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Consideration of freshness and taste of Japanese tomatoes - Comparison of laser biospeckle, and different sensing technologies with human perception

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This article is dedicated to Prof Kehar Singh for his significant contributions to Optics and Photonics

There has been a growing interest in the application of non-invasive laser biospeckle activity in the assessment of agricultural products such as tomato, Indian fruits, apples and so on and compare with other physical measures such as acid and starch content. In this study, we have compared characteristics of tomatoes by optical sensing along with taste and smell measurements in addition to human taste perception. We have employed non-invasive optical method of speckle imaging, smell and taste sensor devices for evaluation of freshness of tomatoes stored at room temperature. Tomatoes purchased from a local supermarket were used for measurements. Movies of biospeckle images acquired with a CMOS camera (1024 × 280 pixels) binned to 240 × 320 pixels sampled at the rate of 15 fps were obtained over a period of 14 sec. Calculating cross-correlation coefficient of biospeckle images at different times with that at time 0 and further quantifying the correlation coefficient (r) at 14^{th} sec as a parameter, it has been found that correlation coefficient decreased as a function of days matching the expectations due to reduction in the cellular activity within the tomato sample due to aging of the sample. We also conducted smell and taste measurements by electronic nose and lipid based taste sensor in addition to human sensing evaluations both of which revealed that the older tomatoes (15 days old) to be tasting better. Comparison of freshness and taste revealed that freshness and taste quality do not always agree. Biospeckle can detect deterioration as early as third day. At the same time, both taste measurement and human perception results suggest for a longer storage to be delicious.

Keywords: Biospeckle, Tomato, Perception, Freshness, Scattering

1 Introduction

With growing health concerns about the food, it is necessary that different attributes such as safety, health, appearance, freshness, packaging and many other factors need to be taken into account. As a way to evaluate one attribute, the freshness or aging, non-invasive methods such as vis/NIR spectrophotometry, time-resolved reflectance spectroscopy, hyperspectral backscattering imaging, laser-induced light backscattering or chlorophyll fluorescence have been used. Laser biospeckle is a method that had been investigated as a viable non-invasive method to estimate aging of different fruits and vegetables [1,2].

Biospeckle is produced when a biological living object is illuminated by laser light. The light gets scattered by the different organelles and structures within the biological objects and gets scattered in three dimensions. The backscattered light interferes and produces a speckle pattern in an observation plane. Actually, the speckle pattern from the living thing consists of two components with the static one from stationary components of the tissue and the dynamic one from moving structures of the tissue. The time

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varying speckle pattern is a characteristic of the biological object and is known as the biospeckle [1]. Here variation of intensity of the biospeckle is determined by the moving structures within the tissue. Braga *et al* [3] have summarized 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, as possible candidates for biospeckle activity (BA). Brownian motions should also be considered as a source of BA [4].

For example, it could be from the flow of water in the plant material or the deterioration with age resulting in refractive index variations. This in turn could mean biospeckles could be used as a tool to distinguish age related effects of the biological material such as human skin, fruits and vegetables. From analyzing the temporal characteristics of the speckle signals, for example using the correlation coefficient of the time varying signal as a parameter of evaluation, we can estimate the age of fruits or vegetables [1].

Till now, there have been studies done with Indian fruits such as apple, pear and tomato by Ansari *et al* [5], Polish apples [6], tomatoes by Romero *et al* [7], and lemon [8]. Most of the studies compare the correlation of the biospeckles obtained on different storage days and consider the deterioration with the passage of time. Some studies, for example, study by Zdneck *et al* [6] considered different varieties of apple and also compared the firmness, soluble solids content, titratable acidity and starch content in relation to the shelf life. In order to quantify biospeckles, different parameters such correlation coefficient, co-occurance matrix, Fuji difference and generalized difference and so on have been used [2]. In this study, we have used correlation as a parameter to characterize the biospeckle for the evaluation of the freshness of tomatoes and considered it in relation to taste and smell as measured respectively, by taste sensor and human perception and smell sensor.

Section 2 describes materials and methods used in experiments with results and discussion given in section 3 followed by summary in section 4. Appendix I gives the survey done in Japanese on the perception of taste of tomatoes felt by the subjects with storage of tomatoes at the room temperature.

2 Experimental

BA, smell, taste and perception investigations were done with tomatoes to investigate freshness and its relation to taste. They (Kumamoto Genki tomato) were purchased from a nearby supermarket with the product brand being the same all the time every third day for use in the experiments and they were stored at room temperature over a period of more than half a month and different measurements were done with separate sets.

2.1 Biospeckle measurement

Figure 1 shows the experimental system. Collimated light from a laser diode of wavelength 635 nm (4mW) (Thorlabs, USA) was used to illuminate a tomato sample and light scattered from the surface of the sample generating speckle at the CMOS camera (Thorlabs, USA) plane. The spot size was 4 mm and the distance between the sample and the camera was fixed to be 30 cm. Movies of biospeckle images were recorded at a frame rate of 15 frames per sec (fps) and acquired for a total time of a 14 sec to be analyzed by PC. First, biospeckle images (1024×1280 pixels) were binned to 240×320 pixels and cross-correlation of biospeckle images acquired at different times with that at time 0 was calculated. Further, the correlation coefficient at 14^{th} sec was used for quantifying the biospeckle activity (BA). A total of three tomatoes with three different points for each one were measured at every 3^{rd} day over a period of more than half a month.

2.2 Smell measurement

Smell or odor measurement was done with a device (Shimadzu) FF-2A (Fig 2a) also known as electronic nose. It can recognize different odors and characterize them with odor indices. The device contained ten metal oxide semiconductors sensor array and nine standard gases hydrogen sulphide, methylmercaptan, ammonia, trimethylamine, propionic acid, butylaldehyde, butylacetate, toluene and heptane were used to calculate nine standard gas vectors to be constructed in a multidimensional space of ten dimensions using multi-variate analysis [9]. Next, the extract of tomato samples (Fig 2b) was used and again the smell measurements were done. The information obtained was compared with the standard gas vectors and it was described in terms of the standard gas categories as radar plots (odor indices in standard gas categories).

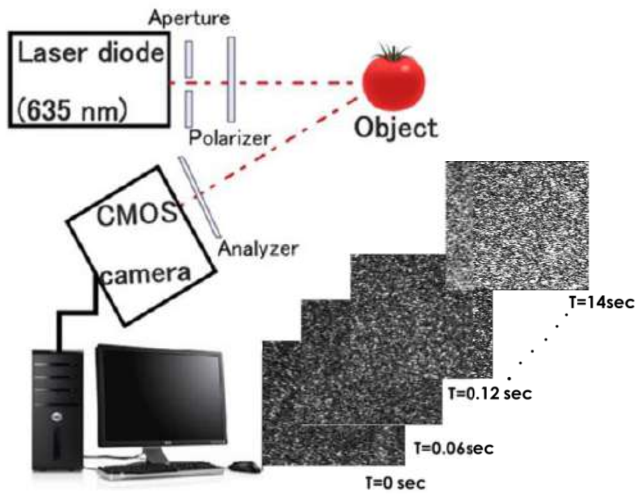

Fig 1. Experimental system for acquiring biospeckles.

Fig 2 (a). Artificial nose FF-2A for measuring smell.

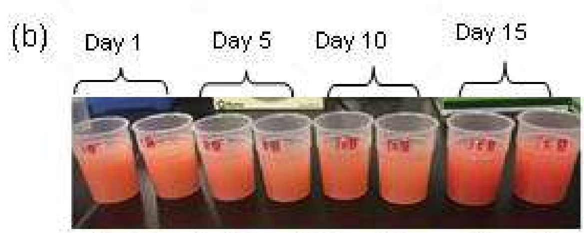

Fig 2 (b). Extracts of tomoto for smell and taste measurements.

2.3 Taste measurement

Taste measurement was done with a device TSZ-5000 (Fig 3) that has an artificial lipid membrane as a taste sensor and it interacts with taste materials through electrostatics and hyperphobic reactions [10,11]. Due to these interactions, there is a change in the potential of the membrane and this change is recorded as a sensor output. The actual procedure involves four processes [10,11]; first, the taste sensor is immersed in a reference solution of 30 mM KCl and 0.3 mM tartaric acid to obtain the membrane potential, Vr. The reference solution has almost no taste and is used in this system as an alternative to human saliva. Next, the taste sensor is immersed in the sample solution to obtain the potential, Vs. The difference in potential (Vs – Vr), the relative value, should approximate the initial taste upon sensory evaluation, including its sourness and saltiness. In the third process, the taste sensor is rinsed lightly with the reference solution and again immersed in the reference solution to obtain the potential Vr'. The difference in potential (Vr' – Vr). This would provide data on the adsorption of bitter and astringent substances. Finally, the taste sensor is rinsed well to remove adsorbed substances from the membrane in alcohol solution so that the next measurement can be done.

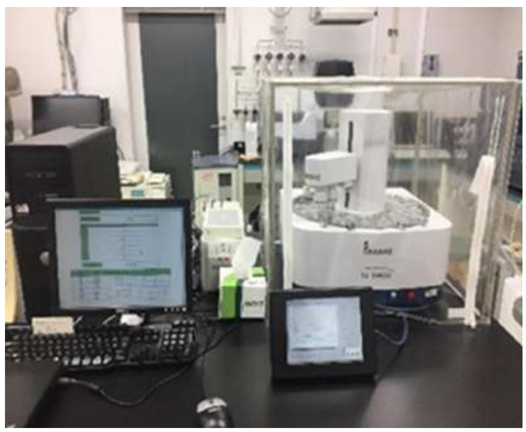

Fig. 3 Taste measurement system TSZ-5000.

3 Results and Discussion

3.1 Biospeckle activity

Biospeckles have been characterized by correlation coefficient measured between the zeroth frame and the rest of the frames with the movie recorded for 14 sec at a rate of 15 fps. The cross-correlation coefficient (r) was calculated using Matlab (2010) and using the following equation:

$$r = \frac{\sum_{m}\sum_{n}(A_{mn} - \bar{A})(B_{mn} - \bar{B})}{\sqrt{\left\{\sum_{m}\sum_{n}(A_{mn} - \bar{A})^2\right\}\left\{\sum_{m}\sum_{n}(B_{mn} - \bar{B})^2\right\}}}$$

where A and B are the zeroth and the subsequent biospeckle intensity frames between which correlation is calculated. $\bar{A}$ and $\bar{B}$ are the mean of each biospeckle intensity frame. m and n are the pixel indices of a frame. The result of speckle correlation as function of time is given in Fig (4). The correlation coefficient r^{14} at 14th sec was used for quantitative comparison of BA across different days and is given in Fig (5). As can be seen from Fig (5), the value of the correlation coefficient decreases monotonously with increasing storage time of the tomato. There is decrease in r^{14} even before 3rd day. Measurements were done every third day and so there is no data available before third day. However, we can see a clear relationship existing between the storage time and BA activity; BA activity reduces with increasing storage time of the tomatoes.

3.2 Results of smell measurements

FF-2A electronic nose (Shimadzu Corporation, Kyoto, Japan) was used to conduct smell measurements. Results of the calculated odor indices with respect to standard gases are given in a radar chart of Fig 6. Blue indicates the valued obtained with day 1 while the orange one is for day 15 tomatoes. Day 1 tomatoes have a higher concentration of aldehydes and low or almost no concentration of aromatics and esther. On the other hand, on day 15, there is increased concentration of aromatics. This would imply that with ripening of the fruit, there is increase of ethylene release and this in turn increases the concentration of aromatics and esther agreeing with the existing knowledge on fruits ripening [12].

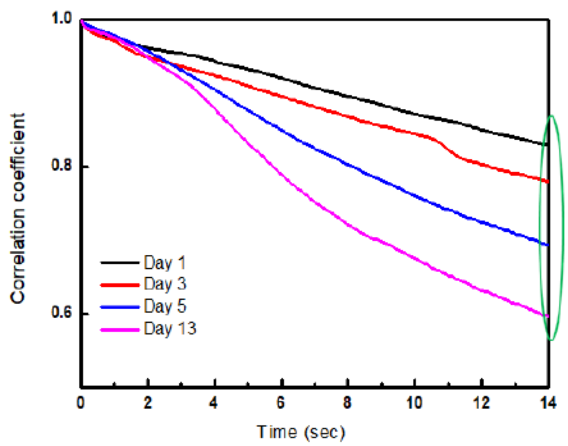

Fig 4. Correlation coefficient between the zeroth and the subsequent frames obtained over a period of 14 sec.

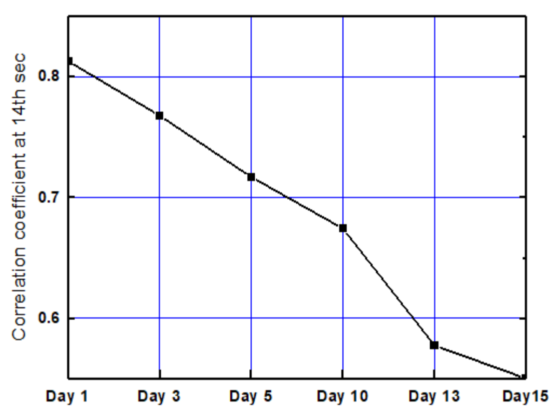

Fig 5. Correlation coefficient at 14th sec as a function of days of storage of the tomato at room temperature.

3.3 Results of taste measurements and taste perception

In order to conduct taste measurements, electronic tongue or TSZ-5000 (Intelligent Sensor Technology, Inc.) was used. Tomato samples of day 1 was used as the standard and a total of eight taste component indices were calculated. Results obtained for day 15 with day 1 used as standard are shown in Table 1. In this table, positive and negative signs respectively indicate increase and decrease in the taste component in comparison to that of day 1 values. Hence, day 15 tomatoes were less soury and less astringent and had more umami component. These results were next compared with human perceptions.

Table 1. Comparison of eight components of taste obtained with electronic tongue TSZ-5000 for day1 and day15 tomatoes α (a corresponds to the aftertaste; Note the mean error measured is much smaller than the taste measured)

Quantity	Sourness	Bitter+a	Astringence+a	Umami	Salty	Bitter	Astringence	Umami+a
Mean error in measurement	1.56	0.04	0.09	0.18	0.19	0.02	0.02	0.09
1st day	0	0	0	0	0	0	0	0
15th day	-10.77	0.74	-0.37	5.14	-0.38	0.07	-0.22	0.09

A total of 15 subjects participated in the study and the participants ranked tomatoes in the order of 1 to 4 with larger digit indicating better taste. The scores were calculated from the survey organized in Japanese that included questions about subjects' likeness, frequency of consumption, type of taste, smell, deliciousness. Table 2 shows the calculated score as a result of the survey with samples from days of 1, 5,10 and 15 days. The scores were obtained by summing up the scores for each of the day. As can be seen, score of day 1 is smaller as compared to score of day 15. These were found to be statistically significant. This in turn indicates that humans perceive 15 days old tomato to be tastier than day 1 tomato. This agrees with the result of taste sensor which indicates for the increased umami and decreased sourness and astringence with longer storage at room temperature.

Table 2. Comparison of perceived taste of different aged tomatoes by human subjects (N = 15)

Parameter	Sample			
	Day 1	Day 5	Day 10	Day 15
Total score across all subjects	29	44	31	46
Score per subject (Max 4)	1.93	2.93	2.07	3.07
Standard deviation	1.0629	1.1235	0.9286	0.8537

3.4 Comparison of different methodologies

Tomatoes stored at room temperature over different number of days have been studied for the BA and correlation coefficient of BA was used a parameter for comparison. Calculated cross-correlation was found to be lower for 15 day old tomato in comparison to day 1 tomato. Now let us consider about the physiological origin of BA.

The BA is a function of mobility of scatterers within the tissue and thus the vitality of a tissue. The mobility is believed to be the result of cytoplasmic movement or cyclosis and this movement happens to maintain optimum conditions for tissue life. During cyclosis, there is transport of nutrients, enzymes and exchange of materials between organelles, as well as between cells and this happens especially in the peripheral parts of plant, for example the cell wall or close to the surface, the region investigated by biospeckle measurement.

On the surface, there could be scatterers of different sizes that are almost the size of the wavelength of light used or much smaller than wavelength. Depending on the size, we would have elastic scattering either Rayleigh (smaller than wavelength) or Mie (almost or a little larger than wavelength) on each boundary, like intracellular membranes, organelles and other particles that could be in motion due to cyclosis. Such scattered fields from different scatterers would interfere to produce speckle pattern and due to their movement, there would be a dynamic biospeckle pattern. With increasing number of days from harvest, the movement deteriorates and thus there would be lower mobility or exchange of particles within the cell wall leading to lesser changes in the dynamics of the speckle pattern. In other words, there would be a decrease in the sustained correlation obtained over a longer duration as in our case 14 sec with increasing age. The correlation coefficient at 14th sec or BA was found to be lower even on third day and biospeckle could detect deterioration at a very early stage. BA could provide information about the freshness of the tomato but could not give any information relation to taste.

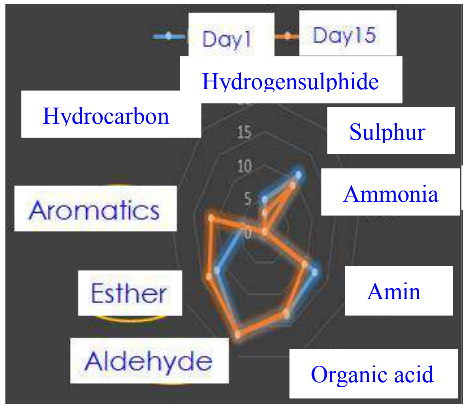

Fig 6. Results of smell measurement. Blue and orange correspond to Day1 and Day 15, respectively. With storage, there is increase in aromatic compounds.

Now, comparing the result of BA with the smell and taste measurements, we could see that being fresh did not necessarily mean tasty. Both the objective measurements of taste sensor and subjective human perception revealed that tomatoes stored over longer periods tasted better than fresh or tomatoes stored for shorter duration. This was also true due to the smell as there were more aromatic compounds with increasing storage.

4 Summary and conclusions

Laser biospeckles have been used for evaluation of freshness of tomatoes stored at room temperature. In order to quantitatively compare BA on different days, correlation coefficient was used as parameter and it revealed that increasing number of storage days decreased correlation coefficient or lesser BA. On the other hand, objective measurement of taste sensor as well as subjective human perception revealed that there was improved taste with increasing storage. The current study reveals that not just the freshness that could be an important attribute in evaluation of vegetables and fruits but also the relation between storage and taste has to be taken onto account.

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