (3)$$

where: im = 1,…I,…, 2I,…, MI and jn = 1,…, J,…, 2J,…, NJ.

III. Material and Methods

In order to study the biospeckle temporal properties of the fruits experimental setup was mounted. An expended He-Ne laser (2mW) with λ = 632.8 nm, beam was used for recording the biospeckle patterns of the fruits with a CCD camera connected to PC. The optical part of the setup was kept on the vibration free table for decreasing the influence of external perturbations. For the experiment, the recording time was equal to 15s with the frame rate equals to 20fps. The observation area was marked on each of them. Measurements were performed every day on the same places of the fruits.

The images of speckles were obtained as a movie. The movies were then recorded as speckle pattern series (series of images) and they were processed using PC by special code developed for this purpose. The code calculated the correlation coefficients $C^{k\tau}$ according to Eq. (3). These data were used to plot the temporal dependencies of these coefficients.

We planned our study as follows - we selected three types of different climacteric (with starch reserves) Indian fruits namely apple, pear and tomato. The fruits were fresh and conditioned at room temperature for one day before a shelf life program consisting of 1, 2, 3, 4, 5, 6 and 7 days of storage. We have plotted cross correlation coefficients for all the three fruits on inter-day basis. We have taken a series of observations for the fruits and repeated these observations three times almost in identical situation with temperature in the range of 24 - 27^0 C and humidity in the range of 62 – 65%.

IV. Results

Biospeckle activity (BA) was calculated using the correlation coefficient $C^{k\tau}$, where k = 0, 1, 2 …. and τ = 1/15 s. $C^{k\tau}$ was calculated as the correlation coefficient of data matrix of the first frame (k = 0) with the data matrixes of the following frames (at kτ) from the images of the biospeckle. In this study, C^{14} was analyzed only as the correlation coefficient between the first frame kτ = 0 and the frame at kτ = 14 s. Then, biospeckle activity BA = 1 - C^{14} value was determined. We are presenting the representative typical graphs for the fruits.

Table I show that BA (biospeckle activity) for different fruit commodities decreases during their storage. The change is gradual and significant. There is a fast decrease in pear. This rate is comparatively lower in the case of apple and tomato.

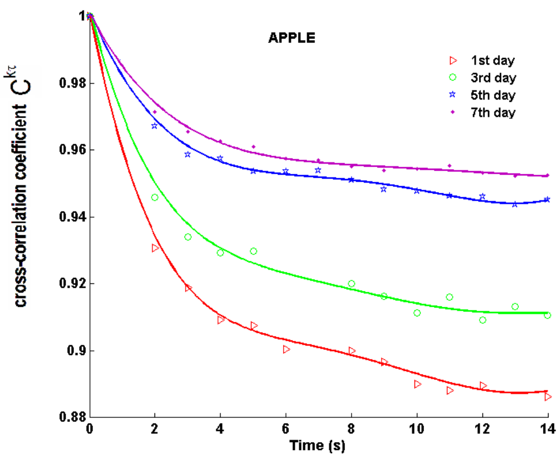

Fig. 1 Temporal changes of cross-correlation coefficients during shelf-life storage for apple

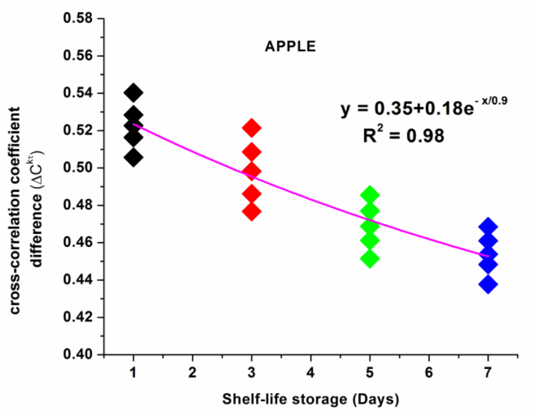

Fig. 2 Cross-correlation coefficient difference $\Delta C^{k\tau}$ as a function of shelf- life storage day for apple

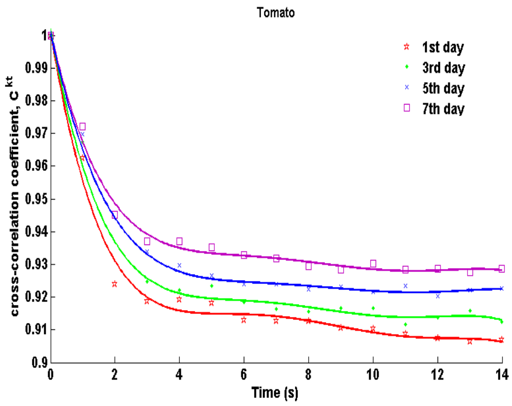

Fig. 5 Temporal changes of cross- correlation coefficient during shelf- life storage for tomato

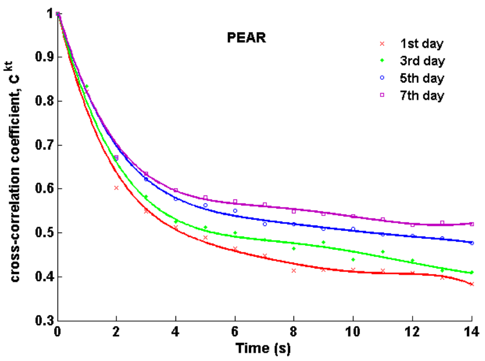

Fig. 3 Temporal changes of cross-correlation coefficient during shelf-life storage for pear

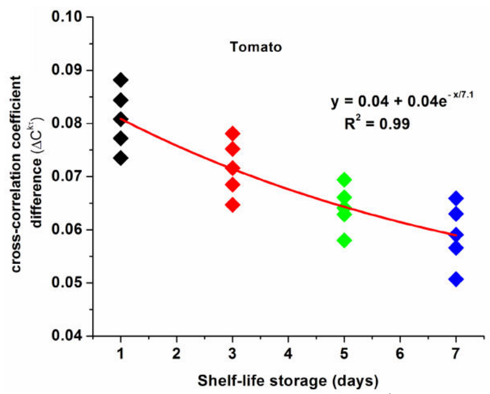

Fig. 6 Cross-correlation coefficient difference $\Delta C^{k\tau}$ as a function of shelf- life storage day for tomato

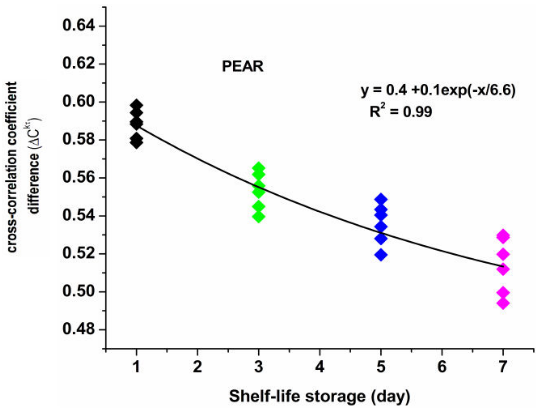

Fig. 4 Cross-correlation coefficient difference $\Delta C^{k\tau}$ as a function of shelf-life storage day for Pear

TABLE I
BA* OF DIFFERENT INDIAN FRUITS DURING THEIR SHELF LIVES

Fruit commodity	Shelf life (days)	BA* (average)
	1	0.522
	3	0.498
Apple	5	0.468
	7	0.453
	1	0.588
Pear	3	0.552
	5	0.534
	7	0.512
	1	0.081
Tomato	3	0.071
	5	0.064
	7	0.059

*BA- biospeckle activity

V. DISCUSSION

The Laser light falling on the fruit surface is elastically scattered on each boundary, like intracellular membranes, organelles and other particles. If any object due to cyclosis is moving, the scattering causes an unstable biospeckle pattern. So the biospeckle activity is a function of particles activity (mobility) and vitality of a tissue. Climacteric fruits like apple, pear and tomato etc. accumulate starch at early stages of maturation and progressively degrade starch to increase sweetness during ripening [25]-[27]. Starch granules are formed in amyloplasts within cells and have a size from 1 µm to 100 µm. Laser light of 632 nm is scattered on starch granules which are bigger than 2 µm according to Mie's theory. Starch does not move around together with organelles however the cyclosis presumably cause some vibrations of the granules. Thus, apart from other moving organelles, starch granules would give many additional non-stationary scattering centers. In result, more starch particles means higher apparent biospeckle activity. Next it has been studied that less chlorophyll content causes higher apparent biospeckle activity [28]. As the fruit ripens, the incoming light penetrates more deeply into it due to the degradation of chlorophyll (which has an absorption peak close to the wavelength of the He–Ne laser used).

Next the process of Respiration plays a major role in the postharvest life of fresh commodities because it reflects the metabolic activity of the tissue that also includes the loss of substrate, the synthesis of new compounds, and the release of heat energy. It is the oxidative breakdown of complex substrate molecules normally present in plant cells, such as starch, sugars, and organic acids, to simpler molecules such as CO_2 and H_2O.

Generally there is an inverse relationship between respiration rates and postharvest-life of fresh commodities. The higher the respiration rate, shorter postharvest-life, the commodity usually is [29].

Many climacteric fruits are harvested before they reach their best quality and storage conditions are optimized to permit the development of optimum quality. In this case, synthesis of pigments and volatiles, loss of chlorophyll, and the conversion of starch to sugar (eg., sweetening of apples) are necessary for the development of maximum quality.

TABLE II
MEAN RATE OF DECREASE OF BA WITH COMMODITIES.

Commodity	Mean rate of decrease of BA
Pear	0.018
Apple	0.0115
Tomato	0.004

Process of respiration within the fresh commodities results in loss of substrate molecules (e.g,. sugar, starch content), loss of cholorophyll etc. During the development of the flesh of a fruit, nutrients are deposited as starch which during the ripening process is transformed into sugars. The progression of the ripening process leads to decreasing starch levels. Thus the biospeckle activities of the fresh commodities should decrease with the decrease in their rates of respiration.

VI. CONCLUSION

1. The cross-correlation coefficient of biospeckle patterns decreases faster when the fruits are fresher. The cross correlation coefficient continues to decrease faster for first few days and then the decrease is slowest in apple and tomato than in pear.

2. There is a significant decrease in biospeckle activities of the different fruits with increasing storage period. The decrease is more in pear but relatively lower in the case of apple and tomato.

3. The decrease in biospeckle activities can be explained by their respiration rates. The respiration rate is high for pear and low for apple and tomato at temperatures (20 – 25 °C) [29]. So as can be seen from Table II. The biospeckle activity for all the three fruits apple, pear and tomato decreases according to their individual rates of respiration.

4. Thus, biospeckle activity evaluation can provide a method to predict the state of metabolism within the cells and tissues of the fruits.

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