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Full Length Research Paper

Early prediction of internal bruising in potatoes by biospeckle laser technique

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The internal bruising in potatoes is a kind of damage caused by the mechanical impact, which is the major cause of post-harvest losses. This kind of injury is hard to detect because it occurs due to the internal cellular rupture of the tuber. This research aims at evaluating the applicability of the biospeckle laser technique in order to detect and predict the internal bruising in potatoes caused by mechanical impacts during the early stages where there is no visual perception of the injury. The correlation of Pearson between a quantitative index generated from the biospeckle laser images (BSL values) and the enzymatic darkening in the region of the injury have been calculated. The BSL values was used to compare a group of potatoes subjected to mechanical impacts caused by a pendulum and a group of potatoes that did not suffer any sort of mechanical impacts. Comparison was performed by average and the standard error of the BSL values was obtained: Before impact, immediately after, 2, 4, 6, 24, 48 and 72 h after the mechanical impact. Results showed that the BSL values correlated significantly with the progressive darkening of the potato tissue (r = - 0.79) and this allowed the detection of the formation of internal bruising from 4 hours after the occurrence of a mechanical impact.

Key words: Mechanical impacts, injury detection, optical technique.

INTRODUCTION

As a result of the reduction in the loss of food worldwide, it is essential to improve food security and reduce pressure for natural resources (FAO, 2013). The study also states that in low-income countries, food losses are related to the management of the harvesting and post-harvesting stages of food in general (Sharpley et al., 2015).

Concerning the losses of fruits, vegetables and tubers, one of the major problems is related to the injuries originated by mechanical impacts caused by falls, collisions and handling of fruits, vegetables and tubers. These injuries lead to physiological, metabolic, flavor and

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quality modifications (Li and Thomas, 2014).

In potatoes, the losses by mechanical injuries occur during every stage of production, from harvest to consumption. However, as the injuries are cumulative, the whole process should be monitored, and there should be actions to prevent damages (Shepherd et al., 2015). Ferreira and Netto (2007) reported that in potatoes the superficial damages and internal bruising can get to 40% of the whole production, reducing the sale price and even causing the rejection of the product.

The internal bruising is a common kind of injury in vegetables generated by mechanical impacts during the post-harvest (Opara and Pathare, 2014). Fruits, vegetables and tubers submitted to impacts, even without any apparent damage, develop internal cellular ruptures on tissues. The damaged membranes allow enzymatic oxidation of phenols and the production of melanin, causing internal and external browning of vegetables, called the bruising phenomenon (Hussein et al., 2018).

The detection of internal bruising in vegetables and fruits allows the control of their losses, therefore generating an increase in the efficiency of agricultural production (Li et al., 2013; Bugaud et al., 2014). The potential of the use of non-destructive approaches can be considered a key factor in the development of techniques to get reliable information on monitoring and predicting the internal bruising in tubercles (Rady and Guyer, 2015; López-Maestresalas et al., 2016), and the effects of the mechanical impacts on potatoes (Danila, 2015).

Optical methods present a capacity to provide information on physical, mechanical and chemical attributes of agricultural products due to the fact that the light has a great interaction with the cellular structure of biological materials (Hu et al., 2015). The laser light, more specifically, presents a great power of penetration in cellular tissues and the response of its dispersion after the incidence in foods can be associated to several quality attributes to these products (Romano et al., 2011).

Biospeckle laser technique is based on an optical interference phenomenon that occurs when one beam of coherent light spreads on a surface of the biological sample. Over time, the successive patterns of biosepckle become susceptible to be tracked, and the changes of biospeckle patterns are associated with biological activity (Braga, 2017; Stoykova et al., 2017). The correlation between the biological activity of a biological materials and variation of the biospeckle pattern over time can be observed in several relevant researches in medicine that studied the monitoring of melanoma (González-Peña et al., 2014) in the evaluation of the activity of bacteria and parasites (Ansari et al., 2016; Grassi et al., 2016) and other variety of applications in agriculture (Zdunek et al., 2014; Retheesh et al., 2016; Sutton and Punja, 2017; Arefi et al., 2018). Szymanska-Chargot et al. (2012) and Gao et al. (2016) presented the biospeckle laser as a feasible way to detect bruising process in apples and potatoes after mechanical impacts.

This research presents a non-destructive optical protocol, based on the biospeckle laser technique, to detect and predict internal bruising in potatoes caused by mechanical impacts during the early stages where there is no visual perception of the injury.

MATERIALS AND METHODS

Samples

The potatoes (Monalisa cultivar) used in this experiment were obtained from a commercial bag of 25 kg. The potatoes were acquired after harvesting and before washing, transportation and commercialization to avoid the occurrence of mechanical damages in these steps. The biospeckle laser tests used 15 potatoes previously submitted to a mechanical impact and 5 potatoes as a control, without the occurrence of mechanical impacts.

The determination of pulp firmness, mass and diameter were performed to characterize the preconditions of the amount of 25 kg of potatoes. Firmness was determined using a digital penetrometer with 5-mm diameter tip. The values of firmness for each fruit were calculated using the mean of three readings for each potato. The mass of the potatoes was determined through a precision weight scale and the diameter was determined through a digital caliper. The mechanical impacts were simulated using a pendulum with an arm of 600 mm and positioned at an angle of 90 degrees according to the plane of the sample. On the arm was placed a steel sphere of 180 g with a radius of 20 mm. Similar protocols to realization of the impact in potatoes can be observed in the research developed by Danila (2015). All impacts were located in the central region of the potatoes and were sufficient to cause internal bruising. The damage was black spot of blue-grey pigmentation, with a hollow concavity in the center whit diffuse edges (Noble, 1985). Figure 1 shows an internal bruising after 72 h, resulting in the impact of the pendulum.

A digital colorimeter was used to quantify the darkening of the impact region during the evolution of the damage before each biospeckle laser test. The darkening scale colorimetric had a range of 0-100, where 0 corresponded to the maximum absence of color (total black) and 100 corresponded to the maximum white color (total white).

Biospeckle laser test

After the impacts, the data acquisition of images was carried out during the early 6 h in intervals of 2 h, and after the early 6 h, there were acquisitions after 24, 48 and 72 h from the impact.

After 72 h from the impact, the potatoes were cut in order to check the presence of any dark area. In addition, the control potatoes were also illuminated over the same period and at the end of the experiment were cut to confirm the absence of bruising.

In the experimental configuration, the camera was a CCD (JAI VGA 480 x 640 pixels), with a pixel size of 7.4 µm, using a macro zoom lens in order to focus the Region of Interest (ROI) within the tuber, defined by a square area of 400 mm^2 . The magnification and the distance of the camera (300 mm) was adjusted to guarantee a speckle grain higher (many times) than the pixel size, and with a well-defined area, that is with high contrast. For the illumination, laser HeNe/632 nm with 36 mW was used, with an angle of incidence between the beam and the sample of approximately 45°. The backscattering configuration (camera caught the scattering of the laser light reflected after the incidence on the sample) was completed with polarized filters to the reduction of the illumination intensity, expander lenses,(20x, 20 mm) and mirrors, to target the beam (Figure 2).

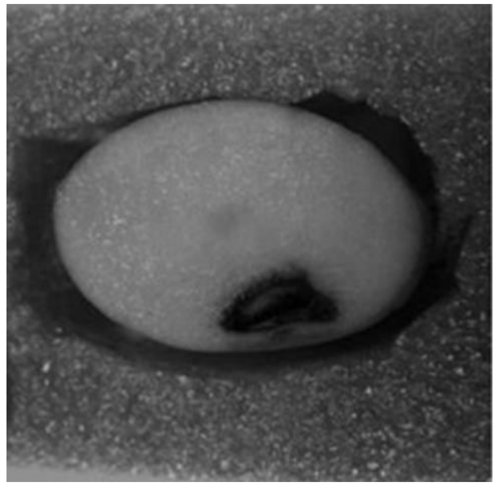

Figure 1. Internal bruising of the type internal crushing in potatoes after 72 h caused by a mechanical impact.

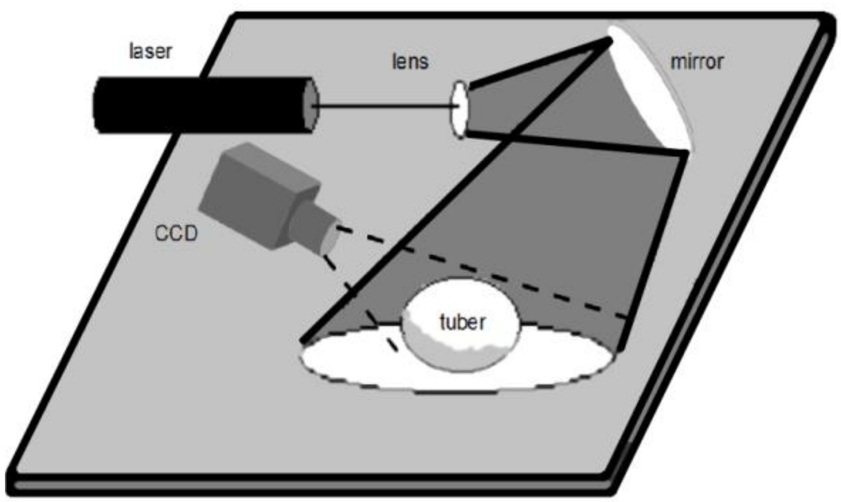

Figure 2. Experimental configuration with a computer, CCD, laser set, lenses and the samples.

Data analysis

A collection of 128 images in 8 bits of speckle patterns was assembled with a time rate of 0.08 s, and the images processed in areas within and out of the damage, and as well with the damaged area merged in the normal tissues (in this latter case the ROI was increased to 2500 mm²). A temporal speckle pattern was built, from the collection of images, generating the Time History Speckle Pattern - THSP (Arizaga et al., 1999). The time history information within the THSP was extracted using a co-occurrence matrix according to Equation 1:

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

Where, the values of Nij are the occurrences of the consecutive gray levels throughout the lines of the THSP.

The algorithm of absolute value of the differences (Cardoso and Braga, 2014) was applied to quantify the intensity of the variations present in the COM, based on Equation 2:

$$AVD = \sum_{ij} \frac{N_{ij}}{\sum_{ij} N_{ij}} |i-j| \quad (2)$$

Where, the variables i and j represent the cells in the COM matrix.

Original values of absolute differences (BSL values) were normalized to minimize the effect of variance in the behavior of the values obtained from each sample. The normalization was performed by the relation between the real value of the BSL values at a given instant and the BSL values before the impact occurs.

The values of the darkening scale colorimetric and BSL values were correlated through the Pearson coefficient to 0.05 significance. For statistical analysis, a completely randomized

Table 1. Characterization of 25 kg of potatoes where the samples were selected for the biospeckle laser test.

Attributes for characterization	Range
Firmness (Kgf)	4.00 - 4.75
Mass (g)	70 - 135
Diameter (mm)	60 - 110

design obtained in 8 instants over time (before impact, immediately after, 2, 4, 6, 24, 48 and 72 h after the mechanical impact) with 3 treatments corresponding to the condition of the samples (samples control, impact region and outside of impact region), 15 repetitions of impacted tubers and 5 repetitions of witnesses (control) were used. Measurements in regions outside the impact were obtained on surfaces adjacent to the impact region. Standard error (ϵ) for each treatment was calculated from the Equation 3:

$$\epsilon = \sqrt{\frac{MSE}{r}} \quad (3)$$

Where, MSE represents the mean square of the error obtained in the analysis of variance of instants in each treatment and r represents the number of repetition of the treatment.

RESULTS AND DISCUSSON

Characterization of samples

The characterization of the amount of 25 kg of potatoes allowed the estimation of the conditions of the samples before being submitted to the mechanical impact. The potatoes used in the biospeckle laser test were selected within this amount, and therefore, the firmness, mass and diameter were within the ranges shown in Table 1.

When analyzing the ability of biospeckle laser to evaluate the development of damage caused by a mechanical impact in potatoes, there was a significant correlation (Figure 3) between the darkening colorimetric of the injury and the BSL value in the region of impact, where the increase of the darkening colorimetric in the region and the impact is associated to the decline of the BSL value.

The mechanical impact on potato causes a rupture in the cell membrane allowing the entry of oxygen and resulting in darkening in the damaged region (Opara and Pathare, 2014). Quantitative levels of BSL values results from metabolic, enzymatic and respiratory changes due to cell degradation (Zdunek et al., 2014). The gradual reduction of cellular metabolism during oxidation in the impact region was responsible for the decline of BSL values. This is due to low variation of the scattering of light reflected when analyzing the biospeckle patterns over time.

Potatoes subjected to impact reduced BSL values over time, while potato control response remained in a range of constant values (Figure 4). The maximum BSL value in the region of impact, immediately after the impact (0h), is explained by the intense oxygen inflow due to the breakage of cell membranes (Hara-Skrzypiec and Jakuczun, 2013). Between 2 and 72 h, the hypothesis is that the BSL values were associated with cell oxidation in the region of impact over time, which resulted in the decline of cellular metabolism and in the darkening region. The BSL values outside the impact showed a similar response to the impact region, increasing the activity immediately after impact and reducing activity over time. The distinction between the group of control potatoes and the group of potatoes subjected to impact occurred after 6 hours; demonstrating that biospeckle laser has the potential to detect potato damage in the early stages after impact.

The comparison of standard error (ϵ) in the three groups of potatoes presented in Figure 4 indicates the regions where there are developing injury (ϵ = 0.04). BSL values tend to present a common response to the biological reactions resulting from cell oxidation. The peripheral regions or absence of injury (ϵ = 0.08 and ϵ = 0.06, respectively) presented major variation in repeatability in BSL values because they do not characterize a specific biological reaction, which makes the scattering of the laser light more random.

As also shown in Figure 4, biospeckle laser showed the ability to distinguish control potatoes and potatoes with injury, even by analyzing the region outside the impact. Metabolic processes involved in the development of injury and enzymatic darkening can cause a systemic effect in the whole potato as observed in plants by introducing external elements, systemically increasing resistance against diseases and pests (Mehari et al., 2015) or on the effect of toxins in animal cells (Iacobellis, 2015). Thus, when analyzing a potato with internal bruising, it is expected that its final metabolism is lower due to energy loss caused by the emergence of internal bruising. This information is relevant since the visual analysis of potato surface cannot be conclusive on the exact location of internal bruising.

Since it would be impossible to know during inspections if there is an impacted area in the tuber, an analysis was performed comparing healthy and damaged potatoes, but this time with no distinction of areas; however with a larger region of interest being used to compute the BSL values. Comparisons between control and damaged potatoes, with data inside and outside the damaged area are presented at Figure 5, being possible to observe the distinction in 4 h.

The result is an indication that the prediction of the development of internal bruising in potatoes by biospeckle laser can be obtained in moments close to the occurrence of the mechanical impact, and no need to distinguish regions that suffered impacts from regions that have experienced only systemic effects. Despite the ability of the biospeckle laser to be computed in small areas (Braga et al., 2007), the increase of the analyzed area improved the ability of the biospeckle laser to follow

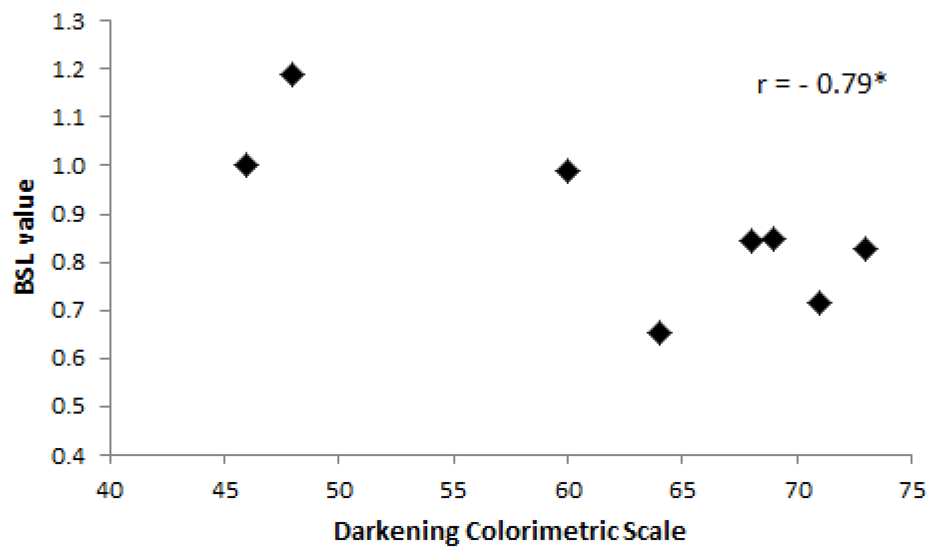

Figure 3. Correlation (r) between darkening colorimetric and BSL value in region of the impact. Significant at 0.05 level (*)

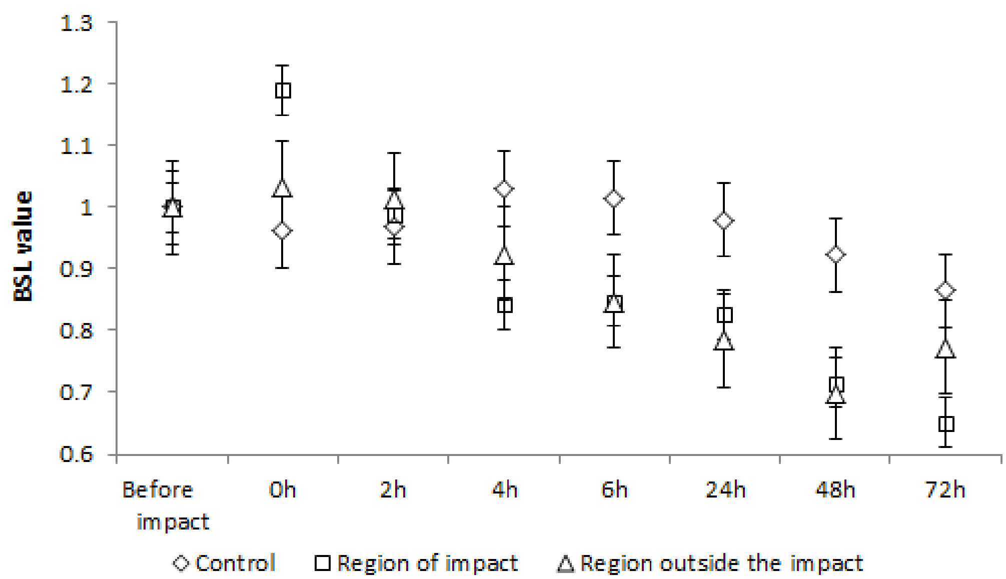

Figure 4. Average response and standard error variation of BSL value in the control samples, region of impact and region outside the impact in potatoes with the occurrence of internal bruising.

the biological phenomena, particularly reducing the dispersion of the results (ϵ = 0.03).

Similarly, López-Maestresalas et al. (2016) were successful in detecting bruising in potatoes, 5 h after impact using of VIS- NIR and SWIR hyperspectral imaging; demonstrating that parameters obtained by optical techniques can be efficient indicators for the detection and prediction of internal bruising. Surface injury on potato tubers on surface skinning was also identified by biospeckle laser within 24 h after the occurrence of an impact (Gao et al., 2016).

Once verified, the early differentiation between healthy potatoes and potatoes that were submitted to some impact during the stages of post-harvest, storage or transportation. A potential tool to detect and predict from the early stages (hours) a bruising process that would cause darkening was presented. From this information, more effective quality control measures can be taken and the percentage of product losses can be reduced, since more rigorous methods of selection and control will lead

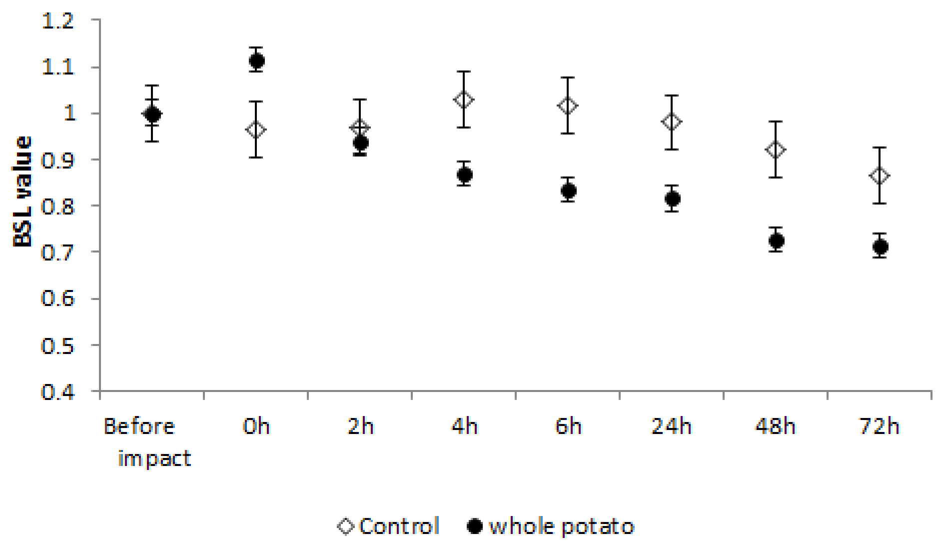

Figure 5. Standard error variation of BSL value without distinction of regions in potatoes with the occurrence of internal bruising and control potatoes (without the occurrence of internal bruising).

to greater care in the handling of products from harvesting to market shelves. In addition, the adoption of a contactless technique with reliable results reinforces the relevance of the research.

Conclusion

BSL values correlated significantly with the progressive darkening of the potato tissue, where the increase of the darkening colorimetric in the region and the impact is associated with the decline of the BSL value. The development of internal bruising was detected from 4 h after the occurrence of a mechanical impact, demonstrating that biospeckle laser technique is a non-destructive alternative to prediction of the occurrence of internal bruising in potatoes, even at times of no visual identification of the injuries.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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