Application of electrical bio – impedance for the evaluation of strawberry ripeness

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ABSTRACT

Electrical bio-impedance measurements were conducted on strawberry fruits. In order to obtain the impedance spectrum of the whole fruit, a non destructive device was designed. Three electrical variables were tested: low frequency resistor R_0 (related to extracellular resistances), high frequency resistor $R\infty$ (related to intracellular resistances), and constant phase element (magnitude CPE – T and phase CPE – P, related to the membrane capacitances and the heterogeneity, respectively). In parallel with electrical bio-impedance measurement, color and firmness were correlated to ripeness stage. The results indicated that strawberries from the highest ripeness stage had significantly lower CPE-P and R_0 values.

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Keywords: Electrical bio-impedance, strawberry, fruit ripeness

INTRODUCTION

Strawberry is a non-climacteric fruit that must be harvested at the full ripeness stage to achieve the maximum quality related to flavor and color. Strawberry quality components include appearance (color, size, shape, freedom from defect and decay), firmness, flavor, and nutritional value (1). Firmness affects the susceptibility of strawberries to physical damage and consequently their suitability for shipping (2). The color of fresh strawberries is a highly variable trait in seedling populations and one of the most important traits for selecting commercially acceptable cultivars (3). Electrical bio-impedance is a simple technique that deals with the passive electrical properties of materials and in particular of biological tissues: the ability to oppose electric current flow (4). This technique has been used, in destructive measurements, to characterize the electrical properties of a wide variety of biological materials. However, the same technique has been used in plant tissues, such as fruits, in a non-destructive way (5). One of the most widely used models to analyze electrical bio-impedance data is the Cole-Cole model. Some investigations published in this field are related to: determine the extent of apple damaged tissue caused by bruising (6), evaluate ripening and chilling injury development in persimmon fruit (7), assess the ripening of kiwi fruit (8), and characterize changes in intracellular and extracellular resistance as well as changes in the condition of membranes during the ripening of nectarines (9). The same technique was used with apples stored under room temperature to assess the conservation degree of the fruit (10). Additionally, the electrical bio-impedance method can be used to assess the soluble solids content and the acidity of apples (11), to assess the conditions of unripe and ripe mango fruits to be harvested with a robotic arm (5) or to distinguish the internal quality of healthy and rotten apple fruits (12). Finally, non destructive determination of the impedance spectrum has also been used to assess apple fruit, with and without skin (13). In strawberries, the electrical bio-impedance technique has proven to be sensitive to changes in the electrical resistance of the apoplast and symplast (14).

A recent work on citrus was published by Jaunsah et al. [15] Here the authors deal directly with frequency dependent parameters (resistance and reactance) finding the highest correlations with ripeness at 1 MHz analyzing different parts of the fruit.

Recently, non contact methods, as the Magnetic Induction spectroscopy, for impedance measurements of biological samples, have been addressed [16]. Using magnetic induction rather than electrodes, to get (eddy) currents across the sample, a secondary magnetic field is generated. The impedance of the sample affects the magnitude and phase of the magnetic field due to this secondary field. The technique is more challenging, requiring higher sensitivity, than direct electrical impedance.

In this paper, the variables obtained from the electrical bio-impedance spectrum of strawberries were used to classify them by its ripeness stages.

MATERIALS AND METHODS

Materials

‘Camino real’ strawberries were obtained randomly from a commercial orchard (classified visually by the expert) in the town of Irapuato, in the state of Guanajuato, Mexico. The fruit remained at 4 °C inside a portable fridge throughout the experiment. A graded color chart and a range equatorial diameter table were used to harvest at three ripeness degrees: 2, 4 and 6 (the scale ranges from 0 to 6, where 0 is non ripe and 6 is fully ripe) and two sizes: B and C (from three sizes available A, B and C, where A is the smallest and C the largest) both characteristics are mentioned in the Mexican Norm [NMX-FF-062-SCFI-2002]. Preliminary results showed no significant differences between adjacent stages, therefore we decided to report the differences found for not adjacent stages that includes the last one (2, 4 and 6). Twenty five strawberries were measured per each ripeness degree and size, a total of 150 fruits were tested for this study.

Experimental Setup

Electrical bio-impedance measurements were carried out using an Impedance Analyzer (Solartron SI 1260) in a frequency range (beta dispersion) between 1 Hz and 1 MHz and 1 V_{rms} generator voltage. A circular device, in four electrode configuration, was used with two current stimulus electrodes separated 120° from each other, and two electrodes for the voltage measurement in a symmetrical array as shown in Figure 1. The electrodes gently touched the intact fruit using a conductive gel between the four copper electrodes and the fruit skin to ensure good electrical contact. The strawberries were placed on the container with the stem end-calyx axis vertically so that the electrodes are placed around the equator.

Two impedance spectra were obtained per fruit. Using both the Nyquist plot and the Cole model, four variables were obtained, shown in Fig. 2. The four electrical variables were: low frequency resistor R_0 (related to extracellular resistance), high frequency resistor R_∞ (that includes information about intracellular resistance), and constant phase element (magnitude CPE-T some times called pseudocapacitance and phase CPE-P). The magnitude CPE-T is related to membrane capacitances and the phase CPE-P with heterogeneity of cell sizes and shapes. The mean value of both measurements for each fruit was used for analysis. The external skin color was evaluated on three sides of the fruit using a Konica Minolta spectrophotometer CM-508d and expressed by three parameters using the color space CIELab: L*, a* and b*. The light source used was D65 and it was calibrated with a white standard tile. The average measurements of the three sides evaluated were considered for analysis. Strawberry firmness (Fx) assessments were performed with a TA-XT2 texture analyzer. A 50 kg load cell was used for firmness determination of the fruit. A probe (4 mm diameter) with a flat tip punctured twice at a speed of 1 mm/s into the intact skin fruit (5 mm depth) rotating it 90°. This procedure provided a force-distance curve where the breakpoint was obtained, related with the firmness (N)

Statistical Analysis

Three ripeness degrees: 2, 4 and 6 (the scale ranges from 0 to 6, where 0 is non ripe and 6 is fully ripe) and two sizes: B and C (from three sizes available A, B and C, where A is the smallest and C the largest) applied to one cultivar of 150 strawberries. Statgraphics plus 5.1 was used for analysis of data. Data were analyzed by analysis of variance (ANOVA), test discriminant analysis and multiple linear regression (MLR).

RESULTS

Analysis of variance (ANOVA) was applied to search for statistically significant differences among ripeness stage parameters. No significant differences were found between ripeness stages and weight, and diameters ($p>0.05$) as expected. Moreover, no significant differences were found between sizes and the following variables: color, firmness, and impedance, in agreement with the fact that size is independent of ripeness. Statistically significant differences were found between ripeness stages and color lightness (L*), firmness (Fx) and also impedance resistance (R_{∞}), and CPE-P of the constant phase element, this last variable differs only in the last ripeness stage (Table 1). It is worth to mention that the ripeness stages are visually and manually established by experts according to color.

In order to quantify the capacity of electrical bio-impedance parameters to rank the ripe stage of strawberries, multilinear regression analysis and a forward stepwise discriminant analysis were carried out using all the parameters considered in this work. Using color variables and destructive firmness to segregate ripeness stages, 99.3% of fruits were well classified. This excellent result was expected because the manual classification made by experts, during harvesting, was based on visual inspection of color, and it confirmed the accurate classification done by experts.

In the case of electrical bio-impedance parameters, as a first approach, a multilinear regression analysis was used to predict color variables and firmness in terms of the impedance variables. Color variables, L* and hue angle ($tg^{-1}(b^*/a^*)$), and firmness were significantly related to impedance variables. Just over 76.7 % of the variance of the L* variable and 70.1 % of the variance of the hue angle could be explained by the impedance variables.

On the other hand, a forward stepwise discriminant analysis was carried out in order to classify the ripeness stages using electrical bio-impedance variables, 77.3% of well classified strawberries were obtained, see Table 2. The impedance variables CPE-P, related to the tissue homogeneity, $R\infty$ related to the intracellular resistance, and R_0 related to extracellular resistance were selected for this analysis.

Strawberries from the highest ripeness stage (6) were perfectly classified (100% of fruit correctly classified). However, ripeness stages 2 and 4 (low and intermediate ripeness stages) were misclassified in around one third of the cases. Strawberries from the highest ripeness stage (6) had lower CPE-P and R_∞ values than the other strawberries ($p<0.05$), Fig. 3. That is, more mature fruits had lower tissue homogeneity and lower intracellular resistance due to the texture degradation. In all cases, strawberries from the highest ripeness stage (6) had a CPE-P lower than 0.77 and R_∞ less than 223 ohms. The lack of the tissue homogeneity, detected using only the CPE-P impedance variable could be used to detect fruits in the last ripeness stage.

DISCUSSION

Color and firmness parameters have been, up to now, the best options to classify strawberry fruits according to their ripeness stage. The use of these parameters is expected in order to perform a ripeness classification because these parameters are used by the experts to classify fruit during harvest. Nevertheless, firmness is generally a destructive test, leaving color as one of the option to assess ripeness in a non-destructive way.

The electrical bio-impedance technique has been used in both, destructive and non destructive ways, to evaluate fruit quality, and is proposed as a non-destructive alternative to perform ripeness classification according to color. However, the comparison may not be so obvious, because bio-impedance data give information about the whole fruit (mainly internal quality) but color addresses the external quality of the fruit, and the ripeness process is non-homogeneous. One third of the strawberries, that were classified by impedance as stage 2, had already enough red color on the surface to be classified by color as stage 4, and one third of the fruits classified by impedance as stage 4 did not have enough surface red-color, or maybe non homogeneous surface color, so they were classified by color as stage 2. Further studies should be carried out to correlate whole impedance parameters with the average quality of the fruit (surface color, firmness and internal tissue condition).

CONCLUSIONS

As expert harvesters use visual color assessment to classify strawberries, the fruit evaluation by those parameters discriminates almost completely the samples considered in this work (99.3% of well classified fruits). Strawberries from the low and intermediate ripeness stages were misclassified by bio-impedance parameters. However, the use of the electrical bio-impedance parameters, CPE-P and R_∞, obtained a complete classification of the last ripeness stage (fruits completely ripe). Strawberries from the highest ripeness stage had a significantly lower CPE-P (lower than 0.77) and R_∞ (less than 223 ohms) than the other strawberries. More mature fruits had lower tissue homogeneity and lower intracellular resistance due to texture degradation. The lack of tissue homogeneity, detected using only the CPE-P impedance variable could be used to detect fruits in the last ripeness stage.

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