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Identification of respiration rate and water activity change in fresh-cut carrots using biospeckle laser and frequency approach

Juliana Alvarenga Alves[a,*], Roberto Alves Braga Júnior[b], Eduardo Valério de Barros Vilas Boas[a]

[a] Food Science Department, Federal University of Lavras, Caixa Postal 3037, Lavras 37.200-000, MG, Brazil
[b] Engineering Department, Federal University of Lavras, Caixa Postal 3037, Lavras 37.200-000, MG, Brazil

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ABSTRACT

For consumers, fresh-cut fruits and vegetables are a convenient product and a healthy source of fresh food that has nutritional and sensory characteristics similar to those of intact products. In this paper, a non-destructive method for analyzing fresh-cut fruit and vegetables is described. The biospeckle laser technique is based on the optical phenomenon of interference generated by a coherent light interacting with biological materials or dynamical systems. Although many publications on this technique's biological applications have reported that biospeckle activity corresponds to the activity of biological samples, there is some difficulty in determining the correlation between a particular phenomenon and the activity observed. In this study, we evaluated the use of biospeckle data for measuring the physiological properties of fresh-cut carrots stored at two temperatures (0 and 10 °C). In conjunction with the biospeckle activity, the moisture content, respiration rate, water activity, and mass loss changes were monitored using traditional analytical methods to evaluate the possible correlation of the biospeckle data with any of these phenomena with or without the use of frequency signatures. The results showed that the manifestation of water in the monitored activity was isolated only by removing these high frequencies, thus allowing the activity manifested in the material to be linked to a specific phenomenon, such as respiration. Therefore, we were able to monitor the respiration process in fresh-cut carrots and assign a spectral signature to their water content and respiration.

1. Introduction

Fresh-cut fruits and vegetables have recently become more popular in response to the increasing demand by consumers for fast preparation or ready-to-eat food (Koukounaras et al., 2008), and they have also become more popular in response to the health benefits associated with a diet rich in fruits and vegetables. Several studies have been conducted to investigate the shelf life of fresh fruits and vegetables using chemical, physical, and microbiological methods (Corbo et al., 2006) as well as mathematical models to estimate the stability of the microbial, chemical, and sensory qualities of these products (Montero-Calderon et al., 2008). However, these approaches are expensive, slow, and require considerable analytical skills. Thus, quick, non-destructive methods of assessing the ripening, quality, and senescence of food products have recently become increasingly important, particularly those involving optical techniques.

An optical method that is emerging as a tool for the non-destructive and non-invasive measurement of food quality is based on the interpretation of the optical phenomena that occur when coherent light is focused on a sample. When the light of a laser reaches a material that exhibits some type of activity, this creates changes in the light scattering producing an interference phenomenon that is referred to in the literature as biospeckle or dynamic speckle.

The measurement of a given activity based upon biospeckle data can be performed using graphical and numerical outputs depending upon the application and the characteristics of the illuminated material. One numerical approach to the analysis of images via the laser illumination of tissues consists of creating a space time speckle (STS) pattern, as suggested by Oulamara et al. (1989) and Xu et al. (1995), and this approach is also known as a time history of speckle pattern (THSP) (Arizaga et al., 1999). Thus, the THSP represents the variation in time of the activity in the region of a biospeckle pattern formed by the projection of a laser beam onto the material under analysis. The moment of inertia (whose acronym is usually written as IM) technique, as presented by Arizaga et al. (1999), has been used as a reliable technique for numerically quantifying this

* Corresponding author. Tel.: +55 35 9812 1486; fax: +55 35 3829 1401.
E-mail address: juliana_alvarenga@yahoo.com.br (J.A. Alves).

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activity using the THSP image to return a dimensionless number that indicates low or high activity of the materials monitored.

Several studies have been developed with regard to foods and in plants that employ the biospeckle laser technique, such as its application to seeds, for example, to measure their water content or to detect fungi (Arizaga et al., 1999; Braga, 2000; Moreira et al., 2002; Rodrigues et al., 2005; Braga et al., 2005). In vegetables, interesting insights can be obtained by the biospeckle laser technique because the degree of biological activity of the illuminated surface can have various origins, such as the level of maturity, decay, growth, metabolism, water content, and temperature of the food (Rabal and Braga, 2008). Studies using a biospeckle laser have been undertaken to analyze bruises in apples (Pajuelo et al., 2003), monitor the quality of oranges (Rabelo et al., 2005), assess tomato ripening (Romero et al., 2009), and analyze the pigments in foods (Zude et al., 2008). Other studies have focused on analyzing the process of senescence in plant tissues, fruits and vegetables (Silva and Muramatso, 2006). Additionally, studies have even correlated the biospeckle activity of the chlorophyll content in apples (Zdunek and Herppich, 2012).

The routine methods that have been proposed to analyze such activities using biospeckle are based on the summation of many contributions related to a wide range of phenomena. Thus, these traditional approaches lack the ability to separate or isolate a particular feature.

A method of investigating the further separation of signals with the use of spectral ranges has been considered a feasible alternative (Sendra et al., 2005) that can be improved by the use of the wavelet transform (Limia et al., 2002; Passoni et al., 2005). The advantage of the wavelet transform, as associated with the IM method, is its ability to represent an activity by filtering only the desired signal, which gives the signature of the biological phenomena being studied. Braga et al. (2007) showed that it is possible to isolate certain features using the frequency domain, particularly through the wavelet transform.

Braga et al. (2007) presented the results of a biospeckle data analysis using wavelet transformations, coefficient filtering, and reconstruction in a novel approach to increase the information obtained. The data used were from two distinct biological investigations as follows: crop seeds and animal sperm. With animal sperm, the results showed that filtering makes it possible to identify the effects of dilution of the sperm sample allowing for the isolation of nuisance covariates. Recently, Cardoso et al. (2011) used the wavelet transform to create maps of the frequency of biological materials, particularly maize and bean seeds, in an effort to isolate water activity. Wavelet transform was then used in conjunction with traditional biospeckle laser methods, including Fujii, generalized differences, and THSP. The data analysis allowed for the access of information at different frequencies making it possible to map activities that only occur within certain ranges in the seeds and that are associated with particular areas. These results confirmed that it is possible to identify the frequency bands at which water activity may be taking place, thus creating a signature that could be useful in further studies.

The current search for scientific and technological resources that have lower cost and broad applicability is intense. Due to the complexity of biological materials, it is necessary to develop research processes that ensure greater efficiency in isolating areas of different activities in the same material using biospeckle. The possibility of creating frequency markers related to physical or chemical phenomena during biospeckle laser monitoring opens the way for important applications in the analysis of biological materials (Cardoso et al., 2011). The same issue was raised by Kurenda et al. (2012) in a recently published study where the authors evaluated the effect of temperature on the activity of biospeckle, and they reported that the phenomenon arises mainly due to biochemical metabolic processes. The authors further described the need to determine whether the biological results of biospeckle activity are valid in comparison to actual physiological processes and not only to the theoretical data as was the case in their study.

Thus, this study sought to identify different physiological phenomena in fresh-cut carrots stored at different temperatures using the laser biospeckle technique in association with spectral approaches to isolate particular phenomena, such as respiration and those related to water.

2. Materials and methods

2.1. Samples

Carrots (*Daucus carota* L.) were purchased at a local market, and they were washed in water with detergent, sanitized in a solution of 100 mg L^{-1} sodium hypochlorite for 15 min, and dried at 18 °C. The carrots were manually sliced in the transverse direction (5-mm thick) with a sharp stainless steel knife. The sliced carrots were then immersed in a solution of 100 mg L^{-1} sodium hypochlorite for 5 min, and they were then packed in rigid polypropylene (15 cm × 11.5 cm × 4.5 cm) with a hard cover made of the same polymer. The packages containing the product were stored at 0 ± 1 °C and 85 ± 5% RH or at 10 ± 1 °C and 90 ± 5% RH. The fresh-cut carrots were analyzed at 2-day intervals over a 10-day period. On each sampling day, three and six individual samples were used for chemical and biospeckle laser analyses, respectively.

During the assay, the temperature effect on the moment of inertia (IM) was examined by simulating a practical situation. The trays containing the fresh-cut carrots were removed from cold storage, and the vegetables were maintained at room temperature before the illumination. The experiment aimed to evaluate whether the activity identified by biospeckle might be influenced by the transient time in the temperature before illumination of the vegetables. Therefore, the vegetables were analyzed over time, immediately after being removed from the storage temperature, and the vegetables were also analyzed 30 and 60 min after being exposed to ambient conditions (approximately 20 °C).

2.2. Analyses

- *Mass loss* was calculated as the difference between the initial mass of the fresh-cut carrots obtained within the package and the mass determined at each storage interval using a semi-analytical balance (Mettler Model PC2000).
- *Moisture content* was determined by a gravimetric technique using an oven at 105 °C with spot checks to obtain the constant mass according to AOAC (2005).
- *Water activity* (*Aw*) was determined using AQUALAB equipment (Decagon 3TE model; Decagon Devices, Inc.). The samples were placed in plastic containers, and readings were performed at a controlled temperature of 25.0 ± 0.3 °C. Measurements were performed in triplicate.
- *Respiration rate* was calculated by measuring the amount of CO_2 produced by a known mass of fresh-cut carrots (approximately 80 g) that was conditioned in hermetic glassware of known volume. This method was performed using a gas analyzer (Check Point O_2 and CO_2, PBI Dansensor). The results were expressed in microgram of CO_2 produced per kilogram per second ($\mu g\ kg^{-1}\ s^{-1}$).