ORIGINAL RESEARCH PAPER

EVALUATION OF OHMIC HEATING EFFECTS ON BELL PEPPER PUREE WITH ADDED CITRUS PECTIN

LUCIAN D. OLARU¹, OANA V. NISTOR*¹, DOINA G. ANDRONOIU¹, VIORICA VASILICA BARBU¹, IOANA OTILIA GHINEA², ELISABETA BOTEZ¹

¹Faculty of Food Science and Engineering, "Dunarea de Jos" University of Galati, 111 Domneasca Street, 800201, Romania

²Faculty of Sciences and Environment "Dunarea de Jos" University of Galati, 111 Domneasca Street, 800201, Romania, Phone/Fax +40 236 460165 *Corresponding author: <u>oana.nistor@ugal.ro</u>

> Received on 2nd March 2018 Revised on 25th September 2018

The important potential of the Mintos F1 variety of bell peppers which can be processed into products with special destinations is studied in this work. The main objective of this work is the evaluation of the ohmic heating (OH) effects on bell pepper puree with added citrus pectin in various concentrations (0.1-0.3%). In order to obtain the purees, the vegetable material was blended at 1900 rpm for 2 minutes. The samples were ohmically heated at 20 V/cm for 3 minutes using a batch installation. The electrical conductivity values were measured and also calculated. Antioxidant capacity, Texture Profile Analyses, Fourier transform infrared (FT-IR) spectroscopy and confocal scanning microscopy were used in order to estimate the possible changes induced by the addition of citrus pectin and ohmic heating. The electrical conductivity values demonstrate a linear and continuous data distribution. After 30 days of storage, the acidity of the samples was slightly modified with a pH decrease of 4% for the sample of bell pepper puree with 0.3% citrus pectin addition (APC0.3). The inhibition of free radicals for the control sample was 71%. The rheological behavior of the samples remains non Newtonian after the OH processing. The cohesiveness values confirm the increase in the intensity of the links between the structural elements of the bell pepper puree. The consistency and stability of the samples was improved by the pectin addition. The ready-to-eat products with special destination are important because of their convenience to consumption.

Keywords: ohmic heating, bell pepper puree, citrus pectin, rheology, texture

Introduction

Bell peppers, also named *Capsicum annuum* as species, belong to a genus of plant from the *Solanaceae* family and *Eudicots Angiosperms* clade, native to southern North America and northern South America.

This species was extended to be cultivated all over the world and enjoyed a wide variety of names, according to their location and type. The most familiar peppers names are chilli, bell, red, green or simply pepper (Faustino et al., 2007).

Bell peppers (*Capsicum annuum*) can be used as coloring and flavoring agents. Bell peppers can be consumed fresh, dried, fermented, or processed into different meals or extracts. They can be consumed as a whole, chopped, coarsely ground, or finely ground, with or without seeds. Bell peppers can also be consumed with or in other products that contain a significant proportion of peppers, including fresh and processed salsas, powder products and many other foods (Nadeem *et al.*, 2011).

Peppers are important to the wide array of phytochemicals like neutral and acidic phenolic compounds, which are major nutritional antioxidants that may reduce the risk of degenerative, mutagenic, chronic diseases, as well as carcinogenic activity (Ozgur *et al.*, 2011).

Purees have gained a major importance in human food mainly on the one hand due to their consistency, which makes the product easy to chew and swallow, and on the other hand their availability. Purees are cohesive, homogenous in texture, smooth and moist. Purees are described in the literature as 'smooth and lump free, with sufficient cohesion to hold its shape on a spoon; when plated they should not "bleed onto the plate" (Atherton *et al.*, 2007).

Pectins have been utilized for their functionality in foods for many years. Pectins are polysaccharides that constitute the matrix network of the cell wall and middle lamella in vegetable tissues, being responsible for the viscoelasticity of the cell walls (Fissore *et al.*, 2012). In the food industry, pectins are used as gelling agents, thickeners, and stabilizers (Siew and Williams, 2008).

Heat transfer to foods is commonplace but critical; heating develops flavour and texture and ensures product safety (Fryer and Robbins, 2005). The quality of the products can be affected by the heat treatment, especially viscosity and the nutrients. By using high-temperature, the short-time processing can minimize the heating induced problems (Cho *et al.*, 2016). Ohmic heating (OH) is defined as a process where alternative electric currents pass through a conductive food directly connected to the electrodes to which sufficient power is supplied (Srivastav and Roy, 2014).

Ohmic heating (OH) is a novel thermal technique, which is known for the volumetric heating, which has no limitations regarding the penetration depth, as long as the inherent electrical resistance of the food is not too high. OH can be used for liquids and solids simultaneously, without requiring stirring or mixing, as in conventional heating (Vicente *et al.*, 2006).

The ohmic heating is very popular by being used on several fruits or vegetables purees. Many researchers have chosen this type of foods because of their disponibility for consume. Due to the fact that ohmic heating is a novel technique recognized as being gentle with food materials, it represents a feasible alternative for the conventional heating. Many biological active compounds from vegetables are preserved or potentiated by ohmic heating or by electroporation the antioxidant

activity of the vegetal material is increased. Thus, Nistor et al., (2013) and (2015) for apple puree, Icier, F., Ilicali, C., (2005b) for apricot and peach puree, Moreno et al., (2011), Nistor et al., (2012) and (2015) for pear puree, Cho et al., (2016) for fermented red pepper paste and Farahnaky et al., (2012) for carrot, red beet and golden carrot, have studied the effects of OH on some fruit purees.

The aim of this study was to evaluate the OH effects over the bell pepper puree with added citrus pectin. The electrical conductivity is a specific measure for the ohmic heating process related to the effects of temperature and the electrical field.

Materials and methods

Sample preparation

The bell peppers (*C. annuum*) Mintos F1 variety were purchased from a local producer from Galati city, Romania. The bell peppers were washed, cut into two pieces and the seeds were subsequently removed. The pieces were blended at 1900 rot/min for 2 min by means of a kitchen robot Philips HR 7755 Germany. The samples without added ingredients were considered as the control samples. Citrus pectin (Alfa Aesar, Germany) was added in the bell pepper puree in the range of 0.1-0.3%. The blending was continued for 1 min to hydrate the pectin in the puree.

OH installation

A batch OH installation consisting in a power supply of 10 kW and an ohmic heating cell (a glass thermoresistant tank, two stainless steel electrodes of 5 mm diameter placed at an equal distance of 10 cm) was used. The OH installation is equipped with digital voltmeter, an ampermeter and a thermocouple type T.

Puree OH treatment

After this, the puree was ohmically heated at 20 V/cm for 3 min. The bell pepper puree was refrigerated at 4-6°C in order to be cooled, and stored for 30 days at same temperatures.

Samples encoding is presented as follows: MA-control sample (bell pepper puree), APC0.1- bell pepper puree with 0.1% citrus pectin addition, APC0.2 - bell pepper puree with 0.2% citrus pectin addition, and APC0.3-bell pepper puree with 0.3% citrus pectin addition, respectively.

Physical measurements

The electrical conductivity values were measured with a conductometer Lutron Electronics USA, model YK-2005CD, and were also calculated in the following formula of Sastry & Salengke (1998); Wang & Sastry (1993):

$$\sigma = \frac{L}{Ae \cdot R} \tag{1}$$

where:

L –the distance between the electrodes, m:

Ae – the section between the electrodes, m^2 ;

R – the electrical resistance value, Ω .

The temperature of the samples was measured with a type T thermocouple during the ohmic heating process.

Chemical determinations

The stability of the samples during storage was monitored by measuring the pH values by means of a WTW Inolab pH 7310 Germany.

The antioxidant capacity was determined by using 2 common methods, i.e. ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenyl1-picrylhydrazyl) assays.

Trolox equivalent antioxidant capacity (TEAC)

For the assay the method of Huang et al. (2005) with some changes was used. A stable stock solution of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical (ABTS) was produced by reacting a 7 mM of aqueous solution of ABTS with 2.45 mM potassium persulfate, as final concentrations.

This mixture was placed in the dark at 25°C for 16 h before use. The working solution was obtained by diluting the stock solution in 50 mM sodium phosphate, pH 7.4 the absorbance values were read at 517 nm. The results were expressed as TEAC that is, µmol of trolox equivalent per gram of fresh/OH treated matter.

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) assay

The antiradical efficiency was assessed, using the DPPH method, as described by Zhuang *et al.* (2012). In this approach, an ethanolic extract was prepared, fresh bell peppers were grounded, and triplicate samples (5 g) were taken. The samples with citrus pectin addition ranging from 0.1-0.3% were processed in extracts obtained by stirring with 75 mL of 80% ethanol at room temperature for 24 h, and subsequent filtering. The filtrates were concentrated by means of a rotary vacuum evaporator at 40 °C. The resulting extracts were used to determine the antioxidant activities. An aliquot of 0.4 mL of extract was taken and mixed with 2 mL of 0.1 mM DPPH methanol solution. The mixture was kept at room temperature in the dark for 30 min, and its absorbance was read at 570 nm using an UV-VIS spectrophotometer with double-beam Jenway, UK.

The DPPH radical-scavenging activity was calculated according to Equation (2):

% scavenging activity =
$$((A_{control} - A_{extract})/A_{control}) \times 100$$
 (2)

where $A_{control}$ = absorbance of the control, and $A_{extract}$ = absorbance of the extract.

Rheological and textural determinations

The bell pepper puree viscosity was measured by means of a Brookfield DV-E viscometer (Brookfield Viscometers Ltd, Harlow, UK). For the tests a LV2 (Liquid Viscosity) spindle was used, with the following characteristics: 18.72 mm diameter and 115 mm height. To study the rheological behavior of all purees, the viscosity (η) versus the shear rate (γ) was evaluated. The values of dynamic viscosity were measured at 25°C.

Texture measurements were performed at room temperature with a CT3 Texture Analyzer (BROOKFIELD, UK). The tested samples were packed into containers 45mm in diameter and 62 mm in height. An acrylic cylinder 38.1 mm in diameter

was used for the double penetration of the samples until 10 mm depth at a speed of 2 mm/s. The cell loading was 1000 g and the trigger load was 0.02 N. For the Ottawa cell analysis the device with the same name was used. Data registration and processing was possible by means of the CT3 Texture Analyzer software named TexturePro CT V1.5.

The simple compression was conducted by means of an Ottawa cell, which is built from an extrusion box, at the base of which a perforated plate is mounted. At the top of the texture analyzer cell a plunger is attached. During the plunger plunging into the cell, the sample is compressed and then extruded. The extrusion force expresses the firmness of the sample (Kilcast, 2013).

FT-IR analysis

The attenuated total reflectance (ATR) spectra were obtained by means of a Nicolet iS50 FT-IR spectrometer (Thermo Scientific, USA) equipped with a diamond crystal, and were plotted between 4000 and 400 cm⁻¹. The near-infrared (NIR) spectra were obtained by means of a Nicolet iS50 FT-IR spectrometer (Thermo Scientific, USA) equipped with an integration sphere, and were plotted between 10000 and 4000 cm⁻¹.

Confocal scanning laser microscopy

The microscopic analyses were assessed using a Zeiss confocal laser scanning system (LSM 710) equipped with a diode laser (405nm), Ar-laser (458, 488, 514 nm), DPSS laser (diode pumped solid state - 561nm) and HeNe-laser (633nm), allowing to have a deeper understanding of how the minimal non-invasive treatments affect the microstructure and texture of the purees. The samples stained with fluorescent dye Congo red (40 μ M) and DAPI (1 μ g/mL) (ratio 3:1:1) were observed with a Zeiss Axio Observer Z1 inverted microscope equipped with a x 40 apochromatic objective (numerical aperture 1.4). The 3D images were analyzed by ZEN 2012 SP1 software (black edition). All experiments were conducted in triplicate.

Statistical analysis

The values for the textural analysis were statistically analyzed using the T-test from Microsoft Excel.

Results and discussion

Physical measurements

The physical properties of foods are very important to the ohmic heating processing, which is based on the use of the electric field.

Electrical conductivity is the main specific property of the ohmic heating process. It is dependent on the material characteristics and the processing temperatures. The electrical conductivity measurement is also highly demanded because of the lack of similar studies on bell peppers. The determinations were conducted at 20 V/cm until the boiling temperature was reached.

For the same samples using the theoretical values of the characteristic data (L- the distance between the electrodes, Ae – the section between the electrodes and R –

the electrical resistance value) the values for electrical conductivities were calculated.

Also, to find out the correlation between the measured and the calculated electrical conductivities values the statistical method proposed by Bower (2009) was used.

The bell pepper purees with similar soluble contents were ohmically heated. The electrical conductivity of the bell pepper purees increased with temperature rise, linearly (Figure 1). The linear model has also been suggested by other researchers to describe the ohmic heating of apricot and peach purees by Icier and Ilicali, (2005b); red apple, golden apple, peach, pear, pineapple and strawberry by Sarang et al. (2008) and orange juice by Icier and Ilicali, (2005a); and lemon juice by Darvishi et al., (2011). The values of the correlation coefficient ($R_2 = 0.9298-0.9403$) are the main criterion for selecting the best model to evaluate the type of distribution for the electrical conductivities. This phenomenon can be sustained by the existence of the solid particles from the purees and the citrus pectin addition. The electrical conductivity values are influenced by the citrus pectin addition. Similar results have been reported by Goullieux and Pain, 2014, classifying hydrocolloids as substances with high electrical conductivity.

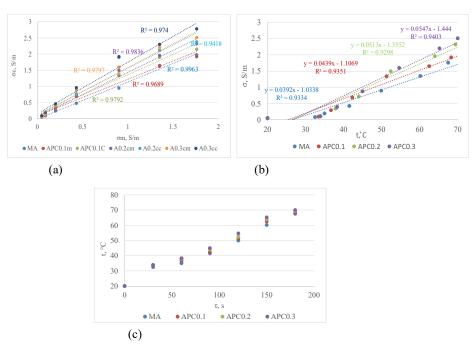


Figure 1. Electrical conductivity variation and correlation during ohmic heating: (a) electrical conductivity variation depending on temperature; (b) correlation of the calculated versus measured electrical conductivity; (c) temperature distribution during ohmic heating

Remarkable values of the electrical conductivity were measured in the case of the bell pepper purees with 0.3% citrus pectin addition (σ APC0.3 = 2.51 S/m). The correlation coefficient registered the highest values for all the samples, for the control sample (R²=0.9411) and APC0.3 (R²=0.9179).

The correlation between the measured and the calculated electrical conductivities seen in Figure 1.b. demonstrates the normal and continuous data distribution from a mathematical point of view. The correlation coefficients endorse the OH treatment efficiency. A good correlation of the data near to +1, indicates a very good data reproducibility.

The temperature values were measured during the ohmic heating for 180 seconds as it is shown in Figure 1.c. Since the electrical energy is converted into thermal energy during the heat treatment, the increase in temperature is dependent on the voltage gradient applied (20 V/cm). The rising temperature in the range 20-70° C depends on the OH time and exhibits a linear trend. It can be noticed that the processing time of bell peppers is reduced to 180 s. The evolution of the temperature according to the processing time remains stable correlated with the temperature gradient on each level, which is about 5-10°C. The uniformity of temperature distribution generates the heat in the entire product mass. In accordance with Icier in (2012), it was observed that this type of uniformity represents the main advantage of OH, which does not impose extremely high temperatures nor other limitations determined by the heat transfer coefficients.

Chemical determinations

The pH values for all the samples were registered for 30 days with a frequency of 5 days (Figure 2).

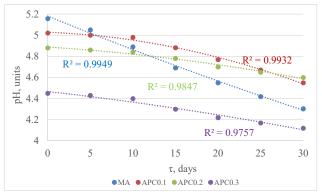


Figure 2. pH values registered during storage

According to other studies, the pH values varied from 6.2-5.1 9 (Shaha *et al.*, 2013 and Vega-Gálvez *et al.*, 2009). The sharpest decrease of the pH values is seen in the control sample (16%), while the sample with 0.3% citrus pectin addition (APC0.3) showed a decrease of 4%. The slight increase of the pH values are an expected effect after the 30 days of storage.

Total antioxidant activity

Table 1 shows the antioxidant capacity of the bell puree samples using the ABTS and DPPH assays. The antioxidant capacity of the puree extracts determined as the inhibition of the free radicals using the ABTS assay showed that the inhibition power is influenced by the ohmic heating. The citrus pectin addition generates stability in the antioxidant capacity of bell pepper purees. As it can be seen, in Table 1 the antioxidant activity of the ohmically heated samples ranged between almost 44 to 54% for ABTS assay, and from 29 to 50% for DPPH inhibition. The inhibition value for the raw control sample is almost 71%, and 44% for the ohmically treated sample respectively.

Table 1. ABTS and DPPH antioxidant capacity

Samples encoding	Inhibition of ABTS, %		Inhibition of DPPH, %		
	Raw samples	Ohmically heated samples	Raw samples	Ohmically heated samples	
MA	71.04 ± 0.0076	43.95±0.0238	71.46 ± 0.0035	28.53 ± 0.0068	
APC0.1	55.38 ± 0.0095	46.21 ± 0.0018	50.10 ± 0.0078	40.72 ± 0.0088	
APC0.2	70.90 ± 0.0047	51.76 ± 0.0019	53.00 ± 0.0012	46.99 ± 0.0089	
APC0.3	68.23 ± 0.0081	53.85 ± 0.0089	59.27 ± 0.0058	49.89±0.0029	

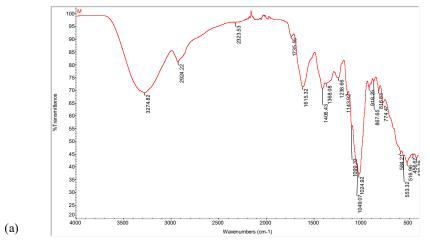
These results were similar with those obtained by Arslan and Özcan, (2011) for red bell-peppers, which are 25.37% higher than the results shown in Table 1 for sample APC0.3. It can be stated that after performing the experiments, the high antioxidant activity is due to the bioactive compounds, which are naturally present in bell peppers, like: carotenes, ascorbic acid, lycopene, resveratrol and lutein. The data obtained are comparable to those obtained by Chávez-Mendoza *et al.*(2015) in a study over different types of bell peppers. In a similar manner to the results obtained by the ABTS assay, the citrus pectin addition stabilizes the antioxidant content.

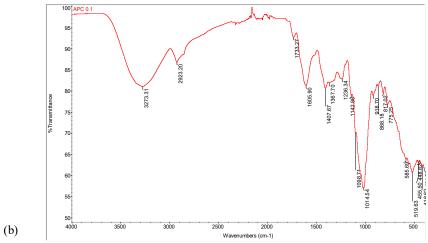
Analysis of FT-IR spectra

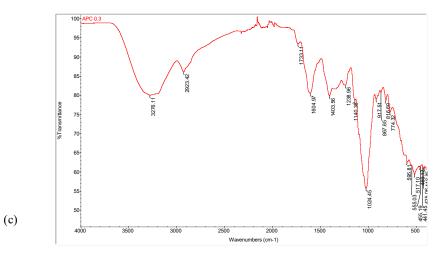
The OH induces a few changes in the bell pepper puree fingerprint region (1500-900 cm⁻¹), where the bands cannot always be attributed. Four new bands are observed, at 1049, 1099, 1143 and 1368 cm⁻¹, compared to the untreated sample (Figure 3).

The 0.3% pectin addition contributes to the disappearance of some of these bands. There is a singular band at 1143 cm⁻¹which is maintained. The molecular structure of the bell pepper puree was protected by the citrus pectin addition.

Specific augmentations are visible with the increased concentration of pectin, in the bands at 1605 and 1735 cm⁻¹. These are carboxyl and carbonyl groups from the pectin structure and the results are similar to those found by Manrique and Lajolo (2002), Kyomugasho *et al.*(2015). These findings confirm the fact that the OH does not affect the pectin structure, nor its functions.







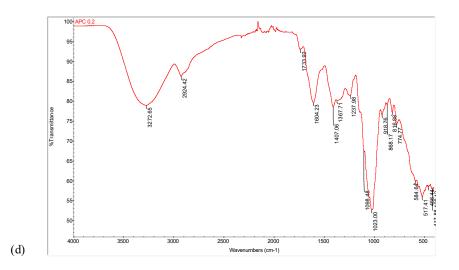


Figure 3. FT-IR spectra for (a) control sample, (b) bell pepper puree with 0.1% citrus pectin, (c) bell pepper puree with 0.2% citrus pectin, (d) bell pepper puree with 0.3% citrus pectin.

Rheological and textural determinations

The OH process efficiency can be observed in the behavior of the purees, which remains steady (Cogne *et al.*, 2003; Ditchfield *et al.*, 2004). These kinds of vegetable products, which cannot be defined by a single viscosity value at a specified temperature, are called non-Newtonian. The viscosity of these materials must always be correlated with a corresponding temperature and shear rate (Bolmstedt, 2000).

The ohmic heating process guarantees the volumetric transfer of the heat in products like puree characterized by high consistency (Icier and Tavman, 2006).

It is well known that the bell pepper naturally contains pectins, but their content decreases dramatically over the maturity period, causing a decrease in the dynamic viscosity of products like creams, purees, pastes (Ramos-Aguilar *et al.*, 2015). For this reason the citrus pectin addition is quite imperatively needed, especially for the consumers with dysphagia or other gastric diseases. The flow curves obtained at higher concentrations of citrus pectin are shown in Figure 4. As the citrus pectin concentration is increased, there is progressive development of 'shear thinning' (reduction in viscosity with increasing the shear rate).

This behavior could be explained by the structural breakdown of the molecules, due to the hydrodynamic forces generated, and the increased alignment of the constituent molecules just like Izidoro *et al.* stated in (2008). The highest concentration of pectin is represented by the highest value of dynamic viscosity APC0.3 (66.8 Pa·s) compared to MA (36.83 Pa·s).

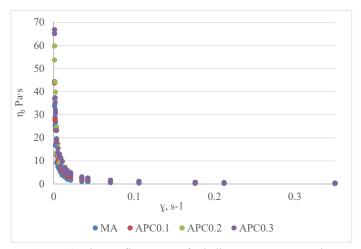


Figure 4. Viscous flow curves for bell pepper puree samples

In order to establish the influence of the thermal treatment (OH) and the pectin addition on the textural properties of the bell pepper purees, the following were tested: the control sample (MA) and the samples with 0.1-0.3% pectin addition (APC0.1, APC0.2, APC0.3).To test the firmness and the adhesiveness of the samples, the uniaxial double compression (TPA), and a simple compression (using the Ottawa cell) were used.

The Texture Profile Analysis revealed an increase in the firmness values by 115-275% (Table 2). Similar results were registered for the Ottawa cell analyses. The results reported by Ismail &Revathi (2006); Cepeda &Collado (2014) are in accordance with the results presented in this paper. The TPA values for firmness are smaller as compared to the same textural parameter determined by Ottawa cell, due to the difference of the dimensions of the contact surface between the plunger and the sample. The citrus pectin addition influenced the growth of the energy necessary to detach the sample from the plunger surface (adhesiveness). The TPA analysis allowed the determination of two other important textural parameters: cohesiveness and springiness.

Table 2. TPA and Ottawa cell parameters for bell pepper purees

Firmness, g	MA	APC0.1	APC0.2	APC0.3
TPA	0.40 ± 0.0033	0.86 ± 0.0066	1.37 ± 0.0025	1.5 ± 0.0065
Ottawa cell	0.7 ± 0.0025	1.00 ± 0.0058	1.66 ± 0.0016	2.23 ± 0.0084
Adhesiveness, mJ	MA	APC0.1	APC0.2	APC0.3
TPA	0.55 ± 0.0065	0.97 ± 0.006	1.33 ± 0.0019	2.13 ± 0.0067
Ottawa cell	1.07 ± 0.01	1.48 ± 0.0048	1.74 ± 0.0021	2.42 ± 0.0019

The cohesiveness values were influenced by the citrus pectin addition, which led to the increase of the bindings between the structural elements in the bell pepper purees (Table 3). So, the values were 10-60.7% higher than the control sample. Ismail and Revathi (2006) reported similar results for chili puree. The pectin addition improved the samples capacity to return to the initial form after the first compression cycle (springiness). The best results were obtained for the sample (APC0.3) with 0.3% citrus pectin addition.

Table 3. Textural parameters determined by TPA

Textural parameter, mu	Samples				
	MA	APC0.1	APC0.2	APC0.3	
Cohesiveness	0.28 ± 0.03	0.31 ± 0.06	0.39 ± 0.02	0.45 ± 0.08	
Springiness, mm	5.32 ± 0.65	6.88 ± 0.32	7.18 ± 0.58	7.6 ± 0.12	

Confocal scanning laser microscopy

Confocal laser scanning microscopy was used to visualize the ohmic heating effects on vegetable matrix components (Figure 5).

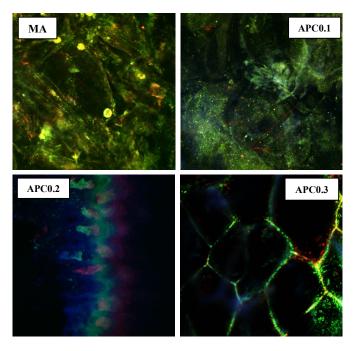


Figure 5. Confocal laser scanning images for bell pepper puree samples

The control bell pepper sample (MA) contains fragments of plant tissue with giant polygonal cells ($l=223.73\mu m \ x \ l=129.50\mu m$). The cellular components (nucleus, vacuoles, chromoplasts and chloroplasts) are also observed. In the APC0.1 sample, intact cells ($l=85.54 \ \mu m$) from the mesocarp with cytoplasmic organelles are visible. In the APC0.2 sample epidermis fragments can be observed, with a thick

cuticle layer (in green) located at the external, periclinal cell wall of epidermal cells. Formed on the outside of the primary cell wall, the cuticle has a complex and heterogeneous chemical composition: polysaccharides, mineral and phenolic elements (Guzmán-Delgado et al., 2016). The major components are cutin and cutan. Cutin is a polyester mainly formed of C16 and C18 fatty acid monomers (Fernández et al., 2016). Cutan is an insoluble compound made up of polymethylenic and polysaccharide fragments linked by non-hydrolyzable bonds, which also contains aromatic domains or carboxylic groups linked by ether or ester bonds with long-chain alcohols and long-chain carboxylic acids, respectively (Deshmukh et al., 2005). Cuticular waxes (in red on the right) are a mixture of compounds, such as long-chain fatty acids, alcohols, alkanes, esters or triterpenoids (Jetter et al., 2006). The parenchymatic cells from the bell pepper mesocarp with the chromoplasts are also present in APC 0.3, so we can say that the ohmic heating treatments are gentle, do not destroy the components of the vegetable matrices, and preserve their properties.

Statistical analysis

All experiments were conducted in triplicate and mean values were reported. Four groups of data were tested. The four groups consist in data for blank sample and the samples with 0.1-0.3% citrus pectin addition. The followed hypothesis is to see if the data are different from each other and if the differences are significant or not. It is important to know if the differences are influenced by the concentration of citrus pectin or the results are happening by chance. Hence, the hypothesis that the two sample sizes are equal and the data distributions have the same variance. The results showed significant differences between the expected values, due to the different pectin addition.

Conclusions

Bell peppers are a good source of antioxidants, carotenoids, flavonoids and polyphenols, which recommends them as a great vegetal raw material. The using of ohmic heating as an alternative heating method for bell pepper purees has enlighten some new findings. The electrical conductivity of the samples is strongly dependent on temperature variation and on citrus pectin concentration. Even so, there are still different aspects which are necessary to be known for the electrical properties of ohmic heated bell pepper purees. The natural bioactive (antioxidants) and biological (cell constituents) compounds are protected by the gentle effects of OH. It appears that the citrus pectin addition has a benefic influence on the rheological and textural properties of the bell pepper purees. Further clinical studies are needed in order to extend the study from laboratory to pilot and then industrial scale.

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