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Microwave inactivation of red beet (*Beta vulgaris* L. var. conditiva) peroxidase and polyphenoloxidase and the effect of radiation on vegetable tissue quality

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ABSTRACT

The inactivation of polyphenoloxidase (PPO) and peroxidase (POX) in red beet by traditional and microwave (MW) blanching was studied. Microwave heating effects on color and texture were also studied.

Red beet subjected to MW blanching for 5 min at 100–200 W resulted in large weight losses accompanied by a high degree of shrinking. POX was the more heat resistant enzyme. The 90% destruction (*D* value) of the activity of both enzymes could be achieved only at 200 W within the 5 min frame employed for the tests.

When the samples were immersed in water and both the food sample and the water were submitted to MW exposure at 250–450 W or variable power with a maximum at 935 W, shrinking was avoided. The *D* value at 90 °C (reference; $D_{T_{ref}}$) and *z* could be determined after time–temperature corrections, and it was observed that, in general, $D_{T_{ref}}$ values for POX were smaller than for PPO. The microwave treatment (maximum power: 935 W) designed to provide a similar temperature profile to the one observed for traditional blanching (immersion in water at 90 °C), showed the smallest $D_{T_{ref}}$ value for POX inactivation. All treatments reduced elastic characteristics and changed the color of the tissues showing a shift to blue mainly in the case of microwave processes.

1. Introduction

Preservation treatments in use for fruits and vegetables include traditional technologies (i.e. freezing, dehydration, canning). Other alternative technologies, still under study or just of incipient application (i.e. electric pulses, high pressures, light pulses, microwaves) have begun to be internationally applied on vegetable foods (Geveke and Brunkhorst, 2003;Hayashi, 2002; Tewari and Juneja, 2007). The increasing demand by consumers for minimally processed (fresh-cut) fruits and vegetables has prompted the sale of pre-cut red beet roots as well as other vegetables in trays commercialized in markets or sold in bulk for institutions.

The application of microwave energy on agricultural production includes drying, insect control and seed germination (Venkatesh and Raghavan, 2004). In the food processing area, applications such as tempering, vacuum drying, freeze drying, dehydration, cooking, blanching, baking, roasting, pasteurization, sterilization and extraction are increasingly being perfected (Orsat et al., 2005). Microwave use has been studied for the blanching of vegetable tissues (Andres et al., 2004; Roberts et al., 2004), observing that the technique was useful for enzyme inactivation but more research is needed concerning its effect on nutrients, texture and color of fruits and vegetables.

Red beet (*Beta vulgaris* L. var. conditiva) is a traditional and popular vegetable in many parts of the world. It is especially rich in fibers as well as in sugars but with moderate caloric value. It has an important content of B-vitamins (B1, B2, B3 and B6) as well as folic acid. Red beet roots are consumed either fresh or after thermal processing or fermentation. The soluble and cell wall associated phenolics as well as betalains, the main pigments in red beet responsible for its reddish-purple hue, are bioactive compounds (Schwartz et al., 1980), due to their antioxidant capacity that is beneficial for human health (Kanner et al., 2001; Gliszczynska-Swiglo, 2006).

The enzymatic activity of vegetable tissues is one of the principal causes of food nutritional and organoleptical impairment. In the oxidative degradation of the phenolic compounds there are two relevant enzymes involved whose activity can lead to the production of brown polymers. These enzymes are the polyphenoloxidase (PPO) and peroxidase (POX) (Tomas-Barberán and Espin, 2001). The POX (sysname: donor hydrogen-peroxide oxidoreductase; EC 1.11.1.7) is one of the most heat-stable enzymes and as such it is often used as a marker for the adequacy of the blanching process (Barret and Theerakulkait, 1995;Hemeda and Klein, 1990; Flick et al.,

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1978). Tyrosinase or PPO (sysname: monophenol L-dopa:oxygen oxidoreductase; EC 1.14.18.1; PPO) is the key enzyme in melanin biosynthesis and in the enzymatic browning of fruits and vegetables. The role of PPO in the secondary metabolism of plants still remains unclear, but its implication in betalain biosynthesis has been proposed. PPO is a copper enzyme that catalyzes two different reactions using molecular oxygen: the hydroxylation of monophenols to o-diphenols (monophenolase activity) and the oxidation of the o-diphenols to o-quinones (diphenolase activity; Sanchez-Ferrer et al., 1995). This enzyme is widely distributed in plants, microorganisms, and animals where tyrosinase is responsible for melanization. Many studies (Richard-Forget and Gauillard, 1997; Subramanian et al., 1999) have demonstrated that while the PPO develops an oxidative action on the substrate, it gives origin to H_2O_2. Quinones are formed by oxidation and H_2O_2 is used by POX.

Tomas-Barberán and Espin (2001) suggested that one of the major concerns in the food industry is to prevent the development of enzymatic browning prior to or during the processing of fruit and vegetables, because formed products generate organoleptical alterations that preclude consumer acceptance of the product. The primary goal of blanching is the inactivation of undesirable enzymes present in fruits and vegetables and the reduction of the microbial load; at the same time, it aids in removing tissue gases, stabilizing texture, color, flavor and nutritional quality of products (Williams et al., 1986).The blanching process usually involves exposing plant tissue to some form of heat, for example through the use of steam or hot water (Barret and Theerakulkait, 1995). Inactivation of POX and PPO enzymes is often used to determine when the vegetable has been satisfactorily blanched considering the high resistance of both enzymes to thermal processing (Collins and McCarty, 1969; Vural Gökmen et al., 2005).

According to Chen et al. (1971), microwave energy may be used to blanch vegetable tissues as an alternative to conventional steam and water blanching. Microwave heating takes place in dielectric materials such as in foods due to the polarization effect of electromagnetic radiation at frequencies between 300 MHz and 300 GHz. Two frequency bands are allocated in the USA by the Federal Communications Commissions (FCC) for industrial, scientific, and medical applications. The 915 MHz band is used for industrial heating only, and the 2450 MHz band is used both in the industry and in the domestic microwave oven. Dielectric properties of foods play a critical role in determining the interaction between the electric field and the foods, and they are dependent on composition, temperature and microwave frequency. Hence, there is an important influence of the dielectric properties of the material on the efficiency of electromagnetic energy coupled into the materials, electromagnetic field distribution and conversion of electromagnetic energy into thermal energy within material (Tang, 2005). Matrix must contain dipolar or ionic species to enable heating to occur. The heating occurs via dipolar polarization and conduction.

Several researchers have studied the application of microwave radiation for blanching or enzyme inactivation (Quenzer and Burns, 1981; Kermasha et al., 1993a,b; Ramaswamy and Fakhouri, 1998; Begum and Brewer, 2001; Brewer and Begum, 2003; Roberts et al., 2004; Lin and Brewer, 2005; Lin and Ramaswamy, 2011) showing its effectiveness and suitability. From the results of Ramaswamy and Fakhouri (1998) and Ramesh et al. (2002) it is possible to observe that vegetable tissue blanched with microwave radiation retained better its nutritional value. Begum and Brewer (1996) reported that microwave blanched asparagus kept its nutritional value, taste and texture, as well as and often better than, asparagus blanched using traditional methods. In many studies reported, the time–temperature and time-power history of the samples was not properly monitored.

Venkatesh and Raghavan (2004) emphasized in their studies the microwave limitations that include the fact that, as materials are being processed, they often undergo physical and structural transformations that affect their dielectric properties. Thus, the ability of microwaves to generate thermal energy varies during processing. Sharp transformations in the ability of the microwave to generate heat can affect process success. An inherent problem associated with microwave heating is the non-uniformity of heating caused by an uneven spatial distribution of electromagnetic field inside the cavity (Vadivambal and Jayas, 2007). The success of microwave blanching depends mostly on raw material quality, timing and wattage of the process.

Absorption of microwave energy by plant tissue results in chemical and physical changes. The intermolecular friction from microwave heating may cause internal cell pressure leading to rupture resulting in a loss of cell content and organization (Quenzer and Burns, 1981). For that reason it is necessary to perform specific studies for different tissues, trying to state a systematic background of information that allows to find the optimum conditions (i.e. time, power level) that permit to attain the goal of the process with minor nutritional and organoleptical damage.

The aim of this work was to evaluate the characteristics of PPO and POX inactivation in red beet by traditional and microwave blanching. The evaluation of color and texture of the samples was also performed.

2. Materials and methods

2.1. Chemicals

They were of analytical grade unless stated.

2.2. Sample preparation and blanching process

Red beet (*B. vulgaris* L. var. conditiva) roots harvested in Argentina were purchased at the local market. They were carefully cleaned, peeled and cut into 10 mm-thick slices perpendicular to their longitudinal axis. Cylindrical samples with a diameter of 15 mm were then obtained from each slice at about 7–10 mm distance from the periphery, by using a cork borer. This sample geometry was chosen by considering its adequacy for mechanical assays.

2.3. Microwave blanching

Batch microwave processing was carried out in a microwave system ETHOS Plus (Milestone Srl, Sorisole, Italy) with a magnetron of 2450 MHz. The microwave used the ATC-400 system for continuous monitoring and control of the internal temperature. The optical sensor used was fitted in a teflon coated ceramic thermowell. The samples were distributed in six glass vessels which were placed inside a polypropylene container of cylindrical shape having a diameter of 27.9 cm diameter and a height of 19.7 cm (Fig. 1). A 360° alternate movement was programed for the container in the microwave cavity to avoid bending of the sensor con-

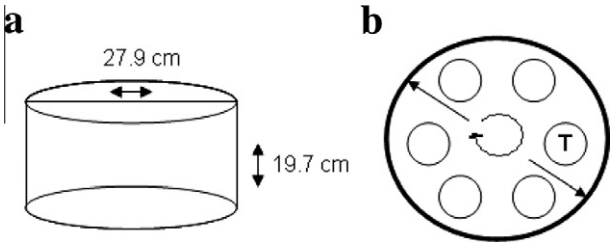

Fig. 1. Scheme of the cylindrical polyprolylene container used into the microwave system (a) and of its upper view (b) which shows the localization of the six vessels. T: means the initial localization of the temperature sensor.

nection during experiments while assuring a more homogeneous treatment of the samples. The container was covered with a lid that served as supporter of the ceramic coated optical fiber.

For each batch, the thermal sensor was placed in the center of one of the tissue samples put inside of one of the vessels, and the temperature profiles were recorded during microwave processing. Previous assays showed that the temperatures in the center and periphery of each cylinder did not show significant differences for sample shape used; this may be ascribed to the small diameter of individual cylinders. It was determined that differences greater than 5% were not observed in the temperature of the different samples of one vessel or for samples placed in different vessels or belonging to different runs performed under the same conditions. Probably, the 360° alternate movement contributed to the homogeneity of temperature.

Two different experimental designs were used. In the first one, cylinders comprising a total weight of ≅36 g were distributed in the six different vessels used (3 cylinders of 1.5 × 1 cm or ≅6 g were placed in each vessel). In the second one, 36 g of material distributed as previously explained were immersed in a 300 ml-total volume of deionized water distributed among the mentioned vessels for performing the experiment. The amount of water used in this experiment was the minimum necessary for the coverage of the tissue in each vessel. The same ratio tissue/water was then used for traditional blanching with the objective of better comparing both treatments.

After each treatment, samples were immersed rapidly in an ice/water bath (for 5 min) to stop the blanching effect. Non-treated cylinders (raw material) were used as control samples.

In the absence of water, treatment was performed at output powers of 100 W, 150 W or 200 W for 5 min. For this treatment, weight loss and diameter change (as an expression of shrinking) were evaluated at the end of the treatment.

In the presence of water, two different experimental situations were evaluated:

a) Constant output powers of 250 W, 350 W or 450 W. Temperature profiles of tissue samples and water bath used for immersion and the power evolution were recorded during, at least, the necessary time for achieving an enzyme activity reduction of 90%, in each case.
b) The equipment was programed with changing powers throughout the experiment so as to obtain, in the center of the tissue cylinders, a similar temperature profile to the one observed for water blanching at a constant temperature of 90 °C. This treatment is named 90 MW. Temperature profiles of sample cylinders and water bath, and the output power evolution were recorded during the time required for achieving an enzyme activity reduction of, at least, 90%.

For all microwave treatments, a minute of venting was applied.

Three replicates per experimental situation were performed. One of them was used for textural and color studies; the other two were used for biochemical determinations.

2.4. Water blanching

Conventional thermal treatment was performed by immersion of tissue cylinders in a water bath at 90 °C-constant temperature. This treatment is named 90C. During heating, temperatures were simultaneously recorded every 10 s for the water bath and for the central point of each tissue cylinder, by using thin-wire copper-constantan thermocouples. These determinations were performed during the time required for achieving an enzyme activity reduction of, at least, 90%. The ratio tissue/water was the same one used for microwave treatments. The assays were carried out in duplicate.

2.5. Enzyme analyses

The samples (control and treated) were directly used to determine enzyme activities.

2.5.1. Acetonic extract for enzyme quantification

Following Walton and Sondheimer (1968), raw tissue of red beet root was homogenized using a Sorvall Omni Mixer (Sorvall, Norwalk, CT, USA) for 2 min, with 4 °C-pre-cooled acetone (Merck Argentina S.A., Buenos Aires, Argentina) in a ratio 1:4 w/v. The homogenate was then filtered through a Büchner funnel using filtering paper (Whatman GF/C, UK). The residue was washed twice with acetone; the solvent was then eliminated, overnight at room temperature and under the laboratory hood. The powder obtained was stored in a freezer (−80 °C) until its usage for enzyme activity determination. An extract was obtained from each replicate.

2.5.2. Protein content

The protein content was determined in each extract obtained according to Lowry et al. (1951) using protein bovine serum albumin (Sigma, St. Louis, MO, USA) as the standard. The determination was performed in triplicate.

2.5.3. Peroxidase (POX) activity

The powder (≅0.5000 g) was suspended in ≅20 ml of 0.05 M phosphate buffer (pH 7.0). Sodium chloride was added until reaching a 1 M-concentration in the suspension. The system was maintained under stirring for 1 h at 5–7 °C and then was centrifuged (Model 5804R, Eppendorf AG, Hamburg, Germany) at 9000 rpm for 15 min (6 °C). The supernatant was assayed for total POX activity, which was determined at 25 °C as described by Marangoni et al. (1995), using guaiacol (o-methoxyphenol; Anedra SA, Buenos Aires, Argentina) as substrate and expressing the activity as the change in absorbance at 470 nm (Absorbance Units, AbU) per minute and milligram of protein (AbU min^{-1} mg^{-1} protein); absorbance was measured with a spectrophotometer (Shimadzu UVmini-1240, Japan). The supernatant of each enzymatic extraction was evaluated in triplicate.

2.5.4. Polyphenol oxidase (PPO) activity

The powder was used for evaluation of PPO activity according to Coseteng and Lee (1987) and Liu et al. (2008a). The powder was suspended in 0.1 M phosphate buffer (pH 6.0) in a ratio 1:40 w/v; sodium chloride was added until reaching a 1 M-concentration in the suspension. The system was maintained under stirring for 45 min at 5–7 °C and was then centrifuged (Model 5804R, Eppendorf AG, Hamburg, Germany) at 9000 rpm for 10 min (7 °C). The supernatant was assayed for total PPO activity, at 25 °C by using 40 mM-pyrocatechol (Merck, Buenos Aires, Argentina) in 10 mM phosphate buffer (pH 7.0). The activity was evaluated following the change in absorbance at 420 nm with a spectrophotometer (Shimadzu UVmini-1240, Japan). One unit of activity was defined as the change in absorbance measured at 420 nm per minute and milligram of protein. The supernatant of each enzymatic extraction was evaluated in triplicate.

2.6. Destruction modeling

The inactivation of enzymes PPO and POX is often described by a first order reaction (Matsui et al., 2008) as can be observed in Eq. (1) where A_0 (AbU min^{-1} mg^{-1} protein) is the initial enzyme activity and A (AbU min^{-1} mg^{-1} protein) is the remaining activity after

blanching at a constant temperature (°C) for a holding time, t (min), being k the inactivation rate constant:

$$\ln\left(\frac{A}{A_0}\right) = -k.t \quad (1)$$

Other parameters are also used to model this destruction such as the D, being $D = 2.303/k$. This model for enzymatic inactivation is presented in Eqs. (2) and (3) for isothermal processing at temperature T and a holding time t (Viera et al., 2002;Matsui et al., 2008):

$$\ln\left(\frac{A}{A_0}\right) = -\frac{2.303}{D}t$$

or

$$\log\left(\frac{A}{A_0}\right) = -\frac{1}{D}t \quad (2)$$

The D value is the time necessary to accomplish 90% reduction of the enzymatic activity under isothermal conditions. The effect of temperature on the D value is expressed as the z value, which is the temperature increase that reduces the D value by 90% as can be observed in the following equation:

$$D = D_{T_{ref}}.10^{\frac{(T_{ref}-T)}{z}} \quad (3)$$

where $D_{T_{ref}}$ is the value of D at the reference temperature (T_{ref}).

According to Matsui et al. (2008), for non-isothermal conditions and assuming first order destruction, the following equation can be used:

$$t_{equiv.} = \int_0^{\infty} 10^{\frac{T(t)-T_{ref}}{z}} dt \quad (4)$$

where $t_{equiv.}$ is the isothermal holding time at a reference temperature (T_{ref}) that results in the same lethal effect on the enzyme as the one observed for the real temperature–time history suffered by the studied product, which is a non-isothermal processing.

As a consequence, it can be said that (Matsui et al., 2008; Deng et al., 2003):

$$\log\left(\frac{A}{A_0}\right)_{\text{model}} = \frac{-t_{equiv.}}{D_{T_{ref}}} = \frac{\int_0^{\infty} 10^{\frac{T_{ref}-T(t)}{z}} dt}{D_{T_{ref}}} \quad (5)$$

In this research, the residual enzymatic activity was numerically evaluated through Eq. (5) using an initial estimate for parameters $D_{T_{ref}}$ and z. Using a slightly different approach, Tajchakavit and Ramaswamy (1997) have obtained time–temperature compensated kinetic parameters by evaluating time corrected D values at each temperature. For the use of Eq. (5), a temperature of 90 °C was used as the reference temperature (T_{ref}) because it was the highest temperature achieved or was within the range of study (Matsui et al., 2008), and the heating and venting periods of the microwave procedure were considered for the calculation. The 90 °C temperature was coincident with the temperature used for the traditional blanching performed. The nonlinear estimation procedure was used to minimize the sum of squared errors (SSE) between experimental $[\log(A/A_0)_{\text{exper}}]$ and predicted $[\log(A/A_0)_{\text{model}}]$ residual enzymatic activities as is defined in Eq. (6), where n is the number of experimental runs (Matsui et al., 2008):

$$\text{SSE} = \sum_{i\to 1}^{n}\left[\log\left(\frac{A}{A_0}\right)_{\text{exper}} - \log\left(\frac{A}{A_0}\right)_{\text{model}}\right]^2 \quad (6)$$

obtaining the $D_{T_{ref}}$ and z for each treatment using all temperature and time experimental data for each case.

The integral in Eq. (4) was evaluated numerically using the trapezoidal method and the reference temperature previously cited and the z calculated as previously indicated. This procedure allowed to obtain the $t_{equiv.}$ or t_{eff} (effective time) for each case of interest.

2.7. Color evaluation

Color evaluation was performed in tissue samples when 90% inactivation of both enzymes was achieved.

The color of red beet samples was assessed, using a colorimeter (Minolta Co. Ltd., Osaka, Japan) with illuminant D65 and 10° observer angle. Each sample was placed onto a white tile and values of Commission Internationale de l'Eclairage (CIE) color space coordinates L^* a^* b^* were acquired, where L^* represents the lightness, a^* the grade of greenness/redness and b^* the grade of blueness/yellowness. The procedure was performed in ten tissue cylinders for each treatment, when 90% inactivation of both enzymes was achieved.

The total color difference (TCD) was evaluated according to Cruz et al. (2007):

$$\text{TCD} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (7)$$

Being:

$$\Delta L^* = L^* - L_0^*$$

$$\Delta a^* = a^* - a_0^*$$

$$\Delta b^* = b^* - b_0^*$$

L_0^*, a_0^* and b_0^* are the readings at zero time (control tissue), and L^*, a^* and b^* are the readings at each processing time ($t > 0$).

2.8. Mechanical assays

Mechanical evaluation was performed in tissue samples when 90% inactivation of both enzymes was achieved.

2.8.1. Compression

A normal uniaxial compression test was performed using an Instron Testing Machine model 3345 (Instron Corp., Norwood, Ma, USA) provided with a 5000 N-load cell and a 30 mm-diameter upper steel plate. For each treatment, ten cylinders (15 mm-diameter; 10 mm-height = H_0) were assayed. Force–deformation curves were recorded up to fracture at a crosshead speed of 10 mm/min. The measurements were normalized calculating:

(1) the tissue firmness (*Firm*) as the ratio between force ($F_{failure}$) and deformation ($d_{failure} = H_0 - H$, being H the final height) at the first peak of failure:

$$Firm = \frac{F_{failure}}{d_{failure}} \quad (8)$$

(2) the ratio of firmness of each sample ($Firm_{treat}$) and firmness of the control (raw) red beet tissue ($Firm_{raw,tissue}$):

$$\frac{Firm_{treat}}{Firm_{raw.tissue}}$$

2.8.2. Relaxation

For relaxation tests, ten specimens were compressed, as above indicated, up to 10% of deformation at a crosshead speed of 10 mm/min. This applied deformation was the lowest one that allowed for the recording of the Instron Machine response without macroscopic tissue failure when raw or treated red beet cylinders were compressed. At this preset deformation level, the crosshead was stopped and the relaxation force $F(t)$ was recorded for, at least, 10 min. Relaxation forces obtained were normalized dividing $F(t)$

by the initial relaxation force (F_0). Curves of normalized forces ($F(t)/F_0$) as a function of relaxation time were fitted to the generalized Maxwell model (Peleg and Calzada, 1976; Nussinovitch et al., 1989):

$$\frac{F(t)}{F_0} = \frac{F_\infty}{F_0} + \sum_{i=1}^{n} \frac{F_i}{F_0} \exp(-t/\tau_i) \quad (9)$$

and the normalized parameters were obtained: the relative force at infinite time of relaxation (F_∞/F_0) which represents the free spring of this mechanical model, the *i-esim* Maxwell relative-force component (F_i/F_0) associated to the corresponding characteristic relaxation time (τ_i) of the *i-esim* Maxwell's body.

2.9. Statistical analysis

The results are reported as the average and standard deviation. Data were subjected to analysis of variance ANOVA (α: 0.05) followed by pair multiple comparisons evaluated by Tukey's significant difference test, using the Statgraphic package (Statgraphic Plus for Windows, version 5.0, 2001, Manugistic Inc., Rockville, MD, USA). Regressions were performed using the same software, or the EXCEL solver (Microsoft, USA).

3. Results and discussion

3.1. Preliminary assays – Microwave treatment without water soaking

When microwave radiation was used for the blanching of red beet tissue cylinders, it was observed that 5 min of treatment in the 100–200 W range were not enough for accomplishing, at least, 90% of POX and PPO inactivation except at 200 W (Fig. 2). As can be observed, POX was the most resistant enzyme at any microwave condition assayed.

The maximum temperature attained increased with the power applied and 73 °C was the highest one observed for 200 W.

Weight losses ranging from 9% to 25% and shrinkages between 6% and 17% occurred after microwave treatments, both increasing with the power applied (Table 1). In particular, at 5 min of microwave treatment at 200 W, a 90% enzyme inactivation was attained (Fig. 2) but the red beet tissue cylinders showed a high degree of deformation that precluded the application of microwave blanching due to tissue damage.

Akarapu et al. (2004) developed an integrated electromagnetic and thermal model for describing the microwave thermal processing. For their experiments, a whey protein gel package of ($140 \times 100 \times 30$) mm^3 was fixed at the center of the bottom part of the applicator. They observed that the microwave power was absorbed at the two ends of the food package and the highest temperatures were achieved in those positions. From the edge towards the middle, the heating intensity was reduced while there seemed to be a relatively strong heating near the center of the food package. When the food package was immersed in water and both, the food package and the water were heated by the microwaves; irradiation was absorbed in the central region of the food package, which caused the hot spot to occur in the central region of the package. The results suggested that the surrounding water helped to redistribute the microwave heating power so that the power was delivered to the central region of the food package. They concluded that the microwave heating is more effective when the food package is not immersed in water. However, with the presence of water, the difference between the highest and lowest temperatures in the package was about 30 °C smaller, in 10 min of treatment. This phenomenon should be expected because water helps to redistribute the microwave energy and makes the field distribution relatively more uniform in the food package, in addition to shifting the heating pattern. According to these conclusions and for the purpose of trying to inactivate enzymes minimizing tissue damage, the following experiments were performed with water present in the cavity.

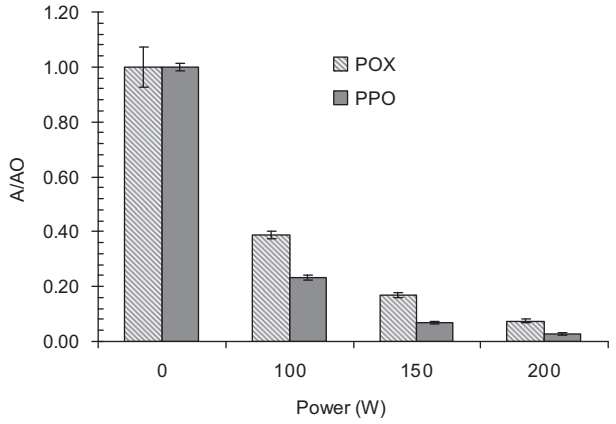

Fig. 2. Remaining peroxidase (POX) and polyphenoloxidase (PPO) activity (A/A_0) determined after 5 min of red beet tissue cylinder exposure to microwave treatment performed either at 100 W, 150 W or 200 W, without immersion in water. Bars indicate the standard deviation for $n = 3$.

Table 1
Shrinkage degree and weight loss suffered by red beet root samples after microwave treatment at 100 W, 150 W or 200 W power for 5 min.

Microwave treatment	Shrinkage degree[a] (%)	Weight loss[a] (%)
100 W	6 ± 3	9.4 ± 0.6
150 W	13 ± 2	17.3 ± 0.3
200 W	17 ± 3	24.7 ± 0.5

[a] Average and standard deviation ($n = 18$) are informed.

3.2. Microwave treatment performed under water soaking

Preliminary tests as well as bibliographic background gave origin to a second set of experiments involving the presence of water for tissue immersion during microwave blanching. In these studies, microwave blanching at 250, 350 or 450 W was carried out, as well as a microwave processing (90 MW treatment) performed programing the equipment for obtaining a similar temperature profile to that recorded during traditional blanching performed by heat conduction in the sample (90C treatment).

3.2.1. Inactivation modelling

From the experimental runs obtained for each enzyme and treatment, the predicted residual enzyme activity was calculated numerically through Eq. (5) using an initial estimate for parameters $D_{T_{ref}}$ and z. The minimization of the error through Eq. (6) produced the optimum values of the parameters, which are reported in Table 2.

Fig. 3 shows the residual activities of POX and PPO vs effective times (t_{eff}) as well as the quality of the model fitting for 450 W and traditional blanching (90C), as an example. Data were, in general, well fitted to semi-logarithmic linear model ($R^2 \geqslant 0.84$; α: 0.05) for all treatments.

Fig. 4 presents the temperature–time profiles observed during blanching of red beet tissue cylinders. Tissue temperature increased faster with the increment in microwave power. There was an upsurge in temperature for traditional blanching (90C) similar to that obtained for 90 MW microwave treatment (changing power with a maximum power of 935 W).

Table 2
Red beet POX and PPO inactivation. For the different treatments, the adjusted kinetic parameters ($D_{T_{ref}}$ and z) are reported. The temperature of 90 °C was used as reference temperature.

Treatment	$D_{T_{ref}}$ (s)[a]	z (°C)[a]	SSE[b]
POX			
250 W	POX: 47	POX: 68	POX: 0.187
350 W	POX: 3	POX: 24	POX: 0.063
450 W	POX: 7	POX: 39	POX: 0.124
90 MW	POX: 2	POX: 5	POX: 0.017
90C	POX: 24	POX: 3	POX: 0.175
PPO			
250 W	PPO: 38	PPO: 57	PPO: 0.220
350 W	PPO: 8	PPO: 30	PPO: 0.037
450 W	PPO: 8	PPO: 39	PPO: 0.169
90 MW	PPO: 8	PPO: 25	PPO: 0.289
90C	PPO: 44	PPO: 80	PPO: 0.029

[a] Calculated according to Eqs. (5) and (6).
[b] Calculated according to Eq. (6).

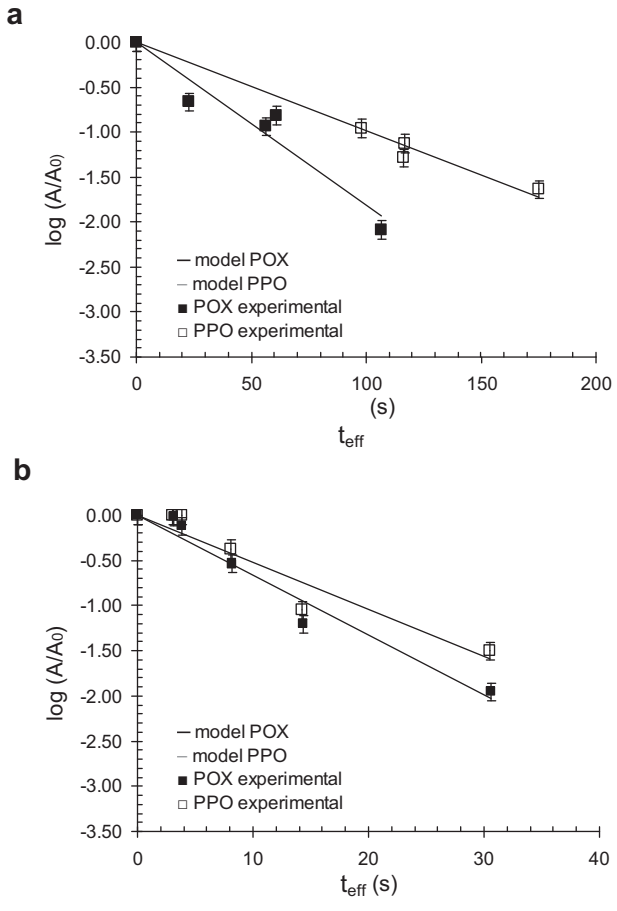

Fig. 3. Residual peroxidase (POX) and polyphenoloxidase (PPO) activity (A/A_0) plotted against the effective time (t_{eff}) calculated from Eq. (4) at a T_{ref} = 90 °C for (a) traditional blanching (90C), and (b) 450 W microwave treatment. Bars correspond to the errors involved in the determination of the enzymatic activity after processing.

As can be observed in Table 2, POX was less resistant to destruction ($D_{T_{ref}} = 24$ s) than PPO ($D_{T_{ref}} = 44$ s) when traditional blanching was performed by immersion in water at 90 °C (90C treatment). When 90 MW microwave processing was used, a similar temperature profile was obtained (Fig. 4) as above mentioned, but the $D_{T_{ref}}$ values for POX and PPO were approximately one order of magnitude smaller than for 90C. As microwaves can transfer

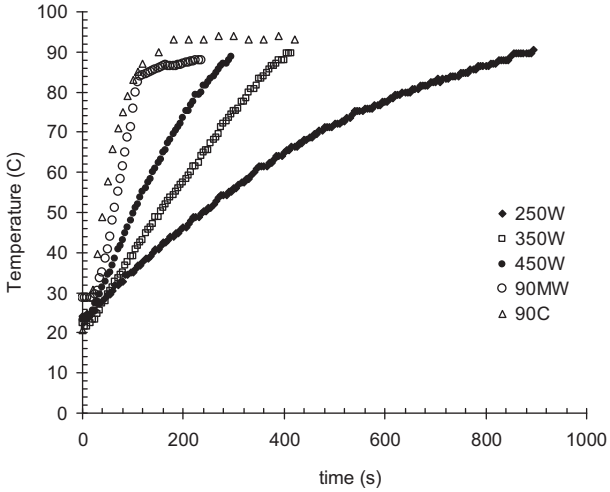

Fig. 4. Thermal profiles recorded during microwave treatments performed on red beet cylinders immersed in water, at 250 W, 350 W, 450 W or under a power managed to accomplish a similar temperature profile (90 MW) to the traditional blanching (90C), whose temperature profile is also shown.

energy throughout the volume of the material, the potential exists to reduce processing time and enhance overall quality to accomplish POX and PPO inactivation, as indicated by Matsui et al. (2008). Since faster enzyme inactivation was found at 90 MW though the temperature profile was similar to that involved in 90C processing, it can be inferred the existence of an additional non-thermal effect of microwaves. When comparing the $D_{T_{ref}}$ for both enzymes, it can be observed that a slightly higher value was obtained for POX at 250 W while PPO showed higher resistance for 350 W, 450 W and 90 MW processing. By using high pressure carbon dioxide (HPCD) treatment at 4.5 MPa, Liu et al. (2008a,b) determined that the time needed for PPO destruction in red beet extracts was 52.6 min, whereas 87.7 min were necessary for POX inactivation, this enzyme being the most resistant. According to Matsui et al. (2008), for temperatures above 77 °C and in coconut water, PPO was more resistant to microwave processing than POX. It can be also observed in Table 2 that the $D_{T_{ref}}$ for POX or for PPO decreased one order of magnitude for output powers higher than 250 W.

It was determined that the temperature profile of water used for tissue soaking was either coincident with the temperature of the center of tissue cylinders (treatment 90 MW) or lower than the one recorded from the central position of tissue cylinders (250 W, 350 W, 450 W treatments). Hence, heat transfer from the water to the tissue samples can be discarded for microwave treatments as a mechanism which might contribute to the increase of tissue temperature. This trend is opposite to the one generally observed during traditional blanching (i.e. treatment 90C) where heat is transferred from the water to the tissue samples due to the higher water temperature.

The ability of a specific solvent to convert microwave energy into heat at a given frequency and temperature is determined by the so-called loss tangent ($tan\,\delta$), expressed as the quotient between ε'' or dielectric loss (indicative of the efficiency by which electromagnetic radiation is converted into heat) and ε' (the dielectric constant describing the ability of molecules to be polarized by the electric field). A reaction medium with a high $tan\,\delta$ at the standard operating frequency of a microwave synthesis reactor (2.45 GHz) is required for good absorption and, consequently, efficient heating (Dallinger and Oliver Kappe, 2007). Water presents a $tan\,\delta$ value of 0.123 (Wathey et al., 2002) whereas ethanol is characterized by a $tan\,\delta$ of 0.941 (ε_r = 30 at 20 °C). The relatively low value of $tan\,\delta$ justifies the smaller temperatures shown by pure

water where red beet tissue cylinders were soaked during microwave treatments, whereas temperature into the central point of the tissue cylinder was higher, since the presence of ions or polar molecules in (cytoplasmic) solution significantly increased the ability of the solvent to convert electromagnetic radiation into heat (Wathey et al., 2002; Dallinger and Oliver Kappe, 2007).

Perreux and Loupy (2001) reported that specific microwave effects which are non-purely thermal can occur. These effects can be understood by consideration of the Arrhenius law [$k = A.\exp(-\Delta G^{\neq}/RT)$] as they can result from modifications in the terms of this equation. These specific effects of microwaves might explain the lower $D_{T_{ref}}$ values observed for treatments 350 W, 450 W and 90 MW in comparison to 90C. Liu et al. (2010) and Heddleson and Doores (1994) also reported non-thermal effects for enzymes destruction. Other studies, like the one of Shazman et al. (2007) concerning the Maillard reaction, protein denaturalization and polymer solubility, informed that experimental data failed to show significant non-thermal effects.

Table 2 shows the z values (temperature sensitivity) which, in general, decreased with increasing output power. For POX, the 90 MW treatment (maximum power ≅935 W) showed the highest sensitivity to the temperature change (lower z) among microwave processings and its value was similar to the one obtained for 90C blanching. For PPO, the lowest z values observed were those corresponding to 350 W and 90 MW treatments, which were similar one with the other. The traditional blanching (90C) showed the highest z value for PPO. It can be also observed that for 90 MW and 90C treatments, z values for PPO were higher than the ones for POX, showing a higher sensitivity to temperature for the last enzyme.

3.3. Texture

3.3.1. Compression

The ratio of the firmness or resistance to fracture for treated tissue to the firmness of control raw tissue (Fig. 5) did not show significant differences between treatments. All treatments produced a firmness decrease of ≅40–50%. These results of compression test until fracture can be firstly ascribed to the loss in turgor pressure and, secondly, to some additional breaking of cross-links in the cell wall and middle lamellae polysaccharides which are involved in cell-cell adherence (Latorre et al., 2010; Rojas et al., 2011). It is known that temperatures higher than 20 °C (Waldron et al., 1997a,b) can lead to β-elimination in the cell wall-middle lamellae pectins as well as to some degree of glycosidic hydrolysis (Fry, 1986).

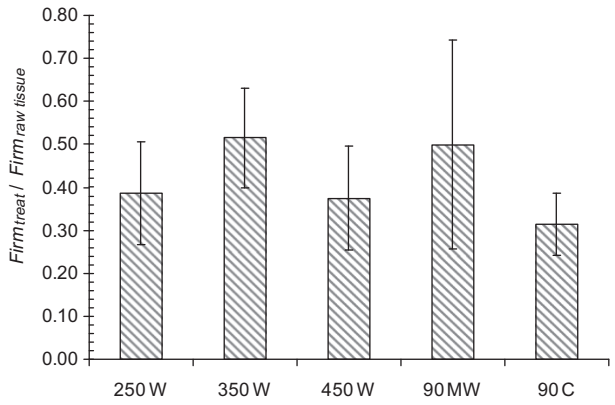

Fig. 5. The ratios between the resistance to failure or firmness of each red beet treated sample ($Firm_{treat}$) and firmness of the control (raw) tissue ($Firm_{raw,tissue}$) are plotted against the treatment performed: traditional blanching by immersion in water at 90 °C (90C), or microwaving at 250 W, 350 W, 450 W or under a power managed to accomplish a similar temperature profile (90 MW) to the traditional blanching. Bars indicate the standard deviation for $n = 10$.

3.3.2. Relaxation

Structural information can be achieved through non linear large deformation assays by using the relaxation test performed with an Instron machine. Relaxation data recorded from non fractured tissue samples allowed to calculate the change of the adimensional residual relaxation force [$F(t)/F_0$] with time for the different treatments. These data fitted to a mechanical model constituted by two Maxwell elements and a free spring (F_∞/F_0) (Fig. 6a). The adimensional residual relaxation force (F_∞/F_0), which is associated to the residual elastic behavior of tissue and mainly ascribed to its turgor pressure, decreased significantly after the processing of tissue, and especially after applying the 90 MW treatment. The Maxwell force component F_1/F_0 associated to the higher characteristic relaxation time (τ_1) significantly increased for all treatments; it showed a maximum value for traditional blanching and non significant differences among microwave treatments. The loss of residual elasticity (F_∞/F_0) that occurred after 90C tissue processing was mainly gained by the F_1/F_0 Maxwell force component with the lower proportion of energy loss by flow. The Maxwell force component F_2/F_0 associated to the fastest characteristic relaxation time (τ_2) was considerable lower than F_1/F_0 for control tissue, whereas showed a significant increase for all treatments, with a maximum for 90 MW blanched tissue, indicating that the latter was the lowest structured viscoelastic material. The adimensional residual (F_∞/F_0) as well as the first (F_1/F_0) or second (F_2/F_0) Maxwell relaxation forces associated to each characteristic relaxation time (τ_1) were inversely related and their changes with processing can be ascribed

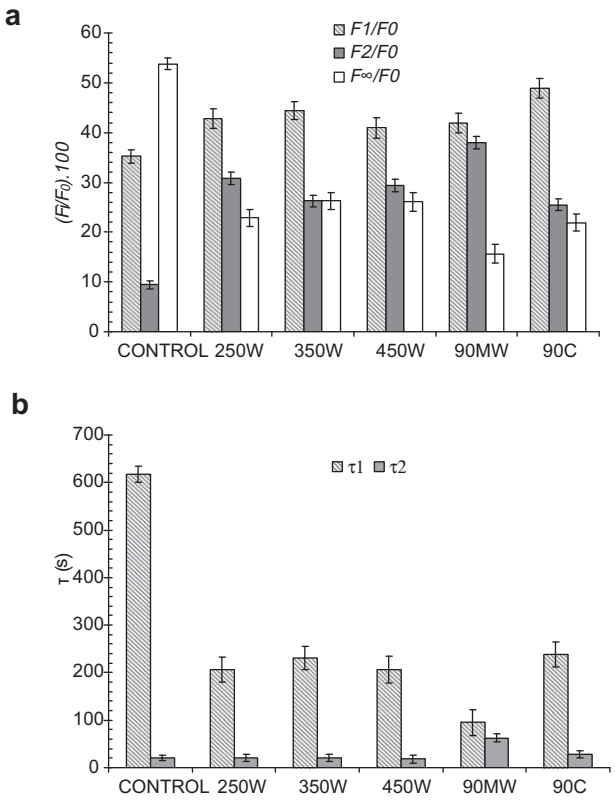

Fig. 6. Relaxation parameters obtained from adjustment to the Maxwell model of the force experimental data [$F(t)$] recorded against time at a 10% constant deformation: normalized relaxation forces (a) and characteristic relaxation times (b). Treatments informed are those performed with microwave radiation at 250 W, 350 W, 450 W or under a power managed to accomplish a similar temperature profile (90 MW) to the traditional blanching (90C). Parameters obtained from control or raw (untreated) tissue are also shown. Bars indicate the standard deviation for $n = 10$.

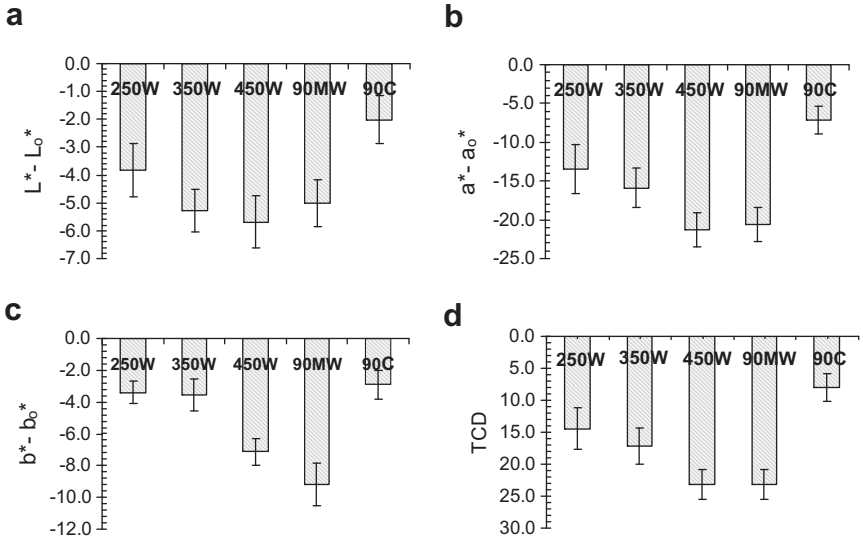

Fig. 7. Change in (CIE) color space coordinates L^* (lightness; a), a^* (grade of greenness/redness; b) and b^* (grade of blueness/yellowness; c) as well as in the total color difference (TCD) evaluated through Eq. (8), for red beet root tissue treated by conventional (90C) or microwave blanching at 250 W, 350 W, 450 W or under a power managed to accomplish a similar temperature profile (90 MW) to the traditional blanching (90C). Bars indicate the standard deviation for $n = 10$.

to decrease in crosslinking density associated with changes in middle lamellae and cell wall polysaccharides as well as to turgor pressure loss (Fry, 1986; Latorre et al., 2010).

For all treatments, the higher characteristic relaxation time (τ_1) decreased in comparison with control tissue (Fig. 6b). Moreover, 90 MW blanched tissue showed the lowest value of the characteristic relaxation time τ_1 and the highest value of τ_2, both factors indicating that a higher proportion of the total stress applied was lost through flow. Although the tissue submitted to 90 MW or 90C treatments showed similar temperature profiles (Fig. 4), a greater damage was observed for the former and this trend can be ascribed to the non-thermal effect of microwaves, at powers higher than 900 W. Somani Omprakash et al. (2003) mentioned the possibility of the rupture of hydrogen bonds after microwave treatments, which might proceed as a consequence of the decrease in the energy barrier of reactions or of the pre-exponential related factor, as above mentioned (Bohr and Bohr, 2000; Perreux and Loupy, 2001).

3.4. Color

Red beet accumulates betacyanins in the store root (Sepúlveda-Jiménez et al., 2004). Betalains (betacyanins and betaxanthins), the main pigments of red beet roots, protect plants from damage caused by UV and visible light, in addition to other functions (Yoshikazu et al., 2008). Betalains are located in the vacuole of these Caryophyllales plant cells. Thus, as turgor pressure is lost as a consequence of the processing of red beet tissue which primarily produces cell membrane denaturalization, color can be affected as a result of the pigment decompartimentalization.

As can be observed in Fig. 7, all treatments, in general, produced a decrease in the L^* (lightness), a^* (greenish shift) and b^* (blue shift) parameters. This trend seemed to be enhanced by power increase between 250 and 450 W although differences are not always significant. The same trend was observed for tissue submitted to traditional blanching (90C treatment) but L^*, a^* and b^* showed the smallest change with respect to control tissue, for this treatment. As a consequence, the total color difference (TCD) was lower for traditional blanching in water bath at 90 °C, whereas TCD increased with the microwave power between 250 and 450 W, with no additional change for 90 MW treatments.

4. Conclusions

Direct microwave blanching on red beet resulted in large weight loss and tissue shrinking. Microwave blanching in tissue immersed in water was explored as an alternative. The kinetic parameters $D_{T_{ref}}$ (T_{ref} = 90 °C) and z could be determined observing that, in general, PPO was more resistant and less sensitive to temperature than POX. All treatments reduced the elastic characteristics and changed the color of the tissues. It is important to state that trends reported for texture and color are specific for the experimental conditions used.

The present work showed the feasibility of using microwaves for blanching of red beet root tissue. The organoleptic quality of the final product, with special reference to color and texture, must be taken into account when selecting the process conditions used to accomplish enzyme inactivation.

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