Journal of Food Engineering 133 (2014) 16–22

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Application of nondestructive impedance spectroscopy to determination of the effect of temperature on potato microstructure and texture

Ana Fuentes [a,*], José L. Vázquez-Gutiérrez [a], María B. Pérez-Gago [b], Erica Vonasek [c], Nitin Nitin [c], Diane M. Barrett [c]

[a] Departamento de Tecnología de Alimentos, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain
[b] Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Tecnología Poscosecha, Apartado Oficial, 46113 Moncada, Valencia, Spain
[c] Food Science and Technology Department, University of California-Davis, Shields Avenue, Davis, CA 95616, United States

ARTICLE INFO

Article history:
Received 3 September 2013
Received in revised form 13 February 2014
Accepted 14 February 2014
Available online 24 February 2014

Keywords:
Potato
Impedance spectroscopy
Microstructure
Texture
Water heating

ABSTRACT

The objective of this study was to use impedance spectroscopy to evaluate microstructural changes in potato tissue caused by heat treatments. Potato samples were subjected to 30 min treatments at room temperature, 30, 40, 50, 60 and 70 °C. Changes in microstructure were evaluated by texture, electrolyte leakage and color measurements, and confocal laser scanning microscopy (CLSM). Samples processed at 60 and 70 °C showed significantly different module of impedance and phase values than those processed at ⩽50 °C. Texture decreased and electrolyte leakage increased after treating potato at temperatures above 60 °C, indicating loss of turgor pressure and rupture of both membranes and cell walls. Significant changes in tissue microstructure and progressive cell wall degradation as temperature increased were visualized using CLSM. Real-time nondestructive impedance spectroscopy proved to be a quantitative means of following the effects of process temperature on potato cell walls and starch, and subsequently on textural properties.

1. Introduction

The potato (*Solanum tuberosum* L.) is an important vegetable grown around the world. It can be consumed in different forms, either fresh or processed into various products, such as French fries, mashed potatoes, potato chips and dehydrated granules. During industrial processing or cooking, the potatoes are subjected to a variety of thermal treatments that affect the final quality of the product. For consumers, the main quality attributes of cooked potato are color and texture (Bordoloi et al., 2012). The textural changes occurring during thermal processing and cooking of potato tubers have been associated with the gelatinization and retrogradation behavior of starch (Alvarez et al., 2001; Kaur et al., 2002; Ormerod et al., 2002) and breakdown of the cell wall and middle lamellae structural components (Alvarez and Canet, 1998; Ormerod et al., 2002).

Starch is the major component of the dry matter of potatoes. Therefore, its molecular organization and interactions with non-starch polysaccharides and sugars are important factors influencing sensory attributes of processed potatoes (Lisinska and Leszczynski, 1989). A better understanding of the physicochemical and structural properties of starch when potato is processed will offer the possibility of controlling the quality of potatoes and potato products, and producing new potato starches with added value. Therefore, it is critical to understand and apply advanced analytical techniques to characterize potatoes and potato starch. The gelatinization of starch has been widely studied through a variety of techniques. Calorimetry techniques provide valuable insight into the order–disorder transition phenomenon of granular starches. Microscopic examination of granules undergoing gelatinization allow observation of the degree and duration of swelling as well as the integrity and size of the swollen granules (Liu et al., 2002). Spectroscopic techniques have been used to study starch crystallinity, the extent of starch gelatinization and the retrogradation process. However, some of these methods are experimentally limited by certain parameters, such as starch/water ratio and the temperature range over which gelatinization can be studied (Liu et al., 2009). Moreover, these methods are tedious, expensive, time-consuming and require skilled personnel.

Electrical properties of food materials are known to provide information about cell structure, for this reason electrical measurements have been used in the past to assess the cell structure of agricultural products (Ohnishi et al., 2004). In this way, the use of electronic sensors such as systems based on impedance spectroscopy are an alternative to conventional methods of food

* Corresponding author. Tel.: +34 96 387 70 07x73664; fax: +34 96 387 73 69.
E-mail address: anfuelo@upvnet.upv.es (A. Fuentes).

http://dx.doi.org/10.1016/j.jfoodeng.2014.02.016
0260-8774/

analysis due to their high sensitivity and fast response (Masot et al., 2010). Impedance spectroscopy allows for the analysis of properties of materials and systems through application of alternate electric signals of different frequencies (voltage or current) and measuring the corresponding electric output signals (current or voltage) (Barsoukov and Macdonald, 2005). The ratio of the signal voltage to the signal current is called impedance and it is frequency dependent (Macdonald and Barsoukov, 2005). Impedance can be written in rectangular coordinates or in polar coordinates. In rectangular coordinates (Eq. (1)), R represents the resistive component and X the reactance.

$$Z = R + jX \tag{1}$$

When impedance is expressed in polar coordinates (Eq. (2)) $|Z|$ represents its module and φ its phase. The module is the ratio between the voltage and current signal while the phase corresponds to the phase shift between the two signals.

$$Z = |Z|e^{j\varphi} \quad \begin{cases} |Z| = \frac{|v(t)|}{|i(t)|} & Module \\ \varphi = 2\pi f \Delta t & Phase \end{cases} \tag{2}$$

where $v(t)$ is the voltage signal, $i(t)$ the current signal, f the frequency of the signals and Δt is the time interval between the zero crossing of the voltage and current signals.

Most biological tissues are composed primarily of cells and extracellular fluid. The electrical properties of tissues are significantly dependent on the composition and distribution of these two elements. Both intracellular and extracellular fluids contain water, electrolytes, free ions, salts and other components; therefore their electrical behavior is mainly resistive. However, the cell membrane which surrounds the cell consists of a double lipid layer, which serves as an interface between the intracellular and the extracellular media. Due to the presence of this double layer, the cell membrane has a capacitive behavior. These two behaviors affect the electrical impedance of biological tissues (Yu et al., 2004). Previous studies have shown that impedance measurements are appropriate for assessing the effect of freezing and cold injury on agricultural products (Ohnishi et al., 2004; Wu et al., 2008), assessing fruit quality (Rehman et al., 2011) or ripening of fruits (Harker and Forbes, 1997; Bauchot et al., 2000). However, the use of impedance spectroscopy in the analysis of the structural and physicochemical changes in potatoes after heat treatment has not been studied. Therefore, the objective of this study was to use impedance spectroscopy to evaluate microstructural changes in potato tissue caused by heat treatments and to analyze these changes using confocal laser scanning microscopy (CLSM) and physicochemical measurements.

2. Materials and methods

2.1. Sample preparation

'Russet' potatoes were obtained from a local supermarket. Forty potato tubers were washed under running water, wiped with blotting paper, hand-peeled and cut in cross section, yielding 3–4 slices per potato of 15 mm in thickness. From the center of each slice, one cylindrical piece of 30 mm diameter was obtained with a cork borer. A total of 150 potato disks (30 mm diameter and 15 mm in thickness) were randomly distributed into 6 batches of 25 disks, with each batch assigned to a different temperature treatment (room temperature (RT), 30, 40, 50, 60, and 70 °C). The heat treatment was carried out by immersing potato samples into a beaker with distilled water (ratio 1:5 w/w) which had been previously introduced into a thermostatically controlled water bath (Model SWB1122A-1, Lindberg, U.S.A.) at the required temperature. Processing time was 30 min, starting the process when the temperature in the center of the sample reached the set temperature. Temperature was monitored by a thermocouple during the entire heating process. After heating, the samples were cooled down to room temperature during a 30 min period, covered with plastic paraffin film in order to avoid dehydration and kept at 10 °C until analysis. From each batch (25 disks per treatment), 10 disks were used for texture and color analyses, 4 disks were used for electrolyte leakage evaluation, 2 disks for confocal microscopy observation and 9 disks for impedance spectroscopy measurements. This experimental design was performed in triplicate.

2.2. Texture analysis

A puncture test with a 5-mm diameter flat-tipped cylindrical probe was performed on the potato disks with a universal testing machine (model TA.XT2 Texture Analyzer, Stable Microsystems, Haslemere, England). The test was performed to a 90% strain using a test speed of 1 mm/s. Force–deformation relationships were analyzed and the parameters studied were: initial slope or stiffness (Mohsenin, 1986), calculated as the gradient of the line connecting the origin of the curve to 20% maximum force (N mm^{-1}), and maximum force (N) or hardness (Bourne, 2002).

2.3. Electrolyte leakage determination

Measurement of the intactness and permeability of the cell membranes of horticultural commodities is carried out using the electrolyte leakage parameter, which measures the amount of ion efflux through ruptured plant cells into a solution (Murray et al., 1989; Vasquez-Tello et al., 1990). For determination of electrolyte leakage, four potato disks were equilibrated at 25 °C for 1 h. One cylindrical piece per potato disk (5 mm in height and 5 mm in diameter) was obtained with a cork borer from the center of the disk and placed into 50-mL centrifuge tubes containing 20 mL of an isotonic solution (0.35 M mannitol) pre-equilibrated at 25 °C. Electrolyte leakage was measured as electrical conductivity (σ) in 0.35 M mannitol solution using a conductivity meter (Accumet portable AP65 Fisher Scientific) over a 2 h time period (t_i) at 25 °C (Saltveit, 2002) in a shaking water bath system (Model SWB1122A-1, Lindberg, U.S.A.). Total conductivity of the samples was measured after freezing (−18 °C) and thawing them twice, resulting in 100% ruptured cells. Electrolyte leakage at time t_i was calculated as a percentage of the total conductivity of the sample, as shown in the Eq. (3).

$$\text{Electrolyte leakage } (\%) = \frac{\text{Conductivity } (t_i)}{\text{Total conductivity}} \times 100 \tag{3}$$

2.4. Color evaluation

Color of the potato samples was measured in the CIELab system with a Hunter colorimeter (HunterLab, Reston, Va., U.S.A.) using the D65 light source and a 10° observer. L^* (white to black or light to dark), a^* (green to red), and b^* (blue to yellow) coordinates were recorded. The instrument was calibrated against a standard white tile. One measurement was taken from each disk.

2.5. Confocal laser scanning microscopy

Microstructure and starch distribution in the potato tissue were evaluated by confocal microscopy. Two cylindrical samples (8 mm in diameter) were taken from each potato disk. Two sections (200 μm) were obtained from each cylindrical sample using an oscillating tissue slicer (EMS 5000, Electron Microscopy Sciences Inc., Hatfield, PA). A Zeiss LSM 510 multiphoton microscope

(Carl-Zeiss Inc., Thornwood, NY) was used for high resolution imaging of the potato tissue microstructure. Multiphoton fluorescence images were obtained using 880 nm laser excitation and a 500–530 nm band pass emission filter using a 40× oil objective (NA = 1.4). The Z-projection image was processed using a Zeiss LSM image analysis toolbox. Calcofluor white stain was used for the visualization of cell walls (Keech et al., 2007). A 0.2% rhodamine-B solution was used for the visualization of starch (Van de Velde et al., 2002).

2.6. Impedance spectroscopy measurements

The system and sensor for measuring impedance in potato samples were developed by the Instituto de Reconocimiento Molecular y Desarrollo Tecnológico (IDM) at the Universitat Politècnica de València (UPV) (Masot et al., 2010). The sensor employed in this study was a double electrode composed of two stainless steel needles 1.5 cm long and 1 mm in diameter, separated by a distance of 1 cm in a non-conductive frame. This design keeps the separation between both needles constant during measurements. The impedance measurements were carried out by inserting the sensor perpendicular to the disk and each disk was measured in triplicate. All measurements were carried out at room temperature (20 °C), measuring the temperature of the sample with a standard probe thermometer placed approximately in the center of the potato disk before and after each measurement. The sample temperature never changed ±1.5 °C during the impedance measurements.

2.7. Statistical analysis

Statistical treatment of the data was performed using the Statgraphics Centurion (Statpoint Technologies, Inc., Warrenton, VA, USA). An analysis of variance (One-Way ANOVA) was conducted for each evaluated parameter, to test whether there were significant differences among treatments. The physicochemical parameters (texture, electrolyte leakage and color coordinates) and impedance measurements (module and phase of impedance for each frequency) were considered as dependent variables and treatment temperature (RT, 30, 40, 50, 60, and 70 °C) was the factor in these analyses. The LSD procedure (least significant difference) was used to test for differences among averages at the 5% significance level.

In order to assess the feasibility of the impedance spectroscopy technique to detect changes in potato tissue depending on the temperature employed during processing, a Principal Components Analysis (PCA), followed by a Discriminant Analysis (DA) was conducted. PCA analysis was performed in order to reduce the number of variables and to detect structure in the relationships between variables, since data obtained from impedance measurements were highly correlated. By means of this procedure the 100 variables obtained (50 module values and 50 phase values) were transformed into a small number of uncorrelated variables, principal components. The number of components was selected according to a Kaiser criterion (only eigenvalues at least equal to one were considered (Kaiser, 1960)). A stepwise DA was also performed using Wilks' lambda as the statistical selection criterion for the variables. The analysis was undertaken considering values of the principal components obtained in the PCA as variables and sample type heat treatment conditions as the classification factor.

3. Results and discussion

3.1. Textural changes

Table 1 shows the initial slope and maximum force (F max) values for each thermal condition. The initial slopes were similar for potato pieces heated at temperatures equal to or below 50 °C and significantly decreased at 60 and 70 °C (Fig. 2A). Initial slope has been related to the stiffness of the material under the load (Bourne, 2002). Therefore, the significant drop in the initial slope at temperature of 60 °C indicates a loss of turgor pressure caused by the rupture of the membranes (Gonzalez et al., 2010). Cell wall integrity is indicated primarily by the maximum force measurement.

A significant loss in firmness between 60 and 70 °C was observed (Table 1). Previous authors indicated that both cell wall cleavage and cell separation contribute to reducing the resistance of cooked potato tissue through the dissolution of calcium-pectic gel in the wall matrix and in the middle lamella (Jarvis and Duncan, 1992; Verlinden et al., 1995; García-Segovia et al., 2008). A more detailed study (Alvarez et al., 2001) found that both starch gelatinization and changes in pectic substances influenced the texture of potato tissue at 70 °C and 80 °C. These authors stated that at lower and higher temperatures (50, 60, 90, and 100 °C, respectively), the solubilization of pectic substances was the dominant factor. Initial slope has been related to the stiffness of the material under the load (Bourne, 2002). In this case, the significant drop in the initial slope at temperatures of 60 °C may indicate a loss of turgor pressure caused by the rupture of membranes, as previously suggested by Gonzalez et al. (2010). Other studies describe that pre-heating potato tuber at 47 °C caused the pectic substances from the middle lamella to degrade slightly (Roberts and Proctor, 1955), while the starch was swollen moderately (Shomer et al., 1993). However, this temperature had no effect on potato firmness, presumably because the pectic material was not substantially denatured by the mild heat treatment (Laza et al., 2001).

3.2. Electrolyte leakage

Electron leakage (EL) increased significantly ($P < 0.05$) when potatoes were subjected to thermal treatment above 50 °C (Table 1). This could be due thermal abuse to the membranes and the changes in membrane permeability with increasing temperature, due to phenomena such as lipid phase transitions and protein conformation changes, which would favor the diffusion of intracellular ions to the apoplast (Gonzalez and Barrett, 2010). Roberts and Proctor (1955) described that pre-heating potato tuber at 47 °C caused a slight degradation of the pectic substances from the middle lamella, which could cause an increase in EL. The increase in EL values was greater when potatoes were cooked at 60 °C and 70 °C. These results also correlate with the sharp decrease in initial slope at 60 °C, which indicates a loss of turgor and rupture of membranes, leading to loss of the primarily liquid cell contents.

Chaiwanichsiri et al. (2001) studied the changes in electrical conductivity in 50 g/kg suspensions of starch from different origins during heating from 20 to 95 °C. These authors observed that once the starch granules started to swell (at around 62 °C in the case of the potato starch suspension), the free ions inside the granules, mainly potassium, followed by magnesium, calcium and sodium (Noda et al., 2005), began to be released, contributing to an increase in electrical conductivity. The electrical conductivity increased progressively as the temperature increased until granules swelled enough to collapse (at temperatures around 72 °C in the case of potato starch suspension). At that moment, all the ions inside were completely released into the suspension. A similar process could take place in potato tissue during the cooking and would explain the sharp increase in EL at 60 and 70 °C. Moreover, the solubilization of pectic substances at high temperatures (Alvarez et al., 2001), leading to the degradation of the cell wall structure, also results in the diffusion of ions to the apoplast, contributing to the increase in EL values.

Table 1
Textural parameters, electrolyte leakge and color coordinates of thermally treated potatoes. [abc]Means with different letters are significantly different ($p < 0.001$).

T (°C)	Initial slope (N mm^{-1})	F max (N)	Electrolyte leakage (%)	L^*	a^*	b^*
RT	20.9 ± 4.4^{ab}	39.6 ± 1.5^{ab}	9.8 ± 1.0^{a}	59.8 ± 2.6^{a}	−2.0 ± 0.8^{a}	11.0 ± 1.1^{a}
30	23.8 ± 3.1^{a}	42.5 ± 3.5^{a}	9.6 ± 1.0^{a}	59.7 ± 4.9^{a}	−2.2 ± 0.5^{a}	11.6 ± 0.8^{a}
40	20.0 ± 3.5^{b}	37.85 ± 2.6^{b}	8.2 ± 0.9^{a}	61.0 ± 2.5^{a}	−2.2 ± 0.2^{a}	10.9 ± 1.1^{a}
50	21.0 ± 3.0^{ab}	36.74 ± 1.3^{b}	17.6 ± 4.7^{b}	59.8 ± 1.9^{a}	−1.1 ± 1.1^{b}	11.5 ± 1.3^{a}
60	6.7 ± 0.9^{c}	36.87 ± 2.8^{b}	91.7 ± 1.1^{c}	64.7 ± 3.4^{b}	−1.9 ± 0.9^{a}	8.3 ± 1.8^{b}
70	7.4 ± 0.8^{c}	30.5 ± 4.4^{c}	95.3 ± 2.0^{c}	53.7 ± 4.4^{c}	−3.8 ± 0.5^{c}	1.7 ± 1.6^{c}

3.3. Color changes

Table 1 shows the effects the thermal treatments had on the color of potato disks, together with a photograph of the samples. No color differences were found in potato disks heated at temperatures of 40 °C or below. At 50 °C, potato disks experienced an intense surface browning after the samples were cooled, which translated into a significant increase in a^* value. At higher temperatures, the samples lost the characteristic yellow color of this cultivar and tissue browning disappeared, which translated into a significant decrease in the color parameters a^* and b^*. These changes in color can be appreciated in the photograph of the disks at the different temperatures (Table 1).

The increase in browning of potato at 50 °C could potentially be attributed to the oxidation of phenolic compounds to quinones, which further polymerize to brown pigments, by the enzyme polyphenol oxidase (PPO) (Friedman, 1997). At this temperature, EL increased (Table 1) and microstructure analysis indicate thinner cell walls (Fig. 1), showing membrane breakdown. This would facilitate the contact between phenolic compounds and the enzyme PPO, inducing enzymatic browning. Yemeniciglu (2002) also described various degrees of browning by heating whole potatoes at 50, 55 and 60 °C, which varied with cultivar and heating time, and this was always associated with a reduction in the internal firmness of the potato. In that work, heating at 50 °C partially reduced the PPO activity in crude potato extracts. However, the degree of inhibition depended on the substrate concentration and extraction technique.

Anthon and Barrett (2002) investigated the heat stability of PPO from potato. Although the PPO inactivation did not conform to simple first-order kinetics, which complicated the determination of the inactivation rate, the results show that potato PPO is not as thermally stable as other enzymes, showing a complete inactivation at temperatures above 75 °C. Duangmal and Owusu-Apenten (1999) also reported the partial inactivation of crude PPO from potatoes at 60 °C and the complete enzyme inactivation at 70 °C. This would explain the color changes observed in our work as temperature increased from 60 to 70 °C, with a significant reduction in a^* and b^* values (loss of yellowness and increase in greenness). Therefore, the observed changes in color at temperatures around 50 °C could be attributed to enzymatic browning facilitated by membrane breakdown, and at temperatures above 60 °C to the inactivation of the PPO.

3.4. Confocal microscopy

The microstructural changes in the potato tissue caused by the cooking process were observed by CLSM (Fig. 1). Cell walls can be observed in blue due to the staining with calcofluor white and starch granules are in yellow due to staining with rhodamine-B. Unheated controls (room temperature, RT) had polyhedral cells (≅200 μm in diameter) with small intercellular spaces and globular to ellipsoid starch granules (20–30 mm) inside the cells.

No microstructural changes in the potato samples were observed after cooking for 30 min at 30 °C and 40 °C. Similar to the RT samples, the cell walls were homogeneously stained and starch granules were organized in clusters inside the cells. Signs of slight hydration and swelling of starch granules could be observed in potatoes cooked at 50 °C. The size of the starch granules appeared to increase when potatoes were cooked at 60 °C, indicating the

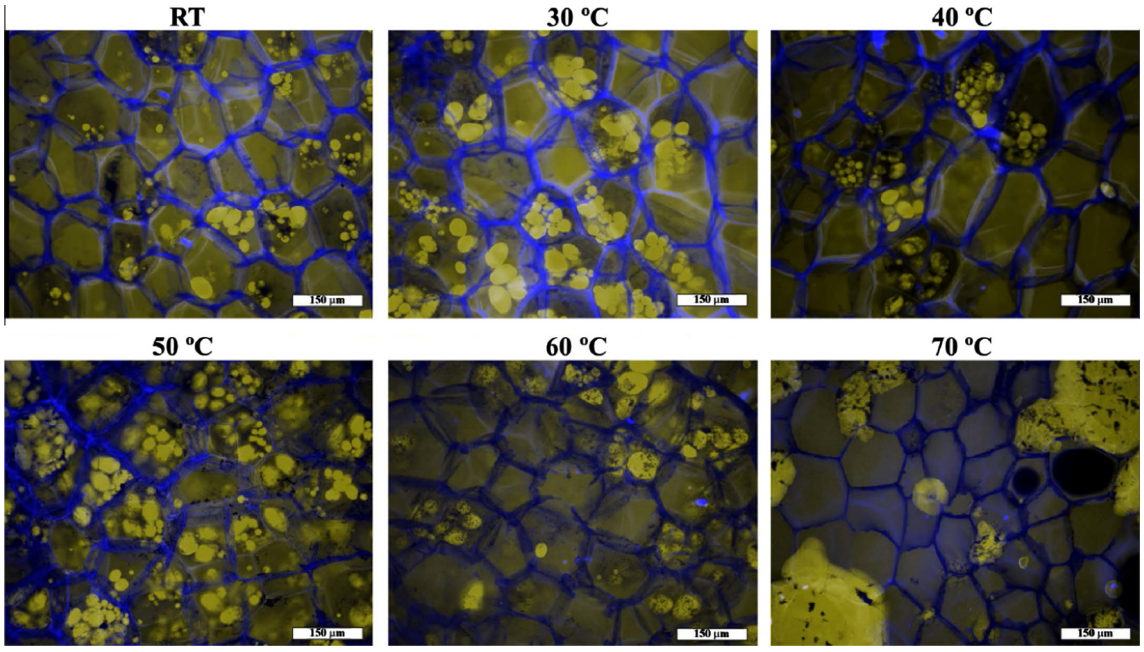

Fig. 1. Confocal laser scanning microscopy (CLSM) micrographs of potato samples subjected to different heat treatments.

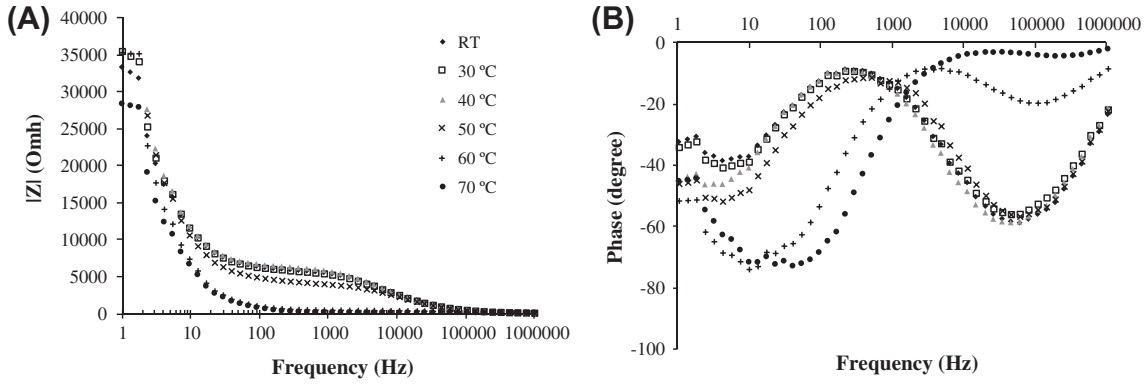

Fig. 2. Module (A) and phase (B) of the impedance of potato samples subjected to different heat treatments.

beginning of starch gelatinization. The starch granules finally ruptured and amylose began to leach out when potato samples were cooked at 70 °C, however the cell walls still appear to be fairly intact, although somewhat thinner (Fig. 1).

CLSM micrographs could explain the texture and EL values obtained. As already discussed, the majority of the ions are released from the starch granules while they swell and collapse during the gelatinization process (Chaiwanichsiri et al., 2001) and the loss of membrane integrity facilitates the diffusion of ions through the porous, partially degraded cell wall to the apoplast. The loss of cell turgor and adhesion between adjacent cells are the main factors that affect firmness of horticultural products (Gonzalez and Barrett, 2010). The thermal breakdown of the pectin in the middle lamella and the cell wall will lead to loss of integrity. The CLSM analysis clearly shows that the potato starch gelatinization process took place between temperatures 60 and 70 °C and in this temperature range indicates changes in the membranes and the cell walls, which correlates with the decrease in firmness and the sharp increase in EL at these temperatures.

3.5. Impedance spectroscopy

The impedance spectra of potato samples processed at different temperatures (RT, 30, 40, 50, 60 and 90 °C) are shown in Fig. 2. In Fig. 2A, the module impedance ($|Z|$) is plotted on a logarithmic scale. In all the samples, independent of the heating treatment employed, the module of impedance at low frequencies was significantly higher ($p < 0.001$) than at high frequencies. This behavior may be explained by the fact that biological tissues are composed of cells that are surrounded by extracellular liquid and the cell membrane acts as an insulator at low frequencies, behaving like a capacitator (Zhang et al., 2010). When low-frequency voltage is applied to the tissue, the main current flows through the extracellular fluid and the cell is bypassed, in this sense, when the voltage frequency increases, part of the current will cross the cell membrane and flow through the intracellular fluid, therefore the overall module impedance will decrease. Low-frequency impedance of biological tissue is larger than high-frequency impedance. For the whole range of frequencies measured, the module of impedance in potato samples decreased as the temperature increased. The heating process provoked the destruction of the cell membranes, which increased the amount of free electrolytes in extracellular spaces in the tissue, increasing its conductivity and producing a decrease in the impedance module. In order to check if there were differences among values obtained with the impedance system, an analysis of variance (One-Way ANOVA) was carried out for each module and phase of impedance. According to the statistical analyses, differences in the module of impedance were observed depending on the temperature employed during the cooking process (ANOVA data no shown). At low frequencies (<1000 Hz), there were significant differences between the module of impedance of samples heated at temperatures below 50 °C and those heated at higher temperatures (60 and 70 °C), indicating that the measurement of impedance at low frequencies could detect the rupture of cell membranes.

Similar differences could be observed among phase values of impedance in potato samples exposed to different temperatures (Fig. 2B). Samples processed at temperatures below 50 °C exhibited a similar behavior through the whole range of frequencies, whereas significant differences were observed as processing temperature increased. Potato samples processed at 60 and 70 °C showed significantly different phase values for the whole range of frequencies than those processed at <50 °C. These differences could be related to the microstructural changes in the potato tissue caused by the heat treatment, as observed by CLSM (Fig. 2). According to results obtained in other studies, rupture of membranes produces a reduction of the capacitive component of biological tissues, which decreases the value of the impedance phase at low frequencies (Fernández-Segovia et al., 2012; Fuentes et al., 2013).

Our results agree with those obtained in previous studies, in which differences in the impedance data depended on the treatment temperature (Dejmek and Miyawaki, 2002; Nyanjage et al., 2001). Thus, Dejmek and Miyawaki (2002) found that at temperatures below 57.7 °C the impedance, resistance and reactance of potato strips remained approximately constant; however, in the range of 60–65 °C a drastic change was observed, while at temperatures above 67.5 °C no further distinct changes were apparent.

Because the modules and phases are highly-correlated variables, a statistical tool which allows for a reduction in the number of study variables is necessary. For this purpose, a PCA was used to obtain a reduced number of orthogonal variables, which are the principal components (PCs). PCA was able to reduce the initial 100 variables to 4 PCs, which explained 98.92% of the total variance. The first two components alone explained 91.32% of the total variance. The results obtained in the PCA showed that in PC1 (which explained 83.30% of the total variance) the highest PC loadings were obtained for all the "module" variables, except in those frequencies between 1 and 10 Hz and higher than 200 kHz. However, in PC2 (which explained 8.02% of the total variance) the higher component loadings were obtained for "phase" variables at low frequencies, especially those in the higher range from 1 to 10 Hz and from 500 to 2000 Hz.

In order to assess the feasibility of the impedance spectroscopy technique in differentiating among potato samples exposed to different heating treatments, a Discriminant Analysis (DA) was

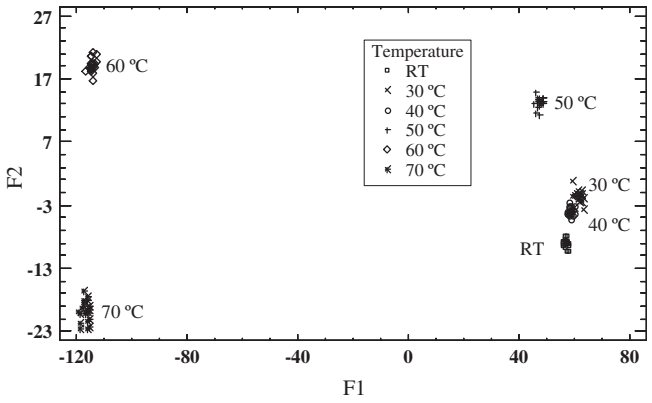

Fig. 3. Discriminant functions plot obtained for potato samples subjected to heating treatments.

Table 2
Discriminant function coefficients in the model to classify potato samples according to heating temperature.

	F1	F2
PC1	1.08707	−0.0261204
PC2	−0.390881	−1.23804
PC3	−0.2555238	−0.413424
PC4	0.536706	1.15232

carried out. The discriminant variables were the PCs obtained in the previous statistical analyses and the treatment temperature was the classification factor.

From this analysis, 5 discriminant functions were obtained. The two first functions (F1 and F2) explained more than 98% of the variance (F1 96% and F2 2.58%). According to these results, 100% of the cases were correctly classified. Fig. 3 shows the distribution of potato samples in the discriminant space. F1 mainly determined the separation of potato samples into 2 groups; one corresponded to those samples subjected to temperatures $\leqslant$50 °C and the other to samples subjected to higher temperatures. No separation among samples subjected to temperatures $\leqslant$40 °C was observed, which agrees with the physicochemical and microstructural studies, where the main changes were observed at temperatures above 50 °C. Function 2 determined the separation of samples exposed to 60 and 70 °C.

Table 2 lists the standardized discriminant function coefficients of the variables. The variable contributing most to the separation of the heating treatment according to F1 was PC1, meanwhile the variable with the most weight was PC2. The results obtained in the statistical analysis confirmed that changes in potato structure caused by the heating treatment could be detected by the response in the impedance measurements, mainly in the module of impedance in the range from 10 Hz to 150 kHz (as it has been explained by PC1).

These results confirm that heating affects the electrical impedance characteristics of potato tissue. This allows the use of impedance spectroscopy as a useful tool for monitoring sequential changes in potato tissue during heating, such as the rupture of membranes and cell walls, the beginning of starch gelatinization, and the collapse of starch granules, which occur at different treatment temperatures.

4. Conclusions

Thermal treatment affects the texture and electrolyte leakage of potatoes, leading to rupture of both membranes and cell walls, loss of turgor pressure and progressive starch gelatinization. These changes in potato microstructure can be detected using real-time impedance spectroscopy. The impedance technique provides an important tool for evaluation of the effects of process temperature on potato cell walls and starch, and subsequently on textural properties.

Acknowledgements

The authors are grateful for the financial support received from the Campus de Excelencia Internacional at the Universidad Politécnica de Valencia for carrying out this research and the Programa Nacional de Movilidad de Recursos Humanos del Plan Nacional de I-D+i 2008–2011 from the Spanish Ministry of Education.

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