Ingeniería de alimentos

Revista Mexicana de Ingeniería Química

Vol. 12, No. 3 (2013) 391-400

EFFECT OF ELECTRIC FIELDS ON THE ACTIVITY OF POLYPHENOL OXIDASES

EFECTO DE LOS CAMPOS ELÉCTRICOS SOBRE LA ACTIVIDAD DE LAS POLIFENOL OXIDASAS

J.H. Castorena-García[1,2], F.J. Martínez-Montes[1], M.R. Robles-López[1,3], J.S. Welti-Chanes[4], H. Hernández-Sánchez[3*] and R.R. Robles-de-la-Torre[1]

[1]*Centro de Investigación en Biotecnología Aplicada, Instituto Politécnico Nacional, Carretera Tepetitla-Sta Inés, Tepetitla, 90700 Tlaxcala, México*

[2]*Laboratorio de Biotecnología, Instituto Tecnológico del Altiplano de Tlaxcala. Carretera Fed. San Martín-Tlaxcala, Km. 7.5, San Diego Xocoyucan, 90122, Tlaxcala, México.*

[3]*Depto. Graduados e Inv. Alimentos, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Carpio y Plan de Ayala, México, DF, México.*

[4]*Departamento de Biotecnología e Ingeniería de Alimentos, Instituto Tecnológico y de Estudios Superiores de Monterrey, Ave. Eugenio Garza Sada 2501 Sur, Col. Tecnológico, 68489, Monterrey, N.L. México.*

Received May 28, 2013; Accepted August 20, 2013

Abstract

The goal of this study was to evaluate the effect of Electric Fields (EF) on the activity of polyphenol oxidase (PPO) enzymes. EF were first applied to a commercial enzyme and then to an enzymatic extract from avocado. A fungal commercial PPO was treated at 10 kV/cm for 0-6 min and frequencies from 0 to 950 Hz; after the treatments the enzyme had a minimal residual activity (RA) of 12% after 6 min at 260 Hz. The avocado extract was treated at 18 kV/cm at frequencies from 0 to 760 kHz, and it was shown that the PPO was insensitive to frequencies higher than 1000 Hz, below this level, the enzyme was inactivated to a RA of about 15%. These results demonstrated the effectiveness of this Electric Field system on enzyme inactivation. It is important to mention that in this system there was no physical contact between the food sample and the electrodes and therefore the temperature remained constant throughout the processes.

Keywords : pulsed electromagnetic field, electric field, nonthermal technologies, avocado, polyphenol oxidase.

Resumen

El objetivo de este estudio fue evaluar el efecto de los Campos Eléctricos (EF) en la actividad de las enzimas polifenoloxidasas (PPO). Los EF se aplicaron primero a una enzima comercial y luego a un extracto enzimático de aguacate. Se trató una PPO comercial de origen fúngico a 10 kV/cm por 0-6 min a frecuencias que iban desde 0 a 950 Hz; después de los tratamientos la mínima actividad residual (RA) de la enzima (12%) se tuvo después de 6 min a 260 Hz. El extracto de aguacate se procesó a 18 kV/cm a frecuencias de 0 a 760 kHz y se pudo demostrar que esta PPO fue estable a frecuencias por arriba de 1000 Hz, por debajo de este valor la enzima pierde actividad hasta una RA del 15%. Estos resultados demuestran la efectividad del sistema de EF para la inactivación enzimática. Es importante mencionar que en este sistema no hay contacto físico entre la muestra de alimento y los electrodos y por lo tanto la temperatura permanece constante durante todo el proceso. *Palabras clave* : campos electromagnéticos pulsantes, campo eléctrico, tecnologías no térmicas, aguacate, polifenol oxidasa.

[*]*Corresponding author. E-mail*: hhernan1955@yahoo.com
Tel. 57-29-60-00 ext 62461, Fax ext 62463

1 Introduction

Heat treatment preserves food through the denaturation of the proteins and DNA of the deteriorative microorganisms present in it, however, heat also damages some of the food components, such as proteins, carbohydrates, vitamins, enzymes and fatty acids, and produces changes in the food quality attributes, e.g. color, flavor and texture (Fennema 1995). On the other hand, the consumers demand fresher and less processed food products. Since the second half of the 20th century, researchers have sought to develop new food preservation processes with less impact on its quality. The last ten years of the 20th century saw the emergence of new technologies that would lead to the introduction of non-thermal food preservation. These emerging techniques primarily include: high hydrostatic pressure, osmotic dehydration, pulsed light systems, ultrasound, irradiation, microwaves, and pulsed electric fields, (Rodríguez *et al*., 2003; Sun, 2005).

Food preservation by Pulsed Electric Field or PEF technology has been the subject of considerable investigation in research centers worldwide over the last twenty years (Toepfl *et al*., 2005), being used mainly for the inactivation of microorganisms and enzyme inhibition without altering the natural attributes of the food (Barbosa-Cánovas *et al*., 2001; Martin-Belloso and Elez-Martínez, 2005). The advantage of PEF treatment for foods is the short pulse duration. PEF treatment was initially applied only to liquids, although the system has now also been tested on semi-solid (Qinghua *et al*. 1994) and solid foods, including meat and seafood (Gudmundsson and Hafsteinsson, 2005), and the process has also been put to other uses (Knorr, 2003), such as the inhibition of fruit fly (Hallman and Zhang, 1997). In spite of the widespread research, by 2005 the process had still not been put into commercial scale (Toepfl *et al*., 2005); however, soon afterwards there were reports that PEF techniques were being used for the commercial preservation of juices (Clark, 2006).

A great deal of literature has now been written about the effect of PEF treatment on microorganisms and there are some very comprehensive revisions (Sitzmann, 1995; Barbosa-Cánovas *et al*., 1998; Sun, 2005; Vega-Mercado *et al*., 2007). Another area of interest on the use of PEF technology was the reduction of enzymatic activity. Other non-thermal technologies have also been used to inactivate enzymes (Serment-Moreno *et al*., 2012). Many papers have been written on the inhibition of enzymes from different sources, e.g. alkaline protease, phosphatase, lactoperoxidase, lipase,-amylase, glucose oxidase, peroxidase, polyphenol oxidase, lizosyme, pepsin, lactate dehydrogenase and pectin methylesterase (Vega-Mercado *et al*., 1995; Grahland, 1996; Ho *et al*., 1997; Yeom *et al*. 2000; Giner *et al*., 2001; Espanchs *et al*., 2003). As far as the action mechanism of PEF on enzyme inactivation is concerned, researchers generally agree that this mechanism is not fully understood. However, more recent studies have shown that the PEF may have an impact on the structure of the native protein, and it has been suggested that the PEF acts on the weaker bonds that give stability to the protein structure, such as the hydrogen bonds, together with other types of non-covalent forces, such as the Van der Waals and electrostatic interaction, leading to the destabilization of the enzyme and a loss of catalytic activity, (Ho *et al*., 1997; Yeom *et al*., 2002; Giner *et al*., 2002; Martin-Belloso and Elez-Martínez, 2005). PEF induce moderate changes in the secondary and tertiary structure of proteins such as α-lactalbumins, as determined by circular dichroism (Robles-López *et al*., 2012).

Another research groups have focused on the use of PEF treatment of plant tissue to induce pore formation and facilitate subsequent operations such as dehydration and solute extraction, resulting in yield increases (Knorr, 2003; Fincan *et al*., 2004; Lebovka *et al*., 2004; El-belghiti and Vorobiev, 2005; Amami *et al*., 2006).

All PEF-generating equipment consists mainly of two metallic electrodes, where the liquid is pumped through them, having a close contact with the food sample, and through which the intermittent electric current or pulses are discharged (Knorr *et al*., 1994; Martin-Belloso *et al*., 1994; Dunn, 1996). This simple description is not to be trivialized, because it accurately describes the type of system being used and defines the type of treatment, namely, as mentioned above, a discrete discharge of electrical current into the liquid, (Sitzman, 1995; McDonald *et al*., 2000; Vega-Mercado *et al*., 2007). However, parallel to PEF technology there have been some articles on a PEF related technology, named only as Electrostatic Field or Electric Field (EF). One of the first applications of EF was electrophoresis. Electrophoresis is a separations technique that is based on the mobility of ions such as proteins or DNA in an electric field (Aguilar *et al*., 2009).

Actually, it is necessary to distinguish between these two applications and understand the similarities

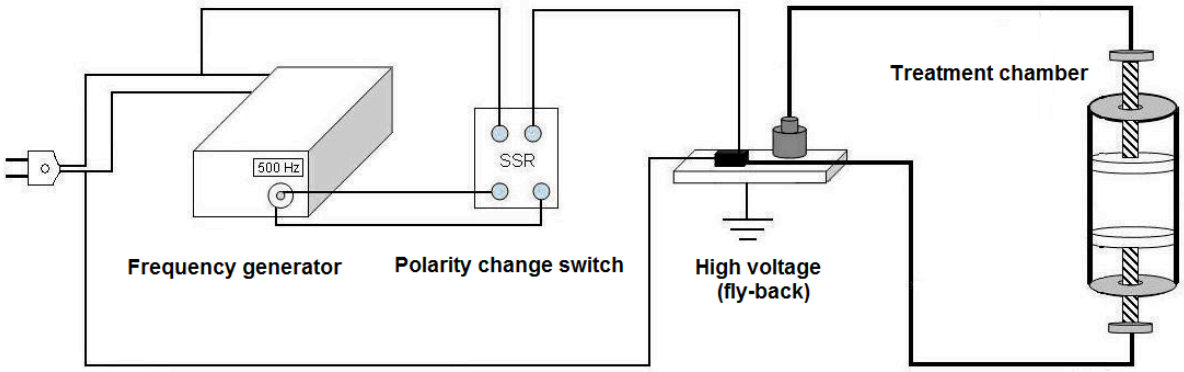

Fig. 1. Diagram of the Electric Field system with constant voltage, (18 kV).

and fundamental differences of these two types of technology based on a field of forces generated by electricity. The main observable difference between the two systems is the contact between the food sample and the electrodes, and as a consequence of that, the time of treatment in every system. EF requires longer application times for food treatment than those of electromagnetic fields or PEF (Esaki *et al*., 1996). Another key difference is that in PEF technology, there are electric current discharges, while in the EF technique there are not; in the EF there is only a field of electrical forces, acting at a distance, similar to the forces in a gravitational field.

Some applications of electric or electrostatic fields (EF) on food processing includes: the effect on wheat dough and bread properties (Aibara *et al*., 1992), effect on evaporation and drying, (Hashinaga *et al*., 1995), induction of structural changes in proteins (Dong *et al*., 1996), influence on the water activity of bread, (Esaki *et al*., 1996), changes in biological products and food preservation (Wei *et al*., 2008; Wang *et al*., 2009).

The aim of this work was to evaluate the effect of a new electric field generating equipment on natural and commercial PPO enzymes.

2 Materials and methods

2.1 Electric field systems

Two Electric Field systems were used in this work, both of them built by personnel of the CIBA-IPN, one of constant voltage (18 kV) shown in Figure 1 and another with variable voltage, using a commercial and integrated device of high voltage (Dielectric Test Set, Megger ®), which releases direct current (DC) in the range of 5 to 80 kV (Figure 2). Both apparatus were coupled with a function generator (BK precision ®) to vary the frequency and choose the waveform together with a solid-state relay (SSR, Crouzet ®) which allows to have, from the DC current, an alternate electric field.

The treatment chamber was made of acrylic with 2 mm thick for isolation, with external diameter of 10 cm and 29 cm long, the chamber allows only a batch operation. The electrodes were built of stainless steel and mirror polished with 0.505 cm thick, diameter of 9.6 cm, the array of these into the chamber was in parallel, one at the top and the other one at the bottom; each electrode was joined to an endless screw, which allowed to close up or down the electrodes to place different amounts of sample placed in a Petri dish. The voltage and frequency were chosen from the control unit to treat the sample. The intensity of the electric field is equal to the ratio of the voltage applied to electrodes to the distance between them.

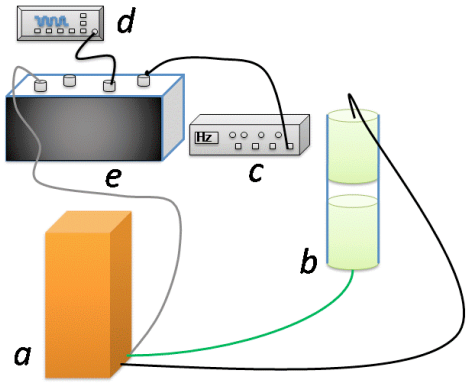

Fig. 2. Electric Field system with variable voltage, (a) source of high voltage, from 5 to 80 kV, (b) treatment chamber with stainless steel circular electrodes, (c) generator and modulator of frequency from 5 to 5,000,000 Hz, (d) oscilloscope, (e) control unit for voltage.

2.2 Test materials and effect of EF

Lyophilized and defatted avocado pulp was used as a source to extract the polyphenoloxidase (PPO). Since a good correlation exists between PPO activity in crude homogenates of the fresh fruit and the enzyme extracted from the acetone powder prepared from different varieties, the concentration of the enzyme in the extract was not determined for these experiments (Kahn, 1975). The enzymatic extract was treated at constant voltage (9 kV/cm) , treatment time (3 min) and wave square form, under a completely random experimental design, with frequency as the variable of study, at levels of 0.00, 0.01, 0.03, 0.05, 0.055 0.076 0.11, 0.14, 0.15, 0.25, 0.29, 0.38, 0.52, 0.56, 0.76, 0.78, 0.99, 1.1, 2.5, 3.8, 5.2, 5.5, 7.6, 11, 25, 38, 52, 76, 110, 380 and 760 kHz. The control sample was the 0.00 time. The sample was placed in a watch glass and taken into the chamber for treatment. The response variable was the residual activity (RA) of the enzyme PPO. The RA was measured adapting a methodology used by González *et al.* (1999), who used catechol dissolved in 0.1 M Tris-HCl buffer (pH 7.1) as substrate (mixture of reaction: 3 ml of catechol 5 mM + 0.2 mL enzyme extract). Absorbance readings were taken at 420 nm every 10 seconds for 2 minutes in a UV-VIS spectrophotometer (Hewlett Packard model 8453). A unit of enzyme activity was defined as the slope of the linear part of the curve of absorbance as a function of time. The RA was calculated according to Eq. (1) (Bendicho *et al.*, 2005).

$$(\%)RA = \left(\frac{p_c}{p_0}\right) \times 100 \tag{1}$$

Where p_c represents the units of enzymatic activity after treatment with the EF, p_o represents the units of enzymatic activity of the enzyme without receiving EF treatment, whereas *RA* sets the percentage of residual activity. The kind of effect (inhibition or activation) was evaluated with Eq. (2):

$$\text{Effect} = \left(\frac{p_c}{p_0} - 1\right) \times 100 \tag{2}$$

This "effect" is a modified residual activity in which the enzyme activity inhibition is indicated by a negative value in Eq. (2). A positive value will designate an activation effect.

Commercial polyphenoloxidase powder from mushroom (SIGMA Aldrich®) was dissolved into sodium and potassium phosphate buffer pH 7.1. Next, it was treated by EF at 10 kV/cm, time (0, 1, 2, 3, 4, 5 and 6 min.) and pulse frequency (0, 25, 100, 260, 540, 730 and 950 Hz), square waveform. In this work experimental data of each frequency were adjusted to first order kinetic model, according to Eq. (3).

$$RA = RA_0 e^{-kt} \tag{3}$$

Where RA_0 is the intercept of the curve, $-k$ is the kinetic constant of exponential decay of first order (min^{-1}) and t is the treatment time (min) for first-order kinetic model. Afterwards, the individual values of k were adjusted to a polynomial model to study the effect of frequency on the rate of enzyme inactivation.

3 Results and discussion

3.1 Effect of EF on the avocado enzymatic extract

The effect of EF on the RA of PPO extract from avocado after treatment at 9 kV/cm, 3 min and frequency from 0 to 760 kHz is shown in Figure 3, which proves that, with some exceptions, frequencies lower than 1000 Hz can inhibit or decrease the activity of polyphenoloxidase, whereas values higher than that can activate the enzyme. This apparently contradictory result has also been reported by Martín-Belloso and Elez-Marínez (2005). They found that some enzymes seem to activate with some PEF treatments; Vega-Mercado *et al.* (2007) attributed the activation or deactivation of enzymes to the conditions of treatments. With this, one can set the hypothesis that there will be a frequency at which a molecule will be more affected while at other frequencies the same molecule will present no sensitivity, or the molecule could be activated; it is possible that each molecule present different conditions of frequency where it can be active or inactive. The effectiveness of EF on the inactivation of enzymes is attributed to the possible conformational changes in the structure of the enzyme after treatment. The inactivation results are in agreement with studies reported by Giner *et al.* (2001), who treated the enzyme PPO from extracts of apple (Golden delicious) and pear (Blanquilla), with PEF intensities up to 22.3 and 24.6 kV/cm, the minimum RA achieved was 97 % in apple extract and 28 % in pear extract. Giner *et al.* (2002) treated PPO extract from peach with PEF at intensities from 2.18 to 24.3 kV/cm; the maximum RA of 30% was obtained with the highest intensity electric field.

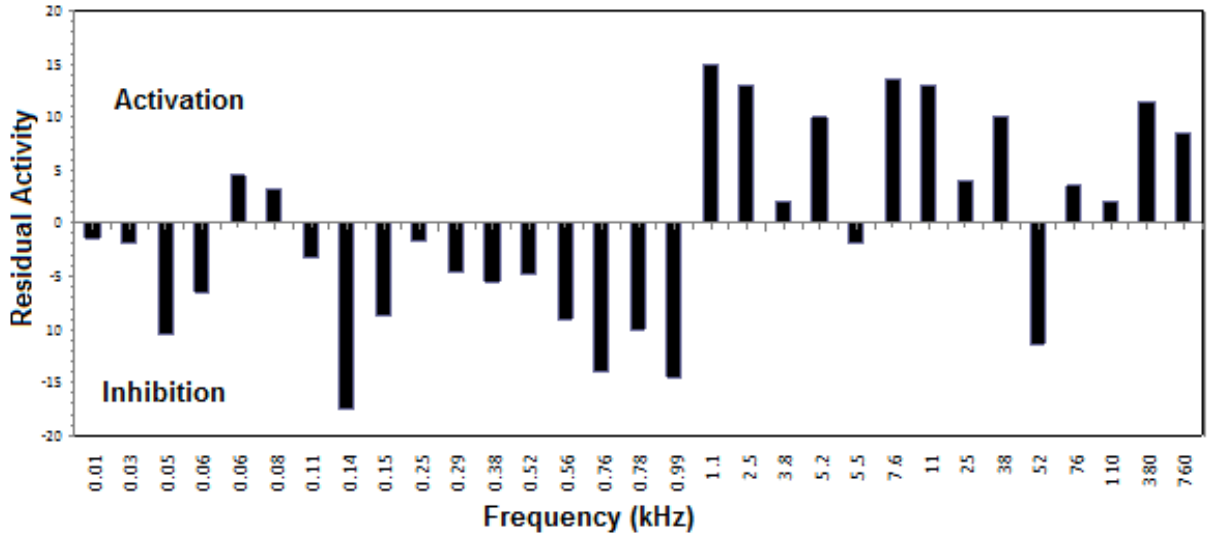

Fig. 3. Modified residual activity ("effect") of polyphenoloxidase extracted from avocado as a function of the EF frequency.

Zhong *et al*. (2007) treated a mushroom PPO in solution with PEF between 15.1 to 25 kV/cm, achieving a RA of 23.8 % at 25 kV/cm.

3.2 Effect of EF treatment on commercial polyphenoloxidase from mushroom

Once the range of working frequencies was defined, commercial PPO was treated at 10 kV/cm of EF and frequencies from 0 to 950 Hz and times from 0 to 6 min. The results of *RA* of commercial PPO are shown in Table 1; the minimal activity was 12% after 6 min and 260 Hz. The PPO inhibition by EF was in the range reported by Yang *et al*. (2004) who reached a RA of 38.2% with mushroom PPO at 33.6 kV/cm^{-1} while Zhong *et al*. (2007) reached 16.9% at 25 kV/cm and 10 Hz. Effects of time and frequency on the RA of PPO after EF treatment (10 kV/cm^{-1}) are shown in Figures 4 and 5. Figure 4 shows an exponential decay of the RA of the PPO enzyme as a function of time for every frequency, the adjustment of these data using equation 2 produces the value of the rate of inactivation of the enzyme, (*k*). The complete regression analysis is shown on Table 2. On the other hand, frequency is considered the third factor of importance after the strength of the EF and time of treatment. There are very few reports about the effect of frequency on inactivation of enzymes with electrostatic field technology; this work is an attempt to analyze the effect of the frequency of the electric field on enzyme inactivation. Figure 5 shows the effect of the EF frequency on the RA of PPO through the k value, the first order constant. The Figure shows a periodical behavior. The polynomial model of k as a function of frequency (f) was:

$$k = 0.18 + 1.44 \times 10^{-4} f + 4.79 \times 10^{-6} f^2 - 1.30 \times 10^{-8} f^3 + 8.45 \times^{-12} f^4 \quad (4)$$

The adjusted determination coefficient was 0.9826. It is important to emphasize that in the EF system used, there are no electrical pulses, there is no electrical current being discharged into the sample, there is no physical contact between electrodes and sample at all and therefore, the word frequency has a rather different meaning to the traditional PEF systems; here, two pieces of the equipment: the function generator and the SSR switch, enables the EF system to change the polarity at the electrodes, we call frequency to this change. In the case of a PEF system, it measures the frequency of the electrical current that is being discretely discharged or pulsed into the sample The physical effect of frequency during PEF treatments has been explained in terms of the polarization of molecules, caused by the electromagnetic field applied. If the change in the frequency applied is very high, the dipoles formed by the PEF or just the EF will not be able to continue to the external field and the polarization of orientation will not be produced. As above mentioned, frequency produced less effect on PPO activity than the electric field strength and time.

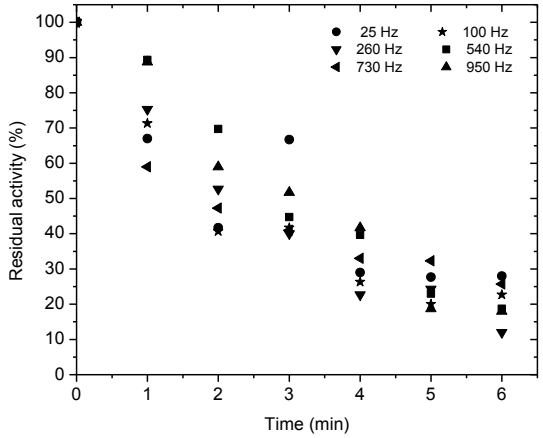

Fig. 4. Residual activity of polyphenoloxidase extracted from avocado as a function of the time of EF treatment at 10 kV/cm.

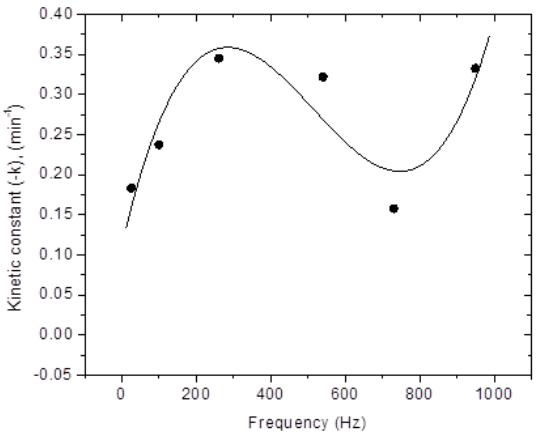

Fig. 5. Behavior of the kinetic constant (min-1) from 25 to 950 Hz.

Jeyamkondan *et al*. (1999) mentioned that the frequency of the pulse applied to foods, plays an important role into the energy added to the medium, an increase in pulse frequency implicates higher power supply consumption of the PEF system and consequently, an increase in the temperature of food as is usually observed. That same year, Fabregat *et al*. (1999) mentioned that sinusoidal factors produce sinusoidal responses. Afterwards, Venkatesh and Raghavan (2005) commented that the answer to the frequency from biological materials, is an electrical feature in EF treatment and depend of composition and environment.

Table 1. Residual activity of polyphenoloxidase in the commercial enzyme preparation under different EF treatment conditions at 10 kV.

Time (min)	Frequency (Hz)	(%) RA±s.d.	Time (min)	Frequency (Hz)	(%) RA±s.d.
1	25	67.0±3.61[a]	4	25	29.0±6.93
1	100	71.3±5.13	4	100	26.3±4.16
1	260	75.3±9.29	4	260	22.7±2.31
1	540	89.3±7.02	4	540	39.7±4.93
1	730	59.0±1.73	4	730	33.0±6.56
1	950	88.7±5.03	4	950	41.7±4.73
2	25	41.7±4.51	5	25	27.7±6.66
2	100	40.7±1.15	5	100	20.0±4.36
2	260	52.7±9.71	5	260	24.3±3.51
2	540	69.7±1.53	5	540	23.0±2.65
2	730	47.3±7.51	5	730	32.3±3.51
2	950	59.0±4.36	5	950	18.7±6.66
3	25	66.7±13.65	6	25	28.0±2.65
3	100	41.7±4.16	6	100	22.7±3.21
3	260	40.0±5.57	6	260	12.0±4.58
3	540	44.7±4.04	6	540	18.7±2.08
3	730	41.0±5.57	6	730	25.7±7.09
3	950	51.7±2.31	6	950	18.0±3.46

(%)RA: Residual Activity percentage

s.d.: standard deviation

[a] : *Mean value of tree data*

Table 2. Regression analysis of the exponential decay kinetics of the polyphenoloxidase activity with time of EF treatment at different frequencies

Treatment frequency (Hz)	$-k$ (min^{-1})	Regression standard error	Coefficient standard probability	Coefficient limit	95% lower confidence limit	95% upper confidence limit	R
25	0.1855	0.2898	0.0399	0.0003	0.1008	0.2702	0.7572
100	0.2401	0.2131	0.0294	0.00001	0.1778	0.3024	0.8363
269	0.3505	0.2404	0.0332	2.8×10^{-8}	0.2802	0.4208	0.9282
540	0.3228	0.1161	0.1161	8.6×10^{-13}	0.2888	0.3568	0.9808
730	0.1615	0.1593	0.0219	1.64×10^{-6}	0.1147	0.2081	0.8783
950	0.3378	0.2280	0.0315	1.01×10^{-8}	0.2711	0.4045	0.9371

Conclusions

By applying current knowledge, technology, and experience, it was possible to design and build electric field generating equipment, which was effective in inhibiting the growth of microorganisms, such as bacteria and fungi spores in real food (unpublished results), here, it was also possible to decrease the activity of the enzyme polyphenol oxidase, pure and from an avocado extract. As noted above, it is possible to develop processes for food preservation by non-thermal methods, preserving the natural qualities of fresh produce through treatments with some of the most promising emerging technologies such as the electric fields. It is emphasized that in this equipment the concept of force field is effectively applied, given the absence of physical contact between electrode and sample, so that in this type of equipment, there is not a temperature rise and therefore, it does not require cooling systems. The equipment built in this project can apply electric fields to liquid, semi-solids and solid foods. The equipment applications can be expanded considerably, for example, to whole nuts, pieces of meat or dairy products and to similar products. In addition, having demonstrated the effect on cellular products, enzymes, whole cells, bacteria, fungi, and fungal spores, it is possible to extend its application to other biological forms such as viruses.

Acknowledgments

The authors of this work acknowledge the financial support of FOMIX- Michoacán State Government through the grant 2005-C01-015. *Author Castorena-Garcia J. Hugo* would like to thank CONACyT for the scholarship for his graduate studies.

Nomenclature

f	electric field frequency KHz
k	enzyme activity exponential decay kinetic constant $min^{-}1$
p_0	enzyme activity of the untreated sample
p_C	enzyme activity after electric field treatment
RA	residual activity of the enzyme %

References

Aguilar, O., Rito-Palomares, M. and Glatz, C.E. (2009). Tridimensional soy protein characterization using two-dimensional electrophoresis and partition in aqueous phases. *Revista Mexicana de Ingeniería Química 8*, 57-65.

Amami, E., Vorobiev, E. and Kechaou, N. (2006). Modelling of mass transfer during osmotic dehydration of apple tissue pre-treated by pulsed electric fields. *Lebensmittel-Wissenschaft und Technologie 39*, 1014-1021.

Aibara S., Hisaki K. and Watanabe K. (1992). Effects of High-Voltage Electric Field treatment on wheat dough and bread-making properties. *Cereal Chemistry 69*, 465-467.

Barbosa-Cánovas, G.V., and Zhang, Q.H. (2001). *Pulsed Electric Fields in Food Processing: Fundamental Aspects and Applications*. CRC Press, Boca Ratón, USA.

Barbosa-Cánovas, G.V., Fernández, M., and Swanson, B. (2001). Pulsed Electric Fields: A novel technology for food preservation. *American Industry Hi Technology March/April*, 9-14.

Barbosa-Cánovas, G.V., Pothakamury, U.R., Palou, E. and Swanson B.G. (1998). *Nonthermal Preservation of Foods*. Marcel Dekker, New York, USA.

Bendicho S., Marsélles-Fontanet, A. R., Barbosa-Cánovas, G.V., and Martín-Belloso O. (2005). High intensity pulsed electric fields and heat treatments applied to a protease from *Bacillus subtilis*. A comparison study of multiple systems. *Journal of Food Engineering 69*, 317-323.

Clark, J. (2006). Pulsed electric field processing. *Food Technology 60*, 66-67.

Dong, X., Phillips, J.C., and Chulten, K. (1996). Protein response to external electric fields: Relaxation, hysteresis, and echo. *Journal of Physics and Chemistry 100*, 12108-12121.

Dunn, J. (1996). Pulsed light and pulsed electric field for foods and eggs. *Poultry Science 75*, 1133-1135.

El-belghiti, K., and Vorobiev, E. (2005). Modeling of solute aqueous extraction from carrots subjected to a pulsed electric field pre-treatment. *Biosystems Engineering 90*, 289-294.

Esaki, K., Ninomiya, F., Hisaki, K., Shibata, K., Murata, K., and Aibara, S. (1996). Effects of High-voltage electric field treatment on the water activity of bread. *Bioscience, Biotechnology and Biochemistry 60*, 1444-1449.

Espanchs, B., Martín-Belloso, O., and Barbosa-Cánovas, G. (2003). Microbial and enzymatic changes in fruit juice induced by high-intensity pulsed electric fields. *Food Reviews International 19*, 253-273.

Fennema, O.R. (1995). *Food Chemistry*. 2nd edition. Marcel Dekker Inc., New York, USA.

Fincan, M., De-Vito, F., and Dejmek, P. (2004). Pulsed electric field treatment for solid-liquid extraction of red beetroot pigment. *Journal of Food Engineering 64*, 381-388.

Giner, J., Gimeno, V., Barbosa-Cánovas, G. V., and Martín-Belloso, O. (2001). Effects of pulsed electric fields processing on apple and pear polyphenoloxidases. *Food Science and Technology International 7*, 339-345.

Giner, J., Ortega, M., Mesegué, M., Gimeno, V., Barbosa-Cánovas, G. V and Martín-Belloso, O. (2002). Inactivation of peach polyphenoloxidase by exposure to pulsed electric fields. *Journal of Food Science 67*, 1467-1472.

González, E., De-Ancos, B., and Cano, M. (1999). Partial characterization of polyphenol oxidase activity in raspberry fruits. *Journal of Agricultural and Food Chemistry 47*, 4068-4072.

Gossling, B.S. (1955). Patent 845,743. United Kingdom.

Grahl, T., Märkl, H. (1996). Killing of microorganisms by pulsed electric fields. *Applied Microbiology and Biotechnology 45, 148-157.*

Gudmundsson, M., Hafsteinsson, H. (2005). Effect of high intensity Electric Field Pulses on solid foods. In: *Emerging Technologies for Food Processing,* (D.W. Sun, ed.), Academic Press. San Diego, USA.

Gutiérrez-López, G. F. and Barbosa-Cánovas, G.V. (2003). *Food Science and Food Biotechnology*, CRC Press, Boca Ratón, USA.

Hallman, G. and Zhang, Q. (1997). Inhibition of fruit fly (Dipteratephritidae) development by pulsed electric field. *Florida Entomologist 80, 239-248.*

Hashinaga, F, Kharel, G.P. and Shintani, R, (1995). Effect of ordinary frequency high electric fields on evaporation and drying. *Food Science and Technology International 1*, 77-81.

Ho, S., Mittal, G. S., and Cross, J. D. (1997). *Effects of high field electric pulses on the activity of selected enzymes. Journal of Food Engineering 31*, 69-84.

Jeyamkondan, S., Jayas, D.S., and Holley, R.A. (1999). Pulsed electric field processing of foods: a review. *Journal of Food Protection 62*, 1088-1096.

Kahn, V. (1975). Polyphenol oxidase activity and browning of three avocado varieties. *Journal of the Science of Food and Agriculture 26*, 1319-1324.

Knorr, D. (2003). Impact of non-thermal processing on plant metabolites. *Journal of Food Engineering 56*, 131-134.

Knorr, D., Geulen, M., Grahl, T., and Sitzmann, W. (1994). Food application of high electric field pulses. *Trends in Food Science and Technology 5*, 71-76.

Lebovka, N.I., Praporscic, I., and Vorobiev, E. (2004). Effect of moderate thermal and pulsed electric field treatments on textural properties of carrots, potatoes and apples. *Innovative Food Science and Emerging Technologies 5*, 9-16.

Martín-Belloso, O., and Elez-Martínez, P. (2005). Enzymatic inactivation by pulsed electric fields. In: *Emerging Technologies for Food Processing*, (D.W. Sun, ed.), Academic Press. San Diego, USA.

Martín-Belloso, O., Zhang, Q., Castro, A., Barbosa-Cánovas, G. V. and Swanson, B. (1994). Revisión: Empleo de pulsos eléctricos de alto voltaje para la conservación de alimentos. *Revista Española de Ciencia y Tecnología de Alimentos 34*, 1-34.

McDonald, C., Lloyd, S., Vitale, M., Petersson, K., and Innings, F. (2000). Effects of Pulsed Electric Fields on microorganisms in orange juice using electric field strengths of 30 and 50 kV/cm. *Journal of Food Science 65*, 984-989.

Qinghua, Z., Chang, F., Barbosa-Cánovas, G. V. and Swanson, B. (1994). Inactivation of microorganisms in a semisolid model food using high voltage pulsed electric fields. *Lebensmittel-Wissenschaft und-Technologie 27*, 538-543.

Robles-López, M.R., Robles de la Torre, R.R., Camarillo-Cadena, M., Hernández-Arana, A., Welti-Chanes, J.S., and Hernández-Sánchez, H. (2012). Effect of pulsed electric fields on the structure of bovine α-lactalbumin. *Revista Mexicana de Ingeniería Química 11*, 373-382.

Rodríguez, J. J., Barbosa-Cánovas, G.V., Gutiérrez-López, G. F., Dorantes-Álvarez, L., Yeom, H. W., and Zhang, Q. H. (2003). An update on some key alternative food processing technologies: microwave, pulsed electric field, high hydrostatic pressure, irradiation and ultrasound. In: *Food Science and Food Biotechnology*, (G.F. Gutiérrez-López. and G.V. Barbosa-Cánovas, eds.), Pp. 279-312. CRC Press, Boca Ratón, Florida, USA.

Serment-Moreno, V., Mújica-Paz, H., Torres, J.A., and Welti-Chanes, J. (2012). Aplicación del método Monte Carlo para simular la inactivación de pectinmetilesterasa (PME) en jugo de naranja con procesos combinados de altas presiones hidrostáticas (APH) y temperatura. *Revista Mexicana de Ingeniería Química 11*, 363-372.

Sitzmann, W. (1995). High-voltage pulse techniques for food preservation, In: *New Methods of Food Preservation*, (G.W. Gould, ed.), Pp. 236-251. Springer, New York, USA.

Sun, D.W. (2005). *Emerging Technologies for Food Processing*. Academic Press. San Diego, USA.

Toepfl, S., Heinz, V., and Knorr, D. (2005). Overview of pulsed electric field processing for food. In: *Emerging Technologies for Food Processing*, (D.W. Sun, ed.), Academic Press. San Diego, USA.

Vega-Mercado H, Gongora-Nieto M.M., Barbosa-Cánovas, G.V., and Swanson, B.G. (2007). Pulsed electric fields in food preservation. In: *Handbook of Food Preservation*, 2nd edition, (M.S. Rahman, ed.), Pp 783-813, CRC Press, Boca Ratón, USA.

Vega-Mercado, H., Powers, J. R., Barbosa-Canovas, G.V., and Swanson, B.G. (1995). Plasmin inactivation with pulsed electric fields. *Journal of Food Science 60*, 1143-1146.

Venkatesh, M.S., and Raghavan, G. S. V. (2005). An overview of dielectric properties measuring techniques. *Canadian Biosystems Engineering/Le génie des biosystèmes au Canada 47 (7)*, 7.15 - 7.30.

Wang, G., Huang, J., Gao, W., Lu, J., Li, J., Liao, R., and Abdul, C. (2009). The effect of high-voltage electrostatic field (HVEF) on aged rice (*Oryza sativa L.*) seeds vigor and lipid peroxidation of seedlings. *Journal of Electrostatics 67*, 759-764.

Wei, S., Xiaobin, X., Hong, Z., and Chuan-Xiang, X. (2008). Effects of dipole polarization of water molecules on ice formation under an electrostatic field. *Cryobiology 56*, 93-99.

Yang, R., Li, S., and Zhang, Q. H. (2004). Effect of pulsed electric fields on the activity of enzymes in aqueous solution. *Journal of Food Science 69*, 241-248.

Yeom, H.W., Streaker, C.B., Zhang, H.Q., and Min, D.B. (2000). Effects of pulsed electric field on the quality of orange juice and comparison with heat pasteurization. *Journal of Agricultural and Food Chemistry 50*, 5107-5114.

Yeom, H.W., Zhang, Q.H., and Chism, G. W. (2002). Inactivation of pectinmethylesterase in orange juice by pulsed electric fields. *Journal of Food Science 67*, 2154-2159.

Zhong, K., Wu, J., Wang, Z., Chen, F., Liao, X., Hu, X., and Zhang, Z. (2007). Inactivation kinetics and secondary structural change of PEF-treated POD and PPO. *Food Chemistry 100*, 115-123.